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ESPECIALIDADE EM NUTRIÇÃO E QUÍMICA DOS ALIMENTOS

Influência do processamento no perfil lipídico de alimentos processados: aspetos nutricionais e toxicológicos

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Tese de Doutoramento

Influência do processamento no perfil lipídico de alimentos processados: aspetos nutricionais e toxicológicos

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Orientação

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Aos meus pais, marido e filha.

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O valor das coisas não está no tempo que elas duram,
mas na intensidade com que acontecem.
Por isso, existem momentos inesquecíveis,
coisas inexplicáveis e pessoas incomparáveis.

Fernando Pessoa

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Resumo

Nas últimas décadas, com a industrialização e a globalização dos sistemas alimentares, o processamento dos alimentos evoluiu rapidamente, contribuindo para a grande variedade de alimentos sujeitos a diferentes tipos de processamento, com diferentes impactos na saúde. Por seu lado, os consumidores contribuem para esta situação pois procuram cada vez mais os alimentos processados por serem práticos, saborosos, atrativos, acessíveis e económicos. O consumo inadequado deste tipo de alimentos está associado ao desenvolvimento precoce de doenças crónicas não transmissíveis, principalmente por terem elevados teores de sal, gordura (saturada e *trans*) e açúcar. Estima-se que os hábitos alimentares inadequados contribuam, na população portuguesa, para a perda de 15,4% de anos de vida saudável.

O processamento dos alimentos está, frequentemente, relacionado com potenciais consequências negativas na qualidade nutricional e segurança dos alimentos. Podem formar-se compostos indesejáveis com potencial toxicológico, como por exemplo, os 4-hidroxi-2-alcenais, cloropropanóis e ésteres glicidílicos de ácidos gordos. O 4-hidroxi-2-nonenal e o 4-hidroxi-2-hexenal são produtos da peroxidação lipídica de ácidos gordos polinsaturados, ómega-6 e ómega-3, respetivamente. Devido à elevada reatividade e atividade biológica destes compostos, os trabalhos de investigação sobre a sua ocorrência em alimentos aumentaram significativamente, especialmente em óleos e gorduras. Os cloropropanóis (3-monocloropropano-1,2-diol e 2-monocloropropano-1,2-diol) e os ésteres glicidílicos de ácidos gordos tornaram-se mais uma preocupação para a saúde, devido à sua presença em alimentos. São considerados tóxicos e potencialmente mutagénicos, sendo contaminantes do processamento alimentar. Estão já definidos teores máximos destes compostos para diferentes categorias de alimentos.

Os objetivos principais desta tese consistiram em desenvolver e aprofundar o conhecimento científico relativo à qualidade nutricional e segurança dos alimentos processados, com vista à identificação de áreas prioritárias de intervenção; aprofundar o conhecimento sobre a formação, ocorrência e mitigação de compostos potencialmente carcinogénicos e tóxicos; e contribuir com informação fundamental para a formulação de políticas alimentares e de nutrição que visem a melhoria de estado de saúde e nutricional da população.

A qualidade nutricional e a conformidade da rotulagem foram avaliadas em 9 categorias de alimentos processados (produtos de pastelaria, produtos de panificação, *snacks*, *fast-food*, batatas, cereais, frutos secos e oleaginosas, refeições prontas a comer e molhos). Sempre

que possível foram adquiridas amostras de produtos similares de marcas diferentes, e também com e sem glúten. Para avaliação da qualidade nutricional determinaram-se analiticamente os teores de sal, gordura, colesterol e composição em ácidos gordos. Foram aplicadas diferentes técnicas de processamento (fritura e/ou confeção no forno) a alimentos com composição nutricional distinta, nomeadamente *nuggets* de frango pré-fritos, óleos alimentares, batatas ultracongeladas e pizas frescas. Para avaliar a conformidade da rotulagem, foram incluídos os resultados obtidos após aplicação dos valores das tolerâncias, definidas na legislação, para o teor de sal, de gordura e de ácidos gordos saturados.

De uma forma geral, a maioria das categorias de alimentos processados continuam a apresentar teores de sal e de gordura saturada acima dos valores desejados. No entanto, os teores de ácidos gordos *trans* encontrados foram baixos, indicando que estes alimentos são seguros relativamente a este parâmetro. Os resultados da avaliação da conformidade dos valores declarados nos rótulos, evidenciam que apenas 50% das amostras apresentaram valores dentro das tolerâncias definidas para o teor de sal, mas a maior parte das amostras estavam em conformidade para os teores de gordura e de ácidos gordos saturados.

Óleos, gorduras e alimentos com alto teor de ácidos gordos polinsaturados, requerem especial atenção relativamente à ocorrência dos 4-hidroxi-2-alcenais. A tendência atual de consumo de alimentos ricos nestes ácidos gordos, poderá representar um aumento no risco de exposição a estes compostos. O óleo de palma e derivados apresentaram uma elevada variabilidade na ocorrência de cloropropanóis e ésteres glicidílicos, que se deve sobretudo a diferentes práticas pré- e pós-colheita do fruto da palma, bem como às condições de processamento. Para ambos os compostos são necessários mais trabalhos sobre o impacto dos métodos de confeção, nomeadamente a fritura, utilizando condições reais (utensílios, temperatura e diferentes tipos de alimentos).

Devido ao elevado consumo dos alimentos processados pelas diferentes faixas etárias da população, e pelo seu impacto na saúde, é fundamental continuar a desenvolver investigação, sem conflito de interesses, promovendo estratégias que envolvam a indústria alimentar e as autoridades com competências em Saúde Pública, visando contribuir para alimentos processados mais seguros e equilibrados nutricionalmente.

Palavras-chave: alimentos processados, qualidade nutricional, cloropropanóis, 4-hidroxi-2-alcenais, segurança alimentar.

Abstract

In the last decades, with the industrialization and globalization of food systems, food processing has grown rapidly, contributing to the wide variety of foods subject to different types of processing, with different health impacts. Furthermore, consumers also contribute to this situation, because they are increasingly looking for processed foods, which are practical, tasty, attractive, and economic. The inadequate consumption of this type of food is associated with the early development of noncommunicable diseases, mainly due to the high levels of salt, saturated and *trans* fats, and sugar. It is estimated that inadequate eating habits contribute, in the Portuguese population, to the loss of 15.4% of healthy life years.

Food processing is often related to potential negative consequences on the nutritional quality and food safety. Undesirable compounds with toxicological potential can be formed, for example 4-hydroxy-2-alkenals, chloropropanols and glycidyl esters of fatty acids. 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal are lipid peroxidation products of omega-6 and omega-3 polyunsaturated fatty acids, respectively. Due to the high reactivity and biological activity of these compounds, research on their occurrence in foods has increased significantly, especially in oils and fats. Chloropropanols (3-monochloropropane-1,2-diol and 2-monochloropropane-1,2-diol) and glycidyl esters of fatty acids have become a health concern due to their presence in food. They are contaminants of food processing, being considered toxic and potentially mutagenic. Maximum levels of these compounds have already been defined for different food categories.

The main objectives of this thesis were to develop and deepen the scientific knowledge regarding the nutritional quality and safety of processed foods, in order to identify priority areas for intervention; to deepen knowledge about the formation, occurrence and mitigation of potentially carcinogenic and toxic compounds; and to contribute with fundamental information for the implementation of food and nutrition policies aiming to improve the health and nutritional status of the population.

The nutritional quality and compliance of the labelling were evaluated in 9 categories of processed foods (pastry products, bakery products, snacks, fast food, potatoes, cereals, nuts and oilseeds, ready-to-eat meals and sauces). Whenever possible samples of similar products from different brands were obtained, as well as foods with and without gluten. To evaluate the nutritional quality, the levels of salt, fat, cholesterol and fatty acid composition were analytically determined. Different processing techniques (frying and/or oven baking) were applied to foods with distinct nutritional composition, namely pre-fried chicken nuggets, edible oils, pre-fried potatoes and fresh pizzas. In order to assess the accuracy of

the labelling, the results obtained after applying the tolerance values defined by the legislation for the salt, fat and saturated fatty acids content were included.

In general, most categories of processed foods continue to have contents of salt and saturated fat above the desired levels. However, the amounts of *trans* fatty acids found were low, indicating that these foods are safe for this parameter. The results of the accuracy assessment of the declared values on the labels show that only 50% of the samples had values within the defined tolerances for the salt content, but most of the samples were in compliance for fat and saturated fatty acids content.

Oils, fats and foods with a high content of polyunsaturated fatty acids require special attention on the occurrence of 4-hydroxy-2-alkenals. The current trend of consumption of foods rich in these fatty acids may represent an increased risk of exposure to these compounds. Palm oil and derivatives showed a high variability in the occurrence of chloropropanols and glycidyl esters, which is mainly due to different pre- and post-harvest practices applied to the palm fruit, as well as the processing conditions. For both compounds, more work is needed on the impact of cooking methods, namely frying, using real conditions (equipment, temperature and different types of food).

Due to the high consumption of processed foods by the different age groups of the population, and their impact on health, it is fundamental to continue developing research, with no conflict of interest, by promoting strategies that involves the food industry and authorities with responsibilities in Public Health, aiming to contribute to safer and nutritionally balanced processed foods.

Keywords: processed foods, nutritional quality, chloropropanols, 4-hydroxi-2-alkenals, food safety.

Publicações e comunicações desenvolvidas no âmbito do projeto de doutoramento

Capítulo de livro

1. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. (2018). 4-Hydroxy-2-Alkenals: A Potential Toxicological Concern of Vegetable Oils?. *Reference Module in Food Science*. Elsevier, pp. 1–8

Publicações de artigos em revistas de circulação internacional

1. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Sanches-Silva, A., Costa, H.S. (2016). Cholesterol determination in foods: Comparison between high performance and ultra-high performance liquid chromatography. *Food Chemistry*, 193, 18–25. DOI: http://dx.doi.org/10.1016/j.foodchem.2014.09.109
2. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Sanches-Silva, A., Bento, A.C., Costa, H.S. (2016). The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. *Food & Function*, 7, 2736–2746. DOI: 10.1039/c6fo00353b
3. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2017). Effects of industrial processing on the quality and safety of ready-to- eat patties. *Brazilian Journal of Food Technology*, 20, e2017012
4. **Albuquerque, T.G.**, Silva, M.A. Silva, Oliveira, M.B, Costa, H.S. (2017). *trans* Fatty Acids Content in Worldwide Edible Fats and Oils: Current Trends and Challenges. *International Journal of Food and Biosystems Engineering*, 3, 56–65
5. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. (2017). 4-Hydroxynonenal: A parameter of quality and safety of vegetable oils. *EC Nutrition*, ECO.01, 27–29
6. **Albuquerque, T.G.**, Santos, J., Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2017). Multivariate characterization of salt and fat contents, and fatty acids profile of pastry and bakery products. *Food & Function*, 8, 4170–4178. DOI: 10.1039/C7FO01191A
7. **Albuquerque, T.G.**, Santos J., Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2018). An update on processed foods: Relationship between salt, saturated and *trans* fatty acids contents. *Food Chemistry*, 267, 75–82. DOI: 10.1016/j.foodchem.2018.01.029
8. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. (2018). Cómo mejorar la calidad nutricional de las galletas tipo María. *Revista Alimentaria*, 493, 40–42

9. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. (2018). 3-MCPD Occurrence in Vegetable Oils**:** Impact on Human Nutrition and Future Challenges. *EC Nutrition*, 13.7, 455–469
10. **Albuquerque, T.G.**, Costa, H.S., Silva, M.A., Oliveira, M.B.P.P. (2018). Are chloropropanols and glycidyl fatty acid esters a matter of concern in palm oil? *(submetido)*
11. **Albuquerque, T.G.**, Costa, H.S., Silva, M.A., Oliveira, M.B.P.P. (2018). Compliance with EU tolerance limits of declared vs. analysed values for mandatory nutrients in prepacked foods *(submetido)*
12. **Albuquerque, T.G.**, Costa, H.S., Oliveira, M.B.P.P. (2018). 4-hydroxy-2-alkenals in foods: State-of-the-art and challenges for the future *(submetido)*

Publicações de artigos em revistas de circulação nacional

1. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2017). Bolacha Maria ou de água e sal: análise comparativa. *Boletim Epidemiológico Observações Especial*, 9, 64–67
2. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2017). Produtos de pastelaria com e sem glúten: descubra as diferenças nutricionais. *Sem Glúten - Revista da Associação Portuguesa de Celíacos*, 46, 22–23
3. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2016). Haverá diferenças nutricionais entre produtos de pastelaria com e sem glúten? *Boletim Epidemiológico Observações Especial*, 8, 21–24
4. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2016). Sementes edíveis: composição em ácidos gordos e impacto na saúde. *Boletim Epidemiológico Observações Especial*, 8, 12–16
5. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. (2016). Consumo de sal e efeitos na saúde na perceção do consumidor: resultados preliminares. *Boletim Epidemiológico Observações*, 15, 9–11

Publicações em actas de encontros científicos

1. Silva, M.A., **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. (2016). Fritura da batata: influência do corte na absorção de gordura. *Ata do 13º Encontro de Química dos Alimentos*, Porto, Portugal. ISBN: 978-989-8124-15-9

Publicações de resumos em revistas internacionais

1. **T.G. Albuquerque**, M.B.P.P. Oliveira, M.A. Silva, J. Santos, H.S. Costa. (2018). Is the nutritional quality of portuguese processed foods a cause of concern from a public health point of view? Translational Research and Innovation in Human and Health Science, *Annals of Medicine*, 50:sup1, S118, DOI: 10.1080/07853890.2018.1427445 DOI: 10.1080/07853890.2018.1427445
2. **T.G. Albuquerque**, M.A. Nunes, M.B.P.P. Oliveira, H.S. Costa. (2018). Accuracy of labeled nutrition declaration among cereal products. Translational Research and Innovation in Human and Health Science, *Annals of Medicine*, 50:sup1, S132, DOI: 10.1080/07853890.2018.1427445 DOI: 10.1080/07853890.2018.1427445
3. **T.G. Albuquerque**, H.S. Costa, M.A. Silva, M.B. Oliveira. (2017). "Snacks" salgados *vs* bolachas: Que tipo de gordura fornecem? *Revista Española de Nutrición Comunitaria*, *Spanish Journal of Community Nutrition*, 1, 46

Publicações de resumos em revistas nacionais

1. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. (2018). Identification of priority categories of processed foods for salt reduction initiatives in Portugal. *Acta Portuguesa de Nutrição*, 13: 48
2. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. (2018). Influence of processing conditions on 4-hydroxy-2-alkenals occurrence in edible oils. *Acta Portuguesa de Nutrição*, 13: 53
3. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. (2017). Cream crackers vs. "Maria" cookies: which are the main diferences? *Acta Portuguesa de Nutrição*, 9: 64
4. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. (2016). Teor de sal e de gordura em refeições prontas a comer. *Acta Portuguesa de Nutrição*, 5: 46

Comunicações orais por convite

1. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Qualidade dos diferentes óleos vegetais. Seminário - Óleos alimentares: Mitos e Realidades*, 16 de fevereiro de 2018, Beja, Portugal

2. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *New sources of edible oils: a sustainability approach*. Encontros com Ciência no DAN. 12 de janeiro de 2018, Lisboa, Portugal
3. **Albuquerque, T.G.**, Costa, H.S. *Rotulagem de alimentos*. I Jornadas de Alimentação e Nutrição, Atlântica University Higher Institution, 20 de abril de 2017, Lisboa, Portugal
4. **Albuquerque, T.G.**, Costa, H.S. *Composição nutricional*. I Jornadas de Alimentação e Nutrição, Atlântica University Higher Institution, 20 de abril de 2017, Lisboa, Portugal
5. **Albuquerque, T.G.**, Costa, H.S., Oliveira, M.B.P.P. *Degradação de gorduras*. Congresso Internacional de Nutrição e Segurança Alimentar, 21-23 de outubro de 2016, Tábua, Portugal
6. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Qualidade dos diferentes óleos vegetais*. Seminário - Óleos alimentares: Mitos e Realidades, 7 de dezembro de 2016, Lisboa, Portugal
7. Costa, H.S., Oliveira, M.B.P.P., **Albuquerque, T.G.** *Promoção de uma Alimentação Saudável: Contributo do projeto PTranSALT*. 2º Simpósio Nacional - Promoção de uma Alimentação Saudável e Segura – Qualidade Nutricional & Processamento Alimentar (SPASS 2015), 26 de novembro de 2015, Lisboa, Portugal
8. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *PTranSALT: Resultados e perspetivas futuras*. *2º* Simpósio Nacional - Promoção de uma Alimentação Saudável e Segura – Qualidade Nutricional & Processamento Alimentar (SPASS 2015), 26 de novembro de 2015, Lisboa, Portugal

Comunicações orais

1. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Salt: Portuguese consumer's perception and its occurrence in processed foods.* 3rd International Congress of CiiEM-2018, 20–22 de junho de 2018, Portugal
2. **Albuquerque, T.G.**, Costa, H.S., Silva, M.A., Oliveira, M.B.P.P. *Are the occurrence of 4-hydroxy-2-nonenal and 4-hydroxy-2-hexenal a safety concern on vegetable oils?* EuroFoodChem XIX Conference, Budapeste, 4–6 de outubro de 2017, Hungria
3. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Santos, J., Costa, H.S*. Is the nutritional quality of Portuguese processed foods a cause of concern from a public health point of view?* 2nd International Congress of CiiEM - Translational Research and Innovation in Human in Health Sciences, 13 de junho de 2017, Monte de Caparica, Portugal

4. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *Application of gas chromatography to evaluate the nutritional quality of processed foods*. XVI Latin-American Congress on Chromatography & 9th National Meeting on Chromatography, 5–9 de janeiro de 2016, Lisboa, Portugal
5. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Fritura das batatas: impacto na qualidade nutricional*. V Jornadas de Ciências da Nutrição do ISCSEM. 25 de fevereiro de 2016, Monte de Caparica Portugal

Comunicações em painel

1. **Albuquerque, T.G.**, Costa, H.S., Oliveira, M.B.P.P. *Occurrence of 4-hydroxy-2-alkenals in foodstuffs: are we facing a challenge?* 32nd EFFoST International Conference, 6–8 de novembro de 2018, Nantes, França
2. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Natural antioxidants in mitigation of vegetable oils oxidation.* 32nd EFFoST International Conference, 6-8 de novembro de 2018, Nantes, França
3. **Albuquerque, T.G.**, Nunes, M.A., Oliveira, M.B.P.P., Costa, H.S. *Prepacked processed foods: accuracy of the nutritional labelled information*. NUTRIMAD 2018, 24–27 de outubro de 2018, Madrid, Espanha
4. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Current situation on trans fatty acids occurrence in processed foods commercialized in Portugal*. Ciência 2018 - Encontro com a Ciência e Tecnologia em Portugal, 2–4 de julho de 2018, Lisboa, Portugal
5. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *Fat and salt content of "Bolas de Berlim": a comparative study*. 3rd International Congress of CiiEM-2018, 20–22 de junho de 2018, Monte de Caparica, Portugal
6. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S*. Influence of processing conditions on 4-hydroxy-2-alkenals occurrence in edible oils*. XVII Congresso de Nutrição e Alimentação e I Congresso Internacional de Nutrição e Alimentação, 10–11 de maio de 2018, Lisboa, Portugal
7. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *Identification of priority categories of processed foods for salt reduction initiatives in Portugal*. XVII Congresso de Nutrição e Alimentação e I Congresso Internacional de Nutrição e Alimentação, 10–11 de maio de 2018, Lisboa, Portugal
8. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S*. A qualidade nutricional de produtos de pastelaria: destaque para os teores de gordura e sal*. 1st International

Meeting on Innovation & Development in the Food Sector, 5 de junho de 2018, Viseu, Portugal

9. **Albuquerque**, T.G., Costa, H.S., Oliveira, M.B.P.P. *Fatty acids analysis to differentiate nutritional quality of similar bakery products*. Analítica 2018, 26–27 de março de 2018, Porto, Portugal
10. **Albuquerque**, T.G., Costa, H.S., Oliveira, M.B.P.P. *Contribution of nuts for the daily intake of salt, fat and fatty acids*. Analítica 2018, 26–27 de março de 2018, Porto, Portugal
11. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. *Are chloropropanols and glycidyl esters a matter of concern in palm oil?* 31st EFFoST International Conference, 13–16 de novembro de 2017, Sitges, Espanha
12. **Albuquerque, T.G.**, Santos, J., Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *Evaluation of the potential impact of processed foods on public health*. 4º Simpósio Nacional "Promoção de uma Alimentação Saudável e Segura, Do Nutriente à Embalagem – Inovação e Desafios" – SPASS 2017, 21 de setembro de 2017, Lisboa, Portugal
13. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *An update on the current salt content of Portuguese processed foods*. Ciência 2017 - Encontro com a Ciência e Tecnologia em Portugal, 3–5 de julho de 2017, Lisboa, Portugal
14. **Albuquerque, T.G.**, Nunes, M.A., Oliveira, M.B.P.P., Costa, H.S. *Accuracy of labelled nutrition declaration among cereal products*. 2th International Congress of CiiEM - Translational Research and Innovation in Human Health Sciences, 11–13 de junho de 2017, Monte de Caparica, Portugal
15. **Albuquerque, T.G.**, Silva, M.A., Costa, H.S., Oliveira, M.B.P.P. *Worldwide comparison on trans fatty acids content in edible fats and oils: current trends and future challenges*. FABE2017 3rd International Conference, 1–4 de junho de 2017, Rhodes, Grécia
16. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Costa, H.S. *Salt and fat contents in a range of portuguese processed foods*. 1st Food Chemistry Conference, 30 de outubro–1 de novembro 2016, Amesterdão, Holanda
17. Silva, M.A., **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Fritura da batata: influência do corte na absorção de gordura*. 13º Encontro de Química dos Alimentos, 14-16 de setembro de 2016, Porto, Portugal
18. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. *Salt content of processed foods available in the portuguese market*. 13º Encontro de Química dos Alimentos, 14–16 de setembro de 2016, Porto, Portugal
19. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Silva, M.A., Costa, H.S. *Teor de sal e de gordura em refeições prontas a comer*. XV Congresso de Nutrição e Alimentação, 19–20 de maio de 2016, Porto, Portugal

20. Silva, M.A., **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Perfil de ácidos gordos de sementes: benefícios para a saúde*. XVIII Congresso Anual da APNEP, 12 de abril de 2016, Porto, Portugal
21. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Chromatographic analysis of hazardous compounds in frying oils*. XVI Latin-American Congress on Chromatography & 9th National Meeting on Chromatography, 5–9 de janeiro de 2016, Lisboa, Portugal
22. Silva, M.A., **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Qualidade nutricional de refeições prontas a comer com base no seu perfil de ácidos gordos*. XVI Latin-American Congress on Chromatography & 9th National Meeting on Chromatography, 5–9 de janeiro de 2016, Lisboa, Portugal
23. Silva, M.A., **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Perfil de ácidos gordos de hambúrgueres e sandes comercializados em cadeias de "fast-food"*. XVI Latin-American Congress on Chromatography & 9th National Meeting on Chromatography, 5–9 de janeiro de 2016, Lisboa, Portugal
24. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Sanches-Silva, A., Costa, H.S. *Avaliação dos teores de gordura e de sal em salgados prontos a comer*. 2º Simpósio Nacional - Promoção de uma Alimentação Saudável e Segura – Qualidade Nutricional & Processamento Alimentar (SPASS 2015), 26 de novembro de 2015, Lisboa, Portugal
25. **Albuquerque, T.G.**, Silva, M.A., Oliveira, M.B.P.P., Sanches-Silva, A., Costa, H.S. *Importância do perfil de ácidos gordos na qualidade nutricional de pizas*. 2º Simpósio Nacional - Promoção de uma Alimentação Saudável e Segura – Qualidade Nutricional & Processamento Alimentar (SPASS 2015), 26 de novembro de 2015, Lisboa, Portugal
26. **Albuquerque, T.G.**, Oliveira, M.B.P.P., Costa, H.S. *Consumo de sal e relação com a saúde: perceção do consumidor*. 2º Simpósio Nacional - Promoção de uma Alimentação Saudável e Segura – Qualidade Nutricional & Processamento Alimentar (SPASS 2015), 26 de novembro de 2015, Lisboa, Portugal

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Índice

Uma vez que o presente documento é bilíngue, o índice geral, de figuras, de tabelas, e a lista de abreviaturas e símbolos, incluem designações em português e/ou inglês de acordo com a forma como aparecem ao longo do documento.

Índice de figuras

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Índice de tabelas

Lista de abreviaturas

ACN	Acetonitrile
AGMI	Ácidos gordos monoinsaturados
AGPI	Ácidos gordos polinsaturados
AGS	Ácidos gordos saturados
AGT	Ácidos gordos *trans*
ANOVA	One-way analysis of variance
BHT	Butylated hydroxytoluene
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide
CONTAM	Panel on Contaminants in the Food Chain
DAD	Diode array detector
DCM	Dichlorometane
DHA	Docosahexaenoic acid
DNPH	2,4-dinitrophenylhydrazine
DRI	Dietary reference intake
E%	Energy intake
ECD	Electrochemical detector
EFSA	Autoridade Europeia para a Segurança dos Alimentos; European Food Safety Authority
EG	Ésteres glicidílicos de ácidos gordos
EI	Electron impact
EPA	Eicosapentaenoic acid
EU	European Union
FA	Fatty acids
FAMEs	Fatty acids methyl esters
FAO	Organização das Nações Unidas para Alimentação e Agricultura; Food and Agriculture Organization of the United Nations
FD	Fluorescence detector
GC	Gas chromatography
GC-ECD	Gas chromatography-electron capture detection
GC-FID	Gas chromatography with flame ionization detection

GC-MS	Gas chromatography with mass spectrometry
GC-MS/MS	Gas chromatography with mass spectrometry/mass spectrometry
GEs	Glycidyl fatty acid esters
GRAS	Generally Recognized as Safe
HPLC	High performance liquid chromatography
4-HOE	4-oxo-nonenal
HDE	4-hydroxy-2-decenal
HHE	4-hidroxi-2-hexenal; 4-hydroxy-2-hexenal
HNE	4-hidroxi-2-nonenal; 4-hydroxy-2-nonenal
HOE	4-hydroxy-2-octenal
HPLC-MS/MS	High performance liquid chromatography-mass spectrometry/mass sepctrometry
HS-SPME-GC-MS	Headspace with solid phase extraction and gas chromatography coupled to mass spectrometry detection
IAN-AF	Inquérito Alimentar Nacional e de Atividade Física
INSEF	Inquérito Nacional de Saúde com Exame Físico
IPA	Isopropanol
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LC-TOF-MS	Liquid chromatography/Time-of-Flight Mass Spectrometry
LDA	Linear Discriminant Analysis
LD_{50}	Median lethal dose
LOD	Limit of detection
LOQ	Limit of quantification
2-MCPD	2-monocloropropano-1,2-diol; 2-monochloropropane-1,3-diol
3-MCPD	3-monocloropropano-1,2-diol; 3-monochloropropane-1,2-diol; 3-monochloro-2-propanediol
nd	Not detected
NP-HPLC	Normal phase high performance liquid chromatography
MS	Mass spectrometry
MUFA	Monounsaturated fatty acids
OMS	Organização Mundial de Saúde
PC	Principal component
PCA	Principal Component Analysis
PHYSA	Portuguese Hypertension and Salt Study

PKO	Palm kernel oil
PO	Palm oil
PUFA	Polyunsaturated fatty acids
RP-HPLC	Reversed phase high performance liquid chromatography
RSD	Relative standard deviation
SCF	Scientific Committee for Food
SD	Standard deviation
SFA	Saturated fatty acids
S/N	Signal-to-noise
TCA	Tabela da Composição de Alimentos
TFA	*trans* fatty acids
TLC	Thin layer chromatography
TTC	Threshold of Toxicological Concern
UE	União Europeia
UHPLC	Ultra-high performance liquid chromatography
UK	United Kingdom
USDA	United States Department of Agriculture
UV	Ultraviolet
WHO	World Health Organization

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CAPÍTULO 1. Introdução geral, Motivação, Objetivos, Organização e Estrutura da tese

Neste capítulo identificam-se as principais motivações para o desenvolvimento desta tese, apresentando uma introdução geral aos temas abordados, nomeadamente a problemática dos alimentos processados (nutrientes e compostos indesejáveis) e a relação do seu consumo com a saúde.

São também apresentados os objetivos, bem como a organização e estrutura desta tese, onde estão descritas as principais tarefas delineadas para este trabalho.

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1.1 Introdução geral e motivação

A relação entre os teores de nutrientes e de compostos indesejáveis em alimentos processados, e o potencial impacto na saúde de quem os consome, motivou o desenvolvimento deste trabalho de investigação.

Nas últimas décadas, com a industrialização e a globalização dos sistemas alimentares, o processamento dos alimentos evoluiu rapidamente, contribuindo para uma imensa variedade de alimentos sujeitos a diferentes tipos de processamento, e que sobretudo têm diferentes impactos na saúde. O consumo inadequado dos alimentos processados está, muitas vezes, associado ao desenvolvimento precoce de doenças crónicas não transmissíveis, principalmente porque estes são reconhecidos como tendo elevados teores de sal, gordura (saturada e *trans*) e açúcar. Estima-se que os hábitos alimentares inadequados contribuem para a perda de 15,4% de anos de vida saudável na população portuguesa (1).

A atual pandemia de obesidade e outras doenças crónicas não transmissíveis relacionadas com os hábitos alimentares da população, constitui uma séria ameaça ao bem-estar futuro e à prosperidade económica, a nível Mundial. Atualmente, assiste-se a um novo paradigma relativamente ao estado de saúde e qualidade de vida da população, uma vez que as pessoas vivem mais anos, mas vivem com mais comorbilidades (diabetes, doenças cardiovasculares, doenças respiratórias, obesidade e doenças oncológicas). Estas doenças estão, frequentemente, associadas a mortalidade e morbilidade precoce, tendo um impacto significativo na economia nacional, principalmente devido à diminuição da produtividade, ao aumento do absentismo laboral e dos encargos com a saúde.

De acordo com a Organização Mundial de Saúde (OMS), as doenças crónicas não transmissíveis matam 41 milhões de pessoas por ano, o que representa 71% de todas as mortes no mundo. Estima-se que o número total anual de mortes por estas doenças aumentará para 55 milhões até 2030, se não forem tomadas medidas de prevenção, nomeadamente através da adoção de um estilo de vida saudável, que inclui uma alimentação variada e equilibrada, bem como a prática de exercício físico e a prevenção do excesso de peso.

Em Portugal, de acordo com os resultados do Inquérito Nacional de Saúde com Exame Físico (INSEF), em 2015, o estado de saúde da população portuguesa entre os 25 e os 74

anos de idade, caracterizava-se pela elevada prevalência de algumas doenças crónicas como a hipertensão arterial (36,0%), a obesidade (28,7%) e a diabetes (9,8%) (2).

Pelos diversos factos já evidenciados na literatura, e devido à necessidade de esclarecer alguns aspetos relacionados com o estudo dos alimentos processados e impacto na saúde, considerou-se de todo o interesse aprofundar o conhecimento nesta área.

Alimentos processados

Os consumidores procuram cada vez mais os alimentos processados sobretudo porque são práticos, saborosos, atrativos, acessíveis e económicos (3,4). Nos últimos trinta anos, o mercado de alimentos processados cresceu como nunca e todos os dias "novos" alimentos processados, com diferentes características, são disponibilizados no mercado (5). Devido à elevada disponibilidade, acessibilidade e variedade, é inevitável a sua presença na alimentação da população de todas as faixas etárias.

O processamento de alimentos evoluiu profunda e rapidamente nas décadas passadas, e está muitas vezes relacionado com potenciais consequências negativas na qualidade nutricional dos alimentos e, por sua vez, nos padrões alimentares da população e no aumento das doenças crónicas não transmissíveis. No entanto, o processamento de alimentos, *per si*, não deve ser encarado como um problema para a nutrição humana. Pelo contrário, desempenhou um papel fundamental na evolução da humanidade e das civilizações, tornando a alimentação mais segura e diversificada. Para além disso, é extremamente importante para prolongar o tempo de vida útil dos alimentos ou simplesmente para os tornar edíveis (6).

Durante muitos anos, não houve consenso sobre a definição de alimentos processados. Em 2010, a *International Food Information Council Foundation* definiu alimento processado como "todo o alimento onde ocorreu qualquer mudança intencional antes de estar disponível para consumo" (6). De acordo com o Departamento de Agricultura dos Estados Unidos da América, os alimentos processados são "qualquer produto agrícola bruto que tenha sido submetido a procedimentos que alterem o seu estado natural" (7). Recentemente, foi introduzido o sistema de classificação NOVA (Figura 1) que agrupa os alimentos, quanto à extensão e o propósito do processamento a que são submetidos, em quatro categorias: (alimentos *in natura* ou minimamente processados, ingredientes culinários processados, alimentos processados e alimentos ultraprocessados) (8). Desta forma, hoje em dia, é possível fazer uma distinção rigorosa dos alimentos por tipo de processamento e uma análise dos seus impactos na saúde (8).

Figura 1. Categorias de alimentos segundo o sistema de classificação NOVA (8).

Sal

O sal é um ingrediente, um condimento e um nutriente, desempenhando um papel fulcral na alimentação humana. No entanto, o seu uso em excesso está associado a um problema de saúde pública, a Hipertensão Arterial. Ao longo dos anos, tem-se verificado um aumento dos casos de hipertensão arterial, estimando-se que esta patologia esteja na origem de 7,5 milhões de mortes por ano (9).

A OMS recomenda um consumo de sal inferior a 5 g/dia para a prevenção de doenças cardiovasculares (10). De acordo com o Regulamento (UE) n.º 1169/2011 do Parlamento Europeu e do Conselho, de 25 de outubro de 2011, relativo à prestação de informação aos consumidores sobre os géneros alimentícios, a dose de referência para um adulto, para uma dieta padrão de 2000 kcal/8400 kJ, é 6 g de sal (11). Também de acordo com esta legislação é obrigatório incluir na declaração nutricional do rótulo o teor de sal dos alimentos pré-embalados, para permitir ao consumidor uma escolha consciente e informada no momento de aquisição dos produtos.

O consumo de sal está profundamente relacionado com o desenvolvimento de doenças cardiovasculares. Os alimentos na sua forma natural geralmente contêm sódio, mas na maioria dos alimentos está presente em baixas quantidades. O sódio é normalmente adicionado aos alimentos na forma de sal. Nos países desenvolvidos, cerca de 75-80% do sal é obtido pela ingestão de alimentos processados, 5-10% ocorre naturalmente nos alimentos e os restantes 10-15% resultam do sal adicionado durante a confeção dos

alimentos ou à mesa (9,10,12). Por outro lado, nos países em desenvolvimento, o sal usado para temperar ou em molhos desempenha um papel muito mais importante.

Do ponto de vista da indústria alimentar, o sal, para além do sabor que confere aos alimentos, desempenha um papel crucial na preservação e na capacidade de processamento dos alimentos (12). Quando se propõe uma redução do teor de sal nos alimentos, deve ter-se em conta que pode estar a quebrar-se a estabilidade microbiológica e a estrutura destes alimentos. Em alguns grupos de alimentos, como por exemplo os produtos de panificação e pastelaria, o sal tem um papel importante na fermentação e na aglutinação (13). Por outro lado, para o consumidor, o sal é um elemento importante para o sabor dos alimentos, para além de suprimir outros sabores menos apreciados. Quando as quantidades de sal são reduzidas drasticamente, o consumidor rejeita esses alimentos.

O consumo de sal em adultos na maior parte dos países Europeus (Figura 2) varia entre 7 e 13 g/dia, de acordo com os valores reportados pelos vários países à Comissão Europeia (14). Por outro lado, Powles et al. (2013) publicou valores para a ingestão de sal notavelmente mais baixos (15). A comparação destes dados está limitada por diferenças relativas ao método utilizado para estimar a ingestão de sal (questionário das 24h anteriores, questionários de frequência alimentar e/ou recolha da urina de 24h). De acordo com a literatura, a ingestão de sal estimada com base em questionários de hábitos alimentares pode traduzir-se em maiores desvios relativamente à realidade (17).

Em Portugal a ingestão de sal é superior aos valores recomendados. De acordo com Polónia et al. (2014), que avaliou a ingestão de sal em 3720 indivíduos (18 aos 90 anos de idade), a população portuguesa em média ingere 10,7 g/dia (16). Mais recentemente, a ingestão de sal foi um dos parâmetros monitorizados na população portuguesa pelo Inquérito Alimentar Nacional e de Atividade Física (IAN-AF) e os resultados são inferiores (7,4 g/dia) aos anteriormente reportados, no entanto continuam a ser superiores aos valores recomendados. Também se verificou que a ingestão de sal é superior nos homens em relação às mulheres (18).

De acordo com o Plano de ação global para a prevenção e controlo de doenças crónicas não transmissíveis (2013 - 2020) a OMS definiu como objetivo reduzir a ingestão de sal em 30% (19). Em 2015, a Sociedade Portuguesa de Hipertensão, em colaboração com as autoridades portuguesas, definiu uma estratégia política para os próximos cinco anos (2015-2020) sobre a redução do teor de sal nos alimentos portugueses. Um dos objetivos é reduzir a ingestão média de sal da população portuguesa abaixo de 10 g/dia até 2020 (20). Portanto, a estratégia desenvolvida baseia-se na reformulação de alimentos.

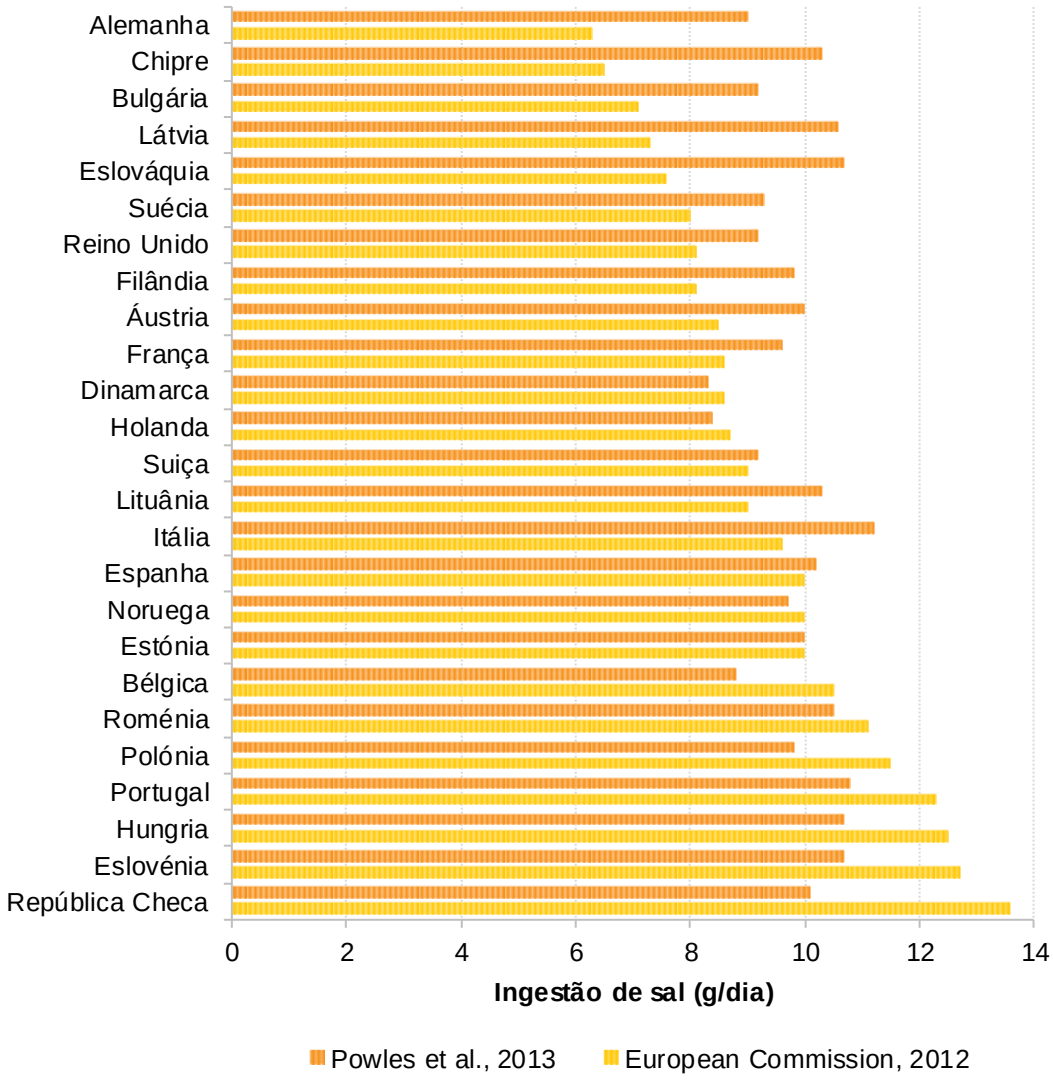

Figura 2. Ingestão média de sal em países Europeus (14,15,17).

No que diz respeito à reformulação de alimentos, em 2009 foi fixado, através de legislação nacional, o limite de 1,4 g/100 g para o teor de sal no pão (21). Recentemente, foi publicado o Despacho n.º 11418/2017 relativo à Estratégia Integrada para a Promoção da Alimentação Saudável, que entre outros temas, incide sobre a questão do sal, referindo que: (i) deve ser monitorizado o teor de sal em diversas categorias de alimentos processados (sopas, refeições, *snacks*, batatas fritas, queijos, conservas, cereais, entre outros); (ii) na sopa e prato de refeição, a quantidade de sal deve ser inferior a 0,2 g de sal/100 g de alimento; (iii) nos outros alimentos a quantidade de sal deve ser inferior a 0,3 g de sal por 100 g ou 100 mL de alimento; e (iv) deve fazer-se com que o consumo de sal *per capita* se aproxime de 5 g/dia em 2020 (22).

Gorduras

De entre os macronutrientes, a gordura dos alimentos tem sido dos mais estudados nas últimas décadas, com uma preocupação não apenas na quantidade ingerida, mas também na sua qualidade (23).

As gorduras dos alimentos são maioritariamente triglicéridos. O tipo de ácidos gordos presentes nos triglicéridos determina não só as características físico-químicas da gordura (como por exemplo a resistência ao ranço) mas também as suas propriedades nutricionais e os efeitos na saúde.

De entre os diferentes tipos de ácidos gordos, saturados (AGS), monoinsaturados (AGMI), polinsaturados (AGPI) e *trans* (AGT), os frequentemente associados a efeitos indesejáveis na saúde da população são os AGS e os AGT. A ingestão inadequada de alimentos ricos neste tipo de ácidos gordos foi fortemente relacionada com o aumento do risco de desenvolvimento de doenças cardiovasculares, obesidade, diabetes e cancro (24–26).

As recomendações para reduzir o risco de doenças cardiovasculares incluem a limitação da ingestão de gordura total, AGS e AGT, e o incentivo à ingestão de AGPI, uma vez que estes têm sido associados a benefícios para a saúde (27). No entanto, cada vez mais as evidências científicas sugerem que os diferentes tipos de AGS têm efeitos na saúde distintos. Por exemplo o ácido láurico (C12:0), o ácido mirístico (C14:0) e o ácido palmítico (C16:0) estão relacionados com o aumento do colesterol das lipoproteínas de baixa densidade, enquanto que o ácido esteárico (C18:0) parece não ter nenhum efeito (24). Considerando os resultados reportados no IAN-AF, 53% da população portuguesa apresenta uma ingestão de AGS superior ao valor recomendado, sendo mais notável nas crianças (73%) e nos adolescentes (72%), do que nos adultos (17).

Relativamente aos AGT existe evidência científica consistente de que uma ingestão inadequada de alimentos com estes ácidos gordos, provenientes de óleos parcialmente hidrogenados está associada ao aumento dos fatores de risco para as doenças cardiovasculares (24). Por este motivo, uma das estratégias que tem sido sugerida à indústria alimentar é limitar a utilização deste tipo de óleos na formulação dos seus produtos e substituí-los por outro tipo de óleos.

Existem três aspetos principais para a presença de AGT nos alimentos (Figura 3). Os AGT mais abundantes na alimentação humana são os isómeros do ácido oleico (C18:1) e do ácido linoleico (C18:2). Relativamente às principais fontes alimentares, os valores mais elevados de AGT foram determinados em produtos que continham na sua composição

óleos vegetais hidrogenados, enquanto os teores mais baixos foram reportados nos produtos lácteos.

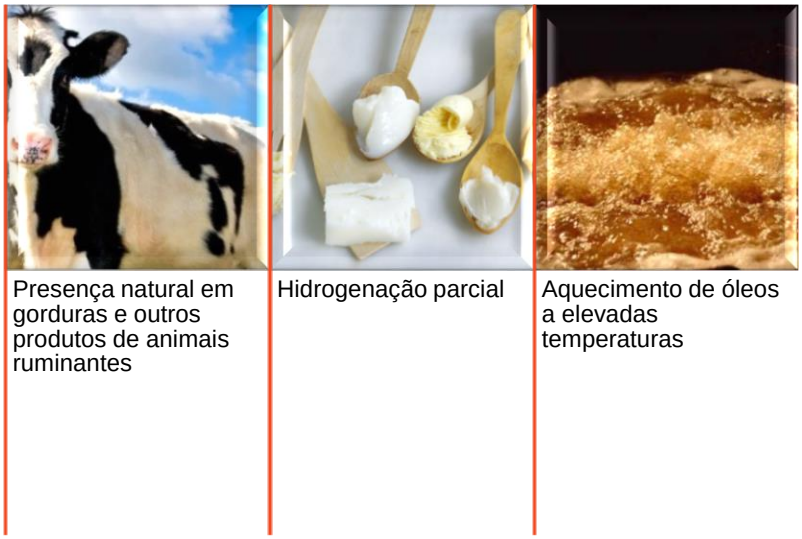

Figura 3. Principais aspetos relacionados com a presença e formação de ácidos gordos *trans* nos alimentos.

A maioria dos países europeus ainda não limita o teor de AGT nos alimentos, exceto Áustria, Suíça, Islândia, Noruega, Dinamarca e Hungria, que definiram um teor máximo de AGT de 2% na gordura total, em alimentos que não contém AGT de ruminantes (28). Além disso, a indicação do teor de AGT na declaração nutricional não é obrigatória, de acordo com o Regulamento (UE) n.º 1169/2011 (11).

A Organização das Nações Unidas para Alimentação e Agricultura (FAO) recomenda que o consumo de AGT deve ser o mais baixo possível (<1% do valor energético total), que para um adulto e uma dieta de 2000 kcal representa cerca de 2 g de AGT/dia (24). A Autoridade Europeia para a Segurança dos Alimentos (EFSA) publicou em 2010 um parecer científico sobre valores de referência para gorduras, incluindo AGS, AGMI, AGPI, AGT e colesterol (27). No entanto, neste documento não é definido um valor de referência para os AGT, sendo apenas recomendando que o seu consumo seja o mais baixo possível num contexto de uma alimentação nutricionalmente equilibrada (27).

De acordo com os resultados do IAN-AF (Figura 4), em média, a população portuguesa ingere cerca de 0,8 g/dia, sendo a maior prevalência verificada no grupo dos adolescentes (1,2 g/dia) (18).

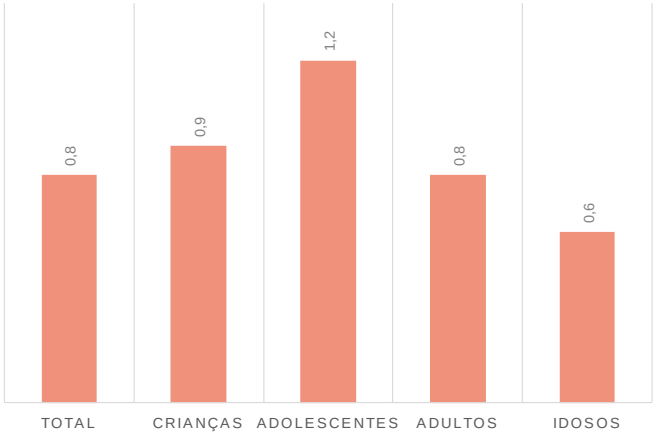

Figura 4. Ingestão média diária de ácidos gordos *trans*, em gramas, por grupo etário (IAN-AF 2015-2016) (18).

De acordo com a Estratégia Europeia para Prevenção e Controlo de Doenças Não Transmissíveis (2012-2016) foi proposta a "eliminação das gorduras *trans* nos alimentos (e a sua substituição por gorduras polinsaturadas)" (29). Para além disso, este objetivo específico também faz parte do Plano de Ação Europeu para Alimentação e Nutrição (2015-2020), cujo objetivo geral é melhorar a gestão do sistema alimentar, a qualidade da alimentação e do estado nutricional da população, e promover a saúde e o bem-estar (30).

Em Portugal, recentemente, também foram definidos objetivos relativamente a esta matéria, nomeadamente: (i) monitorizar o teor de AGT nas seguintes categorias de alimentos: bolachas, produtos de pastelaria, batatas fritas, cereais de pequeno-almoço, cremes de chocolate para barrar e margarinas; (ii) para as gorduras de origem industrial vendidas para o fabrico de produtos alimentares, bem como no produto final, deve ser estabelecido um teor em AGT não superior a 2 g por 100 g de gordura; e (iii) fazer com que o consumo de AGT se aproxime de zero em 2020 (22).

Rotulagem dos géneros alimentícios

A rotulagem nutricional é considerada uma ferramenta de saúde pública, e por esse motivo, entre outros, várias autoridades a nível mundial estabeleceram regulamentações sobre rotulagem nutricional.

O objetivo principal da informação dos rótulos dos alimentos é serem úteis para os consumidores avaliarem a qualidade nutricional de um produto específico. Assim, é possível o consumidor tomar uma decisão baseada numa escolha informada, e considerar as questões relacionadas com a saúde e as suas preferências alimentares (31,32). Por

outro lado, rótulos claros são um compromisso social dos produtores de alimentos e um desafio para desenvolver e/ou reformular produtos com uma qualidade nutricional desejável, por exemplo com menor teor de sal ou açúcar (33).

A consciência das escolhas alimentares saudáveis dos consumidores, e a transparência na relação entre a indústria alimentar e os consumidores, levaram ao desenvolvimento de uma legislação que abrangesse os vários Estados Membros da União Europeia (34).

A Comissão Europeia verificou que a informação nos rótulos dos alimentos pré-embalados, nos vários Estados-Membros, era feita de forma irregular e inconsistente. Por conseguinte, em 25 de outubro de 2011, a União Europeia (UE) publicou o Regulamento (UE) n.º 1169/2011 sobre a prestação de informação aos consumidores, com definições, princípios e procedimentos comuns relativos a este assunto, que possam garantir um equilíbrio entre a proteção do mercado interno e as diferenças de perceção dos consumidores nos Estados-Membros (11). Acima de tudo, a informação sobre os alimentos não deve induzir em erro os consumidores sobre a natureza, características e efeitos na saúde dos alimentos (11). Este regulamento é diretamente aplicável a todos os Estados-Membros da UE e entrou completamente em vigor em 13 de dezembro de 2016. A Figura 5 apresenta a informação obrigatória no rótulo de um alimento pré-embalado e informações opcionais relativas à declaração nutricional.

Os rótulos das declarações nutricionais fornecem informações sobre a composição dos alimentos, permitindo que os consumidores façam uma escolha informada e segura (35). De acordo com o Regulamento (UE) n.º 1169/2011, número 4, artigo 31.º, os valores declarados devem ser valores médios, estabelecidos, conforme o caso, a partir: (i) da análise do género alimentício efetuada pelo fabricante; (ii) do cálculo efetuado a partir dos valores médios conhecidos ou reais relativos aos ingredientes utilizados; ou (iii) do cálculo efetuado a partir de dados geralmente estabelecidos e aceites (11).

É consensual que o método mais preciso para determinar a composição nutricional dos alimentos é a abordagem analítica, preferencialmente por um laboratório acreditado ou laboratórios que participem com sucesso em testes de controlo laboratorial (36). No entanto, o elevado número de “novos” alimentos lançados todos os dias no mercado e o custo/tempo das análises são algumas das razões apontadas, pela indústria alimentar, para justificar a situação real. Portanto, muitas vezes, os produtores de alimentos escolhem calcular ou estimar a composição nutricional de seus produtos alimentares (37).

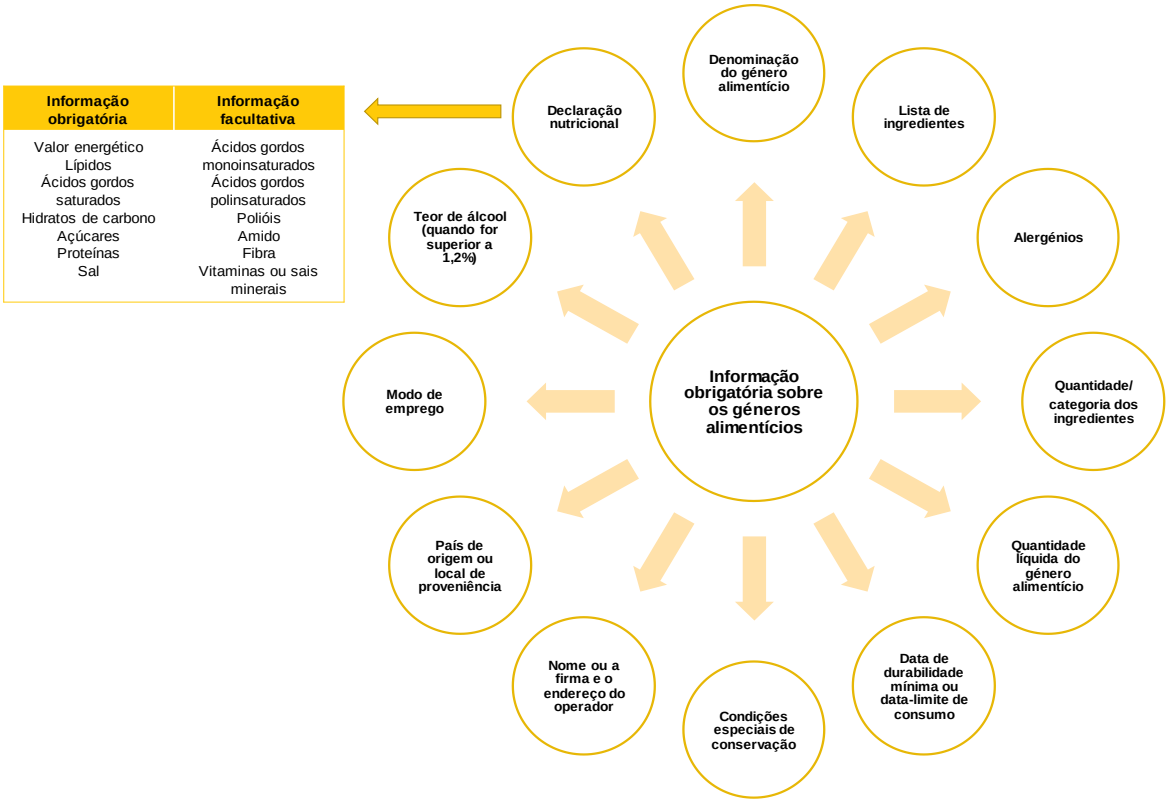

Figura 5. Informação sobre os géneros alimentícios de acordo com o Regulamento (UE) n.º 1169/2011 de 25 de novembro de 2011 (11).

Os alimentos estão naturalmente sujeitos a alterações de composição devido a muitos fatores, não sendo possível garantir que estão sempre presentes as mesmas quantidades de nutrientes. No entanto, as diferenças entre os valores declarados e os teores de nutrientes presentes realmente nos alimentos, não devem desviar-se substancialmente, a fim de evitar induzir o consumidor em erro (38). Por conseguinte, em 2012, a Comissão Europeia divulgou uma orientação às autoridades dos Estados-Membros e aos operadores das empresas do setor alimentar sobre as tolerâncias para fins de rotulagem nutricional (38).

No entanto deve ressalvar-se que sempre que o valor medido estiver fora da tolerância do valor declarado, este facto deve ser objeto de uma avaliação específica para se decidir se devem ser tomadas ações/medidas, nomeadamente considerando os parâmetros indicados na Figura 6.

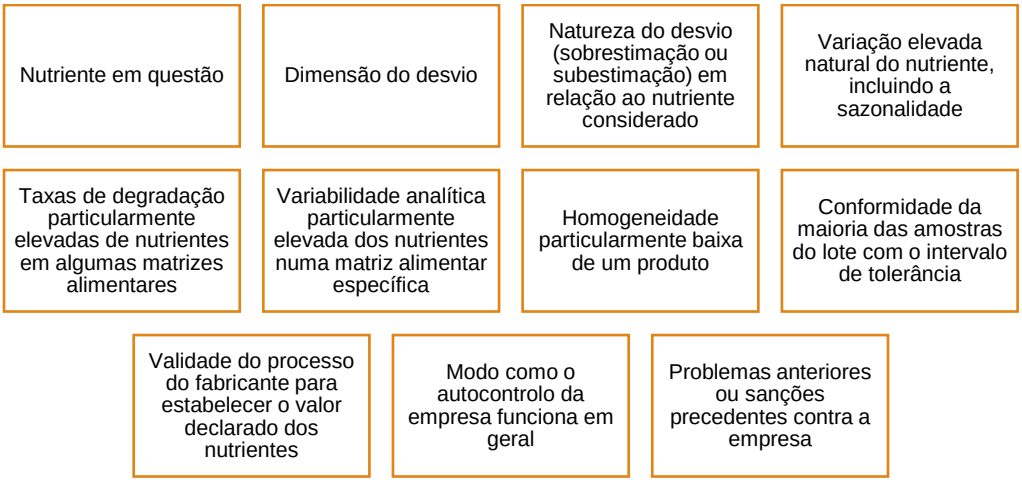

Figura 6. Aspetos a ter em consideração aquando da deteção de valores de nutrientes fora das tolerâncias (38).

4-Hidroxi-2-alcenais

A oxidação lipídica é responsável por perdas relevantes em toda a cadeia alimentar, com impacto em características importantes dos alimentos, como odor, sabor e textura (39,40). Durante a oxidação lipídica de AGPI são formados vários compostos indesejáveis, nomeadamente alcanos, cetonas, álcoois, furanos e aldeídos. Um dos grupos mais reativos de produtos de peroxidação lipídica são os aldeídos α,β-insaturados (39–42).

Os 4-hidroxi-2-alcenais são compostos altamente reativos formados por, pelo menos, três grupos funcionais, aldeído (CHO), alceno (C2 = C3) e um grupo que pode ser um hidroxilo (-OH), um hidroperoxilo (-OOH) ou um grupo oxo (39,42,43).

A presença de 4-hidroxi-2-alcenais em alimentos foi, já, detetada por Schauenstein na década de 1960 (41). Os 4-hidroxi-2-alcenais foram considerados os principais produtos da peroxidação lipídica e, destes, o 4-hidroxi-2-nonenal (HNE) é o mais estudado, seguido do 4-hidroxi-2-hexenal (HHE). HNE e HHE são produtos da peroxidação lipídica secundária de AGPI, ómega-6 e ómega-3, respetivamente (39,41,44).

Os AGPI estão presentes ampla e naturalmente em vários alimentos, mas nos últimos anos, devido à associação entre o consumo de AGPI e benefícios para a saúde, aumentou a tendência de enriquecer os alimentos com este tipo de ácidos gordos. Este comportamento pode levar ao aumento da exposição a estes compostos tóxicos, HNE e HHE. Além disso, os 4-hidroxi-2-alcenais também podem formar-se endogenamente, tendo sido encontrados em vários tecidos, órgãos e fluidos do organismo, em diferentes concentrações (45).

Nos últimos anos, o HNE e o HHE foram relacionados com várias doenças, como cancro, aterogénese, diabetes, inflamação crónica e doenças neurodegenerativas (doença de Alzheimer ou Parkinson), entre outros (41,43,45–47).

Devido à alta reatividade e atividade biológica destes compostos, os trabalhos de investigação sobre a sua ocorrência em alimentos aumentaram, especialmente em óleos e gorduras (48-56), bem como em alimentos enriquecidos com AGPI (57-59). Os resultados indicam que existem outras matrizes alimentares de interesse, nomeadamente peixe e derivados, leite, fórmulas infantis e outros produtos lácteos.

Cloropropanóis e ésteres glicidílicos de ácidos gordos

Nos últimos anos, os cloropropanóis, nomeadamente, o 3-monocloropropano-1,2-diol (3-MCPD) e os ésteres glicidílicos de ácidos gordos (EG) tornaram-se uma preocupação para a saúde, devido à sua presença em alimentos e serem considerados tóxicos e potencialmente mutagénicos.

Os clopropanóis são análogos clorados do glicerol que possuem um átomo de cloro nas posições 1 ou 2, e podem ocorrer em várias formas, como mono- ou diésteres (60). Em 1978, os cloropropanóis foram descritos pela primeira vez em hidrolisados de proteína vegetal, facto que levou ao aumento do interesse nestes compostos.

O 3-MCPD é um contaminante do processamento alimentar classificado como um possível agente cancerígeno para o ser humano. Devido ao seu potencial carcinogénico e genotóxico, a FAO, a OMS, a Comissão Europeia e o Painel de Especialistas em Aditivos Alimentares, estabeleceram uma ingestão diária máxima tolerável para o 3-MCPD de 2 µg/kg de peso corporal (61). Para além disso, para os hidrolisados de proteína vegetal e para os molhos de soja, foi definido pelo Regulamento (CE) n.º 466/2001 da Comissão de 8 de março de 2001, um teor máximo de 0,02 mg/kg de peso fresco, sendo feita a menção que este teor máximo foi definido para o produto líquido contendo 40% de matéria seca, correspondente a um teor máximo de 0,05 mg/kg na matéria seca (é necessário ajustar o teor proporcionalmente, em função do teor de matéria seca dos produtos) (62).

Em 2013, a presença de cloropropanóis foi, também, descrita noutros alimentos, como óleos vegetais, margarinas e alimentos processados a elevadas temperaturas (63). Com base nestes dados, a Comissão Europeia recomendou a monitorização dos teores de 2-monocloropropano-1,2-diol (2-MCPD), 3-MCPD e EG em outras categorias de alimentos (Figura 7).

Figura 7. Categorias de alimentos definidas pela Comissão Europeia para monitorização dos teores de cloropropanóis e ésteres glicidílicos de ácidos gordos.

Os EG são contaminantes do processamento, gerados durante a fase de desodorização, na refinação dos óleos alimentares. Estes compostos indesejáveis foram reportados pela primeira vez por Weißhaar & Perz (2010), como resultado de uma sobrestimação dos teores de 3-MCPD em óleos (64). Portanto, o interesse na investigação dos EG surgiu principalmente, da inconsistência dos dados da literatura para o 3-MCPD. Hoje em dia, estes compostos são considerados os principais contaminantes em óleos processados, formando-se, especialmente, durante as etapas de refinação e desodorização (65,66).

Em 2016, a EFSA, publicou os dados relativos à ocorrência de 3-MCPD, 2-MCPD e EG em alimentos, e os valores mais elevados foram reportados para o óleo/gordura de palma (67). Uma vez que o óleo de palma é dos mais utilizados para vários processos na indústria alimentar, mas também como um ingrediente de inúmeros alimentos processados, a presença destes compostos indesejáveis pode representar uma preocupação acrescida.

Recentemente, foi publicado o Regulamento (UE) n.º 2018/290 da Comissão que diz respeito aos teores máximos (Tabela 1) de EG em óleos e gorduras vegetais, fórmulas para lactentes, fórmulas de transição e alimentos para fins medicinais específicos destinados a lactentes e crianças jovens (68).

Tabela 1. Teores máximos de ésteres glicidílicos de ácidos gordos em diferentes géneros alimentícios (68).

Géneros alimentícios	Teores máximos (µg/kg)
Óleos e gorduras vegetais colocados no mercado para o consumidor final ou para utilização como ingrediente em géneros alimentícios	1000
Óleos e gorduras vegetais destinados à produção de alimentos para bebés e alimentos transformados à base de cereais destinados a lactentes e crianças jovens	500
Fórmulas para lactentes, fórmulas de transição e alimentos para fins medicinais específicos destinados a lactentes e crianças jovens (em pó)	75 até 30.6.2019 50 a partir de 1.7.2019
Fórmulas para lactentes, fórmulas de transição e alimentos para fins medicinais específicos destinados a lactentes e crianças jovens (em forma líquida)	10,0 até 30.6.2019 6,0 a partir de 1.7.2019

Referências

1. Ministério da Saúde. Retrato da Saúde - 2018 [Internet]. 2018. Disponível em: https://www.sns.gov.pt/retrato-da-saude-2018/
2. Barreto M, Gaio V, Kislaya I, Rodrigues AP, Silva AC, Vargas P, et al. 1º Inquérito Nacional de Saúde com Exame Físico. 2016. Disponível em: http://repositorio.insa.pt/bitstream/10400.18/4115/3/1_INSEF_relatório_estado-de-saude.pdf
3. Monteiro CA, Levy RB, Claro RM, Castro R De, Cannon G. Increasing consumption of ultra-processed foods and likely impact on human health: evidence from Brazil. Public Health Nutr. 2010;14(1):5–13.
4. Bielemann RM, Motta JVS, Minten GC, Horta BL, Gigante DP. Consumo de alimentos ultraprocessados e impacto na dieta de adultos jovens Consumption of ultra-processed foods and their impact on the diet of young adults. Rev Saúde Pública. 2015;49(28):1–10.
5. Stuckler D, McKee M, Ebrahim S, Basu S. Manufacturing epidemics: The role of global producers in increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. PLoS Med. 2012;9(6):10.
6. EUFIC. The European Food Information Council. The greatest thing since sliced bread? A review of the benefits of processed. 2010;1–6.
7. Dwyer JT, Fulgoni VL, Clemens RA, Schmidt DB, Freedman MR. Is “Processed” a Four-Letter Word? The Role of Processed Foods in Achieving Dietary Guidelines and Nutrient Recommendations. Adv Nutr. 2012;3(4):536–48.

8. Moubarac J-C, Parra DC, Cannon G, Monteiro CA. Food Classification Systems Based on Food Processing: Significance and Implications for Policies and Actions: A Systematic Literature Review and Assessment. Curr Obes Rep. 2014;3(2):256–72.
9. World Health Organization. A global brief on hypertension - World Health Day 2013 [Internet]. 2013. Disponível em: http://www.who.int/cardiovascular_diseases/publications/global_brief_hypertension/en/
10. World Health Organization. Mapping salt reduction initiatives in the WHO European Region. WHO publications. 2013.
11. Parlamento Europeu e do Conselho da União Europeia. Regulamento (UE) No. 1169/2011 do Parlamento Europeu e do Conselho de 25 de outubro de 2011 relativo à prestação de informação aos consumidores sobre os géneros alimentícios. J da União Eur. 2011;L 304:18–63.
12. Dötsch M, Busch J, Batenburg M, Liem G, Tareilus E, Mueller R, et al. Strategies to reduce sodium consumption: A food industry perspective. Crit Rev Food Sci Nutr. 2009;49(10):841–51.
13. Belz MCE, Ryan LAM, Arendt EK. The Impact of Salt Reduction in Bread: A Review. Crit Rev Food Sci Nutr. 2012;52(6):514–24.
14. European Commission. Survey on Member States' Implementation of the EU Salt Reduction Framework [Internet]. European Commission. 2012. Disponível em: https://ec.europa.eu/health//sites/health/files/nutrition_physical_activity/docs/salt_report1_en.pdf
15. Powles J, Fahimi S, Micha R, Khatibzadeh S, Shi P, Ezzati M, et al. Global, regional and national sodium intakes in 1990 and 2010: A systematic analysis of 24 h urinary sodium excretion and dietary surveys worldwide. BMJ Open. 2013;3(12).
16. Polonia J, Martins L, Pinto F, Nazare J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: Changes over a decade the PHYSA study. J Hypertens. 2014;32(6):1211–21.
17. Kloss L, Meyer JD, Graeve L, Vetter W. Sodium intake and its reduction by food reformulation in the European Union - A review. NFS J. 2015;1:9–19.
18. Lopes C, Torres D, Oliveira A, Severo M, Alarcão V, Guiomar S, et al. Inquérito Alimentar Nacional e de Atividade Fisica [Internet]. 2017. Disponível em: https://ian-af.up.pt/sites/default/files/IAN-AF Relatório Resultados_3.pdf
19. World Health Organization. Global action plan for the prevention and control of noncomunicable diseases 2013-2020 [Internet]. World Health Organization. 2013. Disponível em: http://apps.who.int/iris/bitstream/10665/94384/1/9789241506236_eng.pdf

20. Sociedade Portuguesa de Hipertensão. Estratégia política até 2020 da Sociedade Portuguesa de Hipertensão na luta contra o excesso de teor de sal nos alimentos. Rev Port Hipertens e Risco Cardiovasc. 2013;53(9):1689–99.
21. Assembleia da República. Lei 75/2009 de 12 de Agosto de 2009, estabelece normas com vista à redução do teor de sal no pão, bem como informação na rotulagem de alimentos embalados destinados ao consumo humano. Diário da República. 2009;155(1):5225–6.
22. Gabinetes do Ministro da Agricultura, Florestas e Desenvolvimento Rural, da Ministra do Mar, dos Secretários de Estado dos Assuntos Fiscais, das Autarquias Locais, da Educação, Adjunto e da Saúde e Adjunto e do Comércio e das Secretárias de Estado da Indústria e do Turismo. Estratégia integrada para a promoção da alimentação saudável. Diário da República, 2ª série. 2017;249:29595–8. Disponível em: https://dre.pt/application/conteudo/114424591
23. Linseisen J, Welch AA, Ocké M, Amiano P, Agnoli C, Ferrari P, et al. Dietary fat intake in the European prospective investigation into cancer and nutrition: Results from the 24-h dietary recalls. Eur J Clin Nutr. 2009;63:S61–80.
24. Food and Agriculture Organization of the United Nations (FAO). Fats and fatty acids in human nutrition: report of an expert consultation. Vol. 91, FAO, Food and Nutrition Paper. 2010. 166 p. Disponível em: http://www.who.int/nutrition/publications/nutrientrequirements/fatsandfattyacids_humannutrition/en/
25. Mozaffarian D, Aro A, Willett WC. Health effects of *trans*-fatty acids: Experimental and observational evidence. Eur J Clin Nutr. Nature Publishing Group; 2009;63(S2):S5–21.
26. Willett WC. *Trans* fatty acids and cardiovascular disease-epidemiological data. Atheroscler Suppl. 2006;7(2):5–8.
27. European Food Safety Authority. Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA J. 2010;8(3):1461. Disponível em: http://www.efsa.europa.eu/sites/default/files/scientific_output/files/main_documents/1461.pdf
28. World Health Organization. Eliminating *trans* fats in Europe - A policy brief [Internet]. 2015. Disponível em: http://www.euro.who.int/__data/assets/pdf_file/0010/288442/Eliminating-trans-fats-in-Europe-A-policy-brief.pdf
29. World Health Organization. Action plan for implementation of the European Strategy for the Prevention and Control of Noncommunicable Diseases 2012 – 2016. 2012. Disponível em: http://apps.who.int/medicinedocs/documents/s19752en/s19752en.pdf

30. World Health Organization. European food and nutrition action plan 2015 – 2020. WHO Regional Office for Europe. 2014. Disponível em: http://www.euro.who.int/__data/assets/pdf_file/0008/253727/64wd14e_FoodNutAP_140426.pdf
31. Cheftel JC. Food and nutrition labelling in the European Union. Food Chem. 2005;93(3):531–50.
32. Kleef E Van, Dagevos H. The Growing Role of Front-of-Pack Nutrition Profile Labeling: A Consumer Perspective on Key Issues and Controversies. Crit Rev Food Sci Nutr. 2015;55(3):291–303.
33. Hawley KL, Roberto CA, Bragg MA, Liu PJ, Schwartz MB, Brownell KD. The science on front-of-package food labels. Public Health Nutr. 2013;16(03):430–9.
34. Kasapila W, Shaarani SM. Legislation — Impact and Trends in Nutrition Labeling: A Global Overview. Crit Rev Food Sci Nutr. 2016;56(1):56–64.
35. Himmelsbach E, Allen A, Francas M. Study on the Impact of Food Information on Consumers' Decision Making. 2014.
36. Costa HS, Vasilopoulou E, Trichopoulou A, Finglas P. New nutritional data on traditional foods for European food composition databases. Eur J Clin Nutr. 2010;64:S73–81.
37. Machackova M, Giertlova A, Porubska J, Roe M, Ramos C, Finglas P. EuroFIR Guideline on calculation of nutrient content of foods for food business operators. Food Chem. 2018;238:35–41.
38. Comissão Europeia. Documento de orientação destinado às Autoridades competentes para o controlo do cumprimento da Legislação da UE, no que diz respeito à fixação de tolerâncias aplicáveis aos valores de nutrientes declarados no rótulo. 2012. Disponível em: https://ec.europa.eu/food/sites/food/files/safety/docs/labelling_nutrition-vitamins_minerals-guidance_tolerances_1212_pt.pdf
39. Guéraud F, Atalay M, Bresgen N, Cipak A, Eckl PM, Huc L, et al. Chemistry and biochemistry of lipid peroxidation products. Free Radic Res. 2010;44(10):1098–124.
40. Vieira SA, Zhang G, Decker EA. Biological Implications of Lipid Oxidation Products. J Am Oil Chem Soc. Springer Berlin Heidelberg; 2017;94(3):339–51.
41. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81–128.
42. Guillén MD, Goicoechea E. Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles. Food Chem. 2008;111(1):157–64.

43. Sousa BC, Pitt AR, Spickett CM. Chemistry and analysis of HNE and other prominent carbonyl-containing lipid oxidation compounds. Free Radic Biol Med 2017;111:294–308.
44. Spickett CM. The lipid peroxidation product 4-hydroxy-2-nonenal: Advances in chemistry and analysis. Redox Biol 2013;1(1):145–52.
45. Guéraud F. 4-Hydroxynonenal metabolites and adducts in pre-carcinogenic conditions and cancer. Free Radic Biol Med. 2017;111:196–208.
46. Csala M, Kardon T, Legeza B, Lizák B, Mandl J, Margittai É, et al. On the role of 4-hydroxynonenal in health and disease. Biochim Biophys Acta - Mol Basis Dis. 2015;1852(5):826–38.
47. Pillon NJ, Croze ML, Vella RE, Soulère L, Lagarde M, Soulage CO. The lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE) induces insulin resistance in skeletal muscle through both carbonyl and oxidative stress. Endocrinology. 2012;153(5):2099–111.
48. Guillén MD, Uriarte PS. Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated α,β unsaturated aldehydes. Food Chem. 2012;131(3):915–26.
49. Han IH, Csallany AS. Temperature dependence of HNE formation in vegetable oils and butter oil. J Am Oil Chem Soc. 2008;85(8):777–82.
50. Ma L, Liu G. Simultaneous Analysis of Malondialdehyde, 4-Hydroxy-2-hexenal, and 4-Hydroxy-2-nonenal in Vegetable Oil by Reversed-Phase High-Performance Liquid Chromatography. J Agric Food Chem. 2017;65(51):11320–8.
51. Papastergiadis A, Fatouh A, Jacxsens L, Lachat C, Shrestha K, Daelman J, et al. Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. Food Chem Toxicol. 2014;73:51–8.
52. Papastergiadis A, Mubiru E, Van Langenhove H, De Meulenaer B. Development of a sensitive and accurate stable isotope dilution assay for the simultaneous determination of free 4-hydroxy-2-(E)-nonenal and 4-hydroxy-2-(E)-hexenal in various food matrices by gas chromatography-mass spectrometry. Food Anal Methods. 2014;7(4):836–43.
53. Seppanen CM, Csallany AS. The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of 4-hydroxy-2-trans-nonenal and other α-, β-unsaturated hydroxyaldehydes. J Am Oil Chem Soc. 2006;83(2):121–7.
54. Seppanen CM, Csallany AS. Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature. JAOCS, J Am Oil Chem Soc. 2002;79(10):1033–8.

55. Seppanen CM, Csallany AS. Simultaneous determination of lipophilic aldehydes by high-performance liquid chromatography in vegetable oil. JAOCS, J Am Oil Chem Soc. 2001;78(12):1253–60.
56. Seppanen CM, Csallany AS. Incorporation of the toxic aldehyde 4-hydroxy-2-trans-nonenal into food fried in thermally oxidized soybean oil. JAOCS, J Am Oil Chem Soc. 2004;81(12):1137–41.
57. Surh J, Lee S, Kwon H. 4-Hydroxy-2-alkenals in polyunsaturated fatty acids-fortified infant formulas and other commercial food products. Food Addit Contam. 2007;24(11):1209–18.
58. Surh J, Kwon H. Estimation of daily exposure to 4-hydroxy-2-alkenals in Korean foods containing n-3 and n-6 polyunsaturated fatty acids. Food Addit Contam. 2005;22(8):701–8.
59. Meynier A, Leborgne C, Viau M, Schuck P, Guichardant M, Rannou C, et al. N-3 fatty acid enriched eggs and production of egg yolk powders: An increased risk of lipid oxidation? Food Chem. 2014;153:94–100.
60. Crews C, Chiodini A, Granvogl M, Hamlet C, Hrnčiřík K, Kuhlmann J, et al. Analytical approaches for MCPD esters and glycidyl esters in food and biological samples: A review and future perspectives. Food Addit Contam - Part A Chem Anal Control Expo Risk Assess. 2013;30(1):11–45.
61. Scientific Committee on Food (SCF). Opinion of the scientific committee on food on 3-monochloropropane-1,2-diol (3-MCPD). Updating the SCF opinion of 1994. [Internet]. Scientific Committee on Food. 2001. Disponível em: http://ec.europa.eu/food/fs/sc/scf/out91_en.pdf
62. Comissão Europeia. Regulamento (CE) N.o 466/2001 da Comissão de 8 de Março de 2001 que fixa os teores de certos contaminantes presentes nos géneros alimentícios. 2001.
63. European Food Safety Authority. Analysis of occurrence of 3-monochloropropane-1, 2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment. EFSA J. 2013;11(9):3381. Disponível em: http://doi.wiley.com/10.2903/j.efsa.2013.3381
64. Weißhaar R, Perz R. Fatty acid esters of glycidol in refined fats and oils. Eur J Lipid Sci Technol. 2010;112(2):158–65.
65. Weißhaar R. Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. Eur J Lipid Sci Technol. 2008;110(2):183–6.

66. Cheng WW, Liu GQ, Wang LQ, Liu ZS. Glycidyl Fatty Acid Esters in Refined Edible Oils: A Review on Formation, Occurrence, Analysis, and Elimination Methods. Compr Rev Food Sci Food Saf. 2017;16(2):263–81.
67. European Food Safety Authority. Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. EFSA J. 2016;14(5). Disponível em: http://doi.wiley.com/10.2903/j.efsa.2016.4426
68. Comissão Europeia. Regulamento (UE) 2018/290 da Comissão de 26 de fevereiro de 2018 que altera o Regulamento (CE) No. 1881/2006 no que diz respeito aos teores máximos de ésteres glicidílicos de ácidos gordos em óleos e gorduras vegetais, fórmulas para lactentes, fórmulas de transição e alimentos para fins medicinais específicos destinados a lactentes e crianças jovens [Internet]. Vol. 55, Jornal Oficial da União Europeia. 2018. Disponível em: https://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32018R0290&from=PT

1.2 Objetivos

Apesar da incomensurável literatura relativamente aos alimentos processados e às técnicas de processamento, continua a ser fundamental aprofundar os conhecimentos científicos sobre estes alimentos, que estão amplamente disponíveis e acessíveis, sendo inevitável a sua elevada presença na alimentação da população.

Numa altura em que se enfrenta o desafio de melhorar o estado de saúde da população, nomeadamente na prevenção das doenças crónicas não transmissíveis, é fundamental avaliar o equilíbrio nutricional dos alimentos processados e o seu potencial impacto na saúde. Nesse sentido, este trabalho teve como **objetivos principais**:

- Desenvolver e aprofundar o conhecimento científico relativo à qualidade nutricional e segurança dos alimentos processados, com vista à identificação de áreas prioritárias de intervenção;
- Aprofundar o conhecimento sobre a formação, ocorrência e mitigação de compostos potencialmente carcinogénicos e tóxicos;
- Contribuir com informação fundamental para a formulação de políticas alimentares e de nutrição que visem a melhoria de estado de saúde e nutricional da população.

Para atingir os objetivos gerais supracitados, foram planeadas diversas tarefas, articuladas entre si, procurando uma sequência de trabalho que permitisse atingir os **objetivos específicos** definidos, nomeadamente:

- Determinar os teores de sal, de gordura e o equilíbrio da composição da gordura (ácidos gordos e colesterol) de alimentos processados;
- Estudar a influência de técnicas de processamento alimentar na composição dos alimentos processados;
- Realizar estudos comparativos entre alimentos processados de marca comercial e de marca branca, bem como entre alimentos com características especiais (sem glúten e/ou sem açúcar) e os seus produtos similares;
- Avaliar a conformidade da rotulagem dos alimentos processados;

- Avaliar a segurança de alimentos processados, relativamente à presença de compostos indesejáveis (ácidos gordos *trans*, 4-hidroxi-2-alcenais, cloropropanóis e ésteres glicidílicos);
- Identificar precursores e estratégias de mitigação de compostos indesejáveis presentes nos alimentos processados;
- Transferir os conhecimentos adquiridos para a indústria alimentar e para as autoridades com competências em Saúde Pública, visando contribuir para alimentos processados mais seguros e nutritivos.

1.3 Organização e estrutura da tese

Tendo em consideração os objetivos definidos, bem como as tarefas a seguir para a sua prossecução, a presente tese de doutoramento foi estruturada em cinco capítulos.

No **Capítulo 1** são apresentados a motivação, os objetivos e a organização e estrutura da tese. Neste capítulo pretende-se sumariar os principais motivos de interesse desta tese e quais as questões que estiveram na base do desenho experimental deste trabalho de investigação. A seguir apresentam-se os objetivos gerais e específicos definidos. E por último, descreve-se a organização e estrutura da tese, apresentando os tópicos abordados ao longo dos diferentes capítulos que dela fazem parte integrante.

É de ressalvar que nos capítulos 2, 3 e 4, se optou pela apresentação dos artigos científicos no formato original em que foram publicados ou submetidos para publicação. Por esse motivo há documentos em português, português do Brasil e inglês, além de diferentes estruturas, formatações, regras de nomenclatura e referências bibliográficas. Na Figura 8, estão representadas as principais etapas do trabalho de investigação desenvolvido, incluindo os tipos de amostras, a descrição da atividade experimental e os resultados obtidos.

O **Capítulo 2** engloba as atividades desenvolvidas no âmbito da avaliação da qualidade nutricional e segurança dos alimentos processados, e também a avaliação da conformidade da rotulagem destes alimentos. Este capítulo inclui 10 artigos (7 em revistas internacionais e 3 em revistas nacionais), estando 9 já publicados e 1 submetido. É de referir que dada a grande variabilidade do tipo de amostras estudadas foi necessária a otimização de algumas das metodologias analíticas aplicadas.

Relativamente à qualidade nutricional foram incluídas 9 categorias de alimentos processados e avaliados vários parâmetros (teor de sal, colesterol, gordura total e perfil de ácidos gordos). Atendendo a que, por vezes, as denominações atribuídas às categorias de alimentos processados são ambíguas e demasiado generalistas, a Figura 9 tem exemplos dos alimentos processados por categoria. Por sua vez, relativamente à influência das técnicas de processamento (fritura e/ou confeção no forno) na qualidade nutricional dos alimentos processados, optou-se por incluir tipos de alimentos com composição nutricional distinta, nomeadamente nuggets de frango pré-fritos, óleos alimentares, batatas ultracongeladas e pizas frescas.

Ainda neste capítulo, considerou-se pertinente incluir os trabalhos relativos à conformidade da rotulagem, dada a sua relação com os resultados obtidos na avaliação da qualidade nutricional dos alimentos processados.

Nesta parte do trabalho são apresentados os resultados da comparação da qualidade nutricional de alimentos similares de marca branca e comercial, bem como de alimentos com e sem glúten. No que diz respeito à avaliação da conformidade propriamente dita, foram incluídos os resultados obtidos após aplicação dos valores das tolerâncias, definidos na legislação, para o teor de sal, de gordura e de ácidos gordos saturados. O critério de exclusão aplicado nesta fase do trabalho foi a ausência de declaração nutricional nos rótulos dos referidos alimentos.

O **Capítulo 3** apresenta o trabalho realizado acerca da presença de 4-hidroxi-2-alcenais em alimentos processados, dos quais resultaram 3 publicações (1 capítulo de livro e 2 artigos internacionais, estando 1 submetido). Foi realizada uma revisão bibliográfica sobre as preocupações para a saúde e a avaliação de risco de exposição, bem como metodologias analíticas, condições relacionadas com a formação e mitigação, e sua ocorrência em óleos e gorduras, lacticínios, carne e derivados, peixe e derivados, e outros alimentos processados.

O **Capítulo 4** é dedicado ao trabalho relativo relativamente aos cloropropanóis e ésteres glicidílicos de ácidos gordos, em óleos e gorduras. Neste âmbito foi publicado 1 artigo internacional, resultante da revisão bibliográfica sobre a ocorrência do 3-MCPD em óleos e gorduras. Dada a grande quantidade de trabalhos científicos sobre a incidência deste e outros cloropropanóis, bem como dos ésteres glicidílicos de ácidos gordos em óleo de palma, justificou-se uma revisão bibliográfica sobre estes contaminantes emergentes no óleo de palma. Neste trabalho foram tratados temas como: orientações científicas e legislação, metodologias analíticas, precursores, ocorrência e estratégias de mitigação.

A tese termina com o **Capítulo 5**, que inclui as considerações finais e as perspetivas futuras. Optou-se por esta denominação, uma vez que as publicações apresentadas nos capítulos anteriores incluem conclusões específicas para cada parte do trabalho realizado. Assim, este capítulo constitui não só um sumário dos resultados mais relevantes e das questões a que este trabalho pretendeu responder, mas também um conjunto de reflexões e perspetivas para novos trabalhos e abordagens que permitam ter alimentos processados, nutricionalmente equilibrados e mais seguros, visando a promoção da saúde pública.

Figura 8. Principais etapas do trabalho de investigação efetuado.

Produtos de pastelaria

Pastelaria fina

Biscoitos

Bolachas sem recheio

Bolachas recheadas

Bolachas com cobertura

Bolachas tipo *crackers*

Tortas cobertas e/ou recheadas

Bolachas tipo *wafers*

Produtos de panificação

Pães de leite com e sem recheio

Croissants com e sem recheio

Pães de leite com pepitas

Pães com recheio salgado

Snacks

Aperitivos

Snacks de queijo

Pipocas

Tiras de milho

Fast-food

Hambúrgueres

Sandwiches

Pizas

Figura 9. Exemplos dos alimentos processados incluídos em cada categoria.

Batatas

Batatas fritas (restauração)

Batatas fritas de pacote (com e sem sabores)

Batatas ultracongeladas

Batatas fritas (preparadas em casa)

Cereais

Cereais de pequeno-almoço

Barras de cereais

Granolas

Muesli

Frutos secos e oleaginosas

Frutos secos (ao natural)

Frutos secos (processados)

Sementes

Refeições prontas a comer

Salgados

Pratos pré-preparados

Sopas

Molhos

Cocktail

Ketchup

Maionese

Figura 9. Exemplos dos alimentos processados incluídos em cada categoria (continuação).

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CAPÍTULO 2. Avaliação da qualidade nutricional de alimentos processados e da conformidade da rotulagem

No presente capítulo são apresentados os resultados relativos à avaliação da qualidade nutricional de 9 categorias de alimentos processados, através da determinação analítica de parâmetros como o teor de sal, teor de gordura total, perfil de ácidos gordos e colesterol. São, também, apresentados os resultados relativos à aplicação dos métodos de confeção no forno e fritura e o seu impacto na qualidade nutricional dos alimentos. Com os dados obtidos fez-se uma avaliação da conformidade da rotulagem dos alimentos e aplicaram-se as tolerâncias aos nutrientes em estudo.

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2.1 *trans* fatty acids content in worldwide edible fats and oils: current trends and challenges

Este sub-capítulo consiste numa revisão bibliográfica que incide sobre os teores de ácidos gordos trans em gorduras e óleos, destinados à alimentação humana, sendo também realizada a comparação destes dados entre vários países.

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Trans Fatty Acids Content in Worldwide Edible Fats and Oils: Current Trends and Challenges

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Abstract

trans fatty acids (TFA) have been related with multiple cardiovascular risk factors and higher risk of coronary heart disease. Partial hydrogenation, which converts liquid vegetable oils into solid or semi-solid fats with appropriate melting properties suitable for the production of shortenings and margarines, is one of the sources of TFA. In this study a worldwide comparison regarding the TFA content in edible oils and fats was performed.

Oils and fats, from vegetable or animal origin, are essential ingredients for a variety of food products. The major dietary sources of TFA are foods containing partially hydrogenated vegetable oils, namely, shortenings and/or margarines, and animal fats as butter, while edible vegetable oils, in general have low contents. The content of TFA of vegetable oils can increase when subjected to drastic heating, for example deep-fat frying or oven baking. However, TFA formation strongly depends on several factors, namely, frying conditions (type of fryer, duration and temperature), frying material (oil/fat and the food itself), among others. In conclusion, a great variability between countries was observed for example for margarines, where Canada was one of the countries with the highest percentage of TFA (42.9% of total fatty acids) for margarines produced with partially hydrogenated vegetable oils. On the other hand, in Germany, Portugal, Austria and Canada as well, the identified margarines with lower content of TFA are mainly produced with non-hydrogenated fats. With respect to edible vegetable oils, in general, these have lower contents of TFA than margarines, shortenings and butters, as expected.

Introduction

Oils and fats are usually processed to improve their quality, stability and safety. Despite the removal of a large amount of contaminants from the oil, processing can often originate new compounds that can cause additional health hazards. When the oil and fats are heated for long periods, several complex reactions may occur, resulting in the degradation of the oil/fat. During these reactions, the functional, sensory and nutritional quality of the oil and food can be modified, becoming its quality and safety a concern.

The most frequent chemical reactions that occur in edible oils and fats take place during industrial processing and/or during deep-frying. From the possible reactions that can occur, the most known are: hydrolysis, oxidation, isomerisation and polymerization, which produce volatile or non-volatile compounds. The volatile compounds can contribute to changes in the flavour of fried foods, while the non-volatile compounds that remain in the oil, can change the physical properties and are absorbed by the food (affecting their flavour stability, quality, and texture). All these reactions take place simultaneously and in combination with each other, leading to the formation of several degradation products, namely, free fatty acids, aldehydes, ketones, diglycerides and monoglycerides, *trans* isomers, hydrocarbons, conjugated fatty acids, and cyclic fatty acids (Choe and Min, 2007; Zhang, Saleh, Chen and Shen, 2012; Brühl, 2014).

trans fatty acids (TFA) are not synthesised by the human body and are not required in the diet (EFSA, 2010). According to *Codex Alimentarius*, TFA are “all the geometrical isomers of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) having non-conjugated, interrupted by at least one methylene group, carbon-carbon double bonds in the *trans* configuration” (Codex Alimentarius, 1985). Later, EFSA also defined TFA, specifying that *trans*-PUFA “have at least one *trans* double bond and may therefore also have double bonds in the *cis* configuration” (EFSA, 2010).

The main TFA quantified in foods are elaidic acid (C18:1, n9t) and linoelaidic acid (C18:2, n6t). TFA can be naturally present in foods or can be formed during food processing. One of the sources of TFA is partial hydrogenation which converts liquid vegetable oils into solid or semi-solid fats with appropriate melting properties suitable for the production of shortenings and margarines, increasing oxidation stability and the shelf-life of the product. On the other hand, TFA are naturally present in fats from ruminant animals formed in their stomach by microbial hydrogenation of *cis*-unsaturated fatty acids. Finally, TFA can be produced during heat treatment (Richter, Shawish, Scheeder, and Colombani, 2009; Albuquerque, Costa, Castilho, and Sanches-Silva, 2011). However, TFA formation during deep-frying strongly depends on several factors, namely, frying conditions (type of fryer,

duration and temperature, frying cycles) and frying material (oil/fat and the food itself), among others. The formation of TFA during deep-frying has been shown to be closely related to the temperature and duration of the frying process (Hou, Jiang, and Zhang, 2012).

This review aims to bring a worldwide overview of TFA in edible fats and oils, focusing on the current scientific opinions and recommendations, the advances of analytical methodologies for its quantification, as well as a comparison among different countries on the occurrence of TFA in edible oils and fats.

Scientific opinions and recommendations

In the last years, several international organizations, namely World Health Organization (WHO), Food and Agriculture Organization (FAO) and European Food Safety Authority (EFSA) have published scientific opinions and recommendations regarding TFA occurrence in foods and health concerns (FAO 2010; WHO 2015). Up to now, EU Member States do not have legislation regulating the content of TFA in foods or requiring their labelling (European Parliament/Council of the European Union, 2011). However, Austria, Switzerland, Iceland, Norway, Denmark and Hungary have limited TFA content in foods below 2% of total fat (excluding ruminant TFA) (WHO 2015).

With respect to current dietary recommendations, the report from FAO stated that intake of TFA should be as low as possible (<1% of total energy intake), which for a 2000 kcal diet, represents around 2 g of TFA/day (FAO 2010). In 2010, EFSA has also performed a Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, MUFA, PUFA, TFA, and cholesterol, but no Population Reference Intake, Average Requirement, or Adequate Intake was set, recommending that TFA intake should be as low as possible within the context of a nutritionally adequate diet (EFSA 2010).

Analytical determination

Over the years, several analytical techniques were applied for the identification and quantification of TFA, namely Fourier-Transform Infrared Spectroscopy, reversed phase-high performance liquid chromatography, silver ion chromatography and gas chromatography (GC). Nonetheless, the most widely used technique is GC. In GC, the preparation of volatile derivatives is necessary; therefore a derivatization step prior to GC analysis is required. In this operation, fatty acids are derivatized in the corresponding fatty acids methyl esters (Ruiz-Rodriguez, Reglero and Ibañez, 2010). The saponification can be carried out under acidic or alkaline conditions, being the methanolic sodium hydroxide or methanolic sodium methoxide the most used solvents. Afterwards, an esterification step should be performed and the most widely used catalyst has been boron trifluoride.

GC analysis of TFA isomers is complex and requires an analyst with expertise and with interpretive experience. Another important aspect is the choice of the analytical column to be used. There are several columns that can be used to perform the analysis of TFA, but the most utilized columns are fused silica capillary columns coated with highly polar cyanopolysiloxane stationary phases with 100 m of length, since these reduce the risk of isomers overlap. However, one of the main disadvantages of this analytical technique is the time of analysis, which is usually higher than 60 min (Albuquerque, Costa, Castilho, and Sanches-Silva, 2011). Regarding the type of detector for chromatographic analysis, the most suitable for quantification is flame ionization, however mass spectrometry is also used for confirmation purposes.

TFA occurrence in edible oils and fats

In Table 2, an overview of TFA contents in oils and fats from different countries is shown. One of the main constraints to compare TFA data available in the literature are the units in which those values are presented. Since they can be expressed as % of total fatty acids, % of total fat or even % of fatty acid methyl esters (FAMEs), most of the time it is impossible to compare them.

Oils and fats, from vegetable and animal origin, are essential ingredients for a variety of food products. The major dietary sources of TFA are food containing partially hydrogenated vegetable oils, namely butter, shortenings and/or margarines, while edible vegetable oils, in general have low contents. According to Table 2, a great variability between countries can be observed for example for margarines, where Canada (Ratnayake et al., 2007) was one of the countries with the highest percentage of TFA (42.9 % of total fatty acids) for margarines produced with partially hydrogenated vegetable oils. On the other hand, in Germany (Fritsche and Steinhart, 1997), Portugal (Torres, Casal, and Oliveira, 2002), Austria (Wagner, Auer, and Elmadfa 2000; Wagner, Plasser, Proell, and Kanzler, 2008) and Canada as well (Ratnayake et al., 2007), the identified margarines with lower content of TFA are mainly produced with non-hydrogenated fats. With respect to edible oils, these have lower contents of TFA than margarines, shortenings and butters (Table 2).

The content of TFA from vegetable oils can increase when subjected to heating, for example when cooking methods such as deep-fat frying and baking in the oven are used. However, results provided in the literature are controversial. For instance, Liu, Inbaraj and Chen

Table 2. *trans* fatty acids content (% of total fatty acids) of vegetable oils, margarines and shortenings and other fats from different countries.

Country	Year	Analytical method	Oils and fats	TFA (% of total FA)	Reference
Argentina	2000	GC-FID	Margarine (n=3)	28.6[a]	Tavella et al. (2000)
			Butter (n=1)	4.63	
			Sunflower oil (n=2)	<0.3	
			Corn oil (n=2)	<0.3	
			Mixed seed oil (n=3)	<0.3	
			Rapeseed oil (n=1)	<0.3	
Austria	2000	GC-FID	Margarines (n=9)	0.3 - 3.7	Wagner et al. (2000)
			Plant oils, refined (n=3)	0.0 - 0.8	
			Plant oils, cold pressed (n=6)	0.0 - 0.1	
	2008	GC-FID	Household margarines (n=14)	1.45[a]	Wagner et al. (2008)
			Industrial margarines (n=6)	7.83[a]	
Australia	1993	GC-FID	Margarines (n=13)	8.01 - 14.5	Mansour et al. (1993)
			Butter (n=5)	3.44 - 4.75	
			Lard (n=1)	0.73	
Canada	2007	GC-FID	Margarines of non-hydrogenated vegetable oils (n=15)	0.5 - 1.7	Ratnayake et al. (2007)
			Margarines from partially hydrogenated vegetable oils (n=14)	17.0 - 42.9	
Costa Rica	2007	GC-FID	Corn oil (n=12)	1.41[a]	Baylin et al. (2007)
			Canola oil (n=4)	0.79[a]	
			Palm oil/shortening (n=9)	1.3[a]	
			Partially hydrogenated soybean oil (1995-1996) (n=9)	20.2[a]	
			Partially hydrogenated soybean oil (2000-2003) (n=9)	5.32[a]	
			Soybean oil (n=38)	1.48[a]	
			Sunflower oil (n=4)	2.11[a]	
			Sesame oil (n=4)	1.24[a]	
			Olive oil (n=12)	0.31[a]	
			Oil sprays (n=3)	1.22[a]	
			Regular stick margarine (n=17)	13.3[a]	
			Light stick margarine (n=7)	14.3[a]	
			Regular tube margarine (n=16)	10.8[a]	
			Light tube margarine (n=4)	11.3[a]	
			Liquid margarine (n=6)	10.9[a]	
			Butter (n=10)	6.47[a]	
Czech Republic	2000	GC-FID	Margarines	0.1 - 34.8	Brát et al. (2000)
			Cooking fats	0.2 - 40.7	
			Butter (n=10)	2.4 [a]	
Denmark	1996	GC-FID	Margarine <20% linoleic acid, year of 1992 (n=9)	1.4 - 10.6	Ovesen et al. (1996)
			Margarine <20% linoleic acid, year of 1995 (n=20)	0 - 8.2	
			Margarine 20-40% linoleic acid, year of 1992 (n=17)	13.3 - 42.2	
			Margarine 20-40% linoleic acid, year of 1995 (n=8)	14.8 - 46.0	
			Margarine >40% linoleic acid, year of 1992 (n=14)	0 - 9.6	
			Margarine >40% linoleic acid, year of 1995 (n=6)	0 - 5.6	
			Shortening <20% linoleic acid, year of 1995	0 - 13.7	
Germany	1997	GC-FID	Fat reduced margarines	0.83 - 1.74	Fritsche et al. (1997)
			Vegetable margarines	0.32 - 4.07	
			Diet margarines	0.15 - 0.53	
			Sunflower margarines	3.33 - 4.88	
Mexico	2000	GC-FID	Vegetable oil (n=18)	0.90 - 2.93	Medina-Juárez et al. (2000)
	2011	GC-FID	Spreadable margarines (n=20)	18.0 - 28.2	

			Stick margarines (n=22)	0.24 - 38.9	Hérnandez-Martínez et al. (2011)
New Zealand	2006	GC-FID	Margarines/spreads (n=6)	2.7 - 6.9	Saunders et al. (2008)
Pakistan	2006	GC-FID	Table margarines (n=5)	2.45 - 4.58	Anwar et al. (2006)
			Bakery margarines (n=5)	7.95 - 21.1	
			Butter (n=10)	2.98 - 5.00	
	2008	GC-MS	Margarines (n=10)	2.1 - 33.2	Kandhro et al. (2008)
Portugal	2002	GC-FID	Margarines (n=4)	0.4 - 2.5	Torres et al. (2002)
			Three-quarter-fat margarine (n=1)	5.3	
			Half-fat margarine (n=5)	0.2 - 5.5	
			Fat spread, 70% (n=5)	0.2 - 5.9	
			Fat spread, 38% (n=1)	0.4	
Spain	2000	GC-FID	Sunflower oil and partially hydrogenated sunflower oil (n=1)	18.3	Alonso et al. (2000)
			Vegetable oils and hydrogenated vegetable fats (n=6)	1.01 - 21.3	
			Mixture of vegetable oils and partially hydrogenated vegetable fats (n=3)	0.40 - 10.3	
			Mixture of corn oil and partially hydrogenated vegetable fats (n=1)	1.07	
			Mixture of corn oil and partially hydrogenated corn oil (n=2)	20.5 - 20.8	
	2000	GC-FID	Margarines (n=12)	0.4 – 19.2	Larqué et al. (2003)
			Mixture of vegetable oils and partially hydrogenated vegetable fats (n=3)	0.40 - 10.3	
			Mixture of corn oil and partially hydrogenated vegetable fats (n=1)	1.07	
			Mixture of corn oil and partially hydrogenated corn oil (n=2)	20.5 - 20.8	
			Margarines (n=12)	0.4 – 19.2	
Switzerland	2009	GC-FID	Semi-solid fats (n=10)	0.14 - 28.2	Richter et al., (2009)
			Oils (n=21)	0.04 - 11.4	
Turkey	2002	GC-FID	Margarines (n=12)	7.7 - 37.8	Tekin et al. (2002)
	2006	GC-FID	Shortenings (n=10)	2.0 - 16.5	Karabulut et al. (2006)
			Margarines (n=15)	0 - 39.4	
	2008	GC-FID	Shortenings (n=15)	2.7 - 19.6	Basol et al., (2008)
United Kingdom	2013	GC-FID	Fat spread, 26-39% fat (n=3)	0.17 - 0.60	Roe et al. (2013)
			Fat spread, 41-62% fat (n=3)	0.18 - 0.30	
			Fat spread, 65-75%, fat not polyunsaturated (n=1)	0.27	
			Margarine, hard block (n=1)	0.16	

FA, Fatty acids; TFA, *trans* fatty acids; GC-FID, Gas chromatography with flame ionization detection; GC-MS, Gas chromatography with mass spectrometry.
[a] Results are expressed as mean value.

(2007) have studied non-hydrogenated and hydrogenated soybean oil heated at 160, 180 and 200 ºC for different periods of time. According to their results, no TFA was formed in both soybean oils during heating for 24 h, implying that TFA can only be formed under drastic heating conditions (Liu, Inbaraj and Chen, 2007). Aladedunye and Przybylski (2009) have evaluated the effect of temperature on the degradation of canola oil. Their results have shown that frying temperatures above 195 ºC can increase the amount of *trans* isomers and

the rise of frying temperature to 215 ºC caused extensive *trans* isomerisation of fatty acids, from 2.4 to 5.9% (Aladedunye and Przybylski, 2009).

Concluding remarks and trends

In conclusion, it is possible to confirm that edible oils and fats processing industry has developed efforts in order to lower TFA amount in this type of food, namely in the modification of hydrogenation conditions or by developing genetically modified seeds with a better fatty acids profile. This action is crucial and will also decrease the TFA in other foodstuffs, since edible oils and fats are common food ingredients. Nonetheless, a great variability among countries and within the same country for similar oils and fats was observed. Concerning trends for the future, undoubtedly the current analytical methodologies should be a priority, since these still take too long and the accurate separation and quantification of TFA is sometimes difficult. Another important issue is the mandatory labelling of TFA content in edible oils and fats for all countries, which could increase the awareness of consumers and contribute to perform an informed choice. Moreover, some studies in the literature, reported data on TFA for oils/fats under unrealistic frying conditions, such as temperatures above 185 ºC, which is the recommended frying temperature. Therefore, to investigate what happens during the different stages of frying and under different frying conditions and using the different available oils and fats, as well as their mixtures in different proportions, are also challenges that should be considered in further studies. Finally, authors consider that the research on the development of edible oils and fats with an enhanced fatty acids profile, using genetically modified seeds should also be pointed out.

References

Albuquerque, T.G., Costa. H. S., Castilho, M. C., Sanches-Silva, A., (2011). Trends in the analytical methods for the determination of *trans* fatty acids content in foods. Trends in Food Science & Technology, 22:543-560.

Aladedunye, F. A., Przybylski, R., (2009). Degradation and nutritional quality changes of oil during frying. Journal of the American Oil Chemists' Society, 86:149-156.

Alonso, L., Fraga, M. J., Juárez, M., (2000). Determination of *trans* fatty acids and fatty acid profiles in margarines marketed in Spain. Journal of the American Oil Chemists' Society, 77: 131-136.

Anwar, F., Bhanger, M. I., Iqbal, S., Sultana, B., (2006). Fatty acid composition of different margarines and butters from Pakistan with special emphasis on *trans* unsaturated contents. Journal of Food Quality, 29:87-96.

Basol, B., & Tasan, M., (2008). Fatty acid compositions of Turkish shortenings with emphasis on *trans* fatty acids. Journal of Food Lipids, 15:240-250.

Baylin, A., Siles, X., Donovan-Palmer, A., Fernandez, X., Campos, H., (2007). Fatty acid composition of Costa Rican foods including *trans* fatty acid content. Journal of Food Composition and Analysis, 20:182-192.

Brát, J., Pokorný, J., (2000). Fatty acid composition of margarines and cooking fats available on the Czech market. Journal of Food Composition and Analysis, 13:337-343.

Brühl, L., (2014). Fatty acid alterations in oils and fats during heating and frying. European Journal of Lipid Science and Technology, 116: 707-715.

Choe, E., Min, D. B., (2007). Chemistry of Deep-Fat Frying Oils. Journal of Food Science, 72:77-86.

Codex Alimentarius, (1985). Guidelines on nutrition labelling. CAC/GL 2-1985, http://www.fao.org/ag/humannutrition/33309-01d4d1dd1abc825f0582d9e5a2eda4a74.pdf, (accessed September 19, 2016).

EFSA, European Food Safety Authority, (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal, 8, 1461, http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1461/epdf, (accessed September 19, 2016).

European Parliament/Council of the European Union, (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. Official Journal of the European Union, L304, 18-63.

FAO, Food and Agriculture Organization of the United Nations, (2010). Fats and fatty acids in human nutrition. Report of an expert consultation. Rome, http://foris.fao.org/preview/25553-0ece4cb94ac52f9a25af77ca5cfba7a8c.pdf, (accessed September 19, 2016).

Fritsche, J., Steinhart, H., (1997). *Trans* fatty acid content in German margarines. Fett/Lipid, 99:214-217.

Hernández-Martínez, M., Gallardo-Velázquez, T., Osorio-Revilla, G., (2011). Fatty acid profile including *trans* fatty acid content of margarines marketed in Mexico. Journal of the American Oil Chemists' Society, 88:1485-1495.

Hou, J., Jiang, L., Zhang, C., (2012). Effects of frying on the *trans*-fatty acid formation in

soybean oils. European Journal of Lipid Science and Technology, 114:287-293.

Kandhro, A., Sherazi, S. T. H., Mahesar, S. A., Bhanger, M. I., Talpur, M. Y., Rauf, A., (2008). GC-MS quantification of fatty acid profile including *trans* FA in the locally manufactured margarines of Pakistan. Food Chemistry, 109:207-211.

Karabulut, I., Turan, S., (2006). Some properties of margarines and shortenings marketed in Turkey. Journal of Food Composition and Analysis, 19:55-58.

Larqué, E., Garaulet, M., Pérez-Llamas, F., Zamora, S., Tebar, F. J., (2003). Fatty acid composition and nutritional relevance of most widely consumed margarines in Spain. Grasas y Aceites, 54:65-70.

Liu, W. H., Inbaraj, B. S., Chen B.H., (2007). Analysis and formation of *trans* fatty acids in hydrogenated soybean oil during heating. Food Chemistry, 104:1740-1749.

Mansour, M. P., Sinclair, A. J., (1993). The *trans* fatty acid and positional (sn-2) fatty acid composition of some Australian margarines, dairy blends and animal fats. Asia Pacific Journal of Clinical Nutrition, 3:155-163.

Medina-Juárez, L. A., Gámez-meza, N., Ortega-García, J., Noriega-Rodriguez, J. A., Angulo-Guerreo, O., (2000). *Trans* fatty acid composition and tocopherol content in vegetable oils produced in Mexico. Journal of the American Oil Chemists' Society, 77:721-724.

Ovesen, L., Leth, T., Hansen, K., (1996). Fatty acid composition of Danish margarines and shortenings, with special emphasis on *trans* fatty acids. Lipids, 31:971-975.

Ratnayake, W. M. N., Gagnon, C., Dumais, L., Lillycrop, W., Wong, L., Meleta, M., Calway, P., (2007). *trans* fatty acid content of Canadian margarines prior to mandatory trans fat labelling. Journal of the American Oil Chemists' Society, 84:817-825.

Richter, E. K., Shawish, K. A., Scheeder, M. R., Colombani, P. C., (2009). *Trans* fatty acid content of selected Swiss foods: the TransSwissPilot study. Journal of Food Composition and Analysis, 22:479-484.

Roe, M., Pinchen, H., Church, S., Elahi, S., Walker, M., Farron-Wilson, M., Buttriss, J., Finglas, P., (2013). *Trans* fatty acids in a range of UK processed foods. Food Chemistry, 140:427-431.

Ruiz-Rodriguez, A., Reglero, G., Ibañez, E., (2010). Recent trends in the advanced analysis of bioactive fatty acids. Journal of Pharmaceutical and Biomedical Analysis, 51:305-326.

Saunders, D., Jones, S., Devane, G. J., Scholes, P., Lake, R. J., Paulin, S. M., (2008). *Trans* fatty acids in the New Zealand food supply. Journal of Food Composition and Analysis, 21: 320-325.

Tavella, M., Peterson, G., Espeche, M., Cavallero, E., Cipolla, L., Perego, L., Caballero, B., (2000). *Trans* fatty acid content of a selection of foods in Argentina. Food Chemistry, 69, 209-213.

Tekin, A., Cizmeci, M., Karabacak, H., Kayahan, M. (2002). *Trans* FA and solid fat contents of margarines marketed in Turkey. Journal of the American Oil Chemists' Society, 79:443-445.

Torres, D., Casal, S., Oliveira, M. B. P. P., (2002). Fatty acid composition of Portuguese spreadable fats with emphasis on *trans* isomers. European Food Research and Technology, 214:108-111.

Wagner, K.-H., Auer, E., Elmadfa, I., (2000). Content of *trans* fatty acids in margarines, plant oils, fried products and chocolate spreads in Austria. European Food Research and Technology, 210:237-241.

Wagner, K.-H., Plasser, E., Proell, C., Kanzler, S., (2008). Comprehensive studies on the trans fatty acid content of Austrian foods: convenience products, fast food and fats. Food Chemistry, 108:1054-1060.

WHO, (2015). Eliminating *trans* fats in Europe. A policy brief. http://www.euro.who.int/__data/assets/pdf_file/0010/288442/Eliminating-trans-fats-in-Europe-A-policy-brief.pdf?ua=1, (accessed September 20, 2016).

Zhang, Q., Saleh, A.S.M., Chen, J., Shen, Q., (2012). Chemical alterations taken place during deep-fat frying based on certain reaction products: a review. Chemistry and Physics of Lipids, 165:662-681.

2.2 Consumo de sal e efeitos na saúde na perceção do consumidor: resultados preliminares

Este sub-capítulo apresenta os resultados relativos à aplicação de um questionário que pretendeu avaliar a perceção do consumidor sobre o consumo de sal e os efeitos na saúde, visando sinalizar as principais fragilidades da população portuguesa relativas ao conhecimento sobre esta temática e quais as áreas prioritárias de intervenção para a promoção da literacia em saúde.

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Resumo

O sal é um ingrediente, um condimento e um nutriente, desempenhando um papel fulcral na alimentação humana. No entanto, o seu uso em excesso está associado a Hipertensão Arterial, um problema de saúde pública. Pretendeu-se avaliar a perceção do consumidor sobre o consumo de sal e os efeitos na saúde, visando sinalizar as principais fragilidades da população portuguesa relativas ao conhecimento sobre esta temática e quais as áreas prioritárias de intervenção. Foram inquiridos até ao momento 115 indivíduos, com idades compreendidas entre 16 e 83 anos. Apesar do nível considerável de literacia dos inquiridos, existe ainda algum desconhecimento relativo às alternativas ao uso do sal e à importância da avaliação frequente da tensão arterial. É necessário continuar a tomar medidas que permitam a redução do teor de sal dos alimentos, contribuindo para a melhoria do estado de saúde da população Portuguesa.

Abstract

Salt is an ingredient, a condiment and a nutrient, playing a major role in human nutrition. However, its excessive use is associated with Hypertension, a public health problem. This study intended to evaluate consumers' perception of salt consumption and health effects, in order to signal the main weaknesses of the Portuguese people on this issue and to point out the priority areas of intervention. Up to now 115 individuals have answered the questionnaire (age between 16 to 83 years). Despite the considerable level of literacy of subjects, there is still a lack of knowledge concerning alternatives to the use of salt and the

importance of frequent assessment of blood pressure. It is necessary to continue to take measures for the reduction of salt content in food, contributing to the improvement of health status of the Portuguese population.

Introdução

O sal é um ingrediente, um condimento e um nutriente, desempenhando um papel fulcral na alimentação humana. No entanto, o seu uso em excesso está associado a Hipertensão Arterial, um problema de saúde pública. Ao longo dos anos, tem-se verificado um aumento dos casos de hipertensão arterial, estimando-se que esta patologia esteja na origem de 7,5 milhões de mortes por ano (1). A Organização Mundial de Saúde recomenda um consumo de sal inferior a 5 g/dia para a prevenção de doenças cardiovasculares (1). O consumo de sal está profundamente relacionado com esta patologia, sendo os alimentos processados os que mais contribuem para este consumo. Em Portugal, verificou-se um decréscimo no consumo de sal, mas a população portuguesa ingere sensivelmente o dobro do valor recomendado (2).

Objetivos

Avaliar a perceção do consumidor sobre o consumo de sal e os efeitos na saúde, visando sinalizar as principais fragilidades da população portuguesa relativas ao conhecimento sobre esta temática e quais as áreas prioritárias de intervenção. E assim poder definir estratégias focadas na consciencialização da população portuguesa, aumento do seu conhecimento e, deste modo, promover a saúde pública.

Material e métodos

Durante o ano de 2015, foi desenvolvido um questionário composto por 3 partes: (a) Informação geral; (b) Hábitos alimentares; e (c) Consumo de sal e relação com a saúde. O questionário foi aplicado *online* e foram inquiridos até ao momento 115 indivíduos (idades 16 aos 83 anos). Os indivíduos foram inquiridos relativamente à sua perceção relativa ao teor em sal de alguns alimentos; e teor de sal das refeições servidas em cafés ou pastelarias, cantinas ou bares, restaurantes, restaurantes de *fast-food*, em casa, e em *self-services*. Para além disso foram também colocadas questões relacionadas com a perceção do consumidor relativa ao consumo de sal e efeitos na saúde.

Resultados e discussão

Foram abrangidos 13 distritos de Portugal, embora 52% e 22% dos inquiridos eram residentes no distrito de Lisboa e Porto, respetivamente. Dos 115 indivíduos inquiridos, 80% eram licenciados, mestres ou doutorados (Figura 10).

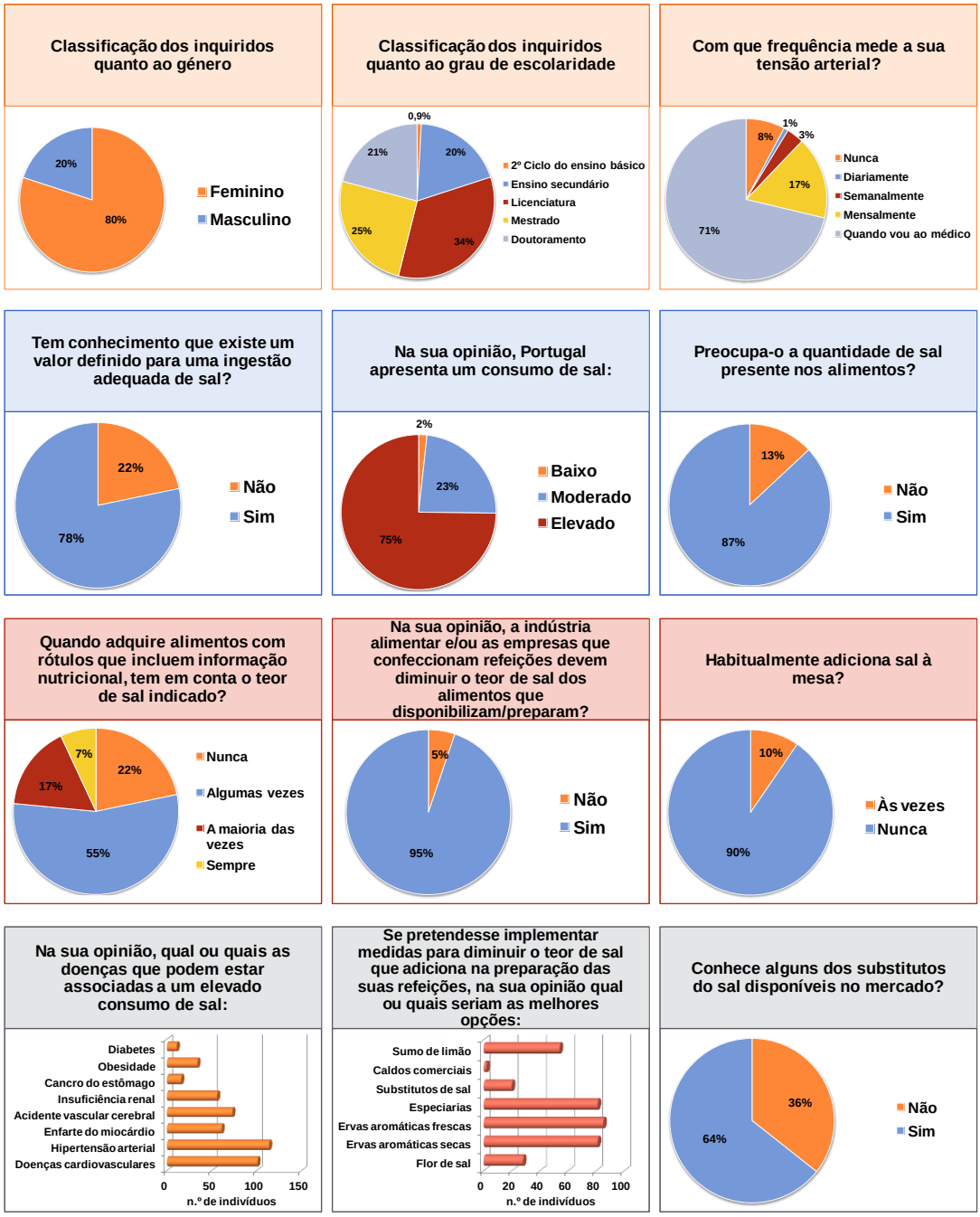

Figura 10. Respostas dos inquiridos a algumas das questões abrangidas no questionário sobre perceção do consumidor sobre o consumo de sal e os seus efeitos na saúde.

Relativamente à frequência de avaliação da tensão arterial, 71% dos indivíduos afirma apenas fazer a avaliação quando vai ao médico. No que diz respeito ao conhecimento de um valor definido para uma ingestão adequada de sal, 78% dos inquiridos reconhece que existe um valor definido, no entanto, cerca de 44% destes indivíduos respondeu que esse valor se situa entre 2 e 3 g/dia. Verificou-se que a maior parte dos indivíduos (54%) considera muito importante indicar o teor de sal no rótulo dos alimentos e 87% dos indivíduos consideram que a indústria alimentar deve reunir esforços para reduzir o teor de sal dos alimentos. No que diz respeito às doenças que podem estar associadas a um elevado consumo de sal, a hipertensão arterial, as doenças cardiovasculares, o acidente vascular cerebral e o enfarte do miocárdio são as mais reconhecidas. Mais de 70% dos inquiridos reconhece que a adição de ervas aromáticas, frescas e secas, e de especiarias são medidas a implementar para diminuir o teor de sal adicionado na preparação das refeições. No entanto, 24% dos indivíduos identificaram o uso de flor de sal como medida para reduzir a adição de sal. No que diz respeito ao local da refeição, 89% afirma que os restaurantes de *fast-food* são aqueles que comercializam alimentos com maiores teores de sal, seguidos pelos outros restaurantes (42%) e *self-services* (35%). Por outro lado, mais de 60% dos indivíduos reconhece que queijos secos, charcutaria, pizas, *fast-food*, *snacks*, salgados e refeições pré-confecionadas, são alimentos com teor elevado de sal.

Conclusões

Os resultados obtidos realçam a importância deste tipo de questionários, porque demonstram que as iniciativas que têm sido realizadas para a consciencialização da população estão a ser eficazes. No entanto, apesar do nível considerável de literacia dos inquiridos, existe ainda algum desconhecimento relativo às alternativas ao uso do sal e à importância da avaliação frequente da tensão arterial. É necessário continuar a tomar medidas que permitam a redução do teor de sal dos alimentos, contribuindo para a melhoria do estado de saúde da população Portuguesa. Pretende-se, num futuro próximo, discutir os resultados de um questionário aplicado a um maior número de indivíduos, focando a população com níveis de literacia inferiores.

Referências bibliográficas

Polónia J, Martins L, Pinto F, Nazare J. (2014). Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. J Hypertens, 32: 1211-1221.

World Health Organization. (2013). Mapping salt reduction initiatives in the WHO European Region. WHO Regional Office for Europe, Copenhagen, Denmark.

2.3 Cholesterol determination in foods: Comparison between high performance and ultra-high performance liquid chromatography

Este sub-capítulo apresenta os resultados relativos ao desenvolvimento, optimização e validação de um método analítico por cromatografia líquida para doseamento do teor de colesterol em géneros alimentícios. Pretendeu-se desenvolver uma técnica com elevada sensibilidade, rápida, económica e com reduzido impacto ambiental.

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Cholesterol determination in foods: Comparison between high performance and ultra-high performance liquid chromatography

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Abstract

Analytical methods for cholesterol evaluation in foods are crucial since this compound was closely related with cardiovascular disease. In the present study, two chromatographic methods were implemented and validated, in order to achieve the ideal analytical method for the quantification of cholesterol in food matrices. The developed methods were applied to different foodstuffs, sour cream, egg, egg yolk and chicken nuggets. Both HPLC and UHPLC methods are rapid, specific, sensitive, precise (RSD < 2.5%) and accurate. The achieved LOD and LOQ for UHPLC were 0.7 and 2.4 µg/mL, respectively, while for HPLC were 3 and 11 µg/mL. UHPLC method allowed reduction of the organic solvents consumption (8 times lower) and decreased analysis time in 4 min, being more eco-friendly, than conventional HPLC methods. Moreover, it will be very useful for the quality control of cholesterol content in food matrices and can be easily adopted by analytical laboratories.

Introduction

Cholesterol (3S,8S,9S,10R,13R,14S,17R)-10,13-dimethyl-17-[(2R)-6-methylheptan-2yl] 2,3,4,7,8,9,11,12,14,15,16,17-dodecahydro-1H-cyclopenta[a]phenanthren-3-ol is a waxy lipid (sterol) whose chemical structure contains multiple hydrocarbon rings. Cholesterol plays an essential role in the structure of many membranes and as a precursor of steroid hormones and bile acids (Connor & Connor, 2002). From a nutritional point of view, cholesterol is not found in significant amounts in plant sources, being mostly present in foods of animal origin, namely cheese, egg, beef, pork, poultry, fish, and shrimp. With

respect to dietary reference values for cholesterol intake, the Panel from European Food Safety Authority has decided not to propose a reference on cholesterol intake beside its conclusion on the intake of saturated fatty acids (EFSA, 2010). However, German-Austrian-Swiss recommendations (D-A-CH, 2008), the World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (WHO/FAO, 2003) have set recommendations for a maximum intake of cholesterol of 300 mg per day for the adult population. In Portugal, for children below 15 years of age the average intake of cholesterol varied between 360 and 378 mg/day, for females and males, respectively. Among Portuguese adults, the values ranged from 302 mg/day (females) to 324 mg/day (males) (EFSA, 2010).

Analytical methods for cholesterol evaluation in foods are crucial due to the relationship of this compound with cardiovascular disease (Dinh, Thompson, Galyean, Brooks, Patterson & Boylan, 2011). Cardiovascular disease is a major cause of disability and premature death throughout the world (WHO, 2007). According to WHO, by 2030 more than 23 million people will die annually from cardiovascular disease. High levels of low-density lipoprotein cholesterol is a major cardiovascular risk factor (Hu, Stampfer, Rimm, Manson, Ascherio, Colditz, et al., 1999). Once dietary cholesterol intake is increasing, the plasma cholesterol levels rise and consequently increases the risk of cardiovascular diseases and atherosclerosis (Connor et al., 2002).

Cholesterol can be determined by several analytical methods, such as, gravimetry, colorimetry, fluorimetry and chromatography. Up to now, in our laboratory an enzymatic method has been used. Nonetheless, this method lacks specificity because other sterols with a 3β-OH group including phytosterols can also be oxidized and form similar pigments (Bragagnolo 2009). Therefore, in processed foods with vegetable lipids, the enzymatic method can overestimate cholesterol content. Gas and liquid chromatography are the most suitable methods for cholesterol determination, due to their ability to separate and quantify this compound from other similar ones (Daneshfar, Khezeli & Lofti, 2009). Gas chromatography (GC) was the most used analytical technique for cholesterol and other sterols quantification. However, GC columns are very efficient for cholesterol separation but sometimes suffer from possible overlapping of cholesterol with other sterols (Fenton, 1992; Dinh et al., 2011). This disadvantage can be easily resolved using high performance liquid chromatography (HPLC), especially reversed-phase HPLC. Moreover, HPLC has the main advantage to be carried out at low temperature and avoid cholesterol oxidation. Most of the HPLC methods previously reported in the literature require long periods of sample preparation and consume large amounts of organic solvents (Ramalho, Casal & Oliveira, 2011). Ultra-high performance liquid chromatography (UHPLC) can be the analytical

technique of choice, since it is more sensitive, cost-effective and less time-consuming than other conventional methods and therefore, ideal for the quantification of cholesterol in food matrices.

The most common detector used in HPLC for cholesterol detection is the diode array detector (DAD). However, other detection techniques have been reported, such as, ultraviolet (UV), fluorescence detection, evaporative light-scattering detection, infrared detection, and electrochemical detection (Dinh et al., 2011; Fenton, 1992; Shimada, Mitamura & Higashi, 2001). Table 3 compares some of the published methods for cholesterol determination in different matrices by liquid chromatography. Reversed-phase HPLC coupled with UV or DAD detector is the most common technique (Maraschiello, Dáz & Regueiro, 1996; Bragagnolo & Rodriguez-Amaya, 2002; Saldanha, Mazalli & Bragagnolo, 2004; López-Cervantes, Sánchez-Machado & Ríos-Vázquez, 2006; Osman & Chin, 2006; Mazalli, Sawaya, Eberlin & Bragagnolo, 2006; Canãbate-Dıáz et al., 2007; Hojo et al., 2007; Lin, Wu & Wu, 2007; Daneshfar et al., 2009; Ahn et al., 2012a). Most of the analytical methods reported in the literature use 5 µm particle size columns, but the use of 3 or 4 µm particle size columns are also described (Saldanha et al., 2004; Mazalli et al., 2006; Saldanha, Samaya, Eberlin & Bragagnolo, 2006; Ramalho et al., 2011). In the present work, a short column, with particle size < 2.0 µm, able to perform analysis under high pressure conditions (15000 psi) was selected, in order to improve parameters such as sensitivity, resolution and analysis time.

In addition to the analytical technique, sample preparation is also very important in cholesterol evaluation. Saponification is one of the most important steps to obtain the cholesterol free from other components. For this procedure, potassium hydroxide is the most common solvent used to separate cholesterol from fatty acids, avoiding also triglyceride interference (Fenton, 1992; Dihn et al., 2011; Ahn et al., 2012b). Then, to remove these compounds the mixture is washed with ultra-pure water and the residuals, such as cholesterol, remain in the extracted solution layer for analysis (Ahn et al., 2012b). Usually potassium hydroxide is prepared with methanol or ethanol, in concentrations ranging from 0.35 M to 2.7 M (Table 3). For the extraction of cholesterol in complex food matrices, such as egg yolk, the most common reagent used is hexane, due to its low polarity compared with toluene, which enables the formation of an emulsion.

The aim of this study was to develop a simple, fast, sensitive and environmental friendly method for cholesterol determination in food matrices. Moreover, two liquid chromatographic systems were compared in order to evaluate the best chromatographic

one. Both methods were validated according to international guidelines and applied to different foods: sour cream, egg, egg yolk and chicken nuggets.

Materials and methods

Standards and reagents

All reagents and standards were of analytical grade. Cholesterol standard (CAS: 57-88-5) was from Sigma-Aldrich (Madrid, Spain) and had a purity ≥99%. Potassium hydroxide (KOH), n-hexane, acetonitrile (ACN), dichloromethane (DCM), ethanol, methanol, and isopropanol (IPA) were purchased from Merck (Darmstadt, Germany). Ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA). Infant/Adult nutritional formula (SRM® 1849) and Fatty acids/cholesterol in frozen diet composite (SRM® 1544) were obtained from National Institute of Standards and Technology (Gaithersburg, MD, USA).

Calibration curves

Calibration curves were performed daily using seven standard concentrations. A stock solution of cholesterol (1.25 mg/mL) was prepared by dissolving 25 mg of cholesterol into 20 mL of ACN/IPA (70:30, v/v). The working standard solutions were prepared from the stock solution by appropriate dilution to obtain the final concentrations of cholesterol (5, 70, 140, 200, 270, 330 and 400 µg/mL).

Analysis of standard reference materials and samples

Samples were randomly collected from major supermarket chains in the region of Lisbon (Portugal). Sour cream, egg and egg yolk were used for the validation of the methods (UHPLC and HPLC). Four brands of frozen chicken nuggets (two commercial brands and two supermarket brands in the pre-fried form); one sample of chicken nuggets from a fast-food restaurant already cooked (baked) and two standard reference materials were used for the application of the chosen validated method.

The chicken nuggets samples acquired in the pre-fried form were submitted to two processing methods: (i) deep fried using a household fryer (180 ºC, 3-5 min), and (ii) baked in a domestic oven (200 ºC, 15 min).

All the samples were homogenized for 1 min at 5600 rpm using a high performance homogenizer (Ultra Turrax®, IKA, Staufen, Germany). Afterwards, samples were kept in containers and stored in a deep freezer (at least -20 ºC). Each sample was analyzed in triplicate and chromatographic analyses performed in triplicate. The cholesterol content of the analysed samples are expressed as mg/100 g of edible portion on fresh weight basis.

Table 3. Liquid chromatographic methods for cholesterol determination.

Chromatographic system	Detector	Column	Detection (nm)	Extraction	Mobile phase	Flow rate (mL/min)	Run time (min)	Range (µg/mL)	Determination coefficient (r^2)	LOQ (µg/mL)	LOD (µg/mL)	Reference
RP-HPLC	DAD	SS Exil ODS (250 x 4 mm, i.d., 5 µm)	208	Methanolic KOH (0.5 M); n-hexane	Methanol/ACN/H_2O (68:28:4, v/v/v)	1.4	11	96 - 2402	0.9998	-	1.38	López-Cervantes et al. (2006)
RP-HPLC	DAD	Spherisorb ODS-2 (150 x 4.6 mm, i.d., 5 µm)	210	-	ACN/IPA (70:30, v/v)	1.1	15	-	-	-	-	Bragagnolo et al. (2002)
UHPLC	DAD	Acquity BEH C18 (100 x 2.1 mm, i.d., 1.7 µm)	210	IPA; $(NH_4)_2SO_4$; KOH; NaCl	A - 90% methanol/ ethanol (50:50) and B - 10% water (100)	0.3	-	-	>0.9999	5.13	1.55	Ahn et al. (2012a)
UHPLC	MS	Acquity BEH C18 (50 x 2.1 mm, i.d., 1.7 µm)	-	Petroleum ether and ethyl ether	Methanol with 0.1% of formic acid	-	-	0.1-5	-	-	-	Zhu et al. (2012)
RP-HPLC	UV	Xterra C18 (250 x 4.6 mm, i.d., 5 µm)	210	IPA; $(NH_4)_2SO_4$; KOH; NaCl	A - 90% methanol/ ethanol (50:50) and B - 10% water (100)	1.0	-	10 - 200	>0.9998	7.56	2.27	Ahn et al. (2012b)
RP-HPLC	UV	CLC-ODS-C8 (150 x 6 mm, i.d., 5 µm)	210	Ethanol (with tetrachloride); ACN	ACN/ethanol (50:50, v/v)	1.0	<10	30-10000	0.9996	30	10	Daneshfar et al. (2009)
RP-HPLC	FD	LiChrospher 60 RP-C8 (250 x 4 mm, i.d., 5 µm)	Excitation - 231 Emission - 352	KOH (1 M); toluene; methanol	Methanol/IPA/H_2O (90:5:5, v/v)	1.0	30	0.04 - 0.77	-	0.01	-	Lin et al. (2007)
NP-HPLC	DAD	Supelcosil™ LC-SI (75 x 3 mm, i.d., 3 µm)	210	KOH (0.5 M); n-hexane	n-hexane/1,4-dioxane (97.5/2.5, v/v)	1.0	12	0.4 - 280	>0.999	0.40	0.13	Ramalho et al. (2011)
NP-HPLC	UV	Porosil (300 x 3.9 mm, i.d., 10 µm)	205	Methanol	H_2O/methanol/ACN (5:70:25, v/v/v)	2.0	5	267 - 1017	0.993	-	-	Choudhari et al. (1996)
RP-HPLC	ECD	Develosil C30-UG-3 (150 x 4.6 mm, i.d., 3 µm)	-	Ethanolic KOH (1 M); n-hexane	ACN/IPA (90:10, v/v) containing 50 mM $LiClO_4$	1.0	>30	0.19 - 38.7	-	-	0.14	Hojo et al. (2007)
RP-HPLC	DAD	Octadecylsilica (150 x 4 mm, i.d., 5 µm)	210	Methanolic KOH (0.5 M); NaCl; ether/ n-hexane	ACN:IPA (55:45, v/v)	1.2	<6	-	-	-	-	Maraschiello et al. (1996)
NP-HPLC	UV	Nova Pack CN HP (300 x 3.9 mm, i.d., 4 µm particle size)	210	Ethanolic KOH (15%) Ethyl ether	n-hexane/IPA (96:4, v/v)	1.0	20	2000 - 2500	-	0.09	0.03	Mazalli et al. (2006)
RP-HPLC	MS	Atlantis C18 (150 x 2.1 mm i.d., 5 µm)	-	Ethanolic KOH (2 M); diethyl ether	ACN/H_2O (0.01% acetic acid)	0.5	<34	0.05 - 20	-	-	-	Canãbate-Dıáz et al. (2007)
NP-HPLC	UV	Selectosil (250 x 4.6 mm, i.d., 5 µm)	205	n-hexane/IPA/acetic acid (98.7:1.2:0.1, v/v)	A - IPA in n-hexane (0.1 % acetic acid) and B - n-hexane	0.6	<50	0.8 - 75	-	-		Murphy et al. (1996)
RP-HPLC	UV	Chromolith C18 (100 x 4.6 mm, i.d., 4 µm)	210	KOH (50%); ethanol; n-hexane	ACN:IPA (85:15 and 95:5, v/v)	2.0	4	-	-	-	-	Saldanha et al. (2004)
RP-HPLC	UV	Symmetry C18 (250 x 4.6 mm, i.d., 5 µm)	-	Ethanolic KOH (2%); n-hexane	ACN:IPA (50:50, v/v)	-	<20	25 - 750	0.995	0.60	0.08	Osman et al. (2006)
NP-HPLC	UV	Nova Pack CN HP (300 x 3.9 mm, i.d., 4 µm)	210	KOH (20 - 50%); ethanol; n-hexane	n-hexane/IPA (97:3, v/v)	1.0	30	100 - 2000	>0.996	-	-	Saldanha et al. (2006)

HPLC – high performance liquid chromatography; UHPLC – ultra-high performance liquid chromatography; RP-HPLC – reversed-phase HPLC; NP-HPLC – normal-phase HPLC; DAD – diode array detector; ECD – electrochemical detector; FD – fluorescence detector; MS – mass spectrometry; LOQ – limit of quantification; LOD – limit of detection.

The standard reference materials (Section 2.1) and samples were prepared according to the method described by Bragagnolo & Rodriguez-Amaya (2003) with slight modifications. In a 50 mL tube, 0.25 - 1 g of sample was weighed, 5 mL of ethanolic KOH (0.4 M, w/v) were added and thoroughly mixed in a vortex for 1 min. Afterwards samples were heated in a water bath at 50 ºC for 30 min. Then, the mixture was cooled at room temperature, 5 mL of ultrapure water were added and thoroughly mixed in a vortex. Cholesterol was extracted twice with 10 mL of n-hexane. An aliquot (3 mL) of the combined extracts was dried under nitrogen, redissolved in 3 mL of mobile phase and aliquots analysed in both chromatographic systems.

Instrument and chromatographic conditions

HPLC

An Alliance 2695 HPLC system (Waters, Milford, MA, USA), equipped with a Waters 2996 DAD detector, using a Supelcosil™ LC-18-DB (150 x 4.6 mm I.D., 3.0 µm particle size) analytical column protected with a Supelcosil™ LC-18-DB guard column (20 x 2.1 mm I.D., 5.0 µm particle size), Supelco (Bellefonte, PA, USA) was used for separation and quantification of cholesterol. The detection was achieved at 210 nm and the peak areas were quantified and processed with Empower™ version 2.0 software (Waters, Milford, MA, USA). The mobile phase was ACN/IPA (70:30 v/v), which was filtered under vacuum through a 0.45 µm (Millipore, Bedford, MA, USA) membrane filter and then degassed in an ultrasonic bath for 30 min. Column and auto-sampler temperatures were kept at 20 ºC, the flow-rate was 1.2 mL/min and total run time 8 min. A volume of 10 µL was injected into the chromatographic system.

UHPLC

Separation and quantification of cholesterol were performed on an Acquity UPLC® (Waters, Milford, MA, USA), equipped with a binary pump, an auto-sampler binary solvent manager, a column thermostatting system and a DAD detector. Chromatographic separation was performed with an Acquity UPLC® BEH C18 analytical column (2.1 x 50 mm I.D., 1.7 µm particle size) and an Acquity UPLC® BEH C18 guard column (2.1 x 5.0 mm I.D., 1.7 µm particle size) from Waters (Milford, MA, USA). The detection signal was recorded and the peak areas quantified and processed with Empower™ software version 2.0 (Waters, Milford, MA, USA). The mobile phase (ACN/IPA, 70:30, v/v) was filtered through a 0.22 µm GHP membrane, Pall filter, (Gelman Laboratory, Canada) and then degassed for 30 min. The total run time of analysis was 4 min at a flow rate of 0.3 mL/min. Column and auto-sampler

temperatures were kept at 20 ºC. The injected volume was 10 µL. Cholesterol identification was carried out based on the retention time and UV spectrum of the cholesterol standards.

Method optimization

In order to optimize the extraction procedure, the sample amount was studied. Moreover, saponification reagents (methanolic and ethanolic KOH) and concentrations (0.1 – 0.4 M) were tested. Four different reaction times (30, 60, 90 and 120 min) were evaluated. Results from the studied samples, analyzed in triplicate, were compared.

For the preparation of cholesterol standards, several reagents were used, such as, DCM, methanol, ACN, ACN/DCM (90:10, 70:30, v/v) and ACN/IPA (90:10, 70:30, v/v).

With respect to the chromatographic conditions, the mobile phase, flow rate and column temperature were optimized. The following mobile phases were tested: ACN (100%), ACN/IPA (95:5, 80:20, 70:30, v/v), ACN/DCM (95:5, 80:20, 70:30, v/v) and methanol/ethanol (50:50). For the HPLC system flow rates from 0.6 to 1.2 mL/min were studied and for the UHPLC system 0.3 to 0.5 mL/min. Different column temperatures were tested varying between 20 and 36 ºC.

Method validation

The developed methods were validated according to Food and Drug Administration (Center for Drug Evaluation and Research, 1994) or International Conference on Harmonisation (International Conference Harmonization, 1997) guidelines and the following parameters were determined: sensitivity, specificity, limit of detection (LOD), limit of quantification (LOQ), range, linearity, precision and accuracy. All the experiments were performed using the optimized conditions.

Specificity was assessed by confirming the absence of any interference at the retention time of cholesterol peak and by comparing the cholesterol absorption spectra of the analysed samples with pure standards.

Six calibration curves in the range of 70 – 400 µg/mL for HPLC system and 5 – 400 µg/mL for UHPLC system were performed using six and seven standard concentrations, respectively, injected six times each. Calibration curves linearity was evaluated by linear regression analysis, plotting peak areas versus standard concentrations. Parameters such as determination coefficient (r^2), slope and intercept were calculated. The slope of calibration curve was also used to evaluate method sensitivity.

The LOD was considered to be the lowest analyte concentration, which gives a signal-to-noise (S/N) ratio of at least 3:1 (Center for Drug Evaluation and Research, 1994;

International Conference Harmonization, 1997). The LOQ was determined as the lowest concentration which gives a S/N ratio of at least 10:1 (Center for Drug Evaluation and Research, 1994; International Conference Harmonization, 1997). The achieved limits were confirmed by analyses of five independent standard solutions at LOD and LOQ concentrations.

The repeatability of the equipment was determined by analyzing six dependent replicate solutions at a medium concentration level of 0.2 mg/mL.

The repeatability of the method was investigated by injecting in triplicate, six samples from sour cream, egg and egg yolk on the same day. The intermediate precision was evaluated on three different days by preparing six samples from the same sour cream (n=6), egg (n=6) and egg yolk (n=6) on each day. Mean, standard deviation (SD) and relative standard deviation (RSD) were calculated.

In order to analyse possible losses of cholesterol during the extraction procedure, the accuracy was determined by recovery tests using the standard addition method. The recoveries were measured after spiking samples from sour cream, egg and egg yolk with cholesterol standard using the following concentrations 1.0, 2.0 and 2.5 mg/mL, respectively. The measurements were performed using six determinations for each sample. After addition of standard solutions, the samples were processed according to the method previously described (Section 2.3).

Statistical analysis

Results are expressed as mean ± standard deviation (SD) or as percentage. Statistical analysis was performed using the SPSS for Windows, IBM SPSS Statistics 19.0 (SPSS Inc, Chicago). Differences between HPLC and UHPLC were determined using t-test for related and independent samples. A value of $p < 0.05$ was considered statistically significant.

Results and discussion

Optimization of the sample preparation and chromatographic method

In order to achieve the best results, this work started with the optimization of extraction conditions, followed by the optimization of chromatographic conditions, for cholesterol evaluation in foods. Different sample amounts (0.1, 0.25, 0.5, 0.75 and 1.0 g) were tested. Depending on the expected cholesterol concentration, a different sample amount was used. For sour cream, with lower cholesterol contents, 1.0 g was used. However, because egg, and especially egg yolk, present higher contents of cholesterol, 0.5 and 0.25 g, respectively, were used. Saponification is a critical step in sample preparation for cholesterol

determination in food matrices, since it is essential for the separation of cholesterol from other unsaponifiable components (Fenton, 1992). There are two types of saponification, the indirect (requires a previous Folch extraction) and the direct. The last one according to most of the studies has superior recovery and accuracy values (Dinh et al., 2011). According to the literature (Table 3), ethanolic KOH is the most used reagent for saponification, within a wide range of concentrations. Therefore, the following ethanolic KOH concentrations were tested: 0.1, 0.2, 0.3 and 0.4 M. Moreover, the saponification reaction time was also evaluated (30, 60, 90 and 120 min). As shown in Fig. 11, the optimal conditions were ethanolic KOH (0.4 M) and 30 min of reaction time.

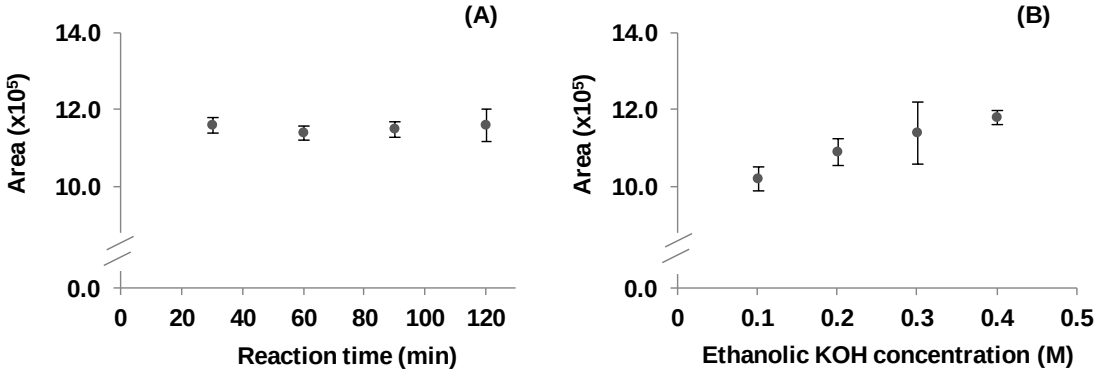

Figure 11. Optimization of the conditions for samples saponification. (A) Reaction time and (B) Ethanolic KOH concentration.

According to our knowledge, up-to-now only one study reported cholesterol determination by UHPLC/DAD (Ahn et al., 2012b). In 2012, another UHPLC method using a mass spectrometry detector was published by Zhu et al. (2012). One of the main objectives of the present study was to develop, validate and compare liquid chromatographic methods for cholesterol determination in food matrices. UHPLC systems allow the use of columns packed with smaller particles (1.7 µm particle size instead of 3 – 5 µm), increasing sensitivity, resolution and selectivity, and reducing solvents consumption and analysis time, being an eco-friendly method (Swartz, 2005). For cholesterol determination by normal phase HPLC, the most commonly used mobile phase is n-hexane (Table 3). However, in reversed-phase HPLC methods, the composition of mobile phases can be very different as shown in Table 3, but most of the mobile phases contain ACN or IPA, with the exception of the method reported by Ahn et al. (2012a and 2012b) that only uses ethanol and methanol. In our study, several mobile phases were tested and the best results for both chromatographic systems were achieved using ACN/IPA (70:30, v/v). Some chromatograms of cholesterol standard (0.40 mg/mL) using different mobile phases are shown in Fig. 12. The selected mobile phase allowed the reduction of the retention time, for example from 6.5 min (ACN, 100%) to 2 min (ACN/IPA, 70:30) allowing also a better

resolution of the compound (Figure 13). As described in the literature, analysis time is directly influenced by the mobile phase flow rate, with direct consequence on solvent consumption, but the temperature of the column can also affect the process. In the case of cholesterol determination the use of low temperatures prevents its oxidation (Ramalho et al., 2011). Therefore, in the present work, different column temperatures as well as different flow rates

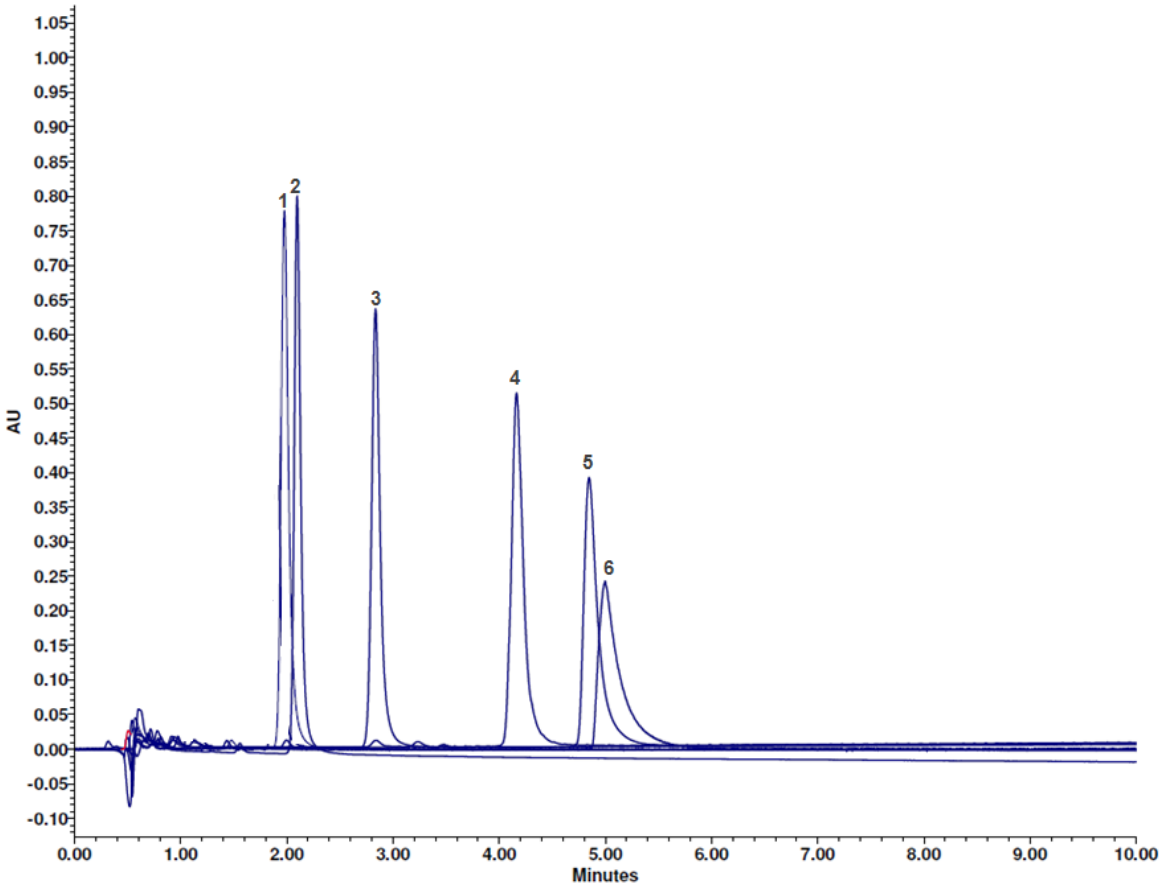

Figure 12. Chromatograms of cholesterol standard (0.40 mg/mL) using UHPLC chromatographic system with different mobile phases. (1) ACN/DCM (95:5, v/v), (2) ACN/IPA (70:30, v/v), (3) ACN/IPA (80:20, v/v), (4) ACN/IPA (90:10, v/v), (5) ACN/IPA (95:5, v/v) and (6) ACN (100%).

were tested (Section 2.4). The following optimum conditions were selected: 0.3 mL/min for UHPLC system, 1.2 mL/min for HPLC system and a column temperature of 20 ºC for both chromatographic systems. Figure 13 illustrates chromatograms from HPLC and UHPLC systems obtained for cholesterol standard (A and B), sour cream sample (C and D), egg sample (E and F) and egg yolk sample (G and H).

Method validation

The present methods were validated for specificity, sensitivity, range, linearity, LOD, LOQ, precision and accuracy. Table 4 presents a comparison of the obtained results regarding validation parameters. To ensure the method specificity, the absence of any interfering peak at the cholesterol retention time was confirmed, and the spectrum from cholesterol in samples and pure standard were compared. The external standard method was used for quantification purposes. Calibration curves were linear over the range 0.07 – 0.4 mg/mL and 0.005 – 0.4 mg/mL, for HPLC and UHPLC systems, respectively. Determination coefficients were always equal or greater than 0.9971 for HPLC and 0.9992 for UHPLC, with RSD ≤ 0.1%, indicating suitability of both systems for cholesterol quantification. The achieved LOD and LOQ for UHPLC were 0.7 and 2.4 µg/mL, respectively, while for HPLC were 3 and 11 µg/mL. A review on the methods to determine cholesterol allowed the comparison of our results (Table 3). Ahn et al. (2012b) that have used UHPLC with the same detector and column, but using a different mobile phase, A – 90 % methanol/ethanol (50:50, v/v) and B – 10% Water, reported a LOD and LOQ values approximately two times higher than the limits achieved within our study.

Table 4. Linearity, LOD and LOQ of HPLC and UHPLC systems used for cholesterol determination.

Chromatographic system		**HPLC**	**UHPLC**
Concentration range (µg/mL)		70 - 400	5 - 400
Slope (n = 6)	Mean ± SD	$2.32 \times 10^6 \pm 2.52 \times 10^4$	$9.93 \times 10^6 \pm 7.02 \times 10^4$
Intercept (n = 6)	Mean ± SD	$4.76 \times 10^4 \pm 6.28 \times 10^4$	$2.31 \times 10^4 \pm 1.11 \times 10^4$
Determination coefficient (r^2)	Mean ± SD	0.9985 ± 0.00	0.9995 ± 0.00
	RSD (%)	0.10	0.02
Equipment repeatability	RSD (%)	0.68	0.12
LOD (µg/mL)		3	0.7
LOQ (µg/mL)		11	2.4

LOQ – limit of quantification; LOD – limit of detection

In order to evaluate the precision of the proposed methods, repeatability and intermediate precision results for both chromatographic systems and for the three tested samples were studied (Table 5). The methods proved to be precise for cholesterol in the analysed food samples and for both systems RSD was always lower than 2% for repeatability and intermediate precision, except for the intermediate precision of egg yolk using the UHPLC system (RSD of 2.5%).

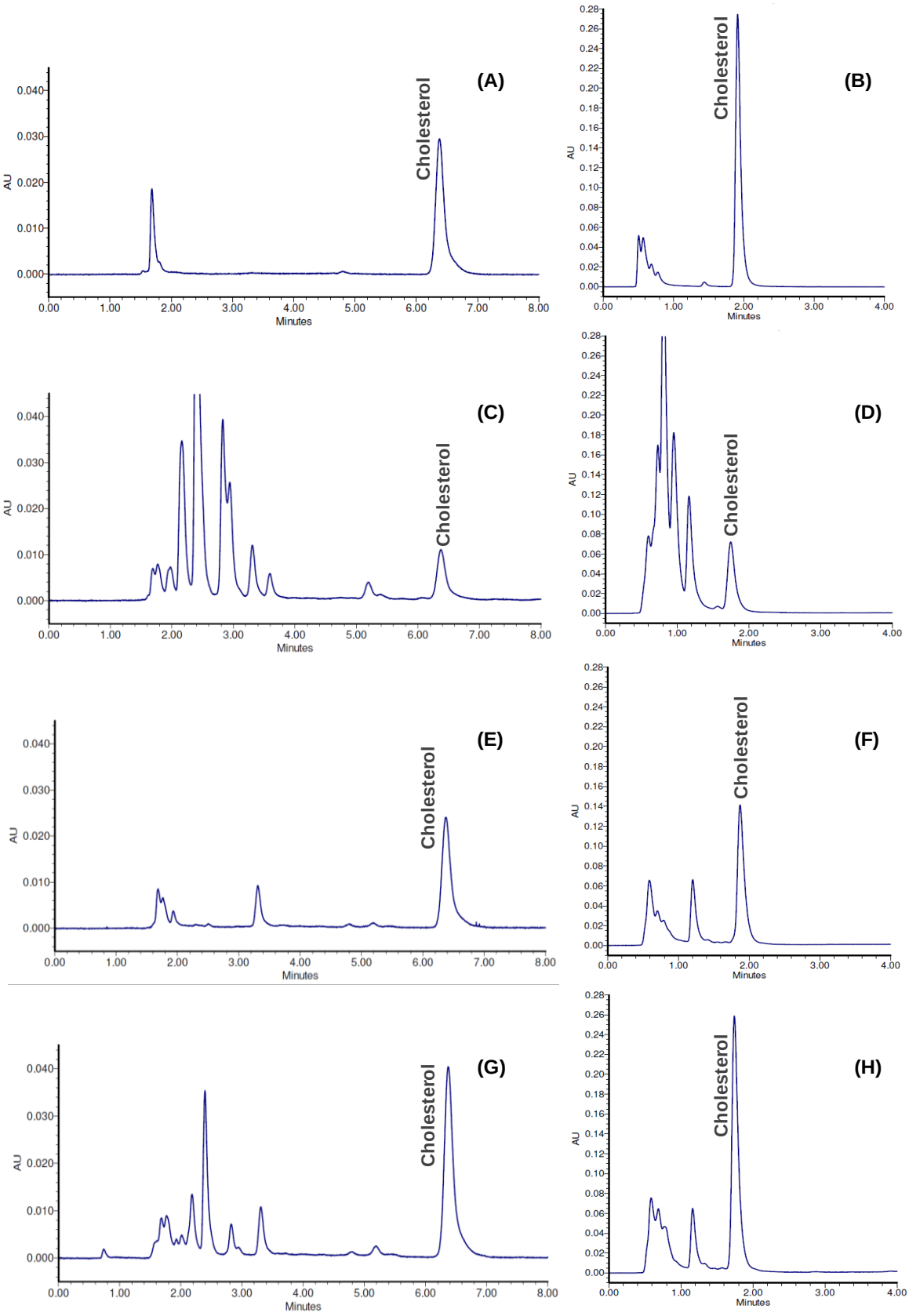

Figure 13. Chromatograms of cholesterol standard (0.14 mg/mL) using HPLC system (A) and UHPLC system (B); and chromatograms from HPLC and UHPLC systems obtained respectively for sour cream (C) 0.05 mg/mL and (D) 0.05 mg/mL; egg (E) 0.11 mg/mL and (F) 0.10 mg/mL; and egg yolk (G) 0.19 mg/mL and (H) 0.16 mg/mL. λ= 210 nm.

The results for accuracy (Table 6) using a standard addition procedure were applied for both chromatographic systems and for the three studied samples. For UHPLC method recovery ranged from 80% to 106%, for egg and egg yolk, respectively, while for HPLC it varied between 111% and 125%, for sour cream and egg yolk, respectively.

Analysis of standard reference materials and samples by the validated UHPLC method

Standard reference materials and chicken nuggets samples were analysed using the validated UHPLC method. As part of the study, two standard reference materials were used for quality control purposes. The certified values of cholesterol content for SRM® 1849 and SRM® 1544 are 13.74 ± 0.29 mg/100 g and 14.83 ± 0.94 mg/100 g, respectively. The obtained cholesterol content for SRM® 1849 was 13.81 ± 0.02 mg/100 g and for SRM® 1544 was 14.87 ± 0.04 mg/100 g.

Nutritional composition data are an essential resource for a range of applications, including public health nutrition, food industry, and food consumption surveys, which investigate the relationship between food and disease in populations and require an accurate estimation of nutrient intake, and are also the basis for the development of dietary recommendations (Costa, Vasilopoulou, Trichopoulou, & Finglas, 2010). Food Composition Databases should contain all nutrients that are important in human nutrition and therefore laboratories that provide consistently reliable and accurate results in food analysis are very important.

In recent years, the consumption of processed foods, breaded fish-based and breaded meat-based foods has increased. Generally, these breaded foods are highly appreciated, especially by young people, since they are considered as very palatable foods. Moreover, there is a growing tendency worldwide to spend less time on food preparation (Varela, Salavador, & Fiszman, 2008; Miranda et al., 2010). One of the main issues related with the consumption of breaded foods, such as chicken nuggets, is the high amount of oil/fat absorbed during the pre-frying and frying processes. However, in the last years due to a high demand for healthier cooking methods, it is also possible to cook chicken nuggets in the oven, which considerable decreases the amount of oil/fat in the food. Up-to-now there are some published studies about the influence of cooking methods on the nutritional composition of foods (Bógnar, 1998; Ngadi, Li, & Oluka, 2007; Weber, Bochi, Ribeiro, Victório, & Emanuelli, 2008; Miranda et al., 2010; Şengör, Alakavuk, & Tosun, 2013). However, there is a lack of data regarding cholesterol content in processed foods subjected to different cooking methods.

Table 5. Validation data for method precision.

Precision		UHPLC						HPLC					
		Cholesterol (mg/100 g of edible portion)											
		Sour cream		Egg		Egg yolk		Sour cream		Egg		Egg yolk	
		Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)	Mean ± SD	RSD (%)
Repeatability	Day 1	101 ± 2	1.52	375 ± 2	1.84	1214 ± 3	0.26	98.4 ± 2	1.50	445 ± 2	1.31	1434 ± 23	1.60
	Day 2	99.0 ± 2	1.67	365 ± 2	1.91	1151 ± 12	1.01	99.9 ± 2	1.63	435 ± 2	1.74	1381 ± 10	0.70
	Day 3	99.4 ± 1	1.42	371 ± 2	1.54	1196 ± 13	1.12	98.4 ± 2	1.51	437 ± 2	1.43	1410 ± 11	0.79
Intermediate precision	Between-day	100 ± 2	1.65	369 ± 7	1.65	1183 ± 30	2.52	98.9 ± 2	1.66	439 ± 8	1.75	1411 ± 28	1.95

Table 6. Validation data for method accuracy.

Chromatographic system	Samples	Cholesterol		Recovery (%)	RSD (%)
		Spiked (mg/mL)	Mean ± SD (mg/mL)		
HPLC	Sour cream	1	2.11 ± 0.02	111	0.69
	Egg	2	4.68 ± 0.05	120	0.54
	Egg yolk	2.5	6.91 ± 0.05	125	1.92
UHPLC	Sour cream	1	1.96 ± 0.02	93	1.33
	Egg	2	3.90 ± 0.06	80	1.24
	Egg yolk	2.5	5.81 ± 0.13	106	1.42

In the present work, four brands (2 commercial and 2 supermarket brands) and one sample from a fast-food restaurant (baked) of chicken nuggets were studied. Afterwards, the four brands were subject to two different cooking methods: deep frying and baked in an oven. In summary, 4 pre-fried samples, 5 baked samples and 4 deep fried samples were analysed for cholesterol content. For all the analysed samples, the highest cholesterol content was found when samples were cooked in the oven (baked), while fried samples had the lowest content, with the exception of one sample from a commercial brand. Baggio et al. (2006) also reported an increase of cholesterol content after meatballs and chicken sausages were subjected to baking.

Within the analysed samples, cholesterol content of the pre-fried samples ranged from 20.1 ± 2 to 47.3 ± 4 mg/100 g of edible portion, while for deep fried samples cholesterol varied between 27.8 ± 2 and 30.8 ± 2 mg/100 g of edible portion. With respect to pre-fried samples, there is a huge difference in the cholesterol content between chicken nuggets from different brands. The lowest cholesterol content for all the applied cooking methods was observed in one sample from a commercial brand. As mentioned before, samples cooked in the oven had the highest cholesterol content (32.3 – 59.1 mg/100 g of edible portion). According to the obtained results, the cholesterol content of baked samples was two times higher than the samples subjected to deep frying. The fast-food sample had a cholesterol content of 41.2 ± 0.1 mg/100 g of edible portion, which is similar to the value presented by the USDA National Nutrient Database for Standard Reference (44 mg/100 g of edible portion). The recovery in samples of chicken nuggets ranged from 80% to 117%.

Conclusion

In summary, the current validated HPLC and UHPLC methods for quantification of cholesterol in food matrices are rapid, specific, sensitive, precise and accurate. Both chromatographic methods were completely validated showing satisfactory data for all the tested parameters. Nonetheless, the new UHPLC method allowed reduction in the consumption of organic solvents (8 times lower) and decreased analysis time (4 min), being more eco-friendly, than conventional HPLC methods.

The method was applied to determine cholesterol content in chicken nuggets, with relatively low amounts of cholesterol, and allowed us to obtain good results. The simple, reliable, economic and rapid UHPLC method will be very useful for the quality control of cholesterol content in food matrices and can be easily adopted by analytical laboratories.

References

Ahn, J., Jeong, I., Kwak, B., Leem, D., Yoon, T., Yoon, C., Jeong, J., Park, J., & Kim, J. (2012a). Rapid determination of cholesterol in milk containing emulsified foods. *Food Chemistry, 135,* 2411-2417.

Ahn, J., Jeong, I., Kwak, B., Leem, D., Yoon, T., Yoon, C., Jeong, J., Park, J., & Kim, J. (2012b). Rapid determination of cholesterol in emulsified confectioneries by ultra-performance liquid chromatography. *European Journal of Lipid Science and Technology, 114,* 1304-1311.

Altunakar, B., Sahin, S., & Sumnu, G. (2004). Functionality of batters containing different starch types for deep-fat frying of chicken nuggets. *European Food Research Technology, 218,* 318-322.

Baggio, S. R., & Bragagnolo, N. (2006). The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products. *Food Chemistry, 95,* 611-619.

Bógnar, A. (1998). Comparative study of frying to other cooking techniques influence on the nutritive value. *Grasas y Aceites, 49,* 250-260.

Bragagnolo, N., & Rodriguez-Amaya, D. B. (2002). Simultaneous determination of total lipid, cholesterol and fatty acids in meat and backfat of suckling and adult pigs. *Food Chemistry, 79,* 255-260.

Bragagnolo, N., & Rodriguez-Amaya, D. B. (2003). Comparison of the cholesterol content of Brazilian chicken and quail eggs. *Journal of Food Composition and Analysis, 16,* 147-153.

Bragagnolo N. (2009). Cholesterol and cholesterol oxides in meat and meat products. In: Nollet LML, Toldra F, editors. Handbook of muscle foods analysis. Florida: CRC Press. p 187–219.

Cañabate-Díaz, B., Carretero, A. S., Fernández-Gutiérrez, A., Veja, A. B., Frenich, A. G., Vidal, J. L. M., & Martos, J. D. (2007). Separation and determination of sterols in olive oil by HPLC-MS. *Food Chemistry, 102,* 593-598.

Center for Drug Evaluation and Research. (1994). Reviewer guidance: validation of chromatographic methods. Rockville: US FDA Federal Register.

Choudhari, K. B., Jayanthi, S., Murty, R. B., & Matharu, R. P. (1996). A high-performance liquid chromatographic method for the analysis of lipids from lyophilized formulations. *Journal of Chromatography A, 724,* 343-347.

Connor, W. E., & Connor, S. L. (2002). Dietary cholesterol and coronary heart disease. *Current Atherosclerosis Reports, 4,* 425-432.

Costa, H. S., Vasilopoulou, E., Trichopoulou, A., & Finglas, P. (2010). New nutritional data on traditional foods for European food composition databases. *European Journal of Clinical Nutrition, 64,* S73-S81.

D-A-CH (Deutsche Gesellschaft für Ernährung - Österreichische Gesellschaft für Ernährung - Schweizerische Gesellschaft für Ernährungsforschung - Schweizerische Vereinigung für Ernährung). (2008). Referenzwerte für die Nährstoffzufuhr. Umschau Braus Verlag, Frankfurt am Main.

Daneshfar, A., Khezeli, T., & Lofti, H. J. (2009). Determination of cholesterol in food samples using dispersive liquid-liquid microextraction followed by HPLC-UV. Journal of Chromatography B, 877, 456-460.

Dinh, T. T. N., Thompson, L. D., Galyean, M. L., Brooks, J. C., Patterson, K. Y., & Boylan, M. (2011). Cholesterol content and methods for cholesterol determination in meat and poultry. *Comprehensive Reviews in Food Science and Food Safety, 10,* 269-289.

EFSA - European Food Safety Authority. (2010). Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. URL http://www.efsa.europa.eu/en/efsajournal/doc/1461.pdf. Accessed 10.12.13.

Fenton, M. (1992). Chromatographic separation of cholesterol in foods. *Journal of Chromatography, 624,* 369-388.

Hojo, K., Hakamata, H., Ito, A., Kotani, A., Furukawa, C., Hosokawa, Y., & Kusu, F. (2007). Determination of total cholesterol in serum by high-performance liquid chromatography with electrochemical detection. *Journal of Chromatography A, 1166,* 135-141.

Hu, F. B., Stampfer, M. J., Rimm, E. B., Manson, J. E., Ascherio, A., Colditz, G. A., Rosner, B. A., Spiegelman, D., Speizer, F. E., Sacks, F. M., Hennekens, C. H., & Willett, W. C. (1999). A prospective study of egg consumption and risk of cardiovascular disease in Men and Women. *JAMA, 281,* 1387-1394.

International Conference on Harmonization. (1997). Guidance for industry Q2b: validation of analytical procedures: methodology. Rockville: US FDA Federal Register.

Lin, Y., Wu, S., & Wu, H. (2007). Highly sensitive analysis of cholesterol and sitosterol in foods and human biosamples by liquid chromatography with fluorescence detection. *Journal of Chromatography A, 1156,* 280-287.

López-Cervantes, J., Sánchez-Machado, D.I., & Ríos-Vázquez, N.J. (2006). High-performance liquid chromatography method for simultaneous quantification of retinol, α-tocopherol, and cholesterol in shrimp hydrolysate. *Journal of Chromatography A, 1105,* 135-139.

Maraschiello, C., Dáz, I., & Regueiro, J. A. G. (1996). Determination of cholesterol in fat and muscle pig by HPLC and capillary gas chromatography with solvent venting injection. *Journal of High Resolution Chromatography, 19,* 165-168.

Mazalli, M. R., Sawaya, A. C. H. F., Eberlin, M. N., & Bragagnolo, N. (2006). HPLC method for quantification and characterization of cholesterol and its oxidation products in eggs. *Lipids, 41,* 615-622.

Miranda, J. M., Martínez, B., Pérez, B., Antón, X., Vázquez, B. I., Fente, C. A., Franco, C. M., Rodríguez, J. L., & Cepeda, A. (2010). The effects of industrial pre-frying and domestic cooking methods on the nutritional compositions and fatty acids profile of two different frozen breaded foods. *LWT - Food Science and Technology, 43,* 1271-1276.

Murphy, E. J., Rosenberger, T. A., & Horrocks, L. A. (1996). Separation of neutral lipids by high-performance liquid chromatography: quantification by ultraviolet, light scattering and fluorescence detection. *Journal of Chromatography B, 685,* 9-14.

Ngadi, M., Li, Y., & Oluka, S. (2007). Quality changes in chicken nuggets fried in oils with different degrees of hydrogenation. *LWT – Food Science and Technology, 40,* 1784-1791.

Osman, H., & Chin, Y. K. (2006). Comparative sensitivities of cholesterol analysis using GC, HPLC and spectrophotometric methods. *The Malaysian Journal of Analytical Sciences, 10,* 205-210.

Ramalho, H. M. M., Casal, S., & Oliveira, M. B. P. P. (2011). Total cholesterol and desmosterol contents in raw, UHT, infant formula powder and human milks determined by a new fast micro-HPLC method. *Food Analytical Methods, 4,* 424-430.

Saldanha, T., Mazalli, M. R., & Bragagnolo, N. (2004). Avaliação comparativa entre dois métodos para determinação do colesterol em carnes e leite. *Ciência e Tecnologia dos Alimentos, 24,* 109-113.

Saldanha, T., Samaya, A. C. H. F., Eberlin, M. N., & Bragagnolo, N. (2006). HPLC separation and determination of 12 cholesterol oxidation products in fish: comparative study of RI, UV, and APCI-MS detectors. *Journal of Agriculture and Food Chemistry, 54,* 4107-4113.

Şengör, G. F. U., Alakavuk, D. U., & Tosun, Y. (2013). Effect of cooking methods on proximate composition, fatty acid composition, and cholesterol content of Atlantic salmon (Salmo salar). *Journal of Aquatic Food Product Technology,* 22, 160-167.

Shimada, K., Mitamura, K., & Higashi, T. (2001). Gas chromatography and high-performance liquid chromatography of natural steroids. *Journal of Chromatography A, 935,* 141-172.

Swartz, M. E. (2005). UPLC™: An Introduction and Review. *Journal of Liquid Chromatography & Related Technologies, 28,* 1253-1263.

U.S. Department of Agriculture, Agricultural Research Service. (2013). USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory. URL, http://www.ars.usda.gov/ba/bhnrc/ndl Accessed 10.12.13.

Varela, P., Salvador, A., & Fiszman, S. M. (2008). Methodological developments in crispness assessment: Effects of cooking method on the crispness of crusted foods. *LWT – Food Science and Technology, 41,* 1252-1259.

Weber, J., Bochi, V.C., Ribeiro, C.P., Victorio, A.M., & Emanuelli, T. (2008). Effect of different cooking methods on the oxidation, proximate and fatty acid composition of silver catfish (*Rhamdia quelen*) fillets. *Food Chemistry, 106,* 140-146.

WHO/FAO (World Health Organization/Food and Agriculture Organization). (2003). Expert Report: Diet, nutrition and prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916. URL http://whqlibdoc.who.int/trs/who_trs_916.pdf. Accessed 10.12.13.

WHO (World Health Organization). (2007). Prevention of cardiovascular disease: guidelines for assessment and management of total cardiovascular risk. Switzerland. URL http://whqlibdoc.who.int/publications/2007/9789241547178_eng.pdf. Accessed 10.12.13.

Zhu, R., Zhang, Y., Yang, M., Chen, H., Qiao, B., & Pu X. (2012). UHPLC-MS/MS determination of cholesterol in edible vegetable oil. *Physical Testing and Chemical Analysis (Part B: Chemical Analysis), 7,* 831-833.

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2.4 The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets

Este sub-capítulo apresenta os resultados relativos à aplicação de diferentes métodos de confeção (forno, pré-fritura e fritura) a nuggets de frango, para posterior avaliação do impacto do processamento na qualidade nutricional destes alimentos. Foram incluídas amostras de marca branca, marca comercial e de fast-food. Para avaliar o potencial impacto dos nuggets de frango na saúde da população aplicaram-se as doses de referência, considerando a porção servida habitualmente.

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The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets

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Abstract

The impact of cooking methods (industrial pre-frying, deep-fat frying and baking) on the nutritional quality and safety of chicken breaded nugget samples from supermarket and commercial brands was evaluated. The changes in quality characteristics (nutritional composition, fatty acids profile, cholesterol and salt) of the fried food and frying oil, after ten consecutive frying operations, were evaluated. Total fat content of nuggets varied between 10.9 and 22.7 per 100 g of edible portion and salt content ranged from 0.873 to 1.63 per 100 g. Taking into account one portion of nuggets, the daily intake of salt can reach 49%, which can have a significant impact on the health of those who regularly consume this type of foods, especially considering the prevalence of hypertension around the world. The analysed chicken breaded nuggets are rich in unsaturated fatty acids, which have been related with potential health benefits, namely regarding cardiovascular diseases. The cholesterol content of baked samples was two times higher when compared with the fried ones. The *trans* fatty acids and polar compounds content of the frying oil used for frying significantly increased, but the values were still away from the maximum recommended by legal entities for its rejection. From a nutritional point of view, it is possible to conclude that the applied cooking methods can significantly influence the nutritional quality and safety of the analysed chicken breaded nuggets. This study will contribute to important knowledge on how the applied cooking methods can change the nutritional quality and safety of foods, namely of chicken nuggets, and can be very useful for dietary recommendations and nutritional assessment.

Introduction

Breaded foods are becoming increasingly popular worldwide, since there has been a tendency to demand food products that require low preparation time. Also, consumers highly appreciate these foods, due to their unique flavour, texture and palatability.[1] Chicken breaded nuggets were introduced by the fast-food industry in 1970's, but now they are sold in fast-food restaurants or they can be purchased in stores all over the world, and they are considered one of the biggest success stories of the poultry industry.[2] Generally chicken breaded nuggets are sold already cooked (fast-food restaurants) or frozen after being pre-fried by the food industry. With respect to the possible cooking methods for this type of food, deep-fat frying or baking in the oven (with or without fat) are the most common.

Consumers are becoming more concerned regarding the consumption of fried foods, which contain high amounts of saturated fat.[3,4] Therefore, the demand for healthier cooking methods is increasing worldwide, in order to improve the health status of those who consume these foods. According to World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO), dietary fat intake, around the world, exceeds the recommendations.[5] It is necessary to evaluate different cooking methods to prepare processed foods that will enhance the nutritional quality and safety of foods, like chicken breaded nuggets.[6]

Deep-fat frying is one of the oldest methods of food preparation, recognized as unique and a highly versatile process.[7] It improves the sensory quality of food by improving the aroma compounds, attractive colour, crispness and texture.[8] During deep-fat frying, several physical, chemical, and nutritional changes occur in foods.

trans fats are the sum of all unsaturated fatty acids (FA) that contain one or more double bonds in a *trans* geometric configuration.[9] *trans* fatty acids (TFA) occurrence in foods can be due to industrial hydrogenation of vegetable oils, deodorisation of unsaturated vegetable oils, bacterial transformation of unsaturated FA in the animal rumen, and by heating oils at too high temperatures.[10] In recent years, the food industry has increased its efforts in order to reduce TFA amounts, especially in processed foods. However, there is still lack of data regarding the TFA content in some of these recently developed foods, especially in what concerns the influence of the cooking method. Consumption of TFA from partially hydrogenated oils adversely affects multiple cardiovascular risk factors and contributes significantly to increased risk of coronary heart disease events.[11]

The aims of the current study were to evaluate the effect of different cooking methods (industrial pre-frying, frying and baking in the oven) on the proximate composition, salt, cholesterol and fatty acids profile of five brands of chicken breaded nuggets, in order to

determine which cooking method will be safer and healthier from a nutritional point of view. Moreover, supermarket and commercial brands of chicken nuggets were compared in order to assess a potential price effect on the nutritional quality and safety of these foods. This study also investigates changes in the quality characteristics of chicken breaded nuggets, and of the frying oil that was subjected to ten consecutive frying operations.

Materials and methods

Standards and reagents

All chemicals and reagents were of analytical grade and were obtained from various commercial sources. Cholesterol standard (CAS: 57-88-5) was purchased from Sigma-Aldrich (Madrid, Spain) with a purity ≥99%. Fatty acids methyl esters (FAMEs) standards mixtures were obtained from Supelco® (Supelco® 37 FAME Mix C4:0 - C24:0, Supelco, CA, USA) and GLC 674 from Nu-Chek-Prep (Elysian, MN, USA). Ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA).

Sample collection and cooking methods

The samples were selected based on production data and market availability. In 2014, four brands of pre-fried chicken nuggets and one brand from a fast-food restaurant were collected at supermarkets and restaurants from the Lisbon region (Portugal). A list of the acquired samples and packaging information is shown in Table 7. After purchase, all samples were transported on ice in cooler boxes directly to the laboratory and samples were kept at -20 ºC. At least 3 kg of each sample was acquired. Chicken breaded nuggets acquired in supermarkets were removed from the freezer and immediately cooked (without thawing), except for the fast-food brand which was already cooked. Oil samples used for the deep-fat frying process were from a major supermarket brand.

Deep-fat frying was performed using a 3 L household deep fryer (Ufesa FR 1510, Spain) equipped with its own thermometer. However an external thermometer was also used to monitor temperature. For this method, the fryer containing the frying oil was pre-heated at 180 ºC, before immersing the samples. For each brand, 10 pieces of pre-fried frozen chicken nuggets were fried for 4 min at 180 ºC using fresh frying oil, according to the manufacturers' recommendations. The remaining oil from the food surface was removed by manually shaking the aluminium basket that contained the chicken nuggets. Afterwards, the samples were immediately removed from the basket and placed under dry tissue paper to remove the excess of oil. Then, the samples were allowed to cool to room temperature.

With respect to the used frying oil, after cooling down, it was kept in a glass container, protected from light and air. For each brand, 10 frying operations were performed using the

same frying oil with no restitution and using 10 new pieces of chicken nuggets for each frying operation. After each frying procedure an aliquot of 10 mL of the frying oil was collected for further chemical analysis.

The oven cooking method was carried out using a conventional household oven (Bosch, Stuttgart, Germany). For this method, the oven was pre-heated at 200 ºC for 10 min. Ten pieces of chicken breaded nuggets from each brand were cooked on a tray for 15 min at 200 ºC, according to the manufacturers' recommendations. Afterwards, the samples were maintained at room temperature to cool down completely. The temperature of the oven was measured using its own and an external thermometer.

Three independent samples were homogenised in a blender (Grindomix, GM200, RETSCH, Germany) for approximately 1 min at 5000 rpm, in order to provide accurate data that cover possible variations among the samples.

Proximate composition

Chicken breaded nuggets were analysed regarding their content of moisture, ash, total protein and total fat. Total carbohydrates (g per 100 g) were calculated as: 100 − [moisture (g) + ash (g) + total protein (g) + total fat (g)]. Energy values were calculated using the following equations: (1) in kJ, 17 × total protein (g) + 17 × total carbohydrates (g) + 37 × total fat (g) and (2) in kcal, 4 × total protein (g) + 4 × total carbohydrates (g) + 9 × total fat (g), according to the conversion factors available in Regulation (EU) no. 1169/2011.[12]

To guarantee the quality of analytical data, the methods used in this study are accredited according to ISO/IEC/17025 (1999) and/or the laboratory successfully participates in proficiency testing schemes.[13]

The moisture content was determined, immediately after the homogenization of samples, by gravimetric method (AOAC 952.08),[14] using a dry air oven (Memmert, Germany). This method consists in the evaporation of water from sample by drying in an oven until constant weight. 5 g of homogenized sample of chicken nuggets were placed in a capsule and were dried at 101 ± 2 ºC, for 2 h. This procedure was repeated until constant weight and the moisture content was calculated based on the weight difference. Total ash analysis was carried out in a muffle furnace M110 (Heraeus Instruments, Hanau, Germany) at 525 ± 25 ºC for 20 h, using 5 g of homogenized sample of chicken nuggets, until constant weight, according to AOAC 923.03.[14]

Each sample was analysed for total nitrogen by the Kjeldahl method in combination with a copper catalyst using a block digestion system Foss Tecator 2006 Digestor (Höganäs, Sweden) and a Foss 2200 Kjeltec AutoDistillation unit (Foss Tecator) (AOAC 991.20).[14] The

total protein content was calculated by using 6.25 as the nitrogen conversion factor, according to Greenfield and Southgate.[15]

Determination of total fat was performed according to Albuquerque *et al.*,[16] where an acid hydrolysis followed by extraction using a Soxhlet apparatus (Soxtec™ 2050, Auto Fat Extraction System, FOSS Analytical, Hillerod, Denmark) with petroleum ether, as the extraction solvent was used.[16] The obtained residue was dried for 1 h 30 min at 101 ± 2 ºC, until constant weight, according to the acid hydrolysis method (AOAC 948.15).[14]

Fatty acids composition

Preparation of FAMEs was performed according to Albuquerque et al.,[16] with a methanolic potassium hydroxide solution (2 M) and *n*-heptane, in accordance with ISO 5509.[17] The experiments for FA determination were performed on an Agilent 6890N Network GC System equipped with an Agilent 7683B Automatic Liquid Sampler (Agilent Technologies, Atlanta, GA, USA) and a flame ionization detector (GC-FID). FAMEs were separated in a Supelco® 2560 column (100 m x 0.25 mm i.d., 0.20 µm film thickness). Helium was the carrier gas. A split ratio of 50 : 1 and an injection volume of 1.0 µL were used. The injector and detector were kept at 240 ºC. The temperature was programmed at 60 ºC for 1 min, then to 168 ºC at 17 ºC/min, held for 28 min, then to 235 ºC at 4 ºC/min, and finally held for 15 min. FAMEs were identified by comparing the retention times of the standards with those of the samples. Whenever necessary, samples were spiked with pure standards. The relative percentage of each fatty acid was calculated by internal normalization of the chromatographic peak area. The conversion of FAMEs to their FA was done according to AOAC 996.06 (2000).[14]

Salt

The salt content was determined by Charpentier-Volhard's titration.[16] This method determines the salt content of foods based on the concentration of the chloride ion titrated with silver nitrate solution. Potassium thiocyanate (0.1 N) and silver nitrate (0.1 N) were prepared in the laboratory. Sodium chloride was estimated from the amount of chloride ion, as determined by the end-point of titration.

Cholesterol

Samples were prepared according to the method described by Bragagnolo *et al.*[18] Separation and quantification of cholesterol in the analysed samples were performed on an UHPLC, Acquity™ (Waters, Milford, MA, USA), according to the previously validated analytical method from Albuquerque *et al.*[19]

Table 7. List of selected chicken nuggets and packaging information.

Identification of samples	Description	Chicken content (%)	Fat used for pre-frying	Nutritional information (per 100 g)						
				Energy (kJ (kcal))	Protein (g)	Carbohydrates (g)	Fat (g)	SFA (g)	Dietary fibre (g)	Salt (g)
Supermarket brand 1 (SB1)	Chicken breast nuggets	74% breast	Rapeseed oil	855 (204)	17.2	14.2	8.3	2.5	1.6	1.2
Commercial brand 1 (CB1)	Chicken breast nuggets	55% breast	Vegetable oil	938 (224)	14.7	18.6	9.9	1.7	0.7	0.9
Supermarket brand 2 (SB2)	Chicken nuggets	42% breast	Sunflower oil	1091 (261)	14.9	17.5	14.2	1.7	1.8	1.8
Commercial brand 2 (CB2)	Chicken nuggets	74% meat	Rapeseed oil	855 (205)	16	8	12	na	na	na
Fast-food brand (FFB)	Chicken nuggets	na	Na	1049 (251)	15.9	16.8	13.1	1.6	1.4	0.9

SFA, saturated fatty acids; na, not available data.

Polar compounds

Total polar compounds content in the frying oils was determined using a CAPSens 5000 Food Oil Sensor (C-CIT Sensors AG, Wädenswil, Zürich, Switzerland).

Statistical analysis

All statistical analyses were performed in SPSS for Windows, version 20.0 (SPSS Inc, Chicago). Results are expressed per 100 g of edible portion, as mean values ± standard deviation (SD) or as percentage, rounded to significant figures. The results were analysed using one-way analysis of variance (ANOVA) followed by Tukey test. A value of $p < 0.05$ was considered statistically significant. The current results are the average of three independent samples (n=3), each sample analysed in duplicate, and are presented on a fresh weight basis.

Results and discussion

Nutritional composition using different cooking methods

The results of the proximate composition, energy value, salt and cholesterol content of the analysed chicken breaded nuggets subjected to three cooking methods are presented in Table 8.

The calculated energy values varied between 218 kcal and 338 kcal, for supermarket brand 1 (SB1) and supermarket brand 2 (SB2), respectively. The samples subjected to deep-fat frying had the highest mean energy value (282 kcal). The moisture content of chicken nuggets ranged from 41.3% to 58.1%, showing a decrease after cooking, whereas the ash content varied between 1.48 ± 0.0 and 2.45 ± 0.0 g per 100 g of edible portion, and an increase after cooking was observed. The USDA National Nutrient Database,[20] reported a range of 44.2 – 53.2 g per 100 g for the water content, and a range of 1.96 – 2.52 g per 100 g for the ash content in chicken breaded nuggets, which is in accordance with the results from the current study. Pre-fried chicken nuggets (SB2) had the lowest protein content (12.0 ± 0.1 g per 100 g of edible portion), while baked chicken nuggets (SB1) had the highest (22.5 ± 0.1 g per 100 g). The observed differences between brands for protein content may be due to the raw meat contents used in the manufacturing of chicken nuggets.[21] Also, according to Gibbs *et al.*,[22] processed chicken products usually are prepared from mechanically recovered chicken meat, with different proportions of white meat, dark meat, other tissues and other ingredients, which can also be a crucial factor for the obtained differences. In the USDA National Nutrient Database,[20] the values for protein content of chicken breaded nuggets ranged from 14.3 to 19.8 g per 100 g, being in agreement with the current study.

Table 8. Proximate composition, energy value, salt and cholesterol (per 100 g edible portion) content of the analysed chicken breaded nuggets. [a,b,c]

Brand	Cooking method	Energy (kJ (kcal))	Water (g)	Ash (g)	Total protein (g)	Total fat (g)	Total carbohydrates (g)	Salt (g)	Cholesterol (mg)
SB1	Pre-fried	888 (213)^{a}	58.1 ± 0.2^{a}	1.92 ± 0.0^{f}	18.5 ± 0.3^{f}	11.6 ± 0.3^{a,b,c,d,f}	9.86 ± 0.5^{a}	1.05 ± 0.0^{b,g,i,k,o}	47.3 ± 4^{e}
	Fried	1132 (272)^{e}	48.2 ± 0.1^{b}	2.30 ± 0.0^{a}	20.6 ± 0.1^{a}	16.1 ± 0.0^{e}	12.7 ± 0.1^{g}	1.20 ± 0.1^{a,d,f,k,r}	28.9 ± 1^{b,c}
	Baked	1072 (256)^{b}	48.6 ± 0.1^{g}	2.39 ± 0.0^{g}	22.5 ± 0.1^{g}	12.6 ± 0.4^{g}	13.9 ± 0.5^{b}	1.15 ± 0.0^{e,f,i,j}	59.1 ± 1^{d}
CB1	Pre-fried	868 (208)^{c}	57.9 ± 0.1^{a}	1.48 ± 0.0^{h}	13.7 ± 0.1^{b}	11.8 ± 0.1^{a,h,i}	15.1 ± 0.2^{c}	0.873 ± 0.0^{g,h,t,u}	20.1 ± 0^{i}
	Fried	1054 (252)^{b}	50.1 ± 0.2^{d}	1.81 ± 0.0^{b,c}	15.0 ± 0.2^{c}	13.6 ± 0.1^{n}	19.6 ± 0.1^{d,e}	1.09 ± 0.0^{d,e,g,l,s}	30.0 ± 2^{c,g,h}
	Baked	988 (236)^{d}	49.8 ± 0.0^{d,e}	1.76 ± 0.0^{b}	15.9 ± 0.4^{d}	11.0 ± 0.2^{b,j,k}	21.5 ± 0.5^{f}	1.01 ± 0.2^{d,h,i,p,q}	32.3 ± 1^{a,c}
SB2	Pre-fried	1044 (251)^{f}	53.0 ± 0.1^{f}	1.86 ± 0.0^{c}	12.0 ± 0.1^{h}	16.8 ± 0.1^{e,l}	16.3 ± 0.3^{h}	1.25 ± 0.2^{a,b,c,e}	33.8 ± 0^{a}
	Fried	1377 (331)^{g}	41.8 ± 0.2^{g}	2.10 ± 0.0^{d}	13.9 ± 0.1^{b}	22.7 ± 0.1^{o}	19.5 ± 0.4^{d}	1.61 ± 0.2^{m}	30.8 ± 1^{a,b,g}
	Baked	1297 (311)^{h}	41.3 ± 0.1^{h}	2.30 ± 0.0^{a}	15.4 ± 0.1^{c,d}	18.2 ± 0.5^{p}	22.7 ± 0.7^{i}	1.50 ± 0.0^{m,n}	40.3 ± 2^{f}
CB2	Pre-fried	894 (214)^{a,c}	58.1 ± 0.2^{a}	2.08 ± 0.0^{d,e}	17.3 ± 0.1^{e}	12.4 ± 0.3^{g,h}	10.1 ± 0.1^{a}	1.05 ± 0.0^{b,j,l,q,r,u}	44.7 ± 1^{e}
	Fried	1150 (276)^{i}	47.6 ± 0.0^{i}	2.21 ± 0.0^{i}	19.4 ± 0.2^{i}	16.5 ± 0.1 ^{kf,l}	14.3 ± 0.3^{b,c}	1.06 ± 0.0^{c,j,k,p,s,t}	27.8 ± 1^{b,h}
	Baked	999 (239)^{j}	48.3 ± 0.1^{b,c}	2.45 ± 0.0^{j}	20.5 ± 0.4^{a}	11.3 ± 0.0^{c,i,j,m}	17.4 ± 0.3^{j}	1.40 ± 0.2^{a,n}	55.8 ± 0^{d}
FFB	Fried	1001 (239)^{d}	49.6 ± 0.1^{e}	2.04 ± 0.0^{e}	16.8 ± 0.1^{e}	10.9 ± 0.1^{d,k,m}	20.6 ± 0.0^{e,f}	1.07 ± 0.2^{c,f,h,l,o}	41.2 ± 0^{f}

[a]SB1 – Supermarket brand 1; CB1 – Commercial brand 1; SB2 – Supermarket brand 2; CB2 – Commercial brand 2; FFB – Fast-food brand. [b]Results are means ± standard deviation of three independent samples (n=3). [c]Means within the same column having different superscripts letters are significantly different ($p < 0.05$).

According to the ingredient information available on the packages of the acquired samples, SB1 and commercial brand 2 (CB2) have 74% of chicken breast or chicken meat, commercial brand 1 (CB1) has 55% and SB2 has 42% (Table 7). The results of our study for protein content are in accordance with this, decreasing in the following order: SB1 > CB2 > CB1 > SB2. Most of the time, it is often assumed that supermarket brand products, are in general less expensive than commercial brands, and have a poorer nutritional quality, especially concerning the amount of meat in the case of chicken breaded nuggets. In our study, this difference was not evident, since SB1 and CB2 have the same amount of meat according to the packaging information (Table 7).

The total carbohydrates in the analysed samples varied between 9.86 ± 0.5 and 22.7 ± 0.7 g per 100 g of edible portion, for SB1 (pre-fried) and SB2 (baked in the oven), respectively. Similar values have been reported for carbohydrate content (7.52 to 26.5 g per 100 g) of pre-fried chicken breaded nuggets.[21]

In our study, the total fat content of pre-fried samples ranged from 11.6 ± 0.3 to 16.8 ± 0.1 g per 100 g of edible portion, while for deep-fat frying the values varied between 10.9 ± 0.1 and 22.7 ± 0.1 g per 100 g of edible portion. Considering the total fat mean value for the applied cooking methods, the total fat content increases in the following order: baked in the oven < pre-fried < fried. Lukman *et al.*[21] reported results for five brands of pre-fried chicken nuggets commercialized in Malaysia and the values for total fat content varied between 18.1 and 25.0 g per 100 g, which are higher than the values obtained in the current study. On the other hand, Gibbs *et al.*[22] also reported values for total fat content of chicken breaded nuggets, ranging from 12.3 to 20.5 g per 100 g. However, the breading of these nuggets was removed before analysis which makes these data not suitable for comparison with our study. The USDA National Nutrient Database[20] reported values for total fat content in different brands of chicken nuggets that varied between 15.4 and 22.6 g/100 g. According to the literature, it is expected that foods subjected to frying have an increase in the fat content due to the oil absorption during and after cooking.[4] In the current study, the fat content has a significant increase ($p < 0.05$) from pre-fried to deep-fat frying for all the studied brands, varying from 2 to 6 g per 100 g of absorbed fat. Also, fat absorption is directly related with water content of the food to be subjected to the frying process. According to the obtained results, it is possible to see that samples SB1 and CB2, which have an equal initial amount of water, have absorbed similar amounts of fat during deep-fat-frying. Nonetheless, previous studies on tortilla chips suggested that most of the oil does not penetrate the product during frying, but does during the cooling period, after removing it from the fryer.[23]

Table 9 shows the major FA of the analysed chicken nuggets according to the cooking method. The most abundant FA in all the analysed samples were palmitic (C16:0), oleic (C18:1,n9c) and linoleic acids (C18:2,n6c). The total saturated fatty acids (SFA) content ranged from 1.21 ± 0.0 to 2.97 ± 0.0 g per 100 g of edible portion, for fast-food brand (FFB) and SB2, respectively, being both samples deep-fat fried. The highest values for monounsaturated FA (7.98 ± 0.0 g per 100 g of edible portion) and polyunsaturated FA (10.7 ± 0.0 g per 100 g of edible portion) were quantified in SB2 subject to deep-fat frying.

With respect to TFA, only elaidic acid (C18:1,n9t) was quantified in the analysed chicken breaded nuggets. The values for this FA varied between 8.21 and 19.6 mg per 100 g of edible portion. Figure 14 shows chromatograms for CB1 subjected to three cooking methods: (A) pre-frying; (B) deep-fat frying; and (C) baking in the oven.

Salt

For the analysed samples, the salt content varied between 0.873 ± 0.0 and 1.63 ± 0.1 g per 100 g of edible portion (Table 8), for CB1 (pre-fried) and SB2 (fried). The USDA National Nutrient Database[20] provides data for the salt content in different brands of chicken nuggets, varying from 1.3 to 1.8 g per 100 g, which is slightly higher than the obtained results. No other data was found in the literature for comparison purposes. Therefore, the results from the current study will bring new information on this area.

The intake of foods rich in salt is one of the major concerns from a public health point of view, because salt intake is a risk factor for high blood pressure. According to WHO recommendations, it should be less than 5 g per person per day for cardiovascular disease prevention.[24] Therefore, in the last few years several strategies to reduce food salt content were adopted by some European Member States. To accomplish this purpose, WHO defined 12 priority categories of food for action, of which meat products and ready meals are included.[24] If we consider the highest salt content of the analysed chicken nuggets and taking into account one portion (150 g or 6 pieces), a daily intake of 49% of the recommended value can be reached, revealing that consumers can easily exceed the dietary reference value for salt intake (Table 10). Moreover, if we consider a complete fast-food meal that includes chicken nuggets, potato crisps and for example a salad, the total salt intake could be two times higher (about 4 g). This high salt content can represent a matter of concern, since chicken breaded nuggets are popular and convenient foods, especially among children and young people.

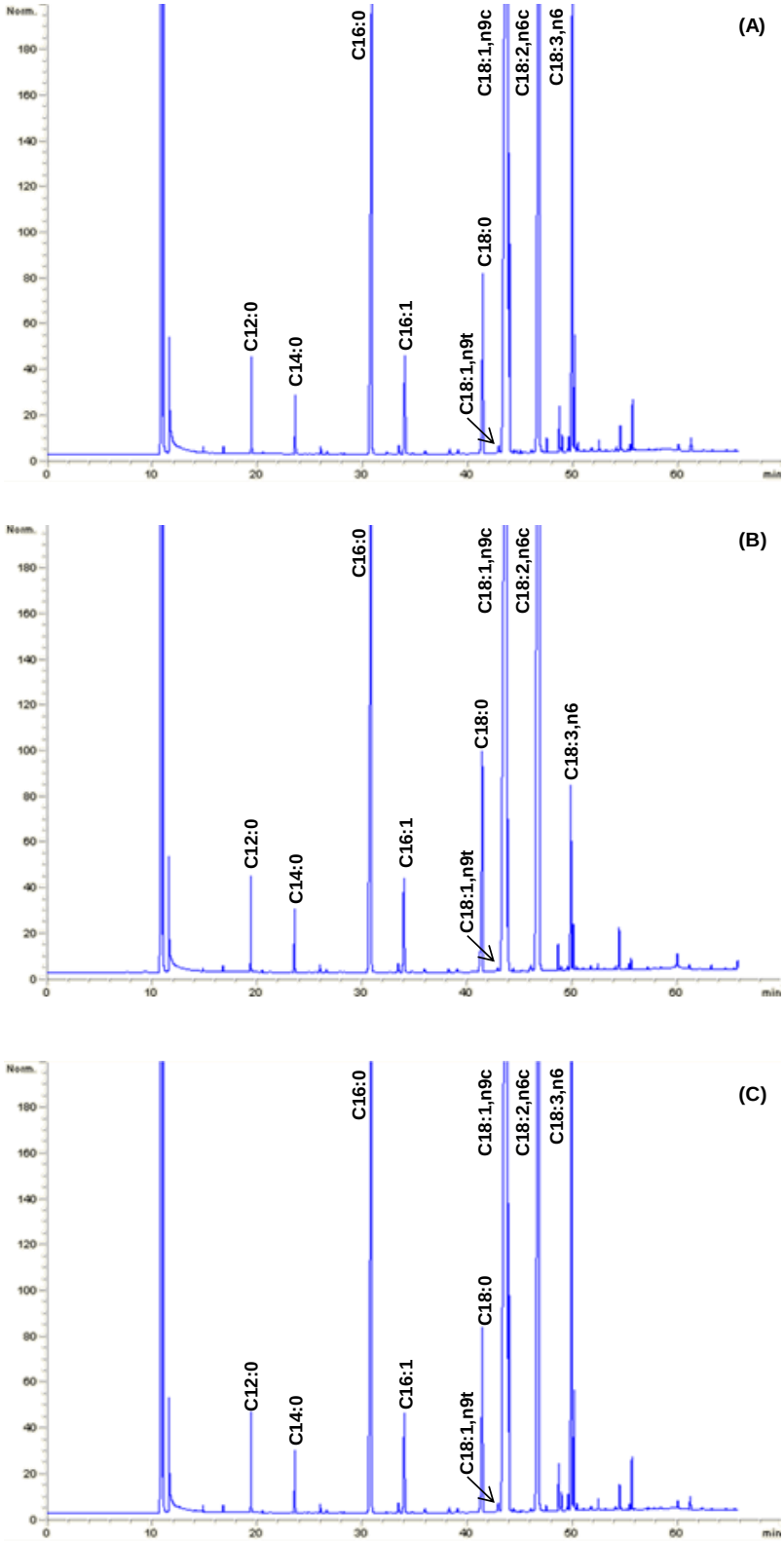

Figure 14. Chromatograms of fatty acids profile of chicken breaded nuggets (sample CB1) subjected to three cooking methods. (A) Pre-frying; (B) Deep-fat frying; and (C) Baking in the oven. C12:0 – lauric acid; C14:0 – myristic acid; C16:0 – palmitic acid; C16:1 – palmitoleic acid; C18:0 – stearic acid; C18:1,n9t – elaidic acid; C18:1,n9c – oleic acid; C18:2,n6c – linoleic acid; C18:3,n6 – linolenic acid.

Table 9. Fatty acids profile (per 100 g of edible portion) of the analysed chicken breaded nuggets. [a,b,c]

Brand	Cooking method	Fatty acids								∑ SFA[d]	∑ MUFA[e]	∑ PUFA[f]
		Myristic (g)	Palmitic (g)	Palmitoleic (g)	Stearic (g)	Elaidic (mg)	Oleic (g)	Linoleic (g)	Linolenic (mg)			
		C14:0	C16:0	C16:1	C18:0	C18:1,n9t	C18:1,n9c	C18:2,n6c	C18:3,n6			
SB1	Pre-fried	0.0208 ± 0.0[b]	1.08 ± 0.0[b]	0.115 ± 0.0[b]	0.404 ± 0.0[b]	8.78 ± 0.0[a,h,i]	3.90 ± 0.0[c]	5.29 ± 0.0[b]	39.2 ± 0.0[b,c]	1.62 ± 0.0[b]	4.08 ± 0.0[d]	5.34 ± 0.0[b]
	Fried	0.0235 ± 0.0[c]	1.39 ± 0.0[c]	0.125 ± 0.0[c]	0.536 ± 0.0[c]	8.21 ± 0.0[c,h,j]	5.45 ± 0.0[b]	7.61 ± 0.0[a]	42.3 ± 0.0[a,b]	2.10 ± 0.0[c]	5.67 ± 0.0[c]	7.65 ± 0.0[a]
	Baked	0.0217 ± 0.0[d]	1.16 ± 0.0[d]	0.114 ± 0.0[b]	0.438 ± 0.0[a]	9.46 ± 0.0[a,f,g,j]	4.23 ± 0.0[d]	5.85 ± 0.0[c]	44.2 ± 0.0[a,b]	1.76 ± 0.0[a,c]	4.43 ± 0.0[e]	5.91 ± 0.0[c]
CB1	Pre-fried	0.0617 ± 0.0[e]	1.13 ± 0.0[a]	0.143 ± 0.1[a]	0.311 ± 0.0[d]	19.6 ± 0.0[d]	6.18± 0.1[e]	2.17 ± 0.0[d]	785 ± 0.1[f]	1.69 ± 0.0[d]	6.57 ± 0.0[f]	2.98 ± 0.0[d]
	Fried	0.0715 ± 0.0[f]	1.45 ± 0.0[e]	0.202 ± 0.0[d]	0.457 ± 0.0[e]	13.0 ± 0.0[b]	5.10 ± 0.1[f]	5.10 ± 0.0[e]	256 ± 0.0[g]	2.18 ± 0.0[e]	5.39 ± 0.1[g]	5.38 ± 0.0[b]
	Baked	0.0585 ± 0.0[g]	1.09 ± 0.0[b]	0.176 ± 0.0[e]	0.295 ± 0.0[f]	19.6 ± 0.0[d]	5.67 ± 0.0[a]	2.04 ± 0.0[f]	717 ± 0.0[h]	1.62 ± 0.0[b]	6.08 ± 0.0[a]	2.78 ± 0.0[e]
SB2	Pre-fried	0.0283 ± 0.0[h]	1.61 ± 0.0[f]	0.192 ± 0.0[a]	0.652 ± 0.0[g]	16.7 ± 0.0[e]	5.42 ± 0.0[b]	7.80 ± 0.0[g]	102 ± 0.0[d]	2.44 ± 0.0[f]	5.72 ± 0.0[b,c]	7.93 ± 0.0[f]
	Fried	0.0330 ± 0.0[i]	2.00 ± 0.0[g]	0.206 ± 0.0[f]	0.746 ± 0.0[h]	12.0 ± 0.0[b]	7.66 ± 0.0[g]	10.5 ± 0.0[h]	108 ± 0.0[d]	2.97 ± 0.0[g]	7.99 ± 0.0[h]	10.6 ± 0.0[g]
	Baked	0.0316 ± 0.0[j]	1.79 ± 0.0[h]	0.217 ± 0.0[g]	0.712 ± 0.0[i]	16.8 ± 0.0[e]	5.86 ± 0.0[h]	8.31 ± 0.0[i]	116 ± 0.0[d,e]	2.69 ± 0.0[h]	6.18 ± 0.0[a]	8.45 ± 0.0[h]
CB2	Pre-fried	0.0156 ± 0.0[a]	0.972 ± 0.0[i]	0.0767 ± 0.0[h]	0.439 ± 0.0[a]	10.3 ± 0.0[f]	3.92 ± 0.0[c]	6.25 ± 0.0[j]	29.0 ± 0.0[b,c]	1.55 ± 0.0[i]	4.07 ± 0.0[d]	6.28 ± 0.0[i]
	Fried	0.0162 ± 0.0[a]	1.12 ± 0.0[a]	0.0697 ± 0.0[i]	0.473 ± 0.0[j]	9.48 ± 0.0[a,c]	4.92 ± 0.0[i]	7.62 ± 0.0[a]	25.9 ± 0.0[a,c]	1.75 ± 0.0[a]	5.07 ± 0.0[i]	7.65 ± 0.0[a]
	Baked	0.0141 ± 0.0[k]	0.877 ± 0.0[j]	0.0659 ± 0.0[j]	0.394 ± 0.0[k]	8.11 ± 0.0[c,g,i]	3.52 ± 0.0[j]	5.68 ± 0.0[k]	26.1 ± 0.0[a,b]	1.39 ± 0.0[j]	3.65 ± 0.0[j]	5.72 ± 0.0[j]
FFB	Fried	0.0102 ± 0.0[l]	0.721 ± 0.0[k]	0.0578 ± 0.0[k]	0.360 ± 0.0[l]	11.8 ± 0.0[b]	5.67 ± 0.0[a]	3.23 ± 0.0[l]	113 ± 0.0[d,e]	1.21 ± 0.0[k]	5.81 ± 0.1[b]	3.35 ± 0.0[k]

[a]SB1 – Supermarket brand 1; CB1 – Commercial brand 1; SB2 – Supermarket brand 2; CB2 – Commercial brand 2; FFB – Fast-food brand. [b]Results are means ± standard deviation of three independent samples (n=3). [c]Means within the same column having different superscripts letters are significantly different ($p < 0.05$). [d]SFA – Saturated fatty acids; ∑SFA = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C17:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. [e]MUFA – Monounsaturated fatty acids; ∑ MUFA = C14:1 + C15:1 + C16:1 + C17:1 + C18:1,n9c + C18:1,n9t + C20:1 + C22:1,n9 + C24:1. [f]PUFA – Polyunsaturated fatty acids; ∑PUFA = C18:2,n6c + C18:2,n6t + C18:3,n6 + C18:3,n3 + c20:3,n6 + C20:3,n3 + C20:4,n6 + C20:5,n3 + C22:2 + C22:6,n3

Cholesterol

The cholesterol content of the analysed chicken breaded nuggets (Table 8) varied between 20.1 and 59.1 mg per 100 g of edible portion, for CB1 (pre-fried) and SB1 (baked in the oven). In the USDA National Nutrient Database,[20] the mean values for cholesterol content in chicken breaded nuggets are 33 mg per 100 g and 50 mg per 100 g, for pre-fried and fried nuggets, respectively. In the analysed pre-fried samples, cholesterol content had a great variability (>20 mg per 100 g) among brands, which could be related with the amount of meat used by the industry for chicken nuggets' manufacture. The mean cholesterol content of baked samples was two times higher than the samples subjected to deep-fat frying (samples SB1 and CB2). These samples had the highest chicken meat content (74%) according to the label (Table 7). Detailed studies on lipid exchanges during frying are scarce, namely regarding cholesterol content in fried foods, such as chicken breaded nuggets. However, according to the literature during frying of fatty foods like meat or chicken, exchanges occur and are explained by the presence of minor compounds (cholesterol, phospholipids, vitamins, etc) in the frying oil.[1] Baggio *et al.*[25] also reported a slight increase of cholesterol content for processed meat products after these foods being baked in the oven. German-Austrian-Swiss recommendations,[26] the WHO and FAO[5] have set recommendations for a maximum intake of cholesterol of 300 mg per day for the adult population. Taking into account, one portion of nuggets (6 pieces/meal - approximately 150 g) and considering the highest obtained value within this study, these will contribute with approximately 30% of the intake of cholesterol (Table 10).

Fatty acids profile of the chicken nuggets and frying oils

The results for fatty acids composition of chicken nuggets after each frying cycle are presented in Figure 15. The same frying oil was used for each brand, for ten successive frying operations, without replacement, and with ten new chicken nuggets. The frying oil is an important ingredient of the fried food and can represent an important issue on the intake of dietary fat.[1,6] According to the obtained results, the SFA content of the chicken breaded nuggets from the different brands increased with the successive frying operations, while unsaturated fatty acids content decreased, namely polyunsaturated fatty acids (PUFAs) (Figure 15A). This can be due to several factors, such as: the food composition of chicken breaded nuggets, oil/fat used for pre-frying operation, product shape, composition of bread coating, surface and porosity of chicken breaded nuggets.[1] With respect to the TFA content, and for all the brands, an increase was observed along the frying operations (Figure 15B). The obtained values for TFA content varied between 8.21 and 26.1 mg per 100 g of edible portion. The TFA content significantly increases ($p < 0.05$) among the frying operations, except for CB2, for the first five frying operations ($p > 0.05$). In Denmark, the TFA content

in foods has been regulated and a maximum of 2% TFA of total fat in products containing non-dairy fat is allowed.[28] Considering this limit, in the current study, TFA content of the analysed chicken breaded nuggets, after ten successive frying cycles, was below.

In this study, for each brand of chicken breaded nuggets around 1.5 L of vegetable oil were used to perform the 10 successive frying operations. The oil was cooled down after each frying operation and kept in a dark container at room temperature.

The obtained results for the oil FA profiles are shown in Figure 16. As it is possible to confirm, the monounsaturated fatty acids (MUFA) content of the oil slightly increased between the 1st and 10th frying operations, while the PUFA fatty acids content decreased (Figure 16A). The linoleic acid content of the analysed frying oils decreased from 51.2 to 49.5 g per 100 g, between the 1st and 10th frying cycle, while oleic acid and palmitic acid, both increased from 33.0 to 34.1 g per 100 g and from 6.29 to 6.56 g per 100 g, respectively. During successive frying operations, the oil can be exposed to several modifications due to cyclization, polymerization and/or oxidative reactions. One of the frequently used indicators to measure the degree of oil degradation is linoleic acid content, due to its high susceptibility to oxidation.[28] On the other hand, usually authors also consider the linoleic/palmitic acid ratio (C18:2/C16:0).[29] In our study, the ratio C18:2/C16:0 significantly decreases ($p < 0.05$) after the successive frying operations, indicating some degradation of the oil. TFA content of the oil subjected to successive deep-fat frying operations (Figure 16B) varied between 16.0 and 64.5 mg per 100 g of edible portion, for SB1 (1st frying) and CB1 (10th frying). Elaidic acid was absent in the fresh oil samples. Between frying operations the elaidic acid (C18:1,n9t) content significantly increases ($p < 0.05$) for all the studied samples but to levels that cannot be considered as harmful. Formation of TFA during frying has been shown to be closely related to the process temperature and time, but it can also be due to the release of TFA from the pre-fried chicken breaded nuggets into the frying oil.

Olive oil, sunflower oil, corn oil, safflower oil and canola oil were studied during the heating process, and a decrease in unsaturation and an increase in *trans* isomers was observed for all these oils.[30] This degradation becomes considerable above 150 ºC, and at higher temperatures a more significant variation is evident.[30] Another crucial aspect is that some studies regarding the formation of TFA are performed without food, by heating only the oil.

Table 10. Nutritional value of the analysed chicken breaded nuggets per portion (150 g) and contribution (%) for the dietary reference intake (DRI).[a,b]

Brand	Cooking method	Energy		Total protein		Total fat		Total carbohydrates		Salt		Cholesterol		SFA	
		Per portion (kJ (kcal))	DRI[d] (%)	Per portion (g)	DRI[d] (%)	Per portion (g)	DRI[d] (%)	Per portion (g)	DRI[d] (%)	Per portion (g)	DRI[d] (%)	Per portion (mg)	DRI[d] (%)	Per portion (g)	DRI[d] (%)
SB1	Pre-fried	1366 (326)	16	27.8	56	17.3	25	14.8	6	1.57	31	69.7	23	2.44	12
	Fried	1750 (418)	21	31.0	62	24.2	35	19.1	7	1.80	36	43.6	15	3.16	16
	Baked	1627 (389)	19	33.7	67	19.0	27	20.8	8	1.73	35	88.7	30	2.64	13
CB1	Pre-fried	1392 (333)	17	20.6	41	17.7	25	22.7	9	1.31	26	30.1	10	2.53	13
	Fried	1634 (391)	20	22.4	45	20.4	29	29.3	11	1.64	33	44.6	15	3.27	16
	Baked	1562 (373)	19	23.8	48	16.5	24	32.3	12	1.51	30	48.3	16	2.43	12
SB2	Pre-fried	1660 (397)	20	18.0	36	25.2	36	24.4	9	1.89	38	50.8	17	3.67	18
	Fried	2120 (507)	25	20.9	42	34.0	49	29.3	11	2.45	49	46.2	15	4.45	22
	Baked	1985 (474)	24	23.2	46	27.3	39	34.1	13	2.24	45	60.3	20	4.04	20
CB2	Pre-fried	1391 (332)	17	26.0	52	18.7	27	15.1	6	1.57	31	67.0	22	2.32	12
	Fried	1776 (424)	21	29.1	58	24.7	35	21.4	8	1.59	32	41.7	14	2.62	13
	Baked	1592 (381)	19	30.8	62	17.0	24	26.1	10	2.08	42	83.7	28	2.09	10
FFB	Fried	1556 (372)	19	25.5	50	16.4	23	30.9	12	1.62	32	61.8	21	1.82	9

[a]SB1 – Supermarket brand 1; CB1 – Commercial brand 1; SB2 – Supermarket brand 2; CB2 – Commercial brand 2; FFB – Fast-food brand; SFA – Saturated fatty acids; DRI – Dietary reference intake. [b] Results are means ± standard deviation of three independent samples (n=3). [c]Dietary reference intake according to Regulation (EU) No 1169/2011.[12] [d]Dietary reference intake according to German-Austrian-Swiss recomendations and WHO/FAO.[24,26]

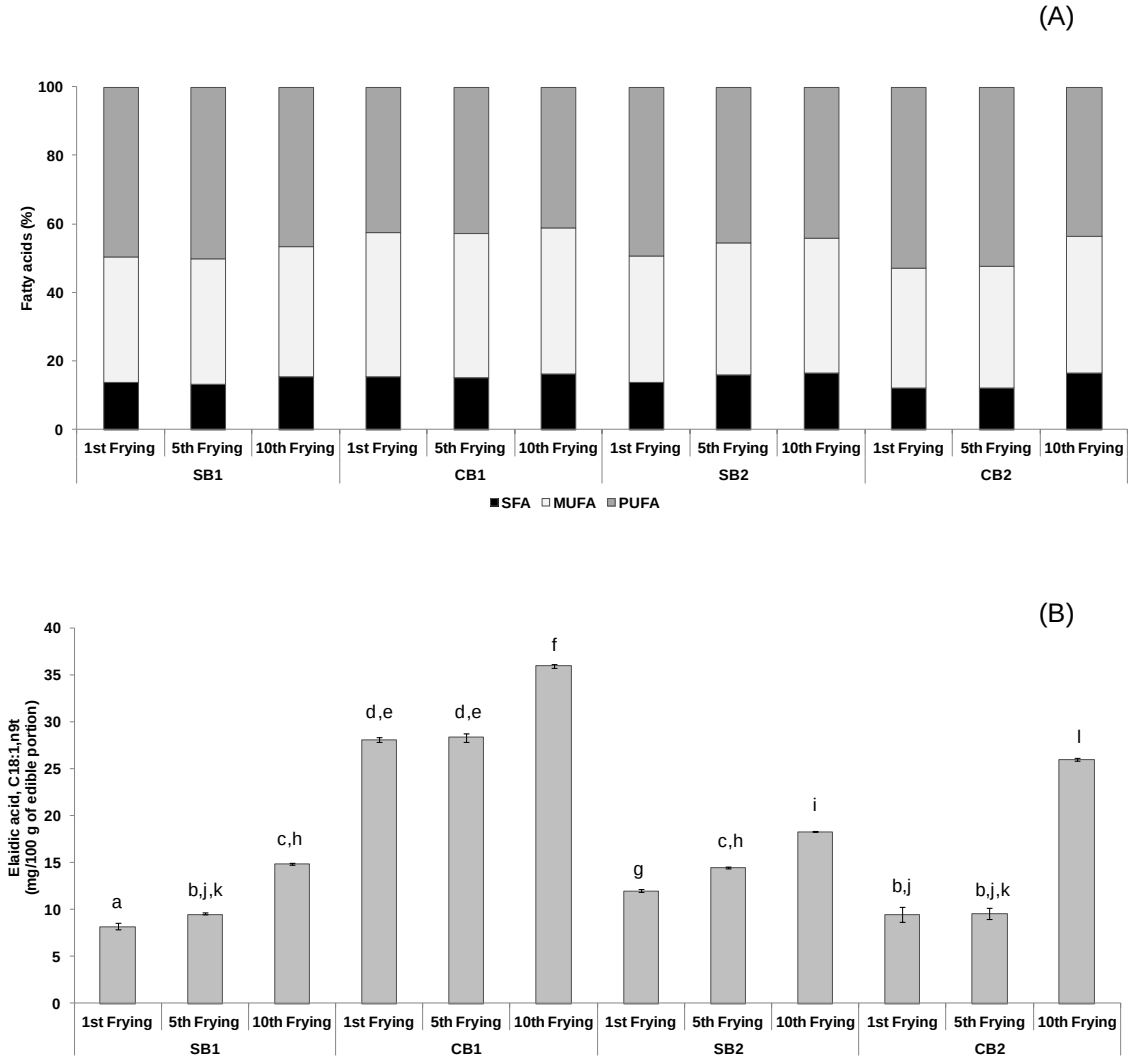

Figure 15. Fatty acids profile of the analysed chicken breaded nuggets subjected to successive deep-fat frying operations. (A) Saturated, monounsaturated and polyunsaturated fatty acids; and (B) Elaidic acid (C18:1,n9t). Different letters above the bars means that values are significantly different ($p < 0.05$).

Total polar compounds of the oil along frying

In accordance with the Portuguese legislation, as well as for several other countries, the legal rejection point for frying oils is 25% of total polar compound content.[31] Within this study, the content of polar compounds of the analysed oils significantly increased between the 1st and 10th frying operations, for all the studied chicken nuggets brands (Figure 17). The total polar compounds content of the frying oils ranged from 4.63 to 15.2%, which does not exceed the maximum legal value (25%).

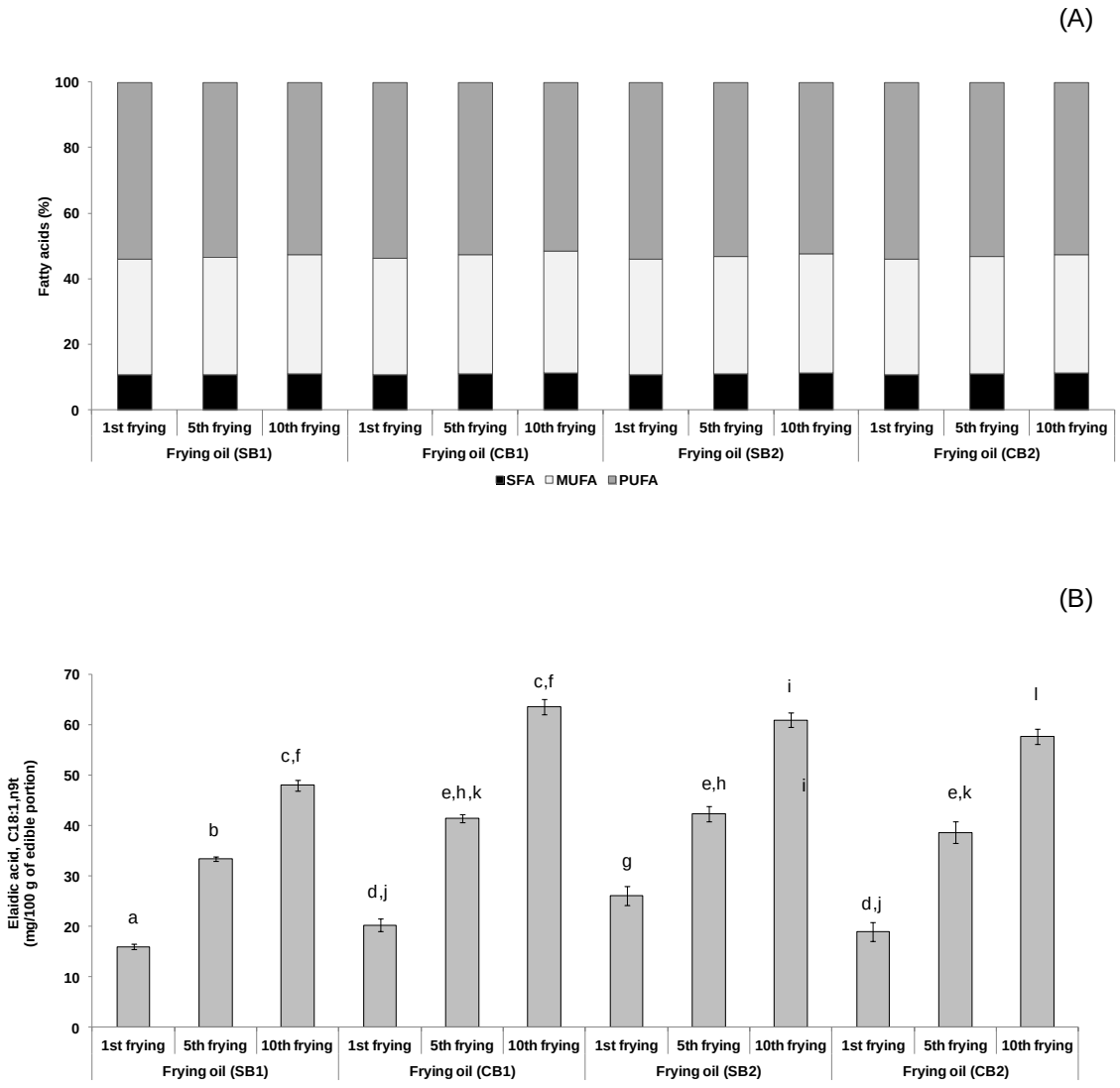

Figure 16. Fatty acids profile of the oils used to fry the chicken breaded nuggets subjected to successive deep-fat frying operations. (A) Saturated, monounsaturated and polyunsaturated fatty acids; and (B) Elaidic acid (C18:1,n9t). Different letters above the bars means that values are significantly different ($p < 0.05$).

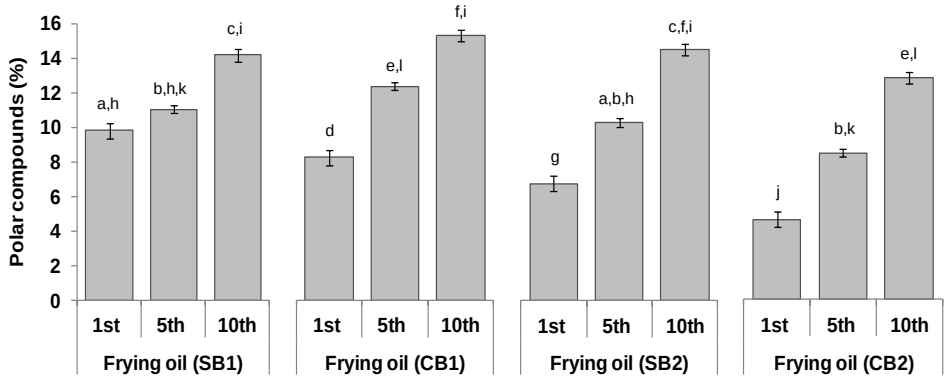

Figure 17. Polar compounds (%) of the oils used to fry the chicken breaded nuggets subjected to successive deep-fat frying operations. Different letters above the bars means that values are significantly different ($p < 0.05$).

Conclusion

From a nutritional point of view it is possible to conclude that the applied cooking methods can significantly influence the nutritional quality and safety of the analysed chicken breaded nuggets. With respect to commercial *vs*. supermarket brand, no considerable differences on the nutritional quality and safety of the analysed nuggets were found, revealing that the cost of chicken breaded nuggets should not represent a factor of choice for consumers. However, the formulation of pre-fried chicken breaded nuggets (*e.g.* chicken meat content) should be taken into account when consumers choose the brand to be acquired. In the current study, the nutritional balance of the cooked nuggets sometimes was questionable, due to the quantity of protein *vs*. fat. Foods like chicken breaded nuggets in a daily meal should represent the source of protein. Therefore, it is not expected that chicken breaded nuggets intake supply similar amounts of protein and fat, as observed in some of the analysed samples. Once more, this is related with the formulation of the pre-fried nuggets. Frequently, health professionals recommend replacement of deep-fat frying by other healthier cooking methods, such as baking. This is mainly due to the uptake of fat during frying, once the intake is closely related with several chronic diseases. One of the most surprising facts for the obtained results is the cholesterol content of baked samples that is two times higher when compared with the other cooking methods. Also, according to the obtained results some of the analysed samples can contribute to almost 50% of the daily intake of salt. This can represent a safety concern for the health status of those who frequently consume this type of foods. Moreover, the content of TFA and polar compounds of the frying oil subjected to ten successive frying operations significantly increased. However, the obtained values are still lower than the maximum recommended by legal entities. The results provided by this study, will contribute to new data on how the applied cooking methods can change the nutritional composition. These can be very useful for further dietary recommendations, as well as for inclusion in the Portuguese Food Composition Database, that up to now has no data regarding this type of processed food.

References

1 C. Dobarganes, G. Márquez-Ruiz and J. Velasco, Interactions between fat and food during deep-frying, *Eur. J. Lipid Sci. Technol.*, 2000, **102**, 521-528.

2 S. Barbut, Convenience breaded poultry meat products – New developments, *Trends Food Sci. Technol.*, 2012, **26**, 14-20.

3 L. Fillion and C. J. K. Henry, Nutrient losses and gains during frying: a review, *Int. J. Food Sci. Nutr.*, 1998, **49**, 157-168.

4 M. Ghidurus, M. Turtoi, G. Boskou, P. Niculita and V. Stan, Nutritional and health aspects related to frying, *Rom. Biotechnol. Lett.*, 2010, **15**, 5675-5682.

5 WHO/FAO, World Health Organization/Food and Agriculture Organization of the United Nations, 2003, Expert Report: Diet, nutrition and prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation. WHO Technical Report Series 916, http://whqlibdoc.who.int/trs/who_trs_916.pdf (accessed January 2016).

6 J. M. Miranda, B. Martínez, B. Pérez, X. Antón, B. I. Vázquez, C. A. Fente, C. M. Franco, J. L. Rodríguez and A. Cepeda, The effects of industrial pre-frying and domestic cooking methods on the nutritional composition and fatty acids profile of two different frozen breaded foods, *LWT--Food Sci. Technol.,* 2010, **43**, 1271-1276.

7 R. Romano, A. Giordano, L. Le Grottaglie, N. Manzo, A. Paduano, R. Sacchi and A. Santini, Volatile compounds in intermittent frying by gas chromatography and nuclear magnetic resonance, *Eur. J. Lipid Sci. Technol.*, 2013, **115**, 764-773.

8 A. Bognár, Comparative study of frying to other cooking techniques influence on the nutritive value, *Grasas Aceites*, 1998, **49**, 250-260.

9 T. G. Albuquerque, A. Sanches-Silva, M. C. Castilho and H. S. Costa, Trends in the analytical methods for the determination of *trans* fatty acids content in foods, *Trends Food Sci. Technol.*, 2011, **22**, 543-560.

10 EFSA, European Food Safety Authority, 2010, Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol, http://www.efsa.europa.eu/en/efsajournal/doc/1461.pdf, (accessed January 2016).

11 D. Mozaffarian, A. Aro and W. C. Willett, Health effects of *trans*-fatty acids: experimental and observational evidence, *Eur. J. Clin. Nutr.*, 2009, **63**, S5-S21.

12 Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011, on the provision of food information to consumers. *Official Journal of the European Union, L304,* 18-63. Retrieved January 5, 2016 from: http://eurlex.europa.eu/LexUriServ/ LexUriServ.do?uri=OJ:L:2011:304:0018: 0063:EN:PDF

13 ISO/IEC, International Organization for Standardisation/International Electrotechnical Commission 17025, *General requirements for the competence of testing and calibration laboratories*, Geneva: International Organisation for Standardisation (ISO), 1999.

14 AOAC, *Official methods of analysis*, Association of Official Analytical Chemists, Washington DC, USA, 17th ed, 2000.

15 H. Greenfield and D. A. T. Southgate, in *Food Composition Data: Production, Management and Use*, 2nd ed., FAO, Rome, 2003, pp. 103.

16 T. G. Albuquerque, A. Sanches-Silva, L. Santos and H. S. Costa, An update on potato crisps contents of moisture, fat, salt and fatty acids (including *trans*-fatty acids) with special emphasis on new oils/fats used for frying, *Int. J. Food Sci. Nutr.*, 2012, **63**, 713-717.

17 ISO, International Standardization Organization 5509, *Animal and vegetable fats and oils – Preparation of methyl esters of fatty acids*, 2000.

18 N. Bragagnolo and D. B. Rodriguez-Amaya, Comparison of the cholesterol content of Brazilian chicken and quail eggs, *J. Food Compos. Anal.*, 2003, **16**, 147-153.

19 T. G. Albuquerque, M. B. P. P. Oliveira, A. Sanches-Silva and H. S. Costa, Cholesterol determination in foods: Comparison between high performance and ultra-high performance liquid chromatography, *Food Chem.*, 2016, **193**, 18-25.

20 U.S. Department of Agriculture, Agricultural Research Service, 2013, USDA National Nutrient Database for Standard Reference, Release 26. Nutrient Data Laboratory, http://www.ars.usda.gov/ba/bhnrc/ndl, (accessed January 2016).

21 I. Lukman, N. Huda and N. Ismail, Physicochemical and sensory properties of commercial chicken nuggets, *Asian J. Food Agro-Industry,* 2009, **2**, 171-180.

22 R. A. Gibbs, C. Rymer and D. I. Givens, Fatty acid composition of cooked chicken meat and chicken meat products as influenced by price range at retail, *Food Chem.*, 2013, **138**, 1749-1756.

23 R. G. Moreira, X. Sun and Y. Chen, Factors affecting oil uptake in tortilla chips in deep-fat frying, *J. Food Eng.*, 1997, **31**, 485-498.

24 World Health Organization, 2013, Mapping salt reduction initiatives in the WHO European Region, http://www.euro.who.int/__data/assets/pdf_file/0009/186462/Mapping-salt-reduction-initiatives-in-the-WHO-European-Region.pdf, (accessed January 2016).

25 S. R. Baggio and N. Bragagnolo, The effect of heat treatment on the cholesterol oxides, cholesterol, total lipid and fatty acid contents of processed meat products, *Food Chem.*, 2006, **95**, 611-619.

26 D-A-CH (Deutsche Gesellschaft für Ernährung – Österreichische Gesellschaft für Ernährung – Schweizerische Gesellschaft für Ernährungsforschung – Schweizerische Vereinigung für Ernährung). *Referenzwerte für die Nährstoffzufuhr.* Frankfurt am Main: Umschau Braus Verlag, 2008.

27 S. Trattner, W. Becker, S. Wretling, V. Öhrvik and I. Mattisson, Fatty acid composition of Swedish bakery products, with emphasis on trans-fatty acids, *Food Chem.*, 2015, **175**, 423-430.

28 E. De Marco, M. Savarese, C. Parisini, I. Battimo, S. Falco and R. Sacchi, Frying performance of a sunflower/palm oil blend in comparison with pure palm oil, *European J. Lipid Sci. Technol.*, 2007, **109**, 237-246.

29 F. A. Aladedunye and R. Przybylski, Degradation and nutritional quality changes of oil during frying, *J. Am. Oil Chem. Soc.*, 2009, **86**,149-156.

30 M. C. M. M. Moreno, D. M. Olivares, F. J. A. López, J. V. G. Adelantado and F. B. Reig, Determination of unsaturation grade and *trans* isomers generated during thermal oxidation of edible oils and fats by FTIR, *J. Mol. Struct.*, 1999, **482**, 551-556.

31 Portaria No. 1135/95. (1995). Diário da República. I Série-B, No. 214, pp. 5836, https://dre.pt/application/file/564538, (accessed January 2016).

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2.5 Efeito do processamento industrial na qualidade e segurança de salgados prontos a comer

Este sub-capítulo apresenta os resultados relativos aos efeitos da fritura industrial na qualidade e segurança de salgados prontos a comer, com base na avaliação do teor de sal, de gordura e composição em ácidos gordos. Foram também estimados os contributos para a ingestão diária destes nutrientes. Verificou-se que a maioria das amostras analisadas neste âmbito são seguras em relação aos teores de ácidos gordos trans. No entanto, continuam a apresentar teores elevados de sal e de gordura, embora maioritariamente insaturada.

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Efeito do processamento industrial na qualidade e na segurança de salgados prontos para comer

Effects of industrial processing on the quality and safety of ready-to-eat savouries

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Resumo

O estilo de vida das famílias tem sofrido alterações profundas, traduzindo-se, entre outros fatores, na redução do tempo para preparação das refeições. Pelo referido, as vendas de alimentos confeccionados, caso dos salgados, têm tido um crescimento contínuo. Este trabalho de investigação teve como objetivos: (1) determinar os teores de sal, gordura total e o perfil em ácidos graxos de salgados prontos para comer; (2) correlacionar os diversos componentes analisados; (3) analisar a evolução da composição dos salgados com base na comparação com os valores da literatura, e (4) estimar os benefícios/riscos para a saúde da população, tendo por base as recomendações de referência. Foram avaliados, em 2015, 12 tipos de salgados prontos para comer. O teor de gordura total foi determinado pelo método de hidrólise ácida, seguido de extração em Soxhlet, com éter de petróleo. O teor de sal foi determinado utilizando-se o método de Charpentier-Volhard. Para o perfil de ácidos graxos, utilizou-se um método de cromatografia gasosa com detecção por ionização em chama. O teor de gordura variou entre 9,14 g/100 g (pataniscas de bacalhau) e 28,9 g/100 g (chamuças de carne). O teor de sal mais elevado foi de 2,13 g/100 g (croquetes de carne). No entanto, uma unidade de empada de requeijão pode contribuir com 45% da ingestão diária de sal recomendada. Os ácidos graxos majoritários, em 58% das amostras analisadas, eram poli-insaturados, e os seus teores variaram entre 3,54 g/100 g (empadas de galinha) e 15,5 g/100 g (chamuças de carne). Todas as amostras analisadas tinham teores de ácidos graxos *trans* inferiores a 2% do teor de gordura total. De uma forma geral, os salgados prontos para comer analisados tinham teores elevados de gordura e de sal, devendo, por isso, o seu consumo ser moderado. No entanto, na maior parte das amostras, o teor de ácidos graxos insaturados era superior aos saturados. No que se refere aos teores de ácidos graxos *trans*, estes alimentos podem ser considerados seguros.

Summary

Family lifestyle has changed greatly over the years, and nowadays there is a lack of time to prepare meals amongst other factors. Hence the sales of ready-to-eat foods, such as savouries, have shown continuous growth. The aim of this study was: (1) to determine the salt and total fat contents and the fatty acid profile of ready-to-eat savouries; (2) to correlate the different components analysed; (3) to analyse the evolution of the composition of savouries based on a comparison with the values found in the literature; and (4) to estimate the health benefits/risks to the population based on the reference dietary recommendations. In 2015, 12 types of ready-to-eat savouries were evaluated. The total fat content was determined by the acid hydrolysis method, followed by the Soxhlet extraction with petroleum ether. The salt content was determined using the Charpentier-Volhard method. Gas chromatography with flame ionization detection was used to determine the fatty acid profile. The fat content varied between 9.14 g/100 g (a cod savoury) and 28.9 g/100 g (a meat savoury). The highest salt content was 2.13 g/100 g (meat croquettes). However, one unit of curd cheese pie can contribute 45% of the recommended daily intake of salt. In 58% of the samples analysed, the major fatty acids were polyunsaturated, and the levels of these fatty acids varied between 3.54 g/100 g (chicken pies) and 15.5 g/100 g (meat savouries). All the samples analysed had *trans* fatty acid contents below 2% of the total fat content. In general, the ready-to-eat savouries analysed showed high levels of fat and salt, and should therefore be consumed in moderation. However, in the majority of the samples the unsaturated fatty acid content was higher than the saturated fatty acid content. With respect to the levels of *trans* fatty acids, these foods could be considered safe.

Introdução

Os avanços tecnológicos, as mudanças nas estruturas domésticas, as sociedades multiculturais, bem como a mudança dos valores sociais, resultaram num aumento da procura por alimentos que sejam de preparação rápida e fácil (Boer et al., 2004; Santos et al., 2012). O estilo de vida das famílias tem sofrido alterações profundas, traduzindo-se essencialmente na falta de tempo para adquirir os alimentos e preparar as refeições. Por esse motivo, adquirem cada vez mais alimentos confeccionados ou pré-confeccionados, também conhecidos por alimentos de conveniência (Albuquerque et al., 2012; Albuquerque et al., 2016a). No entanto, hoje em dia, o consumidor está, também, cada vez mais atento, consciente e preocupado com a sua saúde, exigindo alimentos nutricionalmente adequados e seguros, e reconhece que alguns destes alimentos são ricos em sal e gordura (Berbari et al., 2011).

O consumo excessivo de alimentos com elevados teores de sal está relacionado com um maior risco de desenvolvimento de hipertensão arterial (Ignácio et al., 2013). Neste sentido, têm sido desenvolvidas diversas iniciativas, um pouco por todo o mundo, visando reduzir as quantidades de sal presentes nos alimentos (WHO, 2013). Em Portugal, até a data da realização deste trabalho, apenas estava legislado um valor máximo para o teor de sal no pão (Polónia et al., 2014; SPH, 2016). Apesar disso, esta medida tem tido êxito em Portugal, uma vez que o consumo de sal decresceu de 12,3 para 10,7 g/dia, e pretende-se continuar a implementar medidas legislativas no sentido de fixar limites para outros alimentos, sobretudo os alimentos processados (Polónia et al., 2014; SPH, 2016).

Relativamente à gordura, nos últimos anos, tem-se verificado que os trabalhos de investigação e os guias de recomendações internacionais dão cada vez mais importância ao tipo de gordura presente nos alimentos, nomeadamente a gordura saturada e a *trans*, e não só à quantidade de gordura total. A União Europeia, nos últimos cinco anos, tem desenvolvido diversas iniciativas no sentido de avaliar o teor de ácidos graxos *trans* (AGT) nos alimentos, dado que, em nível Europeu, ainda não existe uma medida legislativa que fixe o limite máximo para os alimentos, nem que obrigue a sua declaração na rotulagem (WHO, 2015; União Europeia, 2011). No entanto, noutros países, como, por exemplo, o Brasil, é obrigatória a declaração dos níveis de gordura *trans* nos rótulos dos alimentos industrializados, quando o teor é superior a 0,2 g/porção (Pinto et al., 2016). Uma ingestão elevada destes ácidos graxos tem sido relacionada com um aumento do risco de desenvolvimento de doenças crônicas, nomeadamente as do foro cardiovascular (Mozaffarian et al., 2006).

Ao longo dos últimos anos, tem-se observado uma maior oferta/disponibilidade de alimentos de conveniência, como, por exemplo, os salgados prontos para comer, estando estes acessíveis em cafés, pastelarias, bares, restaurantes, supermercados, grandes superfícies, entre outros. Os salgados são usados como um “snack” ou como um elemento da refeição principal. Também em relação ao modo de preparação, têm surgido no mercado novas alternativas e, atualmente, além de ser possível continuar a utilizar a fritura, também já é possível encontrar alguns salgados que podem ser adquiridos na forma pré-frita e serem, posteriormente, preparados no forno (Albuquerque et al., 2016a).

No entanto, a fritura continua a ser o método mais usado para a preparação industrial e doméstica de salgados prontos para comer, não só porque é um método de confecção extremamente rápido, mas também por conferir aos alimentos propriedades organoléticas únicas, como o sabor, o aroma e a textura (Damy et al., 2003; Malacrida et al., 2003). Todavia, a utilização desta técnica é muitas vezes questionada, pelo fato de ocorrerem inúmeras reações químicas que colocam em causa a qualidade e a segurança, quer do óleo de fritura, quer do alimento frito (Damy et al., 2003; Malacrida et al., 2003). Alguns dos

fatores identificados como responsáveis por estas alterações são o uso prolongado dos óleos e a utilização de temperaturas elevadas.

Neste trabalho, determinaram-se os teores de sal e de gordura total, e o perfil em ácidos graxos de salgados prontos para comer. Discutem-se os resultados obtidos e correlacionam-se os diversos componentes determinados, analisa-se a evolução da composição dos salgados com base nos valores da literatura e estimam-se os benefícios/riscos do seu consumo, tendo por base as recomendações de referência.

Material e métodos

Amostragem

Foram adquiridos, em 2015, em diversas grandes superfícies da região de Lisboa, 12 tipos de salgados prontos para comer: rolinhos de salsicha, rissóis de camarão, croquetes de carne, chamuças de carne, coxas de frango, pastéis de bacalhau, crepes de legumes, pataniscas de bacalhau, empanadas de atum, empadas de requeijão, empadas de galinha e rissóis de leitão (Figura 18). A seleção das amostras foi realizada com base num estudo de mercado prévio, que avaliou os tipos de produtos disponibilizados ao consumidor nas várias grandes superfícies da região de Lisboa (Portugal). De acordo com a informação fornecida pelos distribuidores, estes produtos foram confeccionados no forno (empanadas de atum, empadas de requeijão e empadas de galinha) ou sujeitos à fritura por imersão, não tendo sido possível obter informação relativa à gordura empregada ou ao óleo utilizado. Para cada tipo de salgado, foram recolhidas diversas unidades, em diferentes grandes superfícies. Após recepção das amostras no laboratório, foram registradas todas as informações consideradas relevantes e as amostras foram homogeneizadas utilizando-se um triturador (GRINDOMIX®, GM200, Alemanha) durante aproximadamente 1 min a 5.000 rpm. Em seguida, as amostras foram acondicionadas em frascos de plástico com tampa, mantidas ao abrigo da luz e do calor, e conservadas numa arca frigorífica a 4 °C. As análises foram realizadas em triplicado para todos os parâmetros.

Determinação do teor de sal

O teor de sal foi determinado pelo método de Charpentier-Volhard (Albuquerque et al., 2012). Este método baseia-se na extração dos cloretos em água ultrapura, seguida de precipitação dos cloretos pelo nitrato de prata, e, por último, a titulação do excesso de nitrato de prata com tiocianato de potássio.

Figura 18. Exemplos das amostras de salgados prontos para comer analisadas.

Determinação do teor de gordura total

O teor de gordura total foi determinado pelo método de hidrólise, com uma solução de ácido clorídrico, seguindo-se uma filtração; posteriormente, procedeu-se à secagem da matéria gorda retida no filtro e à extração em Soxhlet (Soxtec™ 2050, Auto Fat Extraction System, FOSS Analytical, Hilleroed, Dinamarca), com éter de petróleo. Por fim, a eliminação do solvente por evaporação, a secagem e a pesagem do extrato (Albuquerque et al., 2016b).

Determinação do perfil de ácidos graxos

Para a determinação do perfil de ácidos graxos, foi realizada uma extração de gordura utilizando-se éter de petróleo (Albuquerque et al., 2016a). Posteriormente, realizou-se uma transesterificação a frio, com hidróxido de potássio metanólico e n-heptano. Para a análise por cromatografia gasosa acoplada à detecção por ionização em chama, utilizou-se uma coluna capilar Supelco™ 2560 (100 m × 0,25 mm d.i.; 0,2 µm espessura do filme, Supelco™, CA, USA). Foi utilizado como gás de arrasto o hélio. Injetou-se 1 µL de cada amostra a analisar em modo split 50:1. As temperaturas do injetor e do detector foram mantidas a 240°C. Foi utilizada a seguinte rampa de temperatura: 60°C (1 min), aumentar até 168°C a 17°C/min (manter 28 min) e, em seguida, aumentar até 235°C a 4°C/min (manter 15 min).

Análise estatística

Todos os dados foram analisados com os programas Statistica 64 13.0 software (Statsoft Inc., Tulsa, EUA) e Microsoft Office Excel® 2010. Foram efetuados também testes de correlação de Spearman e as médias foram comparadas utilizando-se o teste de Tukey. As diferenças observadas foram consideradas significativas quando o valor de $p < 0,05$. Os resultados estão expressos em g/100 g de amostra e apresentados na forma de média ± desvio padrão (n=3) ou percentagem, utilizando-se algarismos significativos.

Resultados e discussão

Teor de sal e de gordura total

Os resultados relativos aos teores de gordura total e sal (por 100 g) das amostras de salgados prontos para comer apresentam-se na Tabela 11. O teor de gordura total variou entre 9,1 ± 0,1 e 28,9 ± 0,04 g/100 g para as pataniscas de bacalhau e as chamuças de carne, respectivamente (Tabela 11).

Em Portugal, até a atual data, existe pouca informação sobre a composição nutricional dos salgados prontos para comer. Tanto quanto é do conhecimento dos autores, apenas na Tabela da Composição de Alimentos (TCA) é possível encontrar alguma informação relativa a amostras semelhantes (PORTUGAL, 2015). Para efeitos de comparação entre os valores determinados no presente trabalho e a TCA, com a finalidade de fazer um ponto de situação relativa à evolução dos teores de gordura, sal, ácidos graxos saturados (AGS) e AGT, nos últimos anos, apenas foi possível utilizar cinco das amostras. No que diz respeito ao teor de gordura, verificou-se que três das amostras analisadas neste estudo apresentaram teores de gordura inferiores, comparativamente aos valores apresentados na TCA (PORTUGAL, 2015). No entanto, a diferença mais considerável refere-se aos

Tabela 11. Teores de sal, gordura total, ácidos graxos saturados, monoinsaturados, poli-insaturados e *trans* (por 100 g) dos salgados prontos para comer analisados.

Amostras	Sal (g)	Gordura total (g)	AGS (g)	AGMI (g)	AGPI (g)	AGT (mg)
Rolinhos de salsicha	1,67 ± 0,1 ^{g}	13,2 ± 0,02 ^{d}	2,43 ± 0,01 ^{e}	4,05 ± 0,1 ^{d}	6,04 ± 0,04 ^{h}	13,2 ± 0,22 ^{c}
Rissóis de camarão	1,91 ± 0,2 ^{h}	20,1 ± 0,3 ^{j}	2,36 ± 0,01 ^{d}	5,72 ± 0,02 ^{h}	11,0 ± 0,1 ^{k}	9,93 ± 0,13 ^{b}
Croquetes de carne	2,13 ± 0,1 ^{i}	15,5 ± 0,3 ^{g}	3,15 ± 0,002 ^{g}	4,94 ± 0,001 ^{f}	6,59 ± 0,02 ^{i}	15,2 ± 0,16 ^{d}
Chamuças de carne	1,32 ± 0,02 ^{e}	28,9 ± 0,04 ^{k}	3,75 ± 0,001 ^{h}	8,28 ± 0,2 ^{l}	15,5 ± 0,3 ^{l}	14,9 ± 0,38 ^{d}
Coxas de frango	1,29 ± 0,01 ^{e}	10,3 ± 0,4 ^{b}	2,36 ± 0,001 ^{d}	3,73 ± 0,01 ^{c}	3,67 ± 0,01 ^{b}	18,8 ± 0,21 ^{e}
Pastéis de bacalhau	1,01 ± 0,02 ^{c}	11,7 ± 0,6 ^{c}	2,04 ± 0,04 ^{c}	5,33 ± 0,03 ^{g}	3,73 ± 0,02 ^{c}	9,96 ± 0,08 ^{b}
Crepes de legumes	0,762 ± 0,1 ^{a}	14,1 ± 0,1 ^{f}	2,36 ± 0,001 ^{d}	6,23 ± 0,1 ^{i}	4,85 ± 0,001 ^{f}	13,4 ± 0,08 ^{c}
Pataniscas de bacalhau	1,50 ± 0,05 ^{f}	9,14 ± 0,1 ^{a}	1,34 ± 0,01 ^{a}	2,94 ± 0,01 ^{a}	4,41 ± 0,02 ^{e}	7,76 ± 0,13 ^{a}
Empanadas de atum	1,55 ± 0,04 ^{f}	14,2 ± 0,1 ^{f}	1,81 ± 0,004 ^{b}	3,68 ± 0,002 ^{b}	8,00 ± 0,1 ^{j}	13,2 ± 0,72 ^{c}
Empadas de requeijão	1,16 ± 0,1 ^{d}	18,3 ± 0,2 ^{h}	6,61 ± 0,003 ^{i}	6,37 ± 0,01 ^{j}	4,24 ± 0,02 ^{d}	105 ± 0,62 ^{g}
Empadas de galinha	0,877 ± 0,02 ^{b}	19,2 ± 0,6 ^{i}	6,99 ± 0,003 ^{j}	7,65 ± 0,1 ^{k}	3,54 ± 0,01 ^{a}	54,3 ± 0,19 ^{f}
Rissóis de leitão	0,776 ± 0,02 ^{a}	13,4 ± 0,1 ^{e}	2,56 ± 0,002 ^{f}	4,33 ± 0,01 ^{e}	5,82 ± 0,02 ^{g}	15,5 ± 0,02 ^{d}

Os resultados estão apresentados na forma de média ± desvio padrão (n=3); AGS – ácidos graxos saturados; AGMI – ácidos graxos monoinsaturados; AGPI – ácidos graxos poli-insaturados; AGT – ácidos graxos *trans*; Valores na mesma coluna com letras diferentes $^{(a\text{-}l)}$ apresentam diferenças significativas ($p < 0,05$).

rissóis de camarão, apresentando, os salgados desse tipo analisados neste estudo, teores superiores (aproximadamente 10 g) aos descritos na TCA.

O Regulamento (UE) n.º 1169/2011 de 25 de outubro de 2011, relativo à prestação de informação aos consumidores sobre os gêneros alimentícios, determina que a dose de referência para o teor de gordura é de 70 g/dia, tendo por base uma dieta padrão de 8.400 kJ/2.000 kcal, para um adulto (União Europeia, 2011). O contributo (%) de uma unidade de salgados varia entre 7 e 50% da dose de referência de gordura diária, respectivamente para os pastéis de bacalhau e as empadas de requeijão (Tabela 12).
Foi também realizada uma análise comparativa dos salgados confeccionados no forno (empanadas de atum, empadas de requeijão e empadas de galinha) e os produtos sujeitos à fritura, para avaliar se, em termos médios, existia diferença entre os teores de gordura. Verificou-se que a média dos teores de gordura dos alimentos confeccionados no forno é superior (17,2 g/100 g vs. 15,1 g/100 g), mas não se verificaram diferenças significativas.
O teor de sal mais elevado foi de 2,1 ± 0,1 g/100 g (croquetes de carne). Comparando-se os resultados obtidos com os da TCA, verifica-se que as diferenças não são tão evidentes, como no caso do teor de gordura. As diferenças devem-se, sobretudo, à adição de sal durante a preparação dos salgados. A ingestão excessiva de alimentos ricos em sal está diretamente relacionada com o risco de desenvolvimento de Hipertensão Arterial. Os alimentos processados são reconhecidos como alguns dos que mais contribuem para esta ingestão. A Organização Mundial de Saúde recomenda uma ingestão de sal inferior a 5 g/dia, para prevenir doenças cardiovasculares (WHO, 2013). Se considerarmos os resultados obtidos, verifica-se que, por unidade, uma empada de requeijão pode contribuir com 45% do valor recomendado para a ingestão diária de sal, podendo constituir um fator de preocupação para a saúde da população portuguesa.

Composição em ácidos graxos

O teor de AGS variou entre 1,34 e 6,99 g/100 g, para as pataniscas de bacalhau e as empadas de galinha, respectivamente (Tabela 11). Uma elevada ingestão deste tipo de ácidos graxos tem sido relacionada com um aumento do risco de desenvolvimento de doenças cardiovasculares. No entanto, os AGS podem ter efeitos distintos nas concentrações plasmáticas de colesterol. De acordo com a literatura, os ácidos láurico (C12:0), mirístico (C14:0) e palmítico (C16:0) contribuem para o aumento do colesterol das lipoproteínas de baixa densidade, enquanto o ácido esteárico (C18:0) não demonstrou qualquer efeito (EFSA, 2010). Note-se que apenas as empadas de requeijão apresentaram os ácidos graxos saturados como majoritários.

Tabela 12. Composição (g) em gordura total, sal e ácidos graxos saturados dos salgados prontos para comer analisados e contributo para a dose de referência.

Amostras	Unidade (g)	Sal (g)		Gordura (g)		Ácidos graxos saturados (g)	
		Por unidade	Contributo (%)	Por unidade	Contributo (%)	Por unidade	Contributo (%)
Rolinhos de salsicha	80	1,33	27	10,5	15	1,94	10
Rissóis de camarão	45	0,862	17	9,03	13	1,06	5
Croquetes de carne	37	0,784	16	5,70	8	1,16	6
Chamuças de carne	49	0,649	13	14,2	20	1,84	9
Coxas de frango	67	0,859	17	6,86	10	1,57	8
Pastéis de bacalhau	45	0,449	9	5,21	7	0,910	5
Crepes de legumes	62	0,474	9	8,78	13	1,47	7
Pataniscas de bacalhau	80	1,20	24	7,30	10	1,07	5
Empanadas de atum	125	1,94	39	17,8	25	2,27	11
Empadas de requeijão	193	2,24	45	35,3	50	12,8	64
Empadas de galinha	92	0,807	16	17,7	25	6,44	32
Rissóis de leitão	91	0,706	14	12,2	17	2,33	12

De acordo com o Regulamento (UE) n.º 1169/2011, a dose de referência para os AGS é de 20 g/dia (União Europeia, 2011). Nesse sentido, foi realizada uma análise do contributo de uma unidade de cada tipo dos salgados analisados para a ingestão de AGS. Os valores médios das unidades de salgados analisados estão apresentados na Tabela 12. Os salgados (por unidade) que contribuem para uma maior ingestão de AGS são a empada de requeijão (64%) e a empada de galinha (32%), sendo a dose de referência facilmente ultrapassável, num dia. Ou seja: alimentar-se além do limite diário definido para AGS não é difícil, quando se ingerem salgados prontos para comer, como a empada de requeijão e a empada de galinha (Tabela 12).

De forma geral, as amostras analisadas apresentaram majoritariamente ácidos graxos insaturados. Está cientificamente provado que a substituição dos AGS por ácidos graxos insaturados é benéfica para a saúde e diminui o risco de desenvolvimento de algumas doenças crônicas (EFSA, 2010). No caso específico dos salgados prontos para comer, a presença de ácidos graxos insaturados está, em grande parte, relacionada com a qualidade/quantidade de gordura absorvida durante o processo de fritura. Hoje em dia,

cada vez mais as gorduras de origem saturada, nomeadamente banha, manteiga e gordura de palma, são substituídas por óleos vegetais ricos em ácidos graxos insaturados, nomeadamente óleos de girassol, soja e colza, ou misturas de óleos. Estas reformulações e modificações introduzidas pela indústria alimentar podem trazer benefícios à qualidade nutricional dos alimentos, que se traduzem num impacto menos negativo na saúde da população que os consome. Neste trabalho, o teor de ácidos graxos monoinsaturados (AGMI) variou entre 2,94 e 8,28 g/100 g, para as pataniscas de bacalhau e chamuças de carne, respectivamente. Em 50% das amostras analisadas, o teor de AGMI foi superior ou igual a 5 g/100 g de amostra. Os ácidos graxos majoritários em 58% das amostras analisadas foram poli-insaturados, e os teores destes ácidos graxos variaram entre 3,54 g/100 g (empadas de galinha) e 15,5 g/100 g (chamuças de carne).

Relativamente aos AGT, existe evidência científica que fundamenta a necessidade da redução do seu consumo, sobretudo dos que resultam do uso de óleos vegetais hidrogenados ou parcialmente hidrogenados, devido ao aumento do risco de desenvolvimento de doenças cardiovasculares (EFSA, 2010; WHO, 2013). Para 83% das amostras analisadas, os teores de AGT determinados variaram entre 7,76 e 18,8 mg/100 g de amostra. As empadas de galinha e de requeijão foram as amostras que apresentaram os teores mais elevados de AGT (54,3 ± 0,19 e 105 ± 0,62 mg/100 g, respectivamente). De acordo com a Organização Mundial de Saúde, a ingestão de AGT deve ser inferior a 1% do valor energético total. Em Portugal, não existe legislação para o teor deste tipo de ácidos graxos nos alimentos. No entanto, alguns países europeus definem que os teores de AGT devem ser inferiores a 2% do total de gordura nos alimentos que não contêm gordura láctea ou de ruminantes (WHO, 2015). Nesse sentido, no presente trabalho, verificou-se que todas as amostras de salgados prontos para comer estudados apresentam teores de AGT inferiores a 2% do teor de gordura total, podendo, deste ponto de vista, ser considerados seguros.

Correlação entre teores de gordura, sal e composição em ácidos graxos

Neste trabalho, as correlações analisadas, de investigação para os diversos parâmetros em estudo, são, em sua maioria, baixas ou moderadas (Tabela 13). No entanto, também se observaram algumas correlações fortes, nomeadamente para os AGS com os AGT (r=0,81) e com os AGMI (r=0,72).

Tabela 13. Coeficiente de Spearman para as correlações entre os parâmetros analisados (*diferenças significativas p < 0,05).

Variáveis	Gordura	Sal	AGS	AGMI	AGPI	AGT
Gordura	1,00					
Sal	0,08	1,00				
AGS	0,62*	-0,27	1,00			
AGMI	0,80*	-0,34*	0,72*	1,00		
AGPI	0,45*	0,56*	-0,10	0,02	1,00	
AGT	0,32*	-0,40*	0,81*	0,42*	-0,35*	1,00

AGS – ácidos graxos saturados; AGMI – ácidos graxos monoinsaturados; AGPI – ácidos graxos poli-insaturados; AGT – ácidos graxos *trans*.

Foi também realizada uma análise da correlação do sal dos salgados com os outros parâmetros em estudo (Figura 19), no sentido de avaliar se os alimentos ricos em sal têm, por exemplo, um menor teor de gordura. Verificou-se que a correlação do sal foi mais forte para os ácidos graxos poli-insaturados (r=0,56), ou seja, observou-se uma correlação entre estes dois parâmetros nos salgados. Apesar de as correlações do sal com os outros parâmetros terem sido significativas (p < 0,05), exceto para os AGS e a gordura total, estas são fracas ou moderadas, não sendo possível estabelecer uma relação. No entanto, se analisarmos, por exemplo, as chamuças de carne e os croquetes de carne, verifica-se que aquele que tem o maior teor de gordura, tem o menor teor de sal, e vice-versa.

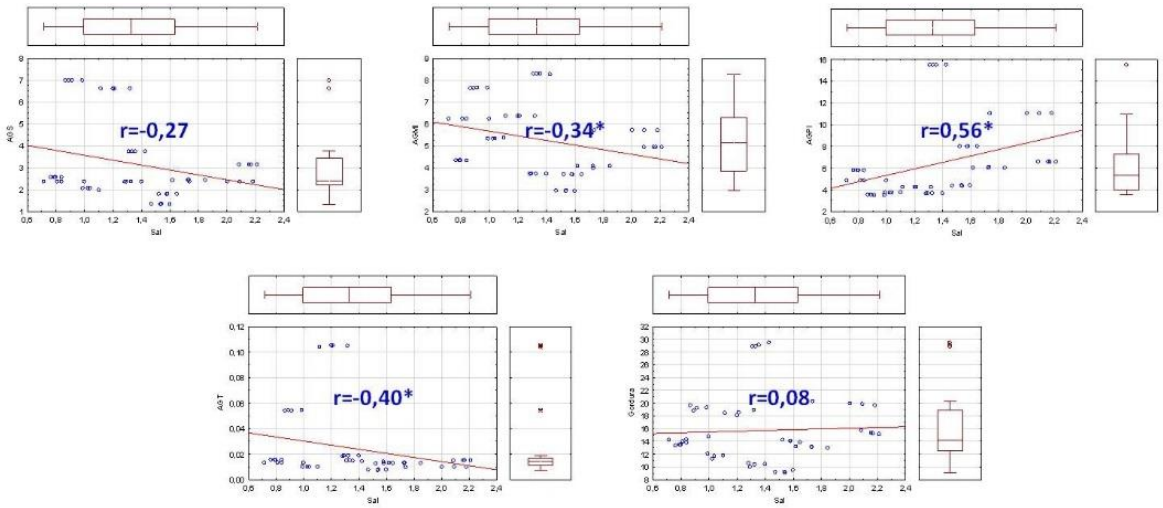

Figura 19. Correlação do teor de sal com os outros parâmetros em estudo (*diferenças significativas p < 0,05). AGS – ácidos graxos saturados; AGMI – ácidos graxos monoinsaturados; AGPI – ácidos graxos poli-insaturados; AGT – ácidos graxos *trans*.

Conclusões

De acordo com os resultados apresentados, pode afirmar-se que, apesar de as amostras analisadas apresentarem teores de gordura elevados, essa gordura é majoritariamente insaturada. Além disso, os teores de AGT foram inferiores a 2% do teor total de gordura, indicando que estes alimentos são seguros, no que se refere a este tipo de ácidos graxos. No entanto, deve reiterar-se que, no que diz respeito ao teor de sal, é necessário reforçar a colaboração com a indústria alimentar, no sentido de desenvolver técnicas que permitam a adição controlada de sal, bem como a redução de forma gradual e progressiva do seu teor neste tipo de alimentos. Até a data atual, tanto quanto é do conhecimento dos autores, não constava nenhum estudo tão detalhado em relação aos salgados prontos para comer. Desta forma, este trabalho constitui uma atualização de informação no que diz respeito à qualidade nutricional deste tipo de salgados, podendo ser muito útil na avaliação de hábitos alimentares e na estimativa de ingestão de alguns nutrientes relacionados com efeitos benéficos/prejudiciais para a saúde da população.

Referências

ALBUQUERQUE, T. G.; OLIVEIRA, M. B. P. P.; SANCHES-SILVA, A.; BENTO, A. C.; COSTA, H. S. The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. **Food & Function**, Cambridge, v. 7, p. 2736-2746, 2016a.

ALBUQUERQUE, T. G.; SANCHES-SILVA, A.; SANTOS, L.; COSTA, H. S. An update on potato crisps contents of moisture, fat, salt and fatty acids (including *trans*-fatty acids) with special emphasis on new oils/fats used for frying. **International Journal of Food Sciences and Nutrition**, London v. 63, p.713-717, 2012.

ALBUQUERQUE, T. G.; SANTOS, F; SANCHES-SILVA, A.; OLIVEIRA, M. B.; BENTO, A. C.; COSTA, H. S. Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefits. **Food Chemistry**, London, v. 193, p. 187-195, 2016b.

BERBARI, S. A. G.; PRATI, P.; FREITAS, D. G. C.; VICENTE, E.; ORMENESE, R. C. S. C.; FAKHOURI, F. M. Utilização de coberturas comestíveis para redução de absorção de gordura em produtos estruturados pré-fritos congelados de mandioca. **Brazilian Journal of Food Technology**, Campinas, v. 14, n. 3, p. 172-180, 2011.

BOER, M.; McCARTHY, M.; COWAN, C.; RYAN, I. The influence of lifestyle characteristics and beliefs about convenience food on the demand for convenience foods in the Irish market. **Food Quality and Preference**, Oxford, v. 15, p. 155-165, 2004.

DAMY, P. C.; JORGE, N. Determinações físico-químicas do óleo de soja e da gordura vegetal hidrogenada durante o processo de fritura descontínua. **Brazilian Journal of Food Technology**, Campinas, v. 6, n. 2, p. 251-257, 2003.

EFSA, European Food Safety Authority. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. **EFSA Journal**, Parma, v. 8, n. 3, 2010.

IGNÁCIO, A. K. F.; RODRIGUES, J. T. D.; NIIZU, P. Y.; CHANG, Y. K.; STEEL, C. J. Efeito da substituição de cloreto de sódio por cloreto de potássio em pão francês. **Brazilian Journal of Food Technology**, Campinas, v. 16, p. 1-11, 2013.

MALACRIDA, C. R.; JORGE, N. Alterações do óleo de soja e da mistura azeite de dendê – Óleo de soja em frituras descontínuas de batatas *chips*. **Brazilian Journal of Food Technology**, Campinas, v. 6, n. 2, p. 245-249, 2003.

MOZAFFARIAN, D.; KATAN, M. B.; ASCHERIO, A.; STAMPFER, M. J.; WILLETT, W. C. *Trans* Fatty Acids and Cardiovascular Disease. **New England Journal of Medicine**, Massachusetts, v. 354, p. 1601-1613, 2006.

PINTO, A. L. D.; MIRANDA, T. L. S.; FERRAZ, V. P.; ATHAYDE, D. D. Determinação e verificação de como a gordura *trans* é notificada nos rótulos de alimentos, em especial naqueles expressos “0% gordura *trans*”. **Brazilian Journal of Food Technology**, Campinas, v. 9, 2016.

POLÓNIA, J.; MARTINS, L.; PINTO, F.; NAZARÉ, J. Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. **Journal of Hypertension**, Philadelphia, v. 32, p. 1211-1221, 2014.

PORTUGAL. Instituto Nacional de Saúde Doutor Ricardo Jorge. **Tabela da Composição de Alimentos**, Lisboa, 2015. Disponível em: http://portfir.insa.pt/foodcomp/introduction. Acesso em: 6 fevereiro 2017.

SANTOS, J. S.; OLIVEIRA, M. B. P. P. Alimentos frescos minimamente processados embalados em atmosfera modificada. **Brazilian Journal of Food Technology**, Campinas, v. 15, n. 1, p. 1-14, 2012.

SPH, Sociedade Portuguesa de Hipertensão. Fórum do sal - estratégia política até 2020 da Sociedade Portuguesa de Hipertensão na luta contra o excesso de teor de sal nos alimentos. **Revista Portuguesa de Hipertensão**, Lisboa, n. 51, 2016.

UNIÃO EUROPEIA. Regulamento (UE) n.º 1169/2011 do Parlamento Europeu e do Conselho de 25 de Outubro de 2011 relativo à prestação de informação aos consumidores sobre os géneros alimentícios. Disponível em: http://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=CELEX:32011 R1169&from=pt. Acesso em: 6 fevereiro 2017.

WHO, World Health Organization. Eliminating *trans* fats in Europe, A policy brief. Copenhagen, 2015. Disponível em: http://www.euro.who.int/__data/assets/pdf_file/0010/288442/Eliminating-trans-fats-in-Europe-A-policy-brief.pdf?ua=1. Acesso em: 6 fevereiro 2017.

WHO. Mapping salt reduction initiatives in the WHO European Region. **WHO Regional Office for Europe**. Copenhagen, 2013. Disponível em: http://www.euro.who.int/__data/assets/pdf_file/0009/186462/Mapping-salt-reduction-initiatives-in-the-WHO-European-Region.pdf. Acesso em: 6 fevereiro 2017.

2.6 An update on processed foods: Relationship between salt, saturated and *trans* fatty acids contents

Este sub-capítulo apresenta os resultados relativos à análise dos teores de sal, de gordura e da composição em ácidos gordos, de 260 alimentos processados agrupados, em 9 categorias. Posteriormente, foi feita uma correlação entre os diferentes nutrientes. Verificou-se que as categorias snacks e fast-food, de uma forma geral, apresentaram teores superiores de sal e de ácidos gordos trans.

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An update on processed foods: Relationship between salt, saturated and *trans* fatty acids contents

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Abstract

To update the current situation on salt, fat and fatty acid composition of processed foods, a study including a wide range of different food categories was conducted in Portugal. Different validated analytical techniques were used, being the fatty acid profile determined by GC-FID and GC-MS. A PCA and correlation analysis were conducted, to establish a trend between the different components of studied in each food category.

The highest salt content was found in snacks, fast-food, sauces and ready-to-eat meals, while the saturated fatty acids were higher ($p < .05$) in bakery products, cookies, biscuits and wafers, and snacks. The highest levels of *trans* fatty acids were found in the fast-food group, followed by the snacks, potato and potato-products and bakery products. A significant positive correlation ($p < .05$) was found between salt and fat content in ready-to-eat meals and in the potato and potato-products. The PCA analysis identified the total fat, and the low *trans* fatty acids and high salt content as distinctive characteristics of some food categories.

Introduction

Processed foods are generally recognized as a source of salt, fat and sugar. An excessive intake of these nutrients is perceived as the leading reason for an increased risk in the development of several chronic diseases (obesity, diabetes, cancer and cardiovascular disease). Generally, food processing is associated with negative effects on the quality and safety of foodstuffs. However, food processing is extremely important to extend shelf-life or just to make them edible (EUFIC., 2010). In 2010, a review on the importance of food

processing and processed foods was published by Floros et al. (Floros, Newsome, Fisher, Barbosa-Cánovas, Chen, Dunne, et al., 2010). Summarizing, this review describes the scientific and technological improvements that allow to have nowadays a production-to-consumption food system able to feed the world population. Moreover, in this review, new tools of biotechnology are addressed focusing on the rapidly growing of the world population for the efficiently and cost-effective production of foods in a sustainable manner (Floros et al., 2010). Some of the main topics focused in this review that are linked with the present research are: preservation of food supply, advantages/disadvantages of food processing and how to solve the diet/disease challenge (Floros et al., 2010). From a nutrition point of view, food processing arises several questions regarding the nutritional quality and safety of food. But, it has also been essential to solve some nutritional barriers, like gluten-free foods, which are crucial for the treatment of coeliac patients.

In the last thirty years the processed foods market has grown like never before and every day "new" processed foods with different features are available on the market (Stuckler, McKee, Ebrhim, & Basu, 2012). There is no consensus regarding the definition of processed foods. In 2010, the International Food Information Council Foundation defined processed food as "*a food where any deliberate change occurs before it's available for us to eat*". Moreover, they defined different categories among processed foods taking into account the extent of processing (International Food Information Council Foundation, 2010). According to the United States Department of Agriculture (USDA), processed food is defined as "*any raw agricultural commodity that has been subject to procedures that alter the food from its natural state"* (Dwyer, Fulgoni, Clemens, Schmidt, & Freedman, 2012). Recently, a new category of processed foods has arisen, the ultra-processed foods, which are formulations developed by the food industry with substances extracted from foods (Louzada, Baraldi, Steele, Martins, Canella, Moubarac, et al., 2015).

Convenience is one of the main reasons to consume processed foods. But it is not the only reason, since frequently processed foods are also more pleasant, due to their sensory and palatability properties (van Langeveld, Gibbons, Koelliker, Civille, de Vries, de Graaf, et al., 2017).

In terms of public health, nutritional quality of foods, dietary intake and mortality are closely linked (Popkin, Adair, & Ng, 2012). Regarding the salt content, a high intake of foods rich in salt is related with an increased risk of developing hypertension, which contributes to the burden of heart disease, stroke and kidney failure, premature mortality and disability (World Health Organization, 2013). Therefore, World Health Organization recommends a population salt intake level of less than 5 g/day for adults and 2 g/day for children, for the prevention of cardiovascular disease (World Health Organization., 2013). According to the European Union strategy for salt reduction, 12 food categories were identified, from which

member states have to select at least 5 to target through their national nutrition action plans and strategies (World Health Organization, 2013). The Portuguese HYpertension and SAlt (PHYSA) study, conducted in 2013, reveals that the Portuguese population has an average salt intake of 10.3 g/day, which is more than the double of WHO recommendations (Polonia, Martins, Pinto, & Nazare, 2014).

Another priority intervention area according to the European Strategy for Prevention and Control of Noncommunicable Diseases 2012-2016 was the "*elimination of trans fats in food (and their replacement with polyunsaturated fats)*" (World Health Organization., 2012). This priority is also part of the European Food and Nutrition Action Plan 2015–2020, whose general objective is to improve food system governance, the overall quality of the population's diet and nutritional status, and to promote health and well-being (World Health Organization, 2014).

Most of the European countries are still not limiting the content of *trans* fatty acids (TFA) in food, except Austria, Switzerland, Iceland, Norway, Denmark and Hungary, which have limited TFA content to below 2% of total fat in foods (excluding ruminant TFA) (World Health Organization, 2015). Moreover, the indication of TFA content on nutritional declaration is not mandatory, according to Regulation (EU) n.º 1169/2011 (European Parliament/Council of the European Union, 2011). Regarding the recommended maximum intake, a report from Food and Agriculture Organization, stated that the intake of TFA should be as low as possible (<1% of total energy intake), which for a 2000 kcal diet, represents around 2 g of TFA intake in a day (FAO., 2010). The European Food Safety Authority (EFSA) has emitted in 2010, a Scientific Opinion on Dietary Reference Values for fats, including saturated, polyunsaturated, monounsaturated and *trans* fatty acids, and cholesterol. However, no Population Reference Intake, Average Requirement, or Adequate Intake was set, recommending that TFA intake should be as low as possible within the context of a nutritionally adequate diet (European Food Safety Authority, 2010). An excessive intake of TFA has been linked with an increase on coronary heart disease risk factors and coronary heart disease events, among other chronic diseases (Souza, Mente, Maroleanu, Cozma, Há, Kishibe, et al., 2015).

Based on the identified priority intervention areas, the aim of this study was to determine salt and total fat contents and fatty acids profile of a selection of processed foods, in order to get an update of the current situation. Also, a novel approach was performed by analysing possible correlations between the different components among the studied food categories, in order to establish a possible trend relative to the relationship between salt, saturated and *trans* fatty acids contents among processed foods.

Materials and methods

Reagents and standards

All chemicals and reagents used in this study were of analytical grade from different suppliers (Merck, Darmstadt, Germany; Panreac, Barcelona, Spain). Fatty acids methyl esters (FAMEs) standards were obtained from Supelco® (Supelco® 37 FAME Mix C4:0 - C24:0, and Linoleic acid cis/*trans* isomers, Supelco, CA, USA) and GLC 674 from Nu-Chek-Prep (Elysian, MN, USA). For quality assurance of analytical data, the Standard Reference Material® 1548a and 1544 were used. Ultrapure water was prepared using a Milli-Q filter system (Millipore, Bedford, MA, USA).

Samples

Sample selection

Between 2014 and 2016, 260 composite samples of different categories of processed foods were collected from retail stores, supermarkets, food chains, restaurants and takeaway in Portugal. Whenever possible, different brands and different retailers for the same type of food were acquired, in order to achieve the best representativeness. The selected food samples were grouped as: cookies, biscuits and wafers (n=61), potato and potato-products (n=47), ready-to-eat meals (n=41), bakery products (n=32), nuts and oilseeds (n=31), fast-food (n=20), snacks (n=13), cereal products (n=11), and sauces (n=4). More detailed information regarding the criteria used for the prioritisation and distribution by food categories is provided in Section Foods prioritisation and distribution by categories.

When the selected items arrived at the laboratory, an excel form was filled out with the following information: (1) identification; (2) description; (3) place, date and time of collection; (4) type of packaging; (5) nutritional declaration (energy, fat, saturated fatty acids, carbohydrates, sugars, fibre, protein and salt); (6) portion size; (7) list of ingredients; (8) expiring date; and (9) other relevant information.

Sample preparation

All the acquired items were stored in accordance with label instructions (when available) and were analysed before their expiry dates. When it was necessary to apply cooking procedures, those items were prepared in accordance with manufacturer's instructions, using domestic cooking equipment. A composite sample of approximately 1 kg was prepared with at least three units of the same item. All the samples were homogenised in a blender (GM200, RETSCH, Germany) during 1 to 3 min at 5000 rpm, depending on the food matrix. Afterwards, samples were properly conditioned according to their perishability (e.g. high perishable foods were frozen at -20 ºC) until analysis.

Total fat

Total fat was determined by an acid hydrolysis method (Albuquerque, Santos, Sanches-Silva, Oliveira, Bento, & Costa, 2016a). Briefly, 75 mL of ultrapure water and 45 mL of hydrochloric acid fuming 37% were added to 2-10 g of sample. Then, this solution was boiled for 20 min and left to cool down until room temperature. Afterwards, the solution was filtered with a filter paper (Whatman® n.º 40, Maidstone, United Kingdom). For the fat extraction, a Soxhlet apparatus (Soxtec™ 2050, Auto Fat Extraction System, FOSS Analytical, Hillerød, Denmark) and petroleum ether were used. The obtained residue was dried for 1h 30 min at 101 ºC ± 2 ºC, until constant weight. The analytical procedure for the determination of total fat content was performed in triplicate. Results are expressed as g/100 g for all the analysed food products.

Fatty acids determination

Fat extraction

For fat extraction, approximately 10 g of sample were weighted and 120 mL petroleum ether was added (Albuquerque, Oliveira, Sanches-Silva, Bento, & Costa, 2016b). The solution was stirred around 60 min, depending on matrices complexity, and a separation of phases was obtained. The upper phase (organic phase) was dried with sodium sulphate (Na_2SO_4) and filtered (Whatman® n.º 42, Maidstone, United Kingdom). Petroleum ether was evaporated using a rotary evaporator (Büchi R-210, Labortechnik AG Switzerland).

Preparation of fatty acid methyl esters (FAMEs)

To determine the fatty acids profile of the selected food samples a cold transesterification method was performed according to the ISO 5509:2000, with slight modifications. 0.2 g of the fat extract were weighed and 2.5 mL of n-heptane and 0.25 mL of methanolic KOH (2 N) were added (European Committee for Standardisation, 2000). After separation of phases, the upper phase was dried with Na_2SO_4 and filtered (Whatman® n.º 42, Maidstone, United Kingdom).

Gas chromatography- flame ionization detection (GC-FID)

A gas chromatograph (Hewlett Packard 6890 series GC-Systems, Waldbronn, Germany) equipped with an autosampler, a programmed split/splitless injector and a flame ionization detector (FID) was used to perform all the gas chromatography analyses. A stabilized non-bonded poly(biscyanopropyl siloxane), SP™ 2560 column (100 m × 0.25 mm i.d. × 0.2 µm film thickness, Supelco™, CA, USA), was employed. Helium was used as carrier gas at 1.0 mL/min flow rate. The split ratio was 50:1 and this was selected after investigating 25:1 and 100:1 split ratios. The oven temperature was programmed as follows: 60 ºC (1 min), then to 168 ºC at 17 ºC/min (held for 28 min), and then to 235 ºC at 4 ºC/min (held for 15 min). The split valve was opened 2 min after injection. The injector and detector temperatures

were 260 ºC and 290 ºC, respectively. The software used to process peak areas was HP Chemstation (Rev. B.02.01-SR2, Hewlett Packard, California, USA). FAMEs were identified by comparing the retention times of the standards with those of the samples. The relative percentage of each fatty acid was calculated by internal normalization of the chromatographic peak area. The conversion of FAMEs to their fatty acids was done according to AOAC 996.06 (2002).

Gas chromatography-mass spectrometry detection (GC-MS)

Whenever necessary, for confirmation purposes, samples were injected in an Agilent 6890N Network GC System equipped with an Agilent 5973 Series GC/MSD, and an Agilent 7683B Automatic Liquid Sampler (Agilent Technologies, Atlanta, GA, USA). Some of the conditions, aforementioned were maintained, namely regarding the column, split ratio, carrier gas and injection volume. Electron impact (EI) mass spectra were obtained at 70eV and detection was carried out in scan mode over a range of 50-550 m/z. The ion source temperature was kept at 230 ºC. The temperature was programmed at 50 °C for 11 min, raised from 50 ºC to 77 ºC at a rate of 25 ºC/min, raised from 77 ºC to 168 ºC at a rate of 17 ºC/min and held for 32 min. Then, the temperature was increased from 168 ºC to 195 ºC at a rate of 1.5 ºC/min, from 195 °C to 199 °C at a rate of 0.5 ºC/min and raised from 199 ºC to 220 ºC at a rate of 1 ºC/min, held for 3 min. Finally, the temperature was increased from 220 ºC to 235 ºC at a rate of 25 ºC/min. The software used to process peak areas was MSD Chemstation (D.01.02.16, Hewlett Packard, California, USA). The FAMEs were identified by comparing the retention times of the standards with those of the samples, and also mass spectra from Wiley mass spectral library (Wiley Registry™, 8th Edition Mass Spectral Library), with an acceptance criterion of a score match above 90%.

Data calculation

Results on fatty acids content were obtained using the sums: Σ saturated fatty acids (Σ SFA = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0); Σ monounsaturated fatty acids (Σ MUFA = C14:1,*cis*-9 + C15:1,*cis*-10 + C16:1,*cis*-9 + C17:1,*cis*-10 + C18:1,*cis*-9 + C20:1,*cis*-11 + C22:1,*cis*-13 + C24:1,*cis*-15); Σ polyunsaturated fatty acids (Σ PUFA = C18:2,*cis*-9,12 + C18:3,*cis*-6,9,12 + C18:3,*cis*-9,12,15 + C20:2,*cis*-11,14 + C20:3,*cis*-8,11,14 + C20:3,*cis*-11,14,17 + C20:4,*cis*-5,8,11,14 + C22:2,*cis*-13,16 + C20:5,*cis*-5,8,11,14,17 + C22:6,*cis*-4,7,10,13,16,19) and Σ *trans* fatty acids (Σ TFA = C18:1,*trans*-6 + C18:1,*trans*-7 + C18:1,*trans*-9 + C18:1,*trans*-11 + C18:2,*trans*-9,12 + C18:2,*cis*-9,*trans*-12 + C18:2,*trans*-9,*cis*-12).

Salt determination

The salt content was determined by Charpentier Volhard's titration (Albuquerque, Sanches-Silva, Santos, & Costa, 2012). This method determines the salt content of foods based on the concentration of the chloride ion. Briefly, 5 g of sample were weighed into a 200 mL volumetric flask. Afterwards, 2.5 mL of potassium ferrocyanide (15% w/v) and 2.5 mL of zinc acetate (30% w/v) were added and ultrapure water was used to complete the volumetric flask volume. The solution was filtered with a 150 mm diameter filter paper (Whatman® n.º1, Maidstone, United Kingdom). Then, the prepared solution was titrated using potassium thiocyanate (0.1N). Sodium chloride was estimated from the amount of chloride ion, as determined by the end-point of titration. Determination of salt content was performed in triplicate.

Statistical analysis

The obtained results were presented on a fresh weight basis and were expressed as mean, range and median, and 25th and 75th percentile, to better represent the data distribution. Further data analysis was performed after data transformation by using first a scaling and log transformation protocol to eliminate the differences caused by the different order of magnitude between variables, allowing to analyse only the relevant variation between the samples and to turn the data distributions more symmetric (van den Berg, Hoefsloot, Westerhuis, Smilde, & Werf, 2006). As the data did not follow a normal distribution, the existence of differences between food categories were evaluated by the non-parametric Kruskal-Wallis ANOVA by Ranks test, followed by post-hoc comparisons of mean ranks of all pairs of groups. To study the relation between the different variables, a correlation study was performed, using the non-parametric Spearman R coefficient. To obtain a global perspective (i.e. all samples and variables analysed simultaneously) a multivariate analysis approach was also performed. A Principal Components Analysis (PCA) was performed to establish which variables were more correlated to each food category and if they permit to obtain a clear distinction between food categories. Statistical significance was defined as $p < .05$ for all the analyses. The statistical analyses were carried out using Statistica 64 13.0 software (Statsoft Inc., Tulsa, USA).

Results and discussion

Foods prioritisation and distribution by categories

The focus of the current study was processed foods. Since up to the start of the research no recent representative national survey was available in Portugal, it was not possible to prioritise food items according to data on nutrient intake. Previous to food prioritisation several supermarket visits were performed, as well as a web search, to compile the

information regarding the potential food items available in the Portuguese food market. Afterwards, the different food items were selected based on their market availability, list of ingredients and knowledge of recent analytical data for the components under analysis. The distribution into the selected food categories was one of the main constraints of this work, since no international agreement exists on a common food grouping (FAO/INFOODS., 2012). Therefore the main ingredients and features of the foodstuffs, as well as the distribution used in other similar studies (Baylin, Siles, Donovan-Palmer, Fernandez, & Campos, 2007; Wagner, Plasser, Proell, & Kanzler, 2008; Saunders, Jones, Devane, Scholes, Lake, & Paulin, 2008; Richter, Shawish, Scheeder, & Colombani, 2009; Roe, Pinchen, Church, Elahi, Walker, Farron-Wilson, et al., 2013), were used to group the selected items in the following categories:

- Cookies, biscuits and wafers: filled cookies (chocolate, strawberry or vanilla); covered cookies (milk chocolate, dark chocolate, or white chocolate); crackers (with salt, without salt or seasoned with rosemary); wafers (chocolate or vanilla); plain (whole, fibre enriched or butter); others.
- Potato and potato-products: potato crisps with and without added flavours, French fries from restaurants; frozen potatoes pre-fried and fried; and homemade French fries.
- Ready-to-eat meals: breaded foods (pre-fried, fried and baked); recipes of beans with meat; recipes with cod; recipes with chicken or turkey; instant soups; pies (chicken, beef, tuna fish, pork, legumes, cottage cheese); croquettes (meat); pastries (shrimp, cod, chicken or pork).
- Bakery products: croissants (puff, filled with chocolate, filled with cream); fine bakery (Portuguese traditional bakery and others); Swiss rolls (filled with chocolate, strawberry, vanilla or covered with chocolate and filled with milk); cakes (marble, yoghurt); sweet breads (covered with chocolate chips, filled with chocolate); and salty breads (garlic, chourizo, pork).
- Nuts and oilseeds: nuts (natural, salty, sweet) and oilseeds.
- Fast-food: pizzas (ham & cheese, four cheeses, smoked ham, bacon & olives, chourizo), burgers (cheeseburger, chicken, fish, beef), and sandwiches (tuna fish, pork & cheese, chicken & brie cheese, seafood sticks).
- Snacks: corn crisps, popcorn (sweet or salty), and cheese & paprika crisps.
- Cereal products: granola, muesli (plain, with nuts or with chocolate), cereal bars (chocolate, strawberry, raisins & coconut or dark chocolate & peanuts), and breakfast cereals (plain, with chocolate, with honey or with fruits & nuts).
- Sauces: cocktail, mayonnaise & garlic, ketchup, and curry & mango.

Some food categories were not included, like edible fats, since a scientific update on TFA content was performed and the contents on these fatty acids were not a concern anymore (Torres, Casal, & Oliveira, 2002).

Fat and salt contents determination of each food category

The fat and salt contents and the fat composition of foodstuffs are within the most studied issues, since they have a great influence on consumers' health. In this study, due to the high number of samples included (n=260), these aspects will be primarily discussed for each food category, detailing it for individual samples only when a distinct result is worth of mention. The results obtained for each category are showed in Table 14. To better represent the data distribution within each category the box-plot for each variable is presented in Figure 20. Due to the high number of different food items included in each category it was possible to note very distinct compositions in terms of fat, salt, SFA, MUFA, PUFA and TFA contents, as well as a wide data range represented in both Table 14 and Figure 20. Yet, some significant differences ($p < .05$) were noticed between the groups in all parameters evaluated (see Table 14). Regarding the salt content four categories, namely, snacks, fast-food, sauces and ready-to-eat meals, presented a significantly ($p < .05$) higher amount than the others. Recently, in November 2015, the Portuguese Society of Hypertension in collaboration with Portuguese authorities has set a political strategy for the next five years (2015-2020) on the reduction of salt content in Portuguese foods. One of the goals is to reduce the mean intake of salt of the Portuguese population below 10 g/day until 2020 (Sociedade Portuguesa de Hipertensão, 2016). Therefore, a strategy was developed to achieve the aforementioned goal and it focuses on the reformulation of foods. Regarding the reformulation of foods, in 2009, a limit of 1.4 g/100 g for the salt content in bread was fixed by legislation implementation (Sociedade Portuguesa de Hipertensão, 2016). Now, a "new" legislative measure to decrease even more the content of salt in bread is necessary, similarly to what happens in other EU countries (Kloss, Meyer, Graeve, & Vetter, 2015; World Health Organization., 2013).

One of the countries with a higher number of reduction initiatives for salt content in food is the United Kingdom (UK). The salt content in bread from UK decreased approximately 20% in ten years (from 1.23 to 0.98 g/100 g) (Brindsen, He, Jenner, & MacGregor, 2013). Comparing it with the current limit in Portugal there is a difference of 43%, which represents a concern and shows that Portugal is still far away and urgently needs to promote more actions regarding the salt content in bread. With respect to worldwide salt reduction initiatives, a review has been recently published (Trieu, Neal, Hawkes, Dunford, Campbell, Rodriguez-Fernandez, et al., 2015). According to the reported data, food reformulation and consumer education were appointed as the most adopted initiatives by WHO regions.

Moreover, bread is the most targeted food, followed by bakery products, sauces and convenience foods (Trieu et al., 2015).

Concerning processed foods and taken into account the above mentioned strategy, efforts should be performed with the engagement of food industries to promote the slow reformulation of these foods in order to progressively reduce salt intake through these products, without affecting their organoleptic properties and without leading to rejection by the consumer. Finally, a progressive reduction of the salt content in the restaurant meals should also be promoted (Sociedade Portuguesa de Hipertensão, 2016).

Therefore, in order to successfully accomplish these objectives, a constant monitoring of the salt content of processed food is needed. This study can be used as a starting point to know the present amount of salt in processed foods, as well as to assess how the reduction of salt content in processed foods is being achieved, or to evaluate the current intake of salt by the Portuguese population.

As aforementioned a high variability among the different analysed food items for the same category was found. Therefore, different approaches and concerns regarding the obtained results are pointed out. For example, in two brands of vanilla wafers, a difference of almost six times on the salt content between brands was observed (0.153 *vs*. 0.834 g/100 g). Therefore, this fact led authors to presume that it is possible to produce similar foods with a lower salt content and that it is feasible their reformulation without rejection by the consumer.

In terms of fat content, nuts and oilseeds group showed the highest contents ($p < .05$), followed by sauces, potato and potato-products, and snacks samples. These were also the groups that showed a higher MUFA and PUFA contents, whose values were significantly higher ($p < .05$) in the nuts and oilseeds category. Although the higher fat content found, especially in nut and oilseed samples, the nutritional quality of this natural fat can be considered more healthy than the one found in cookies, biscuits and wafers, snacks and bakery products group, that had a higher ($p < .05$) SFA content. The obtained results are in agreement with other studies reported in the literature for similar food items (Ansorena, Echarte, Ollé, & Astiasarán, 2013; Dias, Passos, Carmo, Lopes, & Mesquita, 2015; Santos, Cruz, & Casal, 2015; Trattner, Becker, Wretling, Öhrvik, & Mattisson, 2015). TFA were present in almost all food categories, being absent only in samples from the sauces group. The highest levels were found in the fast-food group, followed by the snacks, potato and potato-products and bakery products. From the total of 260 composite samples in this study, 81.9% revealed the presence of TFA in their composition. Although this seems a high presence, the values found in most of them (71.1%) represent less than 1% of the fat content. Regarding the higher contents of TFA, especially those that showed a value above the 2% of the fat content, they only represent 3.5% (9 samples) being determined in snacks,

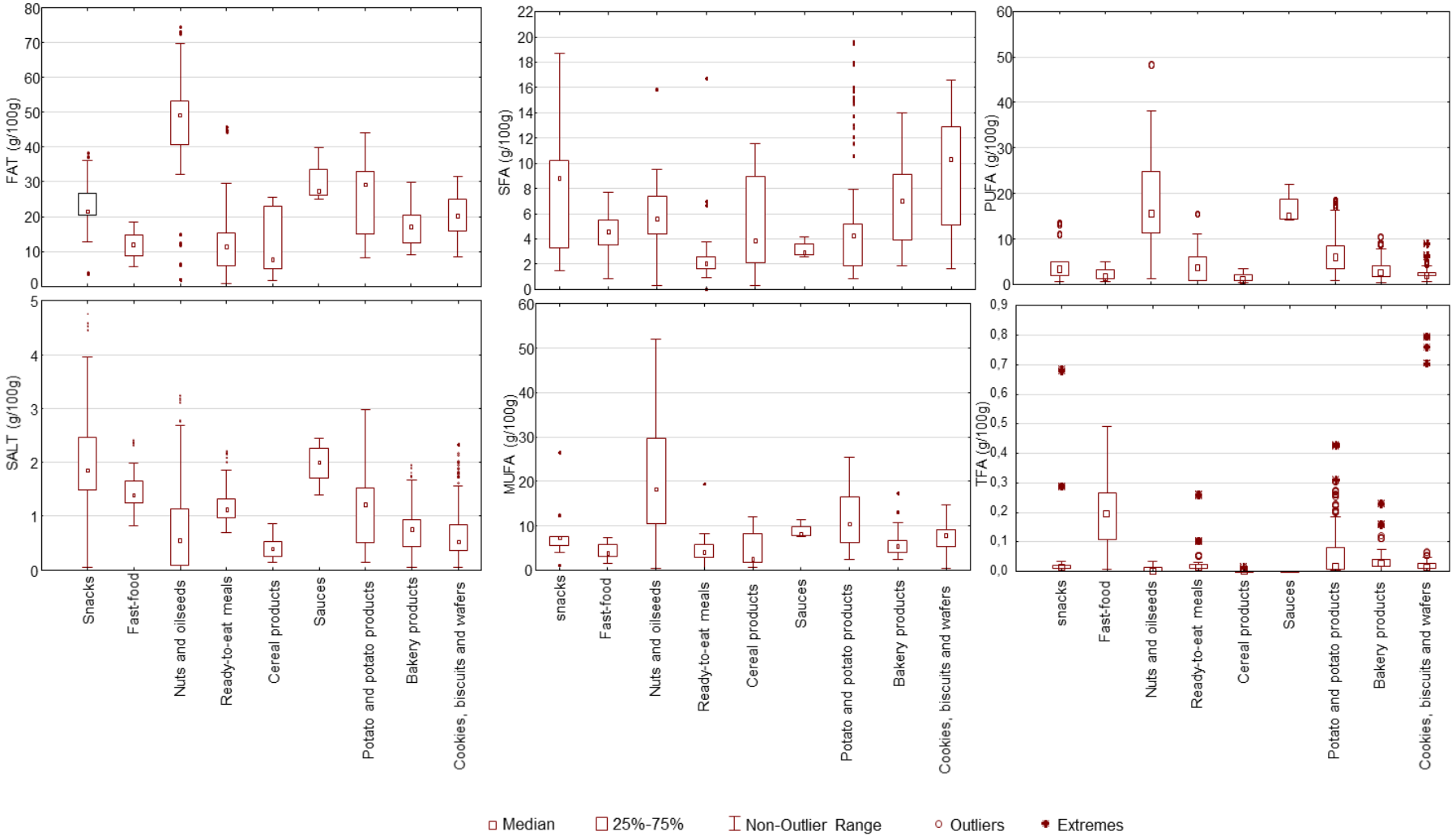

Figure 20. Salt, total fat, SFA, MUFA, PUFA and TFA content (g/100 g of food product) of each food category.

Table 14. Salt, total fat, SFA, MUFA, PUFA and TFA content (g/100 g of food product) found in the different food categories (mean value and range).

Food Categories	*n*	Salt	Fat	SFA	MUFA	PUFA	TFA
Snacks	13	1.94 (0.05-4.77)^{ab}	22.47 (3.67-38.53)^{bc}	7.83 (1.46-18.71)^{ab}	8.41 (1.16-26.6)^{bc}	4.9 (0.57-13.41)^{c}	0.09 (0-0.68)^{bc}
Fast-food	20	1.46 (0.82-2.41)^{ab}	11.66 (5.6-18.51)^{d}	4.53 (0.8-7.69)^{c}	4.24 (1.6-7.22)^{d}	2.05 (0.65-4.92)^{de}	0.19 (0.01-0.49)^{a}
Nuts and oilseeds	31	0.83 (0-3.24)^{d}	45.62 (1.86-74.55)^{a}	5.67 (0.28-15.89)^{bc}	20.21 (0.41-52)^{a}	17.54 (1.18-48.23)^{a}	0.01 (0-0.03)^{d}
Ready-to-eat meals	41	1.18 (0.7-2.21)^{bc}	11.85 (0.85-45.79)^{d}	2.5 (0-16.72)^{d}	4.4 (0-19.5)^{cd}	4.19 (0-15.47)^{c}	0.03 (0-0.26)^{c}
Cereal products	11	0.43 (0.14-0.85)^{d}	11.77 (1.76-25.45)^{d}	5.04 (0.29-11.55)^{c}	4.63 (0.55-12.12)^{d}	1.43 (0.37-3.58)^{e}	0 (0-0.02)^{d}
Sauces	4	1.97 (1.39-2.43)^{a}	29.91 (25.1-39.97)^{ab}	3.13 (2.54-4.18)^{cd}	8.75 (7.48-11.41)^{abc}	16.51 (14.18-21.89)^{a}	0 (0-0)^{d}
Potato and potato products	47	1.16 (0.14-2.98)^{c}	25.58 (8.13-44.15)^{b}	5.57 (0.82-19.59)^{c}	11.71 (2.41-25.43)^{b}	7.15 (0.89-18.53)^{b}	0.07 (0-0.43)^{bc}
Bakery products	32	0.76 (0.05-1.95)^{d}	17.46 (8.97-29.85)^{c}	7.07 (1.89-13.93)^{ab}	5.95 (2.4-17.39)^{d}	3.49 (0.45-10.36)^{c}	0.04 (0-0.23)^{b}
Cookies, biscuits and wafers	61	0.7 (0.05-2.35)^{d}	20.16 (8.61-31.6)^{c}	9.11 (1.62-16.56)^{a}	7.46 (0.45-14.8)^{c}	2.49 (0.58-9.01)^{cd}	0.04 (0-0.8)^{c}

fast-food, ready-to-eat meals and cookies, biscuits and wafers items. From those 9 samples, 7 contained fat of ruminant origin in their composition, leaving only 2 samples (0.8% of the total samples studied, one from the snacks and another from the ready-to-eat meals group) with putative hazardous TFA levels. These results are in accordance with other studies reported in the literature, where TFA content in foods is now lower than the previously reported data (Ritcher et al., 2009; Roe et al., 2013). For instance, Roe et al. reported a significant decrease between 2008 and 2010 on TFA content in some foods, such as mayonnaise, butter, fat-spread, milk chocolate, garlic and herb bread, potato crisps, takeaway chicken pieces, toffees, among others (Roe et al., 2013). However, the comparison with the data reported in the literature sometimes is difficult since authors do not provide enough information to allow it, such as absence of total fat content for the conversion of data reported as % of TFA/total fat; or the data is reported as % of total TFA/FAME and it is necessary to apply conversion factors in order to enable a comparison.

Concerning TFA, in the nineties several studies were performed in order to evaluate the possible routes for their formation in foods, as well as the amounts present in foods. The best known study on the quantification of TFA in foods is TRANSFAIR, which involved fourteen European countries and was led by The Netherlands (van Poppel, van Erp-Baart, Leth, Gevers, van Amelsvoort, Lanzmann-Petithory, et al., 1998). In comparison with the data reported by TRANSFAIR for Portugal, a significant decrease can be observed on TFA content for similar food categories (Aro et al., 1998; van Erp-baart et al., 1998).

However, this subject remains current and very recently several international organizations (EFSA, WHO/FAO, EU) have again performed several studies regarding the occurrence of TFA in food (Europena Food Safety Authority, 2010; FAO/2010; Mouratidou, Livaniou, Saborido, Wollgast, & Caldeira, 2014; WHO, 2015). In 2014, the European Commission published a report on *"Trans fatty acids in Europe: where do we stand? - A synthesis of the evidence: 2003-2013"*, summarising the most recent publicly available data on the content of TFA in foods and on dietary intake of TFA in Europe (Mouratidou et al., 2014). In this report, data on TFA can be found for different food groups by country. However, for Portugal only one study with TFA data on potato crisps (Albuquerque et al., 2012) was considered to be included revealing that there was an urgent need to carry out more studies with different food items, otherwise it is not possible to accurately know the current situation in Portugal. Albuquerque et al., reported that TFA content in potato crisps (n=18) varied between 0.011 and 0.282 g/100 g, while in the current study the TFA content in potato and potato products (n=47) ranged from not detected to 0.43 g/100 g (Albuquerque et al., 2012).

3.3 Correlation between fat composition and salt content of processed foods

Besides the update on fat and salt composition of processed food, one of the main objectives was to investigate the existence of relations between the evaluated parameters. Initially, a correlation study was performed followed by a multivariate analysis approach. The correlation values obtained between the studied parameters in all items are presented in Table 15. As expected, significant correlations ($p < .05$) between the fat related parameters were obtained, being higher between fat and MUFA ($r=0.88$) and PUFA ($r=0.66$) contents. More interestingly, the salt content showed less significant correlations, however, it is worth noticing the significant correlation ($p < .05$) encountered between salt and SFA content ($r=-0.10$), and between salt and TFA content ($r=0.15$) (see Table 15). When looking at these correlations within each food category (results presented in Table 15), the same pattern was followed in almost all groups, especially between the values related to the fat composition. Regarding the correlation between fat and salt contents of the analysed processed foods, this was not significant ($p > .05$) in snacks and bakery products. On the other hand, a significant positive correlation ($p < .05$) was found in ready-to-eat meals

(r=0.49) and in the potato and potato-products (r=0.46), showing a tendency for the food products of these categories to exhibit higher salt values when their fat content increases.

Table 15. Spearman Rank Order Correlations between the TFA, fat and salt content with all parameters evaluated in each food category.

		Fat	Salt	SFA	MUFA	PUFA
All processed foods	TFA	0.21*	0.15*	0.49*	0.12*	-0.20*
	Fat	1.00	-0.04	0.53*	0.88*	0.66*
	Salt	-0.04	1.00	-0.10*	0.03	-0.01
Snacks	TFA	0.19	0.06	0.90*	0.18	-0.68*
	Fat	1.00	-0.22	0.28*	0.94*	0.49*
	Salt	-0.22	1.00	-0.25	-0.22	-0.23
Fast food	TFA	0.67*	-0.33*	0.88*	0.30*	-0.20
	Fat	1.00	-0.54*	0.62*	0.78*	0.29*
	Salt	-0.54*	1.00	-0.13	-0.78*	-0.27*
Nuts and oilseeds	TFA	0.00	-0.05	0.35*	0.12	-0.10
	Fat	1.00	-0.35*	0.42*	0.65*	0.38*
	Salt	-0.35*	1.00	0.26*	0.15	-0.38*
Ready-to-eat meals	TFA	-0.12	-0.33*	0.48*	0.05	-0.42*
	Fat	1.00	0.49*	0.57*	0.78*	0.85*
	Salt	0.49*	1.00	0.11	0.10	0.51*
Cereal products	TFA	-0.71*	-0.69	-0.90*	-0.74*	-0.71*
	Fat	1.00	-0.55*	0.93*	0.96*	0.77*
	Salt	-0.55*	1.00	-0.41*	-0.43*	-0.45*
Sauces	TFA					
	Fat	1.00	-0.71*	0.89*	0.86*	0.86*
	Salt	-0.71*	1.00	-0.76*	-0.61*	-0.71*
Potato and potato-products	TFA	0.69*	0.45*	0.81*	0.60*	-0.01
	Fat	1.00	0.46*	0.76*	0.67*	0.27*
	Salt	0.46*	1.00	0.51*	0.59*	-0.37*
Bakery products	TFA	0.23*	0.12	0.40*	0.06	0.01
	Fat	1.00	-0.05	0.42*	0.88*	0.69*
	Salt	-0.05	1.00	0.18*	0.10	-0.18*
Cookies, biscuits and wafers	TFA	0.41*	-0.26*	0.61*	0.06	-0.02
	Fat	1.00	-0.43*	0.80*	0.67*	0.60*
	Salt	-0.43*	1.00	-0.43*	-0.22*	-0.11

SFA – saturated fatty acids; MUFA – monounsaturated fatty acids; PUFA – polyunsaturated fatty acids; TFA – *trans* fatty acids; *denotes the existence of a significant correlation at $p < 0.05$.

In this sense, the food products of these categories should raise more awareness in public health concerns as they tend to increase simultaneously the fat and salt intake of the consumers. In the majority of the processed foods from the other categories (fast-food, nuts and oilseeds, cereal products, sauces and cookies, biscuits and wafers) the relation

between fat and salt content showed an inverse correlation, representing the tendency of the products to have either more salt or more fat. Regarding the relation between total fat content and the fat quality, the majority of the categories showed a higher positive correlation between the fat and their MUFA content, namely cereals (r=0.96), snacks (r=0.94), bakery products (r=0.88), and sauces (r=0.86). Only samples from ready-to-eat category showed a higher correlation between their fat and PUFA content (r=0.85). In the samples from cereals (r=0.93), sauces (r=0.89), cookies, biscuits and wafers (r=0.80), and potato and potato-products (r=0.76) categories, the fat content showed a higher relation with their SFA content. Concerning the relation of the TFA with the fat composition within each category, it showed a higher and significant correlation ($p < .05$) with their SFA content (see Table 15). This relation was positive for the studied groups, with exception for the items from the cereals products group, that showed less TFA when there was a higher content of SFA in their composition (r=-0.90). Although, in general, the presence of TFA was significantly correlated ($p < 0.05$) with the salt content (r=0.15), that relation was only confirmed in the fast-food (r=-0.33), ready-to-eat meals (r=-0.33), potato and potato-products (r=0.45) and cookies, biscuits and wafers (r=-0.26) food categories.

3.3.1 Principal Component Analysis

This correlation study allowed the analysis of the variable relations by groups of two and showed very specific relations within the established food categories. However, to identify the overall trend that might distinguish the analysed food items, a multivariate analysis was performed. A Principal Components Analysis was applied to obtain an integrated graphic representation of all data at once. The obtained results are presented in Figures 21 and 22. The number of plotted dimensions was chosen accordingly to components eigenvalue (more than 1) and by the total percentage of variance explained by each factor, in order to allow meaningful interpretations of the data.

Through the representation of factor coordinates of the variables in the first 3 principal component (PC) (Figure 21) it is possible to see graphically the variables that are most correlated with each component. In this sense, fat (0.97) and MUFA (0.86) contents are more correlated with PC1, while TFA variation is more represented by the PC2 (-0.85). In PC3, although it only comprises 17.84 % of the data variation, salt content (-0.94) is represented. With the 3 first PC, the model generated by this analysis is able to represent 85% of the data variation, identifying fat, TFA and salt content as the main characteristics to differentiate all the 260 food items.

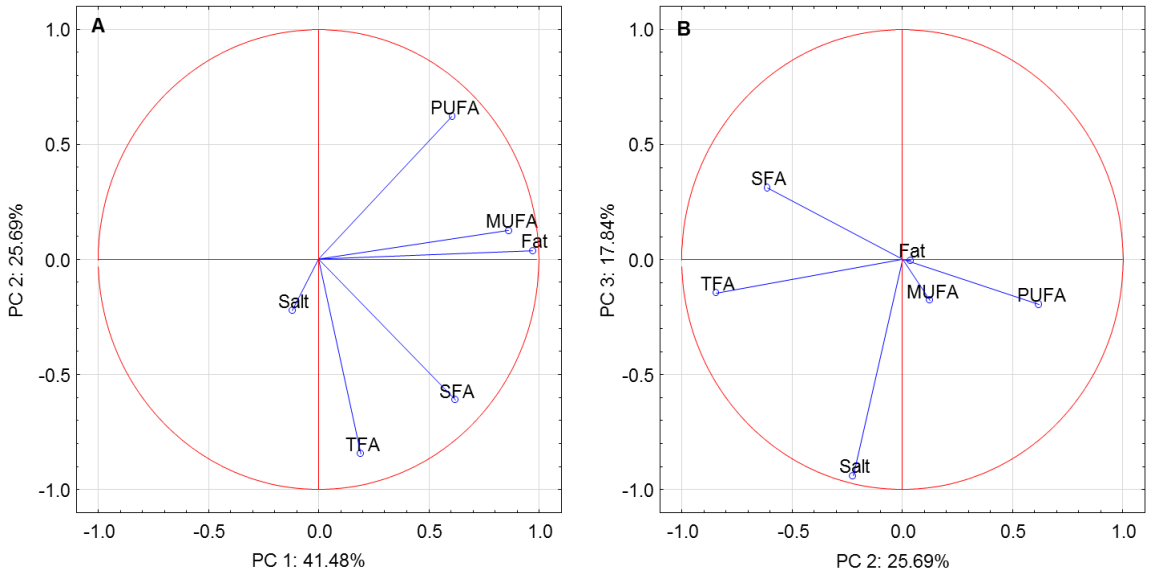

Figure 21. Plot for the projection of each variable on the three main Principal Components (A. factor-plane PC 1 x PC 2; B. factor-plane PC 2 x PC 3).

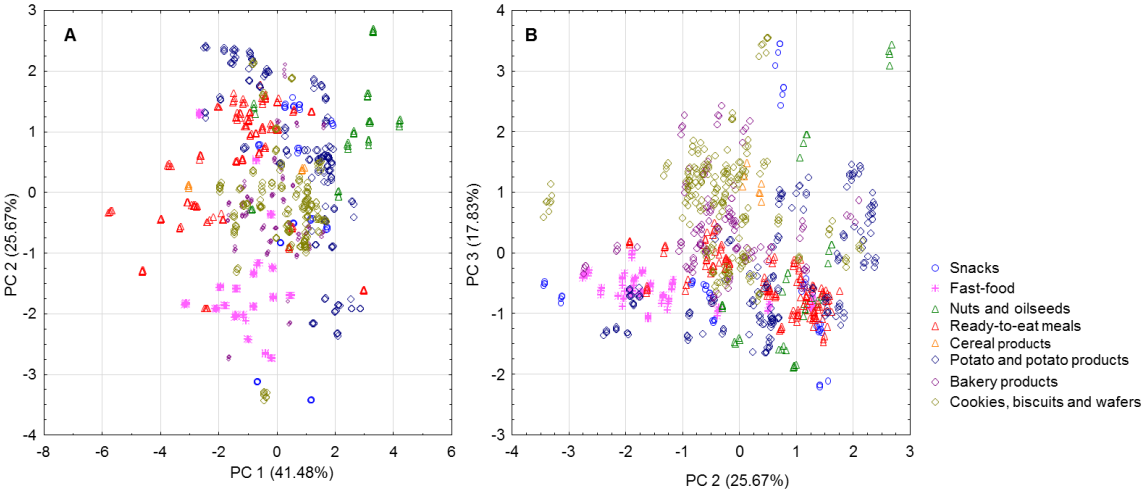

Figure 22. Plots of the all samples scores on the three main Principal Components (A. factor-plane PC 1 x PC 2; B. factor-plane PC 2 x PC 3).

By analysing the graphic representation of the samples in these 3 PC, it is possible to distinguish between several categories of the processed foods, although some overlapping of the groups occurs due to the high variation that each category encloses. However, it is possible to notice that samples from the nuts and oilseeds, potato and potato-products and cookies, biscuits and wafers showed a higher positive correlation with PC1, which identifies their fat and MUFA contents as their main distinguishable traits. Fast-food samples showed a more close relation to negative part of PC2 revealing that a higher presence of TFA is a divergent characteristic of this group. The low content of TFA was an aspect that distinguished most samples of the ready-to-eat meals, potato and potato-products and

some snacks, placed in the positive part of this PC2. In the PC3, the samples that stood out were those from bakery, cookies, biscuits and wafers and cereal products categories due to their generally lower salt content, when compared to the other studied processed samples.

Conclusions

From this study, it is possible to conclude that this subject remains current and new challenges are being pointed out by several national and international organizations. Moreover, the discrepancy of contents for the selected parameters across countries, but also within the same country, stress the importance of continuous research on the nutritional quality of processed foods, namely regarding the content of nutrients linked with potential harmful health effects on consumers.

The presented results allow affirming that we are facing a positive trend in relation to the decrease in the levels of TFA for the processed foods under study. However, with respect to the salt and saturated fat content, it was noticed that new challenges have arisen, especially focusing the food industry and policy makers, and the gradual reduction of the aforementioned components, in some of the food categories analysed within the study, should be a priority.

It is also important to note that most of the works reported in the literature treat TFA as an individual component and as far as authors know the detailed investigation on the relationship of the various food components, such as, total fat, composition of fat and salt, is scarce. Therefore, crucial and significant correlations among the evaluated parameters for processed foods were established, namely between salt and SFA content, as well as between salt and TFA content. Regarding fat content of processed foods, the correlation with salt content was weak, but the correlation with MUFA and PUFA was higher. Moreover, by analysing these correlations within each food category, it was observed that ready-to-eat meals and potato and potato-products categories, exhibit higher salt values when their fat content increases. Industry, researchers and governmental authorities should carefully address this issue, because these nutrients are related with an increased risk for the development of several chronic diseases. Moreover, processed foods are highly appreciated by children and young people, which can be an additional concern from a public health point of view. In summary, this work provides new data of utmost importance for the knowledge on dietary intake and the benefits/risks for human health, as well as to revise and develop new dietary recommendations. Important significant correlations between the studied parameters and within the different food categories were identified, which allows

defining priority areas of intervention. In addition, the current research fully supports the need of further research especially concerning the improvement of nutritional quality of some categories of processed foods.

References

Albuquerque, T. G., Sanches-Silva, A., Santos, L., Costa, H. S. (2012). An update on potato crisps contents of moisture, fat, salt and fatty acids (including *trans*-fatty acids) with special emphasis on new oils/fats used for frying. *International Journal of Food Sciences and Nutrition, 63*, 713-717.

Albuquerque, T. G., Santos, F., Sanches-Silva, A., Oliveira, M. B., Bento, A. C., Costa, H. S. (2016a). Nutritional and phytochemical composition of *Annona cherimola* Mill. fruits and by-products: Potential health benefits. *Food Chemistry, 193,* 187-195.

Albuquerque, T. G., Oliveira, M. B. P. P., Sanches-Silva, A., Bento, A. C., Costa, H. S. (2016b). The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. *Food & Function, 7,* 2736-2746.

Ansorena, D., Echarte, A., Ollé, R., Astiasarán, I. (2013). 2012: No *trans* fatty acids in Spanish bakery products. *Food Chemistry, 138,* 422-429.

AOAC (2002). Official methods of analysis of AOAC International. Gaithersburg, USA: AOAC International.

Aro, A., Amaral, E., Kesteloot, H., Rimestad, A., Thamm, M., van Poppel, G. (1998). *Trans* fatty acids in French fries, soups and snacks from 14 European countries: The TRANSFAIR study. *Journal of Food Composition and Analysis, 11,* 170-177.

Baylin, A., Siles, X., Donovar-Palmer, A., Fernandez, X., Campos, H. (2007). Fatty acid composition of Costa Rican foods including *trans* fatty acid content. *Journal of Food Composition and Analysis, 20,* 182-192.

Brinsden, H. C., He, F. J., Jenner, K. H., MacGregor, G. A. (2013). Surveys of the salt content in UK bread: progress made and further reductions possible. *BMJ Open, 3,* 1-7.

Dias, F. S. L., Passos, M. E. A., Carmo, M. G. T., Lopes, M. L. M., Mesquita, V. L. V. (2015). Fatty acid profile of biscuits and salty snacks consumed by Brazilian college students. *Food Chemistry, 171,* 351-355.

Dwyer, J. T., Fulgoni III, V. L., Clemens, R. A., Schmidt, D. B., & Freedman, M. R. (2012). Is “Processed” a four-letter word? The role of processed foods in achieving dietary guidelines and nutrient recommendations. *Advances in Nutrition, 3,* 536-548.

EUFIC (2010). The greatest thing since sliced bread? A review of the benefits of processed foods. <http://www.eufic.org/en/food-production/article/the-greatest-thing-since-sliced-bread-a-review-of-the-benefits-of-processed> Accessed 18.01.2017.

European Committee for Standardization (2000). ISO 5509:2000. Animal and vegetable fats and oils. Preparation of methyl esters of fatty acids. Brussels.

European Food Safety Authority (EFSA) (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. *EFSA Journal, 8*(3).

European Parliament/Council of the European Union (2011). Regulation (EU) No 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. *Official Journal of the European Union, L304,* 18-63.

FAO (2010). Fats and fatty acids in human nutrition: Report of an expert consultation. Rome, Italy. <http://www.fao.org/3/a-i1953e.pdf> Accessed 20.01.2017.

FAO/INFOODS (2012). FAO/INFOODS Guidelines for checking food composition data prior to the publication of a user table/database version 1.0. Rome, Italy. <http://www.fao.org/docrep/017/ap810e/ap810e.pdf> Accessed 18.01.2017.

Floros, J. D., Newsome, R., Fisher, W., Barbosa-Cánovas, G. V., Chen, H., Dunne, P., … Ziegler, G. R. (2010). Feeding the world today and tomorrow: The importance of food science and technology. *Comprehensive Reviews in Food Science and Food Safety, 9,* 572-599.

International Food Information Council Foundation (2010). What is a Processed Food? You Might Be Surprised!. <http://www.foodinsight.org/sites/default/files/what-is-a-processed-food.pdf> Accessed 20.01.2017.

Kloss, L., Meyer, J. A., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union – a review. *NFS Journal, 1,* 9-19.

Louzada, M. L. C., Baraldi, L. G., Steele, E. M., Martins, A. P. B., Canella, D. S., Moubarac, J. C., … Monteiro, C. A. (2015). Consumption of ultra-processed foods and obesity in Brazilian adolescents and adults. *Preventive Medicine, 81,* 9-15.

Mouratidou, T., Livaniou, A., Saborido, C. M., Wollgast, J., Caldeira, S. (2014). *Trans* fatty acids in Europe: where do we stand?. JRC Science and Policy Reports.

Polonia, J., Martins, L., Pinto, F., & Nazare, J. (2014). Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. *Journal of Hypertension, 32,* 1211-1221.

Popkin, B. M., Adair, L. S., & Ng, S. W. (2012). Now and then: The global nutrition transition: The pandemic of obesity in developing countries. *Nutrition Reviews, 70,* 3-21.

Richter, E. K., Shawish, K. A., Scheeder, M. R. L., Colombani, P. C. (2009). *Trans* fatty acid content of selected Swiss foods: The TransSwissPilot study. *Journal of food Composition and Analysis, 22,* 479-484.

Roe, M., Pinchen, H., Church, S., Elahi, S., Walker, M., Farron-Wilson, M., Buttriss, J., Finglas, P. (2013). *Trans* fatty acids in a range of UK processed foods. *Food Chemistry, 140,* 427-431.

Santos, L. A. T., Cruz, R., Casal, S. (2015). *Trans* fatty acids in commercial cookies and biscuits: An update of Portuguese market. *Food Control, 47,* 141-146.

Saunders, D., Jones, S., Devane, G. J., Scholes, P., Lake, R. J., Paulin, S. M. (2008). *Trans* fatty acids in the New Zealand food supply. *Journal of Food Composition and Analysis, 21,* 320-325.

Sociedade Portuguesa de Hipertensão (2016). Estratégia política até 2020 da Sociedade Portuguesa de Hipertensão na luta contra o excesso de teor de sal nos alimentos. *Revista Portuguesa de Hipertensão e Risco Cardiovascular, 51*.

Souza, R. J., Mente, A., Maroleanu, A., Cozma, A. I., Há, V., Kishibe, T., Uleryk, E., Budylowski, P., Schünemann, H., Beyene, J., & Anand, S. S. (2015). Intake of saturated and *trans* unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *British Medical Journal, 351,* 1-16.

Stuckler, D., McKee, M., Ebrahim, S., & Basu, S. (2012). Manufacturing epidemics: The role of global producers and increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. *PloS Medicine, 9,* 1-8.

Torres, D., Casal, S., Oliveira, M. B. P. P. (2002). Fatty acid composition of Portuguese spreadable fats with emphasis on *trans* isomers. *European Food Research and Technology, 214,* 108-111.

Trattner, S., Becker, W., Wretling, S., Öhrvik, V., Mattisson, I. (2015). Fatty acid composition of Swedish bakery products, with emphasis on *trans*-fatty acids. *Food Chemistry, 175,* 423-430.

Trieu, K., Neal, B., Hawkes, C., Dunford, E., Campbell, N., Rodriguez-Fernandez, R., … Webster, J. (2015). Salt reduction initiatives around the World – A systematic review of progress towards the global target. *PLoS ONE, 10*, 1-22.

van den Berg, R. A., Hoefsloot, H. C. J., Westerhuis, J. A., Smilde, A. K., Werf, M. J. (2006). Centering, scaling, and transformations: improving the biological information content of metabolomics data. *BMC Genomics, 7,* 1-15.

van Erp-Baart, M-A., Couet, C., Cuadrado, C., Kafatos, A., Stanley, J., van Poppel, G. (1998). *Trans* fatty acids in bakery products from 14 European Countries: the TRANSFAIR Study. *Journal of Food Composition and Analysis, 11,* 161-169.

van Langeveld, A. W. B., Gibbons, S., Koelliker, Y., Civille, G. V., de Vries, J. H. M., de Graaf, C., & Mars, M. (2017). The relationship between taste and nutrient content in

commercially available foods from the United States. *Food Quality and Preference, 57,* 1-7.

van Poppel, G., van Erp-baart, M-A., Leth, T., Gevers, E., van Amelsvoort, J., Lanzmann-Petithory, Kafatos, A., Aro, A. (1998). *Trans* fatty acids in foods in Europe: The TRANSFAIR Study. *Journal of Food Composition and Analysis,* 11, 112-136.

Wagner, K. H., Plasser, E., Proell, C., Kanzler, S. (2008). Comprehensive studies on the *trans* fatty acid content of Austrian foods: Convenience products, fast food and fats. *Analytical Methods, 108,* 1054-1060.

World Health Organization (2012). Action Plan for implementation of the European Strategy for the Prevention and Control of Noncommunicable Diseases 2012−2016. <http://apps.who.int/medicinedocs/documents/s19752en/s19752en.pdf> Accessed 20.01.2017.

World Health Organization (2013). Mapping salt reduction initiatives in the WHO European Region. <http://www.euro.who.int/__data/assets/pdf_file/0009/186462/Mapping-salt-reduction-initiatives-in-the-WHO-European-Region.pdf> Accessed 20.01.2017.

World Health Organization (2014). European Food and Nutrition Action Plan 2015–2020. Copenhagen, Denmark. <http://www.euro.who.int/__data/assets/pdf_file/0008/253727/64wd14e_FoodNutAP_140426.pdf> Accessed 20.01.2017.

World Health Organization (2015). Eliminating *trans* fats in Europe. A policy brief, Copenhagen, 2015. http://www.euro.who.int/__data/assets/pdf_file/0010/288442/Eliminating-trans-fats-in-Europe-A-policy-brief.pdf?ua=1. Accessed 20.01.2017.

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2.7 Multivariate characterization of salt and fat content, and the fatty acid profile of pastry and bakery products

Este sub-capítulo apresenta os resultados relativos à determinação dos teores de sal, de gordura total e da composição em ácidos gordos de 91 produtos de pastelaria e/ou panificação. Estes alimentos processados foram agrupados em 11 categorias e distinguidos entre marca branca, marca comercial, com ou sem glúten e com ou sem açúcar. Quando possível foram realizadas comparações entre estas categorias para avaliar o seu impacto na qualidade nutricional e segurança destes alimentos. Os resultados mostram que, na maioria destes alimentos, existem quantidades elevadas de ácidos gordos saturados, mas os níveis de ácidos gordos trans são, em geral, baixos.

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Multivariate characterization of salt and fat content, and the fatty acid profile of pastry and bakery products

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Abstract

Bakery and pastry products are highly appreciated worldwide. Due to consumer demand, a wide diversity of this type of food is available all over the world. The aim of this study was to evaluate the content of salt, fat and fatty acids in the composition of 91 bakery and pastry products commercialised in Portugal. Moreover, a detailed comparison between commercial and supermarket brands of similar foods, as well as between foods with or without gluten was performed. The obtained results allowed us to conclude that most of the analysed foods have a high percentage of saturated fatty acids. Also, considerable amounts of salt were determined, while the *trans* fatty acids content in the analysed foods was in general low (<0.5%). Moreover, differences fin the salt content higher than 6-fold between brands of the same type of foodstuff were observed, which should be considered at the moment of choice. This fact proves that it is possible to produce similar foods which are healthier with better nutritional quality. Since this type of food is highly appreciated by people of all ages, but mostly by children and young people, it is of utmost importance to implement strategies and establish goals, to improve their nutritional quality.

Introduction

Bakery products (cookies, biscuits, cakes, crackers, bread and savoury snacks) are highly appreciated worldwide and are frequently consumed by people of all ages, usually as part of breakfast, mid-morning and afternoon snacks. Due to continuous innovation in the food

market in response to consumer demand, a wide diversity of bakery products are available all over the world. Bakery products are nowadays consumed in significant amounts in several countries and some of the reasons that have been identified for their consumption are availability, sweetness and palatability.[1]

The main ingredients of bakery products are: wheat flour, sugar, fat, water and salt.[2] These ingredients, namely, fat and sugar, give crucial structural and textural characteristics, as well as sensory and hedonic properties to these types of products.[3] In the last few years, great attention has been devoted to the intake of fat, focusing on the type of fat, namely saturated (SFA) and *trans* fatty acids (TFA). Margarine, butter, partially hydrogenated vegetable oils and shortenings are the main fat sources used.[4] Frequently the choice for a source of fat for the production of bakery products is strongly dependent on technological properties and economic parameters, without considering the nutritional impact.[5]

Partially hydrogenated vegetable oils containing TFA are widely used in the food industry, due to their low cost compared to other fats, long shelf life, oxidative stability, as well as to impart desirable characteristics to the food.[6] Recently, the Food and Drug Administration released a final determination that partially hydrogenated oils are not Generally Recognized as Safe (GRAS), since these types of oils are the primary dietary source of artificial TFA in processed foods and were related to serious health concerns.[7] The Food and Agriculture Organization of the United Nations and World Health Organization (WHO) recommended a maximum intake of TFA of 1% of energy intake (*E*%).[8] Several countries have adopted measures to decrease the amount of TFA in different foods.[9] Also, some countries have implemented specific legislation regarding the content of TFA in foodstuffs, Denmark being the first EU country to set up a maximum of 2% TFA of total fat in products.[8] In Portugal, up to now, there has been no specific legislation regarding the limits of TFA content in foods. Nonetheless, the Regulation (EU) no. 1169/2011, from 25th November 2011, states that the labelling of products containing industrial hydrogenated vegetable oils is mandatory.[10]

Since the TRANSFAIR study, carried out in 1998, no other study has been performed with such detailed characterization of TFA content in foods across Europe. In that study, the estimated daily intake of TFA varied between 1.5 and 4.5 g day^{-1}, and the food groups that most contributed to this intake were very different between countries, but bakery products have a remarkable contribution ranging from 6.6 to 20.6% of the daily intake.[11]

The salt content in food is related to the sensorial characteristics, but it can also contribute to increase sweetness, as well as to mask metallic or bitter tastes.[12] Moreover, from the bakery industry perspective, salt addition aims at stabilizing the yeast fermentation rate, strengthening the dough, enhancing the flavour of the final product, and increasing dough mixing time.[13] According to the WHO, salt amounts are consumed far in excess of public health recommendations (5 g day^{-1} for adults and 2 g day^{-1} for children).[14] Therefore, it is

urgent to promote product reformulation, to increase consumers' awareness and education, and to encourage environmental changes.

A high intake of fat, sugar and salt is linked with several chronic diseases, namely obesity, diabetes, hypertension, cerebrovascular diseases and cancer.[15] According to Regulation (EU) n.º 1169/2011, for fat an intake of 70 g day^{-1} is recommended, for a healthy adult, considering an energy intake of 8400 kJ (2000 kcal)day^{-1}.[10]

Rapid urbanization and globalization have contributed to unhealthy lifestyles which are the main causes of the development of these diseases.[16] Food intake is an important issue in the health status of consumers. It is a modifiable determinant of many of the above-mentioned diseases and its role throughout the course of life in the promotion and maintenance of good health is well established.[14]

Cardiovascular disease accounts for approximately 17 million deaths a year, nearly one-third of the total, while being overweight and obesity were estimated to cause 3.4 million deaths, 3.9% of years of life lost, and 3.8% of disability-adjusted life-years worldwide. High-income countries have begun to reduce some of the above-mentioned diseases in their populations through strong public health policies such as the reduction of salt and fat in processed foods.[16] The European Strategy for Prevention and Control of Noncommunicable Diseases 2012-2016, recommended priority interventions, namely: (1) promoting healthy consumption, through fiscal policies and marketing controls; (2) to eliminate TFA from processed foods and replace them with polyunsaturated fats; and (3) to reduce salt intake in the diet to less than 5 g (2000 mg sodium) per person per day.[17] Therefore, it is of utmost importance to continue monitoring the progress, with continuous revision and evaluation of TFA, saturated fat and salt content in several types of foods, including bakery and pastry products. The aim of this study was to evaluate the current situation in a wide range of bakery and pastry products, and to follow trends in the nutritional quality over time.

Different types of fatty acids (saturated, monounsaturated, polyunsaturated and TFA), as well as the salt and total fat content were evaluated in the selected foods, in order to assess the overall nutritional quality of 91 bakery and pastry products. Also, commercial and supermarket brands for the same type of product were compared, aiming to assess the influence of price on the studied components, as well as a comparison between similar foods with and without gluten or sugar, in order to evaluate the diferences in nutritional quality.

Materials and methods

Standards and reagents

All chemicals and reagents were of analytical grade. Fatty acid methyl esters (FAME) standards were obtained from Supelco® (Supelco® 37 FAME Mix C4:0 - C24:0, and linoleic acid cis/*trans* isomers, Supelco, CA, USA) and GLC 674 from Nu-Chek-Prep (Elysian, MN, USA). Ultrapure water from a Milli-Q system (Millipore, Bedford, MA, USA) was used.

Sampling and sample preparation

Between 2015 and 2016, 91 bakery and pastry products were randomly acquired from leading supermarket chains and/or in small bakery stores. Samples were selected considering their expected contente of the focused nutrients, previous studies and market availability, since, up to now, to our knowledge no representative national survey has been available in Portugal. Moreover, the collection was performed from the major supermarkets chains, representing more than 90% of the brands available in the Portuguese market. The majority of the acquired samples are widely available in several countries around the world, since they are produced and commercialised by international companies. Similar samples from different producers/brands were treated as independent samples. Because there is a wide range of products available on the market, 11 different groups from the selected pastry and bakery products were formed, taking into account their similarity, appearance, and nutritional information (Figure 23). The groups were: biscuits (*n*=11), plain sweet cookies (*n*=14), wafers (*n*=6), coated cookies (*n*=11), filled cookies (*n*=8), pastry (*n*=14), Swiss rolls (*n*=4), sweet breads (*n*=7), croissants (*n*=5), plain salty cookies (*n*=8) and salty breads (*n*=3). Due to the wide range of products included in each group, and in order to simplify further comparisons with the results reported in this study, some examples of the samples included in the established groups are provided in the following list:

- Biscuits (B) – palms, cinnamon biscuits, apple biscuits, ties biscuits, among others.
- Plain sweet cookies (PSW) – Maria cookies, Belgium cookies, wholemeal cookies, among others.
- Wafers (W) – chocolate and vanilla wafers.
- Coated cookies (CC) – chocolate cookies coated with white, milk or dark chocolate.
- Filled cookies (FC) – sandwich cookies filled with vanilla or chocolate.
- Pastry (P) – fine bakery products traditionally consumed in Portugal, namely “Pastel de nata”, “Bola de Berlim”, and others such as muffins, rice cake, chocolate éclairs.
- Swiss rolls (SR) – Swiss rolls filled with vanilla, strawberry or milk, and Swiss rolls covered with chocolate and filled with other fillings.

- Sweet breads (SWB) – brioche with chocolate cream filling, plain brioche, and brioche with chocolate chips.
- Croissants (C) – French croissant, chocolate filled croissant and brioche croissant.
- Plain salty cookies (PSA) – cream crackers, wholemeal crackers, among others.
- Salty breads (SAB) – garlic breads, chorizo breads, among others.

From the aforementioned samples, 9 were gluten-free products, 3 were sugar-free products and 2 were from organic production (Figure 23). Upon reception at the laboratory, samples were homogenized in a blender (Grindomix GM200, Retsch, Haan, Germany) at 5000 rpm for 1 min, to obtain composites from each sample. Then, samples were stored in plastic containers under vacuum and kept refrigerated or frozen, according to the samples' perishability. All the analyses were carried out before the expiry date reported on the package.

Analysis of samples

A total of 91 composite samples were analysed regarding their salt, total fat and fatty acids profile. Total fat determination was performed using an acid hydrolysis followed by Soxhlet (Soxtec™ 2050, Foss, Hilleroed, Denmark) extraction with petroleum ether 40 – 60º.[18] The salt content was determined by Charpentier-Volhard's titration.[19] This method determines the salt content of foods based on the concentration of the chloride ion titrated with silver nitrate solution (0.1 N). The sodium chloride amount was estimated from the amount of chloride ion, as determined by the end-point of titration. Fatty acid (FA) transesterification was performed using a methanolic solution of potassium hydroxide and *n*-heptane, according to the method described by Albuquerque *et al.*[20] Afterwards, samples were analysed using a gas chromatograph (Agilent 6890N series GC-Systems, Atlanta, GA, USA) equipped with an Agilent 7683B Automatic Liquid Sampler (Agilent Technologies, Atlanta, GA, USA) and a flame ionization detector. A polar fused capillary column (Supelco® 2560, 100 m x 0.25 mm i.d., 0.25 µm film thickness), a split ratio of 50:1, and an injection volume of 1 µL were used to perform chromatographic separation of FAMEs. With respect to temperature programme, the following oven ramp was applied: 60 ºC for 1 min, then to 168 ºC at 17 ºC min^{-1}, held for 28 min, then to 235 ºC at 4 ºC min^{-1}, and finally held for 15 min. Injector and detector were kept at 240 ºC. The identification of FAMEs was done by a comparison of the retention times of the peaks in the sample with those of pure standards Supelco® 37 FAME Mix C4:0 - C24:0, linoleic acid cis/*trans* isomers (Supelco®, CA, USA) and GLC 674 from Nu-Chek-Prep (Elysian, MN, USA). The conversion of FAMEs to their fatty acids was done according to appropriate conversion factors. Fatty acids were quantified by comparing their peaks with relevant peak areas of the corresponding standard

fatty acids where each fatty acid was then expressed as a percentage of the total fatty acids quantified.

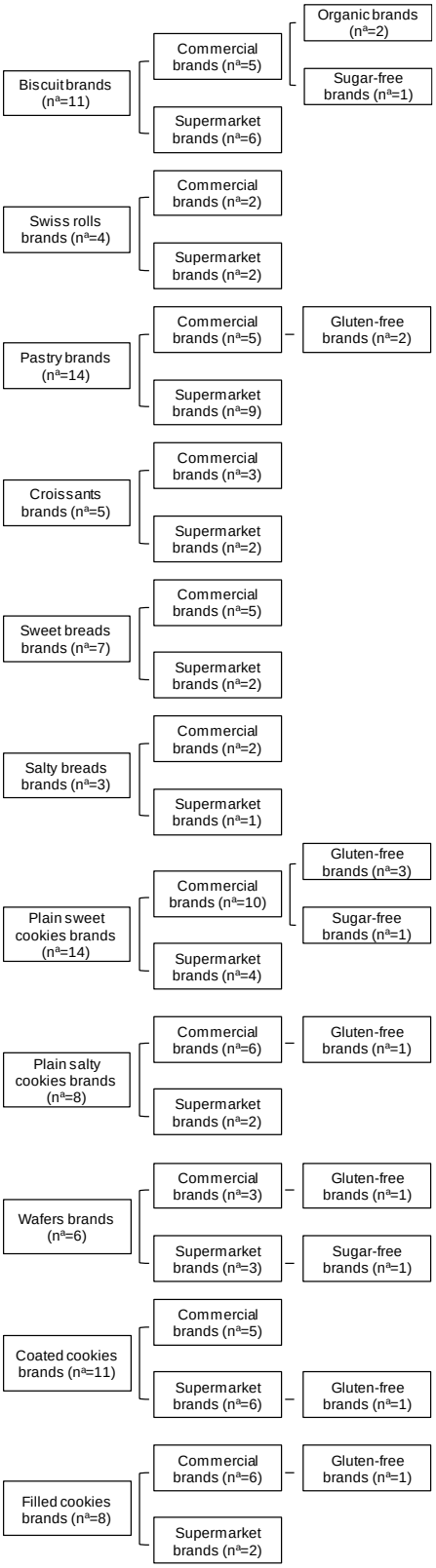

Figure 23. Distribution of 11 bakery and pastry food groups by brand type (commercial or supermarket) and identification of gluten-free, sugar-free or organic production brands. (ªNumber of composite samples each consisting of between 3 and 10 sub-samples for each brand).

Statistical analysis

The obtained results were presented per 100 g of edible portion on a fresh weight basis and were expressed as mean, range and also median, and 25th and 75th percentile, to better represent the data distribution. The results presented in the tables are the average values ± standard deviation (SD). Further data analysis was performed after data transformation to avoid the differences caused by the different order of magnitude of the analysed parameters. The existence of differences between groups was evaluated by one-way ANOVA followed by *post-hoc* Tukey comparison tests. Statistical significance was defined for $p < 0.05$ for all analyses. To obtain a global perspective (*i.e.* all samples and variables analysed simultaneously) a multivariate analysis approach was also performed. A Linear Discriminant Analysis (LDA) was performed using forward stepwise analysis, considering a *p* value of 0.01, to determine which variables contribute more to discriminate between the different food groups studied. This was followed by a canonical analysis to obtain more knowledge about the relationship between the variables and the canonical variates generated in the model. The statistical analyses were carried out using Statistica 64 13.0 software (Statsoft Inc., Tulsa, USA).

Results and discussion

Total fat and salt content

The total fat and salt content determined in the different groups of bakery and pastry products are presented in Figure 24. Wafers and coated and filled cookies had the highest mean fat content, together with croissants and biscuits. The obtained results indicate that the total fat content in croissants ranged from 15.2 to 25.5 g per 100 g. In comparison, Ansorena *et al.* reported a fat content for croissants between 24.3 and 25.7 g per 100 g, Saadeh *et al.*, between 18.3 and 30.4 g per 100 g, while Vicario *et al.* presented a mean fat content of 24.6 ± 3.04 g per 100 g for this kind of foodstuff.[21–23] According to Trattner *et al.* and Vicario *et al.*, the total fat content of “Maria” cookies, ranged from 12.3 to 14.8 g per 100 g, which is slightly lower than the values obtained within our study (8.7 – 19.5 g per 100 g).[23,24] With respect to crackers, the fat content values presented by different authors, namely Tavella *et al.* (12.1 g per 100 g), Daglioglu *et al.* (14.3 g per 100 g) and Vicario *et al.* (22.2 ± 12.5 g per 100 g), were in the same range as the values found in this study, where the fat content of crackers varied between 10.3 and 23.0 g per 100 g.[23,25,26] The total fat content of the analysed wafers, filled with chocolate or with vanilla, varied between 24.8 and 27.7 g per 100 g, which is also similar to the values reported in the literature.[22,24,26,27] The lowest mean fat content was found in salty breads (Figure 24). Roe *et al.* have also analysed similar kinds of salty breads and the values reported for total fat content (16.7 g

per 100 g) are considerably higher than the mean value (9.93 g per 100 g) obtained within our study.[28] The fat content range of each group was very dependent on the similarity found within the samples included (Figure 24) in each group. Broader groups like biscuits or general pastry revealed a wider fat range, while groups like wafers and coated and filled cookies, showed a higher homogeneity of the total fat content. However, some outliers were detected in the coated and filled cookies groups, corresponding to two different samples of cocoa biscuits coated with dark chocolate, while in the filled cookies group, the highest fat value was determined in a chocolate sandwich biscuit filled with vanilla. Another outlier was found in the salty cookies group, where a gluten-free cream cracker revealed a much higher fat content with respect to the other samples included in that group. Other studies reported in the literature have also grouped different bakery and pastry products to make comparisons. For instance, Santos *et al.* reported a higher fat content for wafers (30.8 g per 100 g), for covered/filled cookies (24.7 g per 100 g), as well as for plain cookies (18.2 g per 100 g).[27] Considering the results reported by Vicario *et al.*, chocolate filled cookies had a higher total fat content (31.8 g per 100 g) than the coated cookies (17.9 g per 100 g), while in our study the opposite was observed (24.9 g per 100 g for coated cookies and 20.4 g per 100 g for filled cookies).[23] Recently, in 2016, Pérez-Farinós *et al.*, conducted a research study on the most sold food items in Spain, and a lower value for the total fat content of biscuits (14.6 g per 100 g) was obtained in comparison with our data.[29] On the other hand, the mean fat content found in our biscuits (23.7 ± 5.46 g per 100 g) was more similar to the values reported by Khunt *et al.* that analysed a high number of foods from the German market, including 85 biscuits.[30]

Regarding the salt content, the highest values were found, as expected, in the salty breads and plain salty cookies' samples (Figure 24). However, the salt level found in croissants, sweet breads and biscuits was not significantly different ($p>0.05$) from those with expected higher values (salty breads and plain salty cookies). Wafers and Swiss rolls were the samples that showed the lowest salt content ($p < 0.05$) within the samples under study. The only sample that showed a very different and high content with respect to the other samples of its group (sweet cookies) was a wholemeal cookie that had a salt content at the same level as the salty cookies and breads, being identified as an outlier of the sweet cookies group. Concerning the comparisons with other studies, results for the salt content in these types of foods are scarce, since the main publications concerning bakery and pastry products have only focused on their fatty acid profile. An excessive intake of salt is associated with several chronic diseases and represents a serious public health concern. According to the obtained data, around 21% of the analysed samples showed a salt content higher than 1.0 g per 100 g. Therefore, the implementation of strategies to reach a salt reduction in these types of foodstuffs should be a priority.

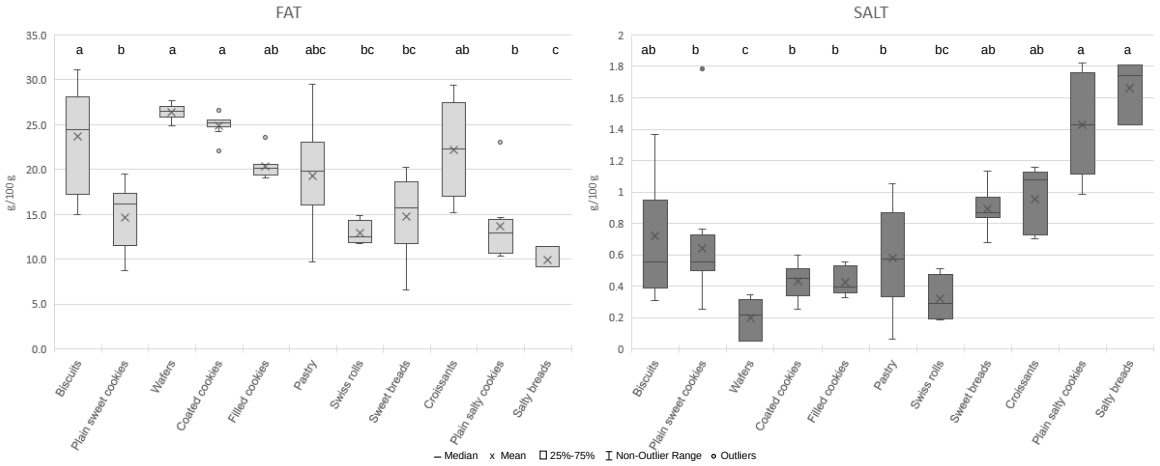

Figure 24. Fat and salt content of the 11 bakery and pastry food groups analysed (mean, median, 25th and 75th percentile and range values) (different letters indicate the existence of significant ($p < 0.05$) differences between the categories).

Fatty acid profile

The mean content of the major FA found in the different groups studied is presented in Table 16 and Figure 25. In Table 16, the sums for SFA, MUFA, PUFA and TFA, which correspond to all the identified fatty acids for each group (please see the footnotes of Table 16), are also included. The majority (88.9%) of the analysed pastry and bakery groups showed a prevalence of SFA on their composition (above 40% of their total fatty acid content) with the Swiss roll samples and the filled cookies showing SFA content of above 57% (see Figure 25). The exceptions were found in the pastry and plain sweet cookies groups that had a higher MUFA than SFA content (38.6% and 46.4%, respectively). Santos *et al.* have also analysed a wide range of commercial cookies and biscuits, and reported a SFA fraction higher than 50% in 30 samples out of 50, with only 6 samples with a SFA content lower than 40%.[27] Trattner *et al.* reported a prevalence of SFA in Swedish bakery in 70% of their samples [24] and Ansorena *et al.* have shown that for all the Spanish bakery products studied (*i.e.* sobaos, mini-ensaimadas, mini-puff pastry palms, mini-croissants and mini-chocolate croissants), the major fatty acids were saturated, with the exception of chocolate nut spreads that had a higher content of MUFA.[21] The same was reported in a

Table 16. Mean content of major fatty acids (g per 100 g) for the different groups of pastry and bakery analysed products (different superscript letters in the same row indicate the existence of significant ($p < 0.05$) differences between the groups).

Fatty acids (g per 100 g)	Biscuits	Plain sweet cookies	Wafers	Coated cookies	Filled cookies	Pastry	Swiss rolls	Sweet breads	Croissants	Plain salty cookies	Salty breads
C12:0	0.35 ± 0.92^{b}	0.32 ± 0.53^{b}	0.81 ± 1.17^{ab}	0.17 ± 0.3^{ab}	1.49 ± 1.04^{a}	0.51 ± 0.97^{ab}	1.37 ± 0.76^{ab}	0.18 ± 0.18^{ab}	0.11 ± 0.1^{ab}	0.03 ± 0.02^{b}	0.14 ± 0.18^{ab}
C14:0	0.26 ± 0.33	0.41 ± 0.6	0.54 ± 0.46	0.25 ± 0.14	0.7 ± 0.39	0.23 ± 0.3	0.62 ± 0.3	0.16 ± 0.08	0.22 ± 0.08	0.12 ± 0.05	0.49 ± 0.53
C16:0	7.27 ± 3.8^{ab}	3.94 ± 1.39^{b}	10.22 ± 1.02^{a}	7.65 ± 1.52^{ab}	7.4 ± 1.6^{ab}	4.74 ± 3.14^{b}	4.11 ± 0.59^{ab}	3.94 ± 1.59^{b}	8.17 ± 2.06^{ab}	4.73 ± 1.39^{ab}	2.56 ± 0.75^{ab}
C18:0	1.08 ± 0.47^{bc}	0.73 ± 0.39^{bc}	1.23 ± 0.24^{bc}	4.41 ± 0.83^{a}	1.09 ± 0.27^{bc}	1.1 ± 0.61^{bc}	0.79 ± 0.36^{bc}	1.32 ± 0.72^{bc}	1.7 ± 1.32^{ab}	0.55 ± 0.18^{c}	1.04 ± 0.03^{bc}
C16:1	0.04 ± 0.02^{b}	0.04 ± 0.07^{b}	0.04 ± 0^{ab}	0.05 ± 0.01	0.04 ± 0.01^{b}	0.05 ± 0.02^{b}	0.02 ± 0.01^{b}	0.02 ± 0^{ab}	0.03 ± 0.01^{b}	0.04 ± 0.04^{b}	0.25 ± 0.03^{a}
C18:1t	0.02 ± 0.01	0.13 ± 0.25	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.05 ± 0.06	0.02 ± 0	0.01 ± 0	0.03 ± 0.02	0.01 ± 0.01	0.06 ± 0.06
C18:1	8.42 ± 2.71	6.37 ± 3.73	9.15 ± 1.39	8.75 ± 1.2	5.88 ± 2.33	6.97 ± 3.79	3.47 ± 0.73	4.78 ± 1.52	7.12 ± 1.89	5.38 ± 1.58	3.15 ± 0.6
C18:2tt, C18:2ct and C18:2tc	0.08 ± 0.02^{a}	0.06 ± 0.05^{ab}	0.1 ± 0.05^{a}	0.06 ± 0.02^{ab}	0.09 ± 0.04^{a}	0.04 ± 0.02^{ab}	0.04 ± 0.01^{ab}	0.04 ± 0.02^{ab}	0.08 ± 0.03^{a}	0.04 ± 0.02^{ab}	0.01 ± 0.01^{b}
C18:2	4.57 ± 1.8^{a}	1.38 ± 0.54^{b}	2.42 ± 0.47^{ab}	1.8 ± 0.4^{ab}	2.02 ± 0.68^{ab}	3.64 ± 2.9^{ab}	1.56 ± 0.14^{ab}	3.31 ± 2.35^{ab}	3.26 ± 1.17^{ab}	1.83 ± 0.78^{ab}	1.06 ± 0.51^{ab}
$\sum_{SFA}{}^{A}$	9.11 ± 4.47^{ab}	5.77 ± 2.67^{b}	13.11 ± 1.56^{a}	12.72 ± 1.84^{a}	11.13 ± 1.47^{ab}	6.67 ± 3.64^{b}	7.13 ± 1.06^{ab}	5.75 ± 2.23^{b}	10.32 ± 2.7^{ab}	5.47 ± 1.63^{b}	4.63 ± 1.99^{ab}
$\sum_{MUFA}{}^{B}$	8.54 ± 2.76	6.44 ± 3.72	9.17 ± 1.4	8.79 ± 1.21	5.91 ± 2.31	7.04 ± 3.84	3.5 ± 0.74	4.78 ± 1.51	7.16 ± 1.89	5.41 ± 1.59	3.5 ± 0.52
$\sum_{PUFA}{}^{C}$	4.65 ± 1.78^{a}	1.52 ± 0.54^{b}	2.69 ± 0.6^{ab}	2.03 ± 0.4^{bc}	2.18 ± 0.72^{ab}	4.43 ± 2.98^{ac}	1.64 ± 0.14^{ab}	3.43 ± 2.35^{ab}	3.44 ± 1.18^{ab}	2.02 ± 0.92^{bc}	1.23 ± 0.56^{bc}
$\sum_{TFA}{}^{D}$	0.09 ± 0.03	0.16 ± 0.28	0.12 ± 0.06	0.08 ± 0.03	0.12 ± 0.05	0.09 ± 0.06	0.06 ± 0.01	0.06 ± 0.02	0.11 ± 0.04	0.05 ± 0.02	0.08 ± 0.07

SFA – Saturated fatty acids; MUFA – Monounsaturated fatty acids; PUFA – Polyunsaturated fatty acids; TFA – *trans* fatty acids. $^{A}\sum_{SFA}$ = C4:0 + C6:0 + C8:0 + C10:0 + C11:0 + C12:0 + C13:0 + C14:0 + C15:0 + C16:0 + C18:0 + C20:0 + C21:0 + C22:0 + C23:0 + C24:0. $^{B}\sum_{MUFA}$ = C14:1,*cis*-9 + C15:1,*cis*-10 + C16:1,*cis*-9 + C17:1,*cis*-10 + C18:1,*cis*-9 + C20:1,*cis*-11 + C22:1,*cis*-13 + C24:1,*cis*-15. $^{C}\sum_{PUFA}$ = C18:2,*cis*-9,12 + C18:3,*cis*-6,9,12 + C18:3,*cis*-9,12,15 + C20:2,*cis*-11,14 + C20:3,*cis*-8,11,14 + C20:3,*cis*-11,14,17 + C20:4,*cis*-5,8,11,14 + C22:2,*cis*-13,16 + C20:5,*cis*-5,8,11,14,17 + C22:6,*cis*-4,7,10,13,16,19. $^{D}\sum_{TFA}$ = C18:1,*trans*-6 + C18:1,*trans*-7 + C18:1,*trans*-9 + C18:1,*trans*-11 + C18:2,*trans*-9,12 + C18:2,*cis*-9,*trans*-12 + C18:2,*trans*-9,*cis*-12

study from Brazil by Dias *et al.* Surprisingly, in Pakistan, Kandhro *et al.* reported a higher proportion of MUFA in twelve brands of biscuits with high consumption.[31] In our study, the MUFA and the TFA content did not show significant differences between the studied groups ($p>0.05$). However, in the case of sweet cookies and salty breads, their TFA content represented about 1% of their fat content, being equal or less than 0.5% in the majority (88.9%) of the groups. Regarding their PUFA content, the highest percentage (around 24%) was found in pastry and sweet breads, while the coated cookies showed a PUFA content inferior to 9%.

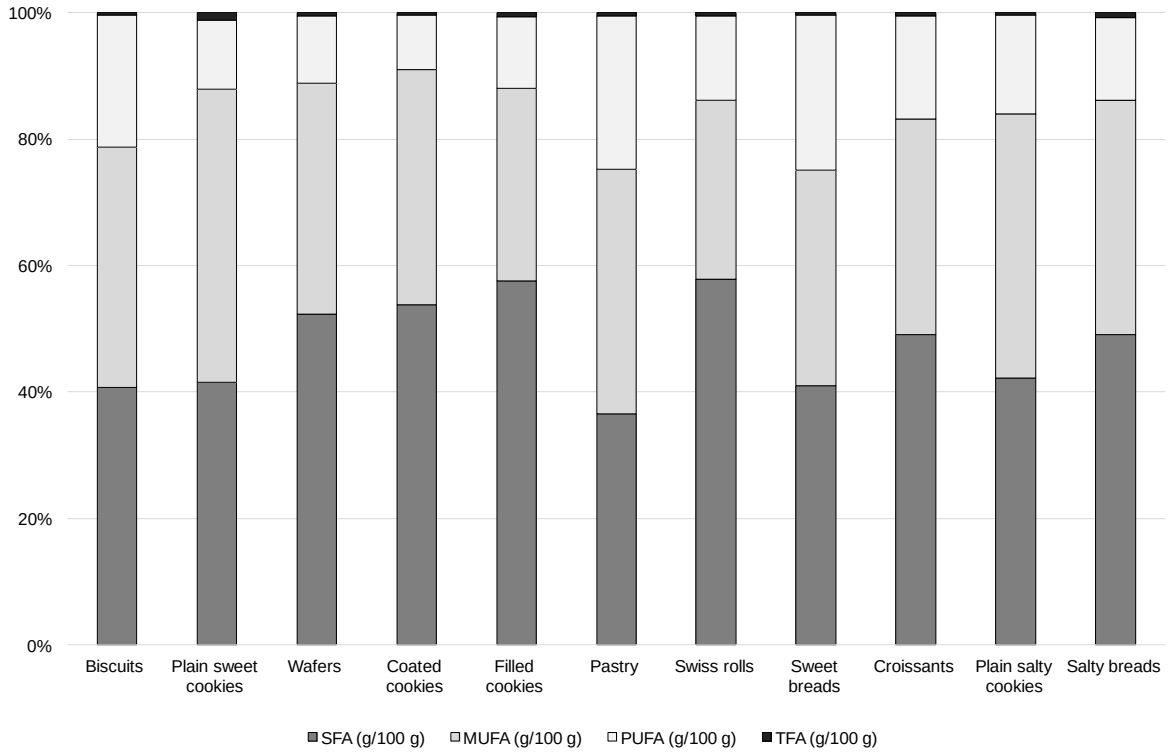

Figure 25. Mean composition of the fat fraction of the different bakery and pastry food groups (SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids).

Regarding the individual FA identified in the samples (see Table 16), the majority of the food groups showed a prevalence of oleic acid (C18:1), representing 33-46% of their total fatty acids, followed by palmitic acid (C16:0) with a 28-37% proportion. The exceptions were found in the Swiss rolls, croissant, wafers and filled cookies groups, where palmitic acid (C16:0) was the main FA (33-41%) followed by the oleic acid (C18:1). Linoleic acid (C18:2) was the third fatty acid in the bakery and pastry products, representing 8-24% of their profile. Only coated cookies had a higher percentage of stearic acid (C18:0, around 19%) than linoleic acid. Another FA that represented about 10% of the mean FA profile of Swiss rolls and filled cookies was lauric acid (C12:0). The minor FA of these products, namely lauric

(C12:0) and myristic acids (C14:0) also showed a high variation (with a RSD higher than 100%) within a great number of the groups. This may be due to the great heterogeneity of food products included in some groups, especially in biscuits, pastry and plain sweet cookies, and to the type of fat used in the production of each product. Regarding the presence of TFA in the studied pastry and bakery food products (C18:1t and C18:2tt, C18:2ct and C18:2tc), all of them showed a prevalence inferior to 0.5% of their total FA. The obtained results are in accordance with other studies from the literature. Ansorena *et al.* reported low TFA amounts in Spanish bakery products in 2012 (0.68 ± 0.15 g per 100 g of total fatty acids),[21] and Dias *et al.* showed higher values in salty snacks, while in biscuits the highest amount of TFA was of 0.86% of total FA.[32] Trattner *et al.* studied the evolution of TFA content in Swedish bakery products, between 2001 and 2007, and concluded that the TFA levels significantly decreased, being mostly replaced by SFA.[24] Roe *et al.* have also evaluated the TFA content in a range of UK processed foods and concluded that the recent industry reformulations have significantly contributed to the decrease of TFA among the studied foods.

Comparison among brands

In general, supermarket brands or own brands are recognised as having poorer quality than similar products from commercial brands.[21] Moreover, supermarket brands are usually cheaper than the other brands. Therefore, in our study, a detailed comparison of the most representative brands and products was made. In the plain sweet cookies (PSW) group, a comparison among “Maria” type cookies (Figure 26A) was made. Two supermarket brands and three commercial brands were considered. Regarding total fat and FA composition, it was possible to observe that most of the samples had a similar profile. However, one commercial brand in this group has a very different FA distribution, with higher MUFA, while all the other samples have a prevalence of SFA. In this case, it was a product from a commercial brand and the observed differences could be related to the type of fat used. According to the label sunflower oil has been used instead of other common fats. For crackers, from plain salty cookies group (PSA), it was possible to perform comparisons among 5 supermarket brands and 3 commercial brands, and the obtained results showed that there are 2 products with a clear distinct fat composition (PSA5, supermarket brand and PSA2, commercial brand), where MUFAs were the major FAs (Figure 26B). Moreover, in this group it was also observed that the commercial brand PSA3 has a much higher total fat content in comparison with the other similar products.

In the group of coated chocolate cookies five commercial brands and four supermarket brands were compared (Figure 26C). All the analysed brands have a very similar fatty acid

profile, with the exception of sample CC9 from a commercial brand, for which the major fatty acids were MUFA instead of SFA. Among the different analysed commercial brands of sweet breads filled with chocolate (SWB2, SWB3 and SWB4), considerable differences were also found (Figure 26D). The product SWB2 had a higher content of PUFA, while the other samples had a higher content of SFA, indicating that it was possible to produce similar foods with a healthier FA profile. In this case, the differences found in the FA profile of SWB2 could be due to the presence of hazelnuts on the ingredient list. Concerning the sweet breads with chocolate chips (Figure 26E), 1 supermarket brand (SWB5) and 2 commercial brands (SWB6 and SWB7) were also compared. In these samples, no notable differences were found among the FA profile. However, the amount of total fat in the supermarket brand was considerably higher than that in the commercial brands. Finally, two samples of French croissants (C1 and C2) were compared (Figure 26F). Besides the total fat content, which was also higher in the supermarket brand, differences in the FA profile were found. Sample C1 had similar amounts of MUFA and PUFA, while the commercial sample had a lower content of PUFA.

With respect to the salt content, the differences among the studied food products were not so evident, as for total fat and FA profile. Nonetheless, in two samples of Swiss rolls with milk filling and chocolate coating, the salt content for the analysed commercial brand was almost double the equivalent product from a supermarket brand.

Although the stated differences exist between the commercial and supermarket brand, these were not sufficient to establish a trend within the entire group of bakery and pastry products analysed in this study (n=91). Therefore, the indication of “supermarket brand” or even the lower price should not be considered as an indicator of nutritional quality.

Comparison among products with gluten and gluten-free

9 gluten-free foods were included in this study, from the most representative categories of bakery and pastry products. The determined parameters were used to evaluate the differences among similar foods with and without gluten, namely “madeleine” cakes, cream crackers, “Maria” cookies, vanilla wafers, chocolate cookies filled with chocolate, sandwich cookies filled with chocolate and other plain sweet cookies. Concerning the salt content, the highest differences (≥ 0.5 g per 100 g) were observed in “madeleine” cakes and in other plain cookies.

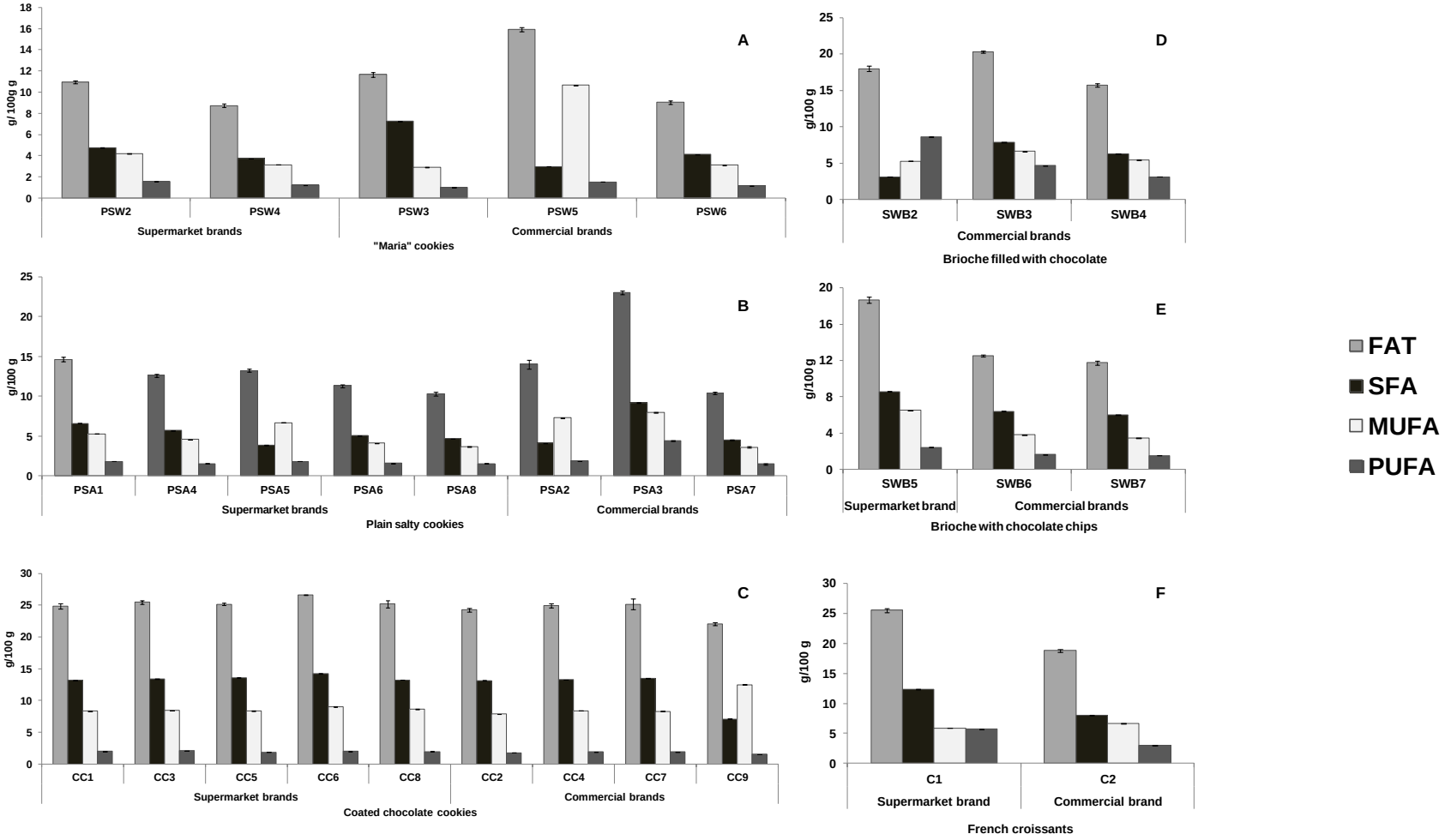

Figure 26. Comparison of the fat content and fatty acid composition of commercial and supermarket brands for different groups of pastry and bakery products (different numbers represent different brands for similar products; PSW, plain sweet cookies; SWB, sweet breads; PSA, plain salty cookies; CC, Coated cookies; C, croissants; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; TFA, *trans* fatty acids).

However, in “madeleine” cakes, the higher salt value was determined in the samples with gluten (0.557 ± 0.06 g per 100 g, with gluten *vs.* 0.06 ± 0.01 g per 100 g, gluten-free) while in other plain cookies it was observed in gluten-free samples (1.17 ± 0.66 g per 100 g, gluten-free *vs.* 0.276 ± 0.03 g per 100 g, with gluten). Regarding the total fat content, the biggest difference was found between cream crackers products, where the gluten-free samples had the highest value (23.0 ± 0.54 g per 100 g, gluten-free vs. 14.3 ± 0.39 g per 100 g, with gluten). Nevertheless, the FA profile was quite similar between them. “Maria” gluten-free cookies also had a higher content of fat (19.5 ± 0.35 g per 100 g) than the ones with gluten (11.3 ± 2.66 g per 100 g).

Nonetheless, the type of fat present in “Maria” gluten-free cookies was mostly MUFA (12.5 ± 3.20 g per 100 g) in contrast to the higher SFA content was found in “Maria” cookies with gluten. In other plain cookies, the opposite was observed, since the gluten-free cookies were the ones with the lower MUFA content (6.00 ± 0.91 g per 100 g).

Linear Discriminant Analysis

The total fat, the salt content and the fatty acid profile of all samples included in this study were analysed by Linear Discriminant Analysis (LDA) to gain more knowledge about the differences between the composition of the different groups. The LDA generated a model that included first stearic acid (C18:0), followed by the total salt and fat content of the samples (LDA result in Table 17). These variables contributed the most to the discrimination between the different groups of bakery and pastry foods. On the other hand, and although it was a major fatty acid of these products, oleic acid (C18:1) was the only variable not included in the LDA model, as it showed a very similar content with no significant differences ($p>0.05$) between groups (see Table 16). A canonical analysis followed the LDA to allow presenting the diferences between the samples in a canonical variate (CV) scatterplot. The first 3 CVs were selected as they comprise 82% of all data information (see Figure 27). In the first dimension (CV 1) 36.3% of the data variation is represented, being the samples separated mostly due to their different fat and salt content (see Table 17). The CV 2 represents 35.2% of the variance, and it is mostly related to the stearic acid content of the samples. In the CV 3 (10.3%), the most correlated data are again the salt and the TFA content (see Table 17).

By analysing the generated scatterplot (Figure 27), it was possible to notice the great influence of the salt content on the separation of the groups, especially salty breads and cookies from the coated, filled and wafer cookies. However, it was also possible to see that most of the samples showed a very similar composition, as no clear separation of all groups was attained in the generated model. Biscuits, pastries and sweet cookies showed a more

disperse distribution on the 3 CVs, revealing the great heterogeneity of products that could be encountered in these groups.

Table 17. Multivariate analysis results.

LDA analysis Summary (N=91, N of variables in model: 10; Wilks' Lambda: .00517 approx. F (100,520) = 5.6509 p<0.0000)							Standardized coefficients for canonical variables		
	Wilks'	Partial	F-remove	p-value	Toler.	1-Toler.	**CV 1**	**CV 2**	**CV 3**
C18:0 (g per 100 g)	0.019	0.275	18.707	0.000	0.389	0.611	0.23	-1.47	-0.03
Salt (g per 100 g)	0.010	0.507	6.911	0.000	0.877	0.123	-0.55	0.01	0.71
Total fat (g per 100 g)	0.007	0.709	2.914	0.004	0.518	0.482	0.53	0.29	0.38
C16:1 (g per 100 g)	0.009	0.566	5.450	0.000	0.759	0.241	-0.47	-0.38	-0.04
C12:0 (g per 100 g)	0.008	0.666	3.554	0.001	0.311	0.689	0.25	0.67	-0.30
C16:0 (g per 100 g)	0.006	0.803	1.739	0.089	0.264	0.736	0.29	0.57	-0.24
C18:1t (g per 100 g)	0.009	0.605	4.640	0.000	0.378	0.622	-0.38	0.59	-0.62
C18:2t, C18:2ct and C18:2tc (g per 100 g)	0.007	0.717	2.798	0.006	0.600	0.400	0.49	0.04	0.52
C18:2 (g per 100 g)	0.007	0.780	2.005	0.045	0.812	0.188	-0.20	0.19	0.35
C14:0 (g per 100 g)	0.006	0.796	1.822	0.072	0.160	0.840	-0.43	-0.32	0.27

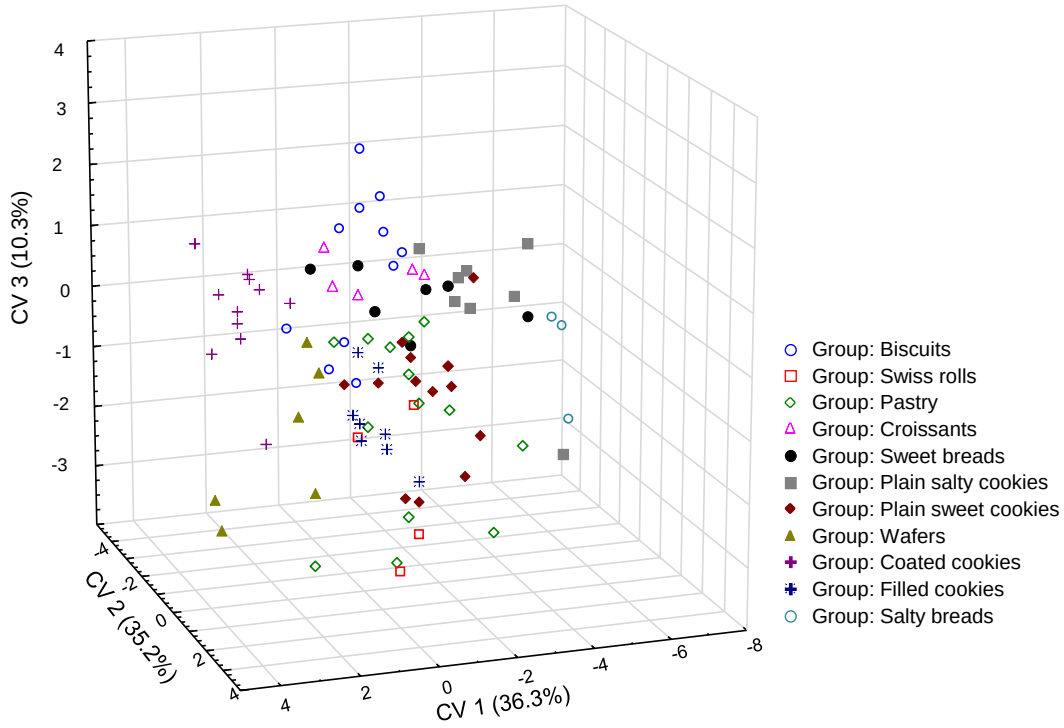

Figure 27. Plot of the first canonical variate (CV1) *versus* the second canonical variate (CV2) *versus* the third canonical variate (CV3) for the 91 bakery and pastry products.

Conclusions

To the best of our knowledge, this is the first study in Portugal that focuses not only on the fat composition (SFA, MUFA, PUFA and TFA), but also on the salt content of pastry and bakery products widely available in the European market. It is possible to check according to the obtained results that a considerable percentage (21%) of the analysed foods have a salt content higher than 1 g/100 g. Also, for almost 90% of the analysed bakery and pastry products the major fatty acids were SFA. Nonetheless, the analysed foods also have a high content (33-46% of total fatty acids) of oleic acid, which is a MUFA associated with several health benefits, namely regarding the decrease of low density lipoproteins cholesterol. Concerning the TFA content, similarly to what happens in other countries, a significant decrease was observed along the years, and the analysed foods had a TFA content lower than 0.5% of total fatty acids. This indicates that the replacement of fats (reported by the food industry) used to produce these types of products, was effective. With respect to the differences found between commercial and supermarket brands of similar foods, they were not sufficient to establish a relationship between brand/price/nutritional qualities. However, they allowed us to conclude that it is possible to produce similar foods which are healthier with a better nutritional quality. The comparison between similar bakery and pastry products with and without gluten revealed in most cases considerable differences in their FA profile. The aforementioned findings highlight the importance of updating the data concerning the composition of foods, to accurately estimate the intake of these nutrients and to evaluate the potential health effects associated with their consumption. Moreover, as these types of foods are highly appreciated by people of all ages, but mostly by children and young people, it is of utmost importance to implement strategies and establish goals, in order to enhance their nutritional quality and decrease their potential health impact.

References

1 S. M. Mohsen, H. H. M. Fadel, M. A. Bekhit, A. E. Edris and M. Y. S. Ahmed, *Int. J. Food Sci. Technol.*, 2009, **44**, 1705–1712.

2 H. Mamat, M. O. A. Hardan and S. E. Hill, *Food Chem.*, 2010, **121**, 1029–1038.

3 C. Biguzzi, P. Schlich and C. Lange, *Food Qual. Prefer.*, 2014, **35**, 41–47.

4 M.-A. van Erp-Baart, C. Couet, C. Cuadrado, A. Kafatos, J. Stanley and G. Van Poppel, *J. Food Compos. Anal.*, 1998, **11**, 161–169.

5 F. Caponio, C. Summo, D. Delcuratolo and A. Pasqualone, *J. Sci. Food Agric.*, 2006, **86**, 356–361.

6 T. G. Albuquerque, H. S. Costa, M. C. Castilho and A. Sanches-Silva, *Trends Food Sci. Technol.*, 2011, **22**, 543–560.

7 Food and Drug Administration, Final determination regarding partially hydrogenated oils, https://www.fda.gov/food/ingredientspackaginglabeling/foodadditivesingredients/ucm449162.htm, (accessed 7 July 2017).

8 Food and Agriculture Organization of the United Nations, *Fats and fatty acids in human nutrition - Report of an expert consultation*, Rome, 2010.

9 European Commission, *Trans fatty acids in Europe: where do we stand? A synthesis of the evidence: 2003-2013*, Luxembourg, 2014.

10 European Parliament and of the Council, Regulation (EU) n.º 1169/2011, on the provision of food information to consumers, http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R1169&from=pt, (accessed 7 July 2017).

11 K. F. A. M. Hulshof, M. A. van Erp-Baart, M. Anttolainen, W. Becker, S. M. Church, C. Couet, E. Hermann-Kunz, H. Kesteloot, T. Leth, I. Martins, O. Moreiras, J. Moschandreas, L. Pizzoferrato, A. H. Rimestad, H. Thorgeirsdottir, J. M. N. van Amelsvoort, A. Aro, A. G. Kafatos, D. Lanzmann-Petithory and G. van Poppel, *Eur. J. Clin. Nutr.*, 1999, **53**, 143–157.

12 M. Mesías, F. Holgado, G. Márquez-Ruiz and F. J. Morales, *LWT - Food Sci. Technol.*, 2015, **62**, 633–639.

13 R. A. Miller and R. C. Hoseney, *Cereal Foods World*, 2008, **53**, 4–6.

14 World Health Organization, *A Global Brief on Hypertension*, Geneva, 2013.

15 World Health Organization, *Diet, nutrition and the prevention of chronic diseases*, Geneva, 2003, vol. 916.

16 World Health Organization, *Mapping salt reduction initiatives in the WHO European Region*, Copenhagen, 2013.

17 World Health Organization, *Action Plan for implementation of the European Strategy for the Prevention and Control of Noncommunicable Diseases 2012-2016*, Copenhagen, 2012.

18 T. G. Albuquerque, F. Santos, A. Sanches-Silva, M. Beatriz Oliveira, A. C. Bento and H. S. Costa, *Food Chem.*, 2016, **193**, 187–195.

19 T. G. Albuquerque, A. Sanches-Silva, L. Santos and H. S. Costa, *Int. J. Food Sci. Nutr.*, 2012, **63**, 713–717.

20 T. G. Albuquerque, M. B. P. P. Oliveira, A. Sanches-Silva, A. C. Bento and H. S. Costa, *Food Funct.*, 2016, **7**, 2736–2746.

21 D. Ansorena, A. Echarte, R. Ollé and I. Astiasarán, *Food Chem.*, 2013, **138**, 422–429.

22 C. Saadeh, I. Toufeili, M. Z. Habbal and L. Nasreddine, *J. Food Compos. Anal.*, 2015, **41**, 81–85.

23 I. M. Vicario, V. Griguol and M. León-Camacho, *J. Agric. Food Chem.*, 2003, **51**, 134–139.

24 S. Trattner, W. Becker, S. Wretling, V. Öhrvik and I. Mattisson, *Food Chem.*, 2015, **175**, 423–430.

25 M. Tavella, G. Peterson, M. Espeche, E. Cavallero, L. Cipolla, L. Perego and B. Caballero, *Food Chem.*, 2000, **69**, 209–213.

26 O. Daglioglu, M. Taşan and B. Tunçel, *Turkish J. Chem.*, 2002, **26**, 705–710.

27 L. A. T. Santos, R. Cruz and S. Casal, *Food Control*, 2015, **47**, 141–146.

28 M. Roe, H. Pinchen, S. Church, S. Elahi, M. Walker, M. Farron-Wilson, J. Buttriss and P. Finglas, *Food Chem.*, 2013, **140**, 427–431.

29 N. Pérez-Farinós, M. Á. Dal Re Saavedra, C. Villar Villalba and T. Robledo de Dios, *Gac. Sanit.*, 2016, **30**, 379–382.

30 K. Kuhnt, M. Baehr, C. Rohrer and G. Jahreis, *Eur. J. Lipid Sci. Technol.*, 2011, **113**, 1281–1292.

31 A. Kandhro, S. T. H. Sherazi, S. A. Mahesar, M. I. Bhanger, M. Y. Talpur and S. Arain, *J. Am. Oil Chem. Soc.*, 2008, **85**, 1057–1061.

32 F. D. S. L. Dias, M. E. A. Passos, M. D. G. T. Do Carmo, M. L. M. Lopes and V. L. V. Mesquita, *Food Chem.*, 2015, **171**, 351–355.

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2.8 Bolacha Maria ou de Água e Sal: análise nutricional comparativa

Este sub-capítulo apresenta os resultados relativos à análise nutricional comparativa entre 15 tipos de bolachas (8 marcas de bolachas de água e sal e 7 marcas de bolachas tipo Maria). Foram também avaliados os contributos de uma porção para a ingestão diária de sal e de gordura, com base nos valores de referência para um adulto. Verificou-se que, entre produtos similares, existem diferenças muito significativas relativas aos teores de sal, de gordura e composição em ácidos gordos. Demonstrou-se assim ser possível reformular alguns destes produtos e torná-los mais equilibrados e seguros.

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Resumo

O estilo de vida da população tem sofrido alterações nos últimos anos, levando à diminuição da realização de atividade física e à improvisação de refeições rápidas, menos variadas e provavelmente menos saudáveis, onde têm papel relevante as bolachas. Estas são caracterizadas por serem ricas em açúcares, gordura e em alguns casos sal. Nos últimos anos, e em resposta ao aumento da incidência de diversas doenças crónicas, decorreram várias iniciativas, no sentido de melhorar a qualidade nutricional deste tipo de produtos. Neste trabalho determinaram-se o teor de sal, gordura total e a composição em ácidos gordos de bolachas Maria e de água e sal. Realizou-se ainda uma avaliação do potencial impacto na saúde da população que as consome, tendo por base as recomendações de ingestão diária dos referidos nutrientes, bem como a porção recomendada. As bolachas de água e sal analisadas apresentam teores superiores de gordura total e de sal, devendo ser estabelecidas metas que permitam a reformulação gradual destes alimentos. Os teores de ácidos gordos *trans* nas amostras analisadas decresceram comparativamente com dados publicados anteriormente. Parece fundamental manter os esforços no sentido de modificar a composição destes alimentos, para diminuir o risco de doenças crónicas e para a promoção da saúde da população.

Abstract

In the last years, there has been a change in the lifestyle of the population, resulting in a lack of time for the preparation of healthy meals and to practice physical activity. Cookies are characterized by being foods rich in sugars, fat and in some cases salt. In recent years,

several initiatives have been developed to improve the nutritional quality of this type of product due to the increased incidence of chronic diseases. In this work the content of salt, total fat and the fatty acid composition of “Maria” cookies and cream crackers were determined; and an evaluation of the potential impact on the health of the population was made, based on the nutrients dietary reference intakes, as well as considering the recommended portion. The analyzed cream crackers present higher levels of total fat and salt, and goals must be established to allow the reformulation of these foods. The levels of *trans* fatty acids in the analyzed samples decreased in comparison with previous data. It is very important to develop continuous efforts to modify the composition of these foods in order to reduce the risk of chronic diseases and to promote the health of the population.

Introdução

Uma alimentação saudável é determinante para um bom desenvolvimento físico e intelectual, e para a promoção da saúde e do bem-estar geral. De acordo com a distribuição apresentada na Roda dos Alimentos Mediterrânica, as bolachas estão incluídas no grupo dos cereais, derivados e tubérculos (1). Os alimentos deste grupo deverão fornecer cerca de 28% da energia total diária, o que corresponde a uma ingestão de 4 a 11 porções por dia, de acordo com a faixa etária, género e estado de saúde dos indivíduos, entre outros fatores. Também neste documento são apresentadas porções para os diferentes alimentos dos diferentes grupos, e a porção para as bolachas é de 6 unidades, que corresponde aproximadamente a 35 g (1). As bolachas são alimentos ricos em açúcares, gordura e em alguns casos sal. Nos últimos anos, têm decorrido diversas iniciativas no sentido de melhorar a qualidade nutricional deste tipo de produtos, devido ao aumento da incidência de doenças crónicas, como a obesidade, a diabetes e a hipertensão arterial (2). No entanto, por serem amplamente disponíveis, de grande diversidade, práticas e saborosas, as bolachas são apreciadas, de uma forma geral, pelas diversas faixas etárias da população. Pelos motivos já referidos é fundamental avaliarmos a sua qualidade nutricional e o potencial impacto na saúde da população portuguesa associado ao seu consumo.

Objetivos

Avaliar a qualidade nutricional das bolachas tipo Maria e de água e sal, com base nos teores de gordura total, sal e composição em ácidos gordos; realizar uma análise comparativa entre os dois tipos de bolachas selecionados; e estimar o potencial impacto na saúde da população, tendo por base as recomendações de ingestão diária dos referidos nutrientes, bem como a porção recomendada.

Material e métodos

Em 2015 foram adquiridos, em grandes superfícies da região de Lisboa, 15 tipos de bolachas (8 marcas de bolachas de água e sal e 7 marcas de bolachas tipo Maria). As amostras foram selecionadas de acordo com a disponibilidade no mercado, sem deixar de incluir produtos de marca branca, de marca comercial, sem glúten e sem açúcar. Para cada marca, foram adquiridas, no mínimo, 3 embalagens pertencentes a lotes diferentes, e sempre que possível provenientes de diferentes superfícies comerciais. Nas amostras foram avaliados o teor de gordura total, o teor de sal e a composição em ácidos gordos, de acordo com os métodos descritos por Albuquerque et al. (3, 4). Para estimar o seu contributo nutricional, utilizaram-se as doses de referência do Regulamento (UE) n.º 1169/2011 e da Organização Mundial de Saúde (OMS), e as porções referidas na Roda dos Alimentos Mediterrânica (1, 5, 6).

Resultados e discussão

O teor de sal determinado foi aproximadamente o dobro nas bolachas de água e sal, comparativamente às bolachas tipo Maria (Tabela 18).

Tabela 18. Teores de sal e de gordura (g/100 g) nas bolachas analisadas e contributo (%) para a dose de referência.

Bolachas		Gordura		Sal	
		g/100 g	Contributo DR (%)	g/100 g	Contributo DR (%)
Água e sal	**A**	14,6 ± 0,3	7	1,41 ± 0,12	10
	B	14,0 ± 0,1	7	0,984 ± 0,12	7
	C	23,0 ± 0,5	12	1,45 ± 0,06	10
	D	12,6 ± 0,2	6	1,62 ± 0,11	11
	E	13,2 ± 0,2	7	1,81 ± 0,10	13
	F	11,3 ± 0,2	6	1,29 ± 0,07	9
	G	10,4 ± 0,3	5	1,05 ± 0,05	7
	H	10,3 ± 0,2	5	1,82 ± 0,12	13
Maria	**A**	11,0 ± 0,1	5	0,539 ± 0,07	4
	B	11,7 ± 0,2	6	0,763 ± 0,05	5
	C	8,73 ± 0,2	4	0,781 ± 0,12	5
	D	15,9 ± 0,2	8	0,646 ± 0,03	5
	E	9,03 ± 0,2	5	0,740 ± 0,06	5
	F	19,5 ± 0,3	10	0.602 ± 0,02	4
	G	17,2 ± 0,1	9	0,559 ± 0,02	4

A OMS recomenda uma ingestão de sal, para adultos, inferior a 5 g/dia, para prevenção de doenças crónicas, nomeadamente a Hipertensão Arterial (6). Uma porção (35 g) das bolachas de água e sal analisadas pode contribuir com 13% da ingestão diária recomendada de sal, enquanto que uma porção de bolachas Maria pode contribuir com 5%. O teor de gordura total nas amostras analisadas variou entre 8,7 e 23,0 g/100 g para as bolachas Maria C e para as bolachas de água e sal C, respetivamente (Tabela 18). Considerando a dose de referência de ingestão diária de gordura, para um adulto (70 g/dia) e, tendo por base uma dieta padrão de 2000 kcal, uma porção de bolachas (35 g) pode contribuir com 12% (5). No entanto, é também importante a qualidade da gordura presente nas amostras analisadas, para poder estimar os benefícios/riscos associados ao seu consumo. Com base na análise da composição em ácidos gordos das amostras (Figura 28) é possível observar que, em 67% dos produtos analisados, os ácidos gordos saturados são os maioritários. Uma alimentação rica neste tipo de ácidos gordos foi correlacionada com um aumento no risco de desenvolvimento de doenças cardiovasculares, entre outras doenças crónicas (7).

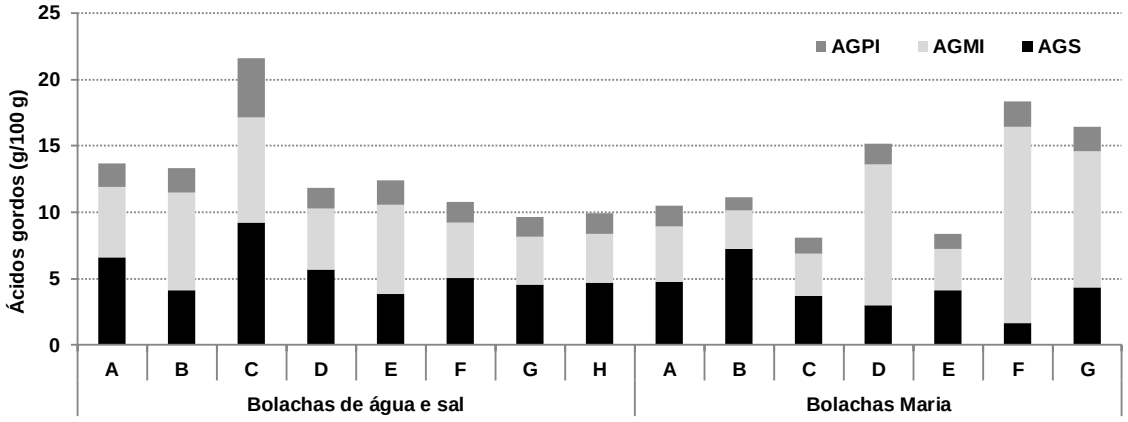

Figura 28. Composição em ácidos gordos (g/100 g) das amostras de bolachas Maria e de água e sal analisadas. (AGS – ácidos gordos saturados; AGMI – ácidos gordos monoinsaturados; AGPI – ácidos gordos polinsaturados).

No entanto, deve reforçar-se que, apesar de não estar totalmente evidenciado, os resultados da literatura indicam que esta relação é mais forte com os ácidos gordos saturados de cadeia média, nomeadamente os ácidos láurico (C12:0), mirístico (C14:0) e palmítico (C16:0) (7). Outro grupo de ácidos gordos que tem sido muito estudado nos últimos anos, não só pelos efeitos negativos que a sua ingestão pode causar, mas também porque têm sido reformulados muitos alimentos processados que continham teores elevados, são os ácidos gordos *trans* (AGT). O teor de AGT nas amostras de bolachas analisadas variou entre 24,5 e 82,4 mg/100 g, para as bolachas Maria G e bolachas de

água e sal H, respetivamente (Figura 29). De acordo com as recomendações de alguns países Europeus, os alimentos processados que não contenham gordura láctea, devem apresentar um teor de AGT inferior a 2% do teor de gordura total (8). Nos anos 90, Portugal participou num estudo Europeu sobre o teor destes ácidos gordos em diversas categorias de alimentos, entre as quais os produtos de pastelaria. Após uma análise comparativa com os dados da literatura e os dados obtidos neste trabalho, para o mesmo tipo de alimentos, verificou-se que os teores de AGT decresceram (9).

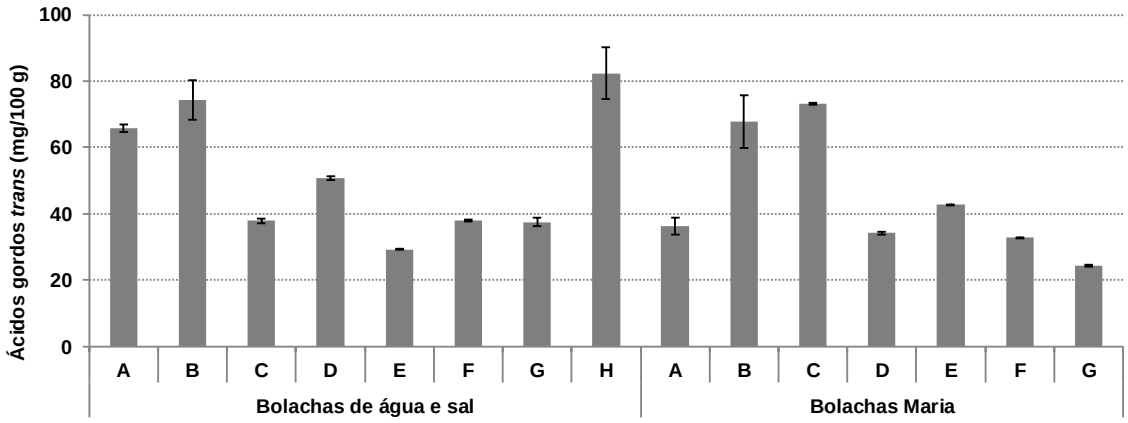

Figura 29. Teor de ácidos gordos *trans* (mg/100 g) das amostras de bolachas Maria e de água e sal analisadas.

Conclusões

As bolachas de água e sal analisadas apresentam teores superiores de gordura total e de sal, devendo ser estabelecidas metas que permitam a reformulação gradual destes alimentos. As bolachas analisadas, de forma geral, continuam a apresentar teores elevados de gordura saturada. No entanto, neste trabalho verificou-se que algumas bolachas apresentam quantidades superiores de ácidos gordos insaturados, que podem contribuir para a prevenção de doenças crónicas e diminuir o impacto na saúde relacionado com o consumo deste tipo de alimentos. Os teores de AGT nas amostras analisadas decresceram em comparação com os dados anteriores existentes na literatura. Esta observação pode estar relacionada com o facto de as gorduras atualmente utilizadas na confeção destes produtos terem sido substituídas.

Referências bibliográficas

1. Roda dos Alimentos Mediterrânica. Direção-Geral da Saúde. 2017. http://www.alimentacaosaudavel.dgs.pt/roda-dos-alimentos-mediterranica/
2. Food and Agriculture Organization of the United Nations. Fats and fatty acids in human nutrition: report of an expert consultation. Rome: FAO, 2010. http://foris.fao.org/preview/25553-0ece4cb94ac52f9a25af77ca5cfba7a8c.pdf
3. Albuquerque TG, Sanches-Silva A, Santos L, et al. An update on potato crisps contents of moisture, fat, salt and fatty acids (including trans-fatty acids) with special emphasis on new oils/fats used for frying. Int J Food Sci Nutr 2012;63(6):713-17.
4. Albuquerque TG, Oliveira MB, Sanches-Silva A, et al. The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. Food Funct. 2016;7(6):2736-46.
5. Regulamento (UE) n.º 1169/2011 do Parlamento Europeu e do Conselho, de 25 de outubro de 2011, relativo à prestação de informação aos consumidores sobre os géneros alimentícios. JO. 22.11.2011: L 304/18-63. http://data.europa.eu/eli/reg/2011/1169/oj
6. World Health Organization. Mapping salt reduction initiatives in the WHO European Region. Copenhagen: WHO Regional Office for Europe, 2013. www.euro.who.int/__data/assets/pdf_file/0009/186462/Mapping-salt-reduction-initiatives-in-the-WHO-European-Region.pdf
7. European Food Safety Authority. Scientific Opinion on Dietary Reference Values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, *trans* fatty acids, and cholesterol. EFSA Journal 2010;8(3):1461. http://onlinelibrary.wiley.com/doi/10.2903/j.efsa.2010.1461/full
8. Mouratidou T, Livaniou A, Saborido CM, et al. *Trans* fatty acids in Europe: where do we stand? European Commission. 2014. http://publications.jrc.ec.europa.eu/repository/bitstream/JRC91353/lbna26795enn.pdf
9. Tabela da Composição dos Alimentos. Instituto Nacional de Saúde Doutor Ricardo Jorge. 2015. http://portfir.insa.pt/

2.9 Haverá diferenças nutricionais entre produtos de pastelaria com e sem glúten?

Este sub-capítulo apresenta os resultados relativos às diferenças nutricionais de 14 produtos de pastelaria (bolachas tipo "Maria", "Crackers", "Wafers", bolachas recheadas e madalenas) dos quais 9 com glúten e 5 sem glúten. Verificou-se que, alguns dos produtos sem glúten, apresentam teores de gordura e de sal superiores aos produtos semelhantes com glúten.

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Resumo

A doença celíaca é uma doença auto-imune, desencadeada pela ingestão de glúten, em indivíduos com predisposição genética. O tratamento desta doença passa, única e exclusivamente, pela prática de uma alimentação isenta de glúten, ao longo de toda a vida do doente celíaco. Por este motivo, a indústria alimentar tem investido no aumento de oferta e variedade de produtos alimentares isentos de glúten. Este trabalho de investigação pretendeu avaliar diferenças, do ponto de vista nutricional, entre produtos de pastelaria com e sem glúten. Em 2015, foram adquiridos 14 produtos, dos quais 9 com glúten e 5 sem glúten. Determinaram-se os teores de gordura total, de sal e o perfil de ácidos gordos. As bolachas tipo “Crackers” e tipo “Maria” sem glúten apresentaram teores de gordura superiores aos produtos similares com glúten. No entanto, é muito importante avaliar que tipo de gordura se encontra nestes alimentos. Nas bolachas tipo “Maria” sem glúten a gordura era maioritariamente monoinsaturada, cuja ingestão está associada a um efeito protetor no desenvolvimento de doença coronária. Sendo este tipo de alimentos, apreciado por todas a faixas etárias, mas sobretudo por jovens, é muito importante alargar este trabalho de investigação a uma maior gama de produtos.

Abstract

The coeliac disease is an autoimmune disease triggered by the ingestion of foodstuffs with gluten in genetically predisposed individuals. The treatment of this disease is based on a gluten-free diet, which should be followed throughout life by the coeliac patient. For this reason, the food industry has developed efforts to increase the supply and variety of gluten-

free foods. This research aimed to evaluate the differences from a nutritional point of view of bakery products with and without gluten. During 2015, 14 products of which 9 were with gluten and 5 were gluten-free were acquired in different food chains. Total fat, salt and fatty acid profile were determined in the selected samples. The "Crackers" and "Maria" gluten-free biscuits present a higher fat content than similar products with gluten. However, it is very important to evaluate what type of fat can be found in these foods. In "Maria" gluten-free biscuits fat is mostly monounsaturated, whose intake is associated with a protective effect on the development of coronary heart disease. As this type of food, is appreciated by all the age groups, but especially by young people, it is very important to extend this research to a wider range of products.

Introdução

A doença celíaca é uma doença auto-imune desencadeada pela ingestão de glúten em indivíduos com predisposição genética (1). Por este motivo a população portadora desta doença não pode ingerir alimentos que contenham trigo, centeio e/ou cevada, sendo que a ingestão de produtos que contém aveia permanece controversa e por isso é muitas vezes restringida (1). O tratamento desta doença passa, única e exclusivamente, pela prática de uma alimentação isenta de glúten que deve ser seguida toda a vida pelo doente celíaco (1). Recentemente surgiram outras situações associadas ao glúten, como por exemplo a sensibilidade ao glúten não celíaca, em que as pessoas apresentam melhorias quando praticam uma dieta isenta de glúten, mas não preenchem os critérios de diagnóstico de doença celíaca. Dada a necessidade de praticarmos uma alimentação equilibrada, diversificada e completa, a indústria alimentar tem-se empenhado no sentido de aumentar a oferta e variedade de produtos alimentares isentos de glúten. As bolachas, biscoitos, bolos, farinhas e massas são alguns dos produtos que podemos encontrar nas superfícies comerciais e para os quais já existe uma grande variedade. Algumas destas categorias de alimentos são consideradas fontes de gordura saturada, sal e açúcar, sendo o seu consumo muitas vezes desaconselhado. Existem algumas publicações científicas que sugerem que uma alimentação isenta de glúten pode apresentar défices do ponto de vista nutricional, nomeadamente em termos de fibra alimentar, vitaminas e minerais (2-4).

Objetivos

Este trabalho de investigação pretendeu avaliar diferenças do ponto de vista nutricional entre produtos de pastelaria com e sem glúten, tendo por base a determinação analítica dos teores de gordura e de sal, e do perfil de ácidos gordos.

Material e métodos

Foram adquiridos, em 2015, 14 produtos de pastelaria (bolachas tipo “Maria”, bolachas tipo “Crackers”, bolachas tipo “Wafers”, bolachas recheadas e madalenas) dos quais 9 com glúten e 5 sem glúten. O teor de gordura total foi determinado utilizando uma hidrólise ácida seguida de extração em Soxhlet com éter de petróleo, e o teor de sal foi determinado por titulação pelo método de Charpentier-Volhard (5). Para a determinação dos ácidos gordos nas amostras selecionadas, utilizou-se uma transesterificação a frio com uma solução metanólica de hidróxido de potássio, seguida de análise por cromatografia gasosa acoplada à deteção por ionização de chama (5).

Resultados e discussão

O teor de gordura total nas amostras analisadas variou entre 11,1 $\pm$ 0,0 e 27,7 $\pm$ 1,0 g/100 g de parte edível, para as bolachas tipo “Maria” com glúten e as bolachas tipo “Wafers”, respetivamente (Figura 30). Tendo em conta que a dose de referência para a ingestão de gordura é de 70 g por dia, uma porção de bolachas recheadas (3 bolachas $\approx$ 75 g) pode contribuir com 22% da dose de referência (6). As bolachas tipo “Crackers” e tipo “Maria” sem glúten apresentaram teores de gordura superiores aos produtos similares com glúten. As bolachas tipo “Crackers” apresentaram os teores de sal mais elevados, com valores a variar entre 1,07 e 1,40 g/100 g de parte edível (Figura 31). A ingestão excessiva de alimentos ricos em sal está relacionada com um aumento na predisposição para o desenvolvimento de doenças crónicas, tais como a hipertensão arterial (7). Nesse sentido têm sido desenvolvidas iniciativas para diminuir o teor de sal dos alimentos. Dos produtos analisados, as madalenas, as bolachas recheadas e as bolachas tipo “Crackers” sem glúten apresentaram teores de sal superiores comparativamente aos produtos similares com glúten. Em 71% dos alimentos analisados neste trabalho, os ácidos gordos maioritários são saturados (Figura 32), sendo os ácidos gordos saturados mais abundantes o ácido mirístico (C14:0) e o ácido palmítico (C16:0). A ingestão de alimentos ricos neste tipo de ácidos gordos está relacionada com um aumento do colesterol das lipoproteínas de baixa densidade e consequentemente com um aumento do risco de desenvolvimento de doença coronária. A bolacha tipo “Maria” sem glúten apresentou um teor de ácidos gordos monoinsaturados cerca de 4 vezes superior aos produtos similares com glúten. Este facto está relacionado com o tipo de gordura utilizado na produção destas bolachas e leva-nos a considerar que é possível produzir produtos similares com melhor qualidade nutricional. Relativamente aos ácidos gordos *trans*, os teores determinados nos produtos analisados são inferiores aos valores recomendados internacionalmente, ou seja, inferior a 2% do teor de gordura total (8).

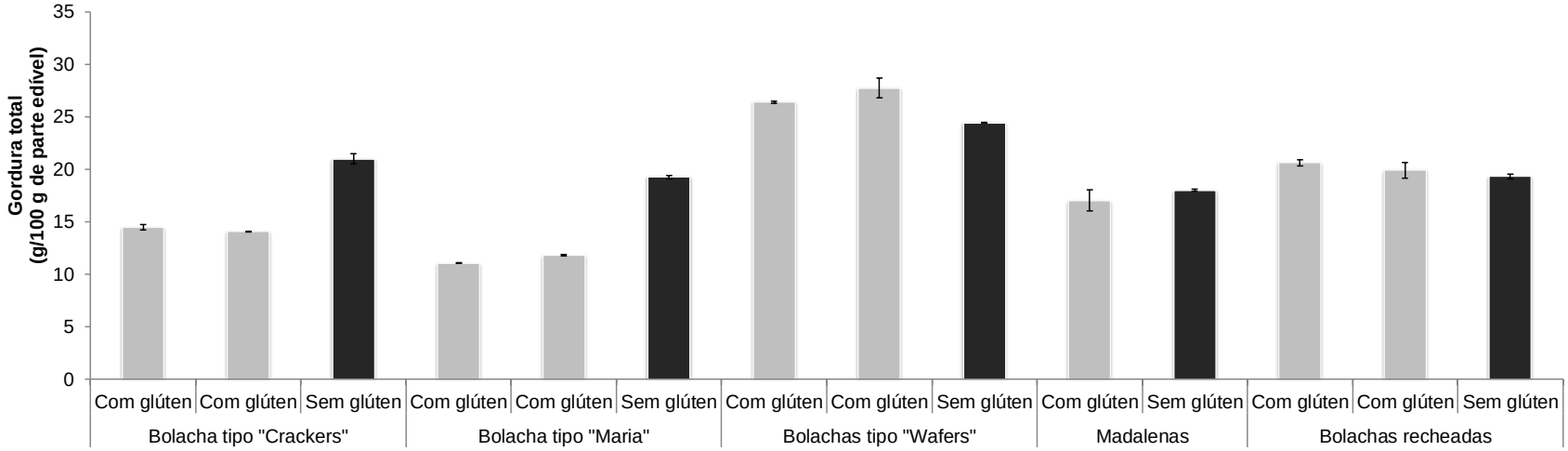

Figura 30. Teor de gordura total (g/100 g de parte edível) nos produtos de pastelaria analisados.

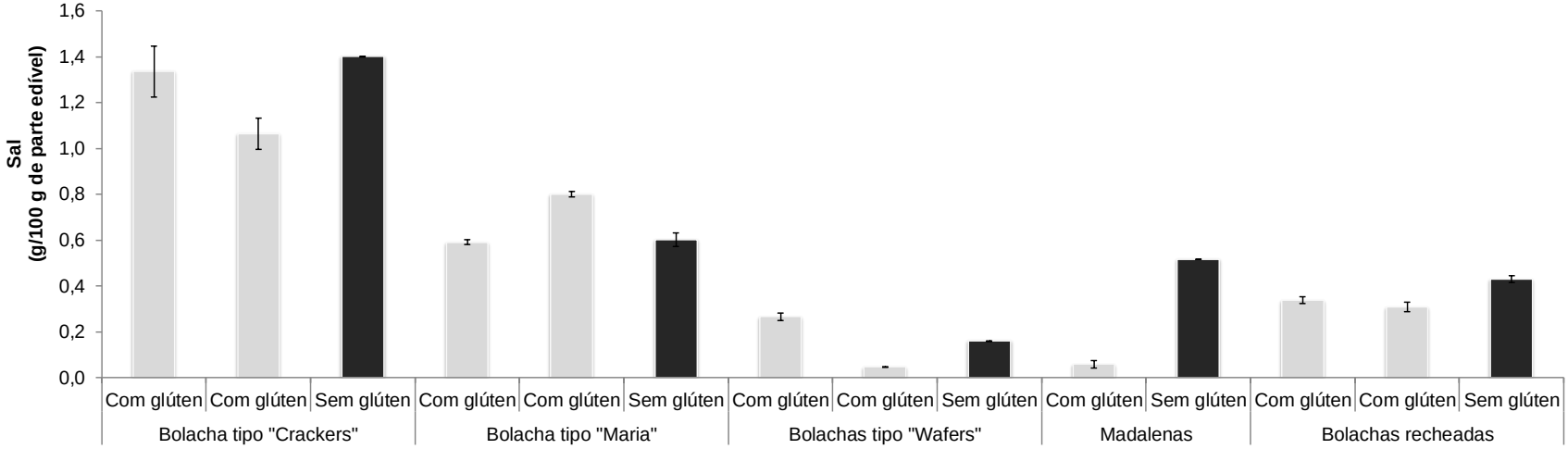

Figura 31. Teor de sal (g/100 g de parte edível) nos produtos de pastelaria analisados.

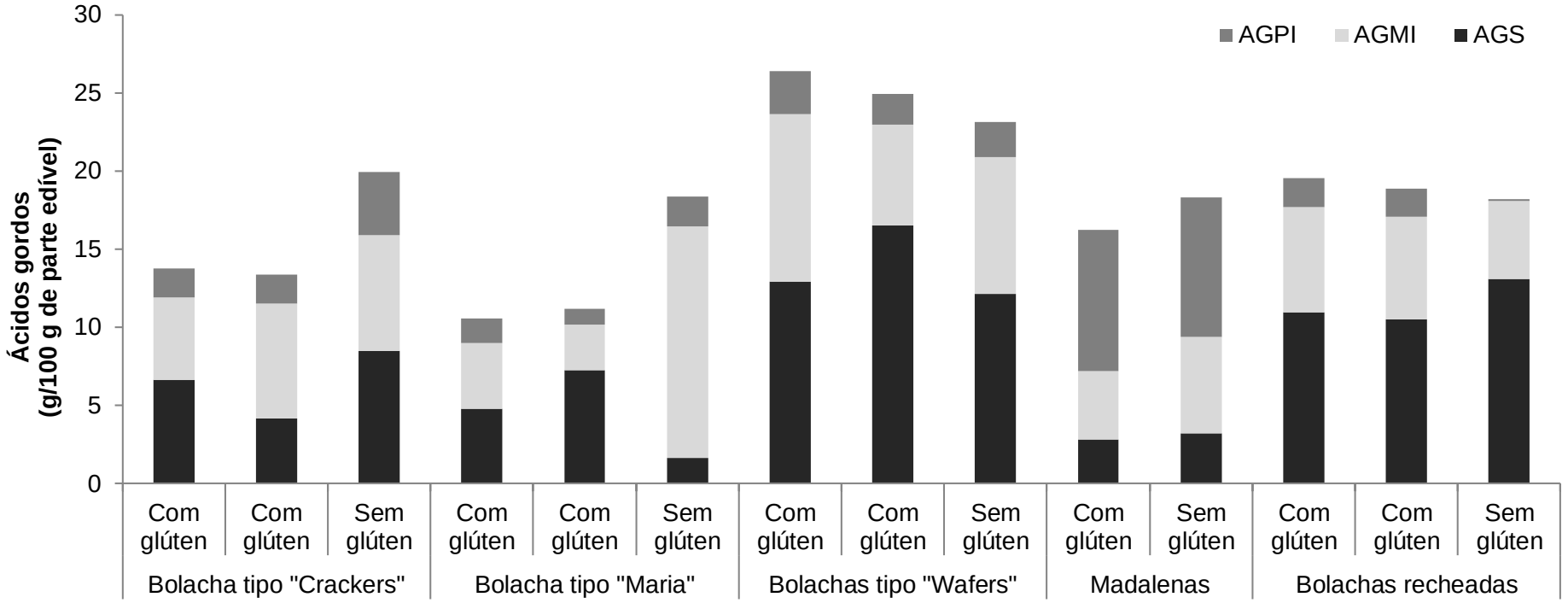

Figura 32. Composição em ácidos gordos (g/100 g de parte edível) nos produtos de pastelaria analisados.

Conclusões

Os resultados obtidos indicam que alguns dos produtos sem glúten analisados apresentam teores de gordura e de sal superiores aos produtos semelhantes com glúten. No entanto, é muito importante avaliar que tipo de gordura se encontra nestes alimentos. Neste trabalho apesar das bolachas tipo “Maria” sem glúten apresentarem um teor de gordura superior às com glúten, essa gordura é maioritariamente monoinsaturada

cuja ingestão está associada a um efeito protetor no desenvolvimento de doença coronária. Relativamente ao teor de sal nos alimentos analisados, considera-se que é muito importante continuar a desenvolver iniciativas no sentido de diminuir os seus teores. Sendo este tipo de alimentos, apreciado por todas as faixas etárias, mas sobretudo por jovens, é muito importante alargar este trabalho de investigação a uma maior gama de produtos.

Referências bibliográficas

1. Mahan LK, Escott-Stump S, Raymond JL. Krause – Alimentos, Nutrição e Dietoterapia. Tradução da 13ª Edição, Elsevier Editora, 2013.
2. Hopman EGD, le Cessie S, von Blomberg ME, Mearin ML. (2006). Nutritional management of the gluten-free diet in young people with celiac disease in The Netherlands. J Pediatr Gastroenterol Nutr, 43: 102-108.
3. Thompson T, Dennis M, Higgins A, Lee AR, Sharrett MK. (2005). Gluten-free diet survey: are Americans with coeliac disease consuming recommended amounts of fibre, iron, calcium and grain foods? J Hum Nutr Dietet, 18: 163-169.
4. Miranda J, Lasa A, Bustamante A, Churruca I, Simon E. (2014). Nutritional differences between a gluten-free diet and a diet containing equivalent products with gluten. Plant Foods Hum Nutr, 69: 182:187.
5. Albuquerque TG, Oliveira MB, Sanches-Silva A, Bento AC, Costa HS. (2016). The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. Food funct, 7: 2736-2746.
6. Regulamento (UE) n.º 1169/2011, do Parlamento Europeu e do Conselho de 25 de Outubro de 2011, relativo à prestação de informação aos consumidores sobre os géneros alimentícios. Jornal Oficial da União Europeia, L 304/18.
7. Polónia J, Martins L, Pinto F, Nazare J. (2014). Prevalence, awareness, treatment and control of hypertension and salt intake in Portugal: changes over a decade. The PHYSA study. J Hypertens, 32: 1211-1221.
8. World Health Organization. (2015). Eliminating *trans* fats in Europe – A policy brief. WHO Regional Office for Europe, Denmark.

2.10 Compliance with EU tolerance limits of declared *vs.* analysed values for mandatory nutrients in prepacked foods

Este sub-capítulo apresenta os resultados relativos à análise da conformidade dos valores apresentados na declaração nutricional de 209 alimentos processados. Foi também realizada uma análise comparativa da conformidade da rotulagem entre as 9 categorias de alimentos selecionados. Verificou-se que as maiores discrepâncias ocorrem para os teores de sal.

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Compliance with EU tolerance limits of declared *vs*. analysed values for mandatory nutrients in prepacked foods

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Abstract

This paper assesses the compliance between the declared and analysed values of prepacked foods, considering the tolerance limits for salt, fat and saturated fatty acids. Additionally, foods were distributed by food categories (e.g. snacks, ready-to-eat meals, potato and potato-products, bakery and pastry products). A total of 209 food products were acquired, analysed and the label checked for compliance with EU tolerance limits. Only 50% of the samples analysed were within the tolerance limits for salt content. The lowest number of samples outside the tolerance limits was observed for fat. For saturated fatty acids, 26% of the samples were outside of the tolerance limits. The category “cereal products” was the only for which 100% of the products were complying for fat and salt. It is of utmost importance that manufacturers continuously updated the declared values for prepacked foods because this information is crucial for consumers and for food policy makers.

Introduction

Nowadays, consumers are more aware of the relationship between diet and health. Therefore, their attention to the choice of an adequate diet to supply their individual dietary needs increased, simultaneously with a healthy lifestyle adoption (Miller & Cassady, 2015). In fact, this is also a concern for policy makers, because scientific evidence indicates that a healthy diet is not only important for individuals, but it also has impact in public health care expenses and in the active population productivity (Albert, 2010).

The European Strategy for Prevention and Control of Noncommunicable Diseases 2012-2016, recommends priority interventions, namely: (i) promoting healthy consumption,

through fiscal policies and marketing controls; (ii) elimination of *trans* fatty acids from processed foods, replacing them with polyunsaturated fats; and (iii) reducing diet salt intake to less than 5 g (2000 mg sodium) per person per day (World Health Organization, 2012).

Considering the nutritional labelling as a public health tool, several worldwide food authorities established regulations on nutritional labelling. In 1985 (later amended in several years) the *Codex Alimentarius* Commission adopted the Guidelines on Nutritional Labelling providing consumers with reliable information about the nutritional profile of the products in the market. Later, several countries developed mandatory nutritional labelling and initiated approaches for specified foods and products bearing claims, including the European Union (EU) legislation (Codex Alimentarius, 2013; Talati et al., 2017).

The foremost purpose of food labels information is to help consumers to understand the nutritional profile and quality of a specific product. Hence, better decisions based on informed choices and health/diet related can be made by consumers (Cheftel, 2005; Kleef & Dagevos, 2015). On the other hand, clear labels are a societal compromise of food manufactures and a challenge to develop and/or to reformulate products with a favourable nutritional profile *e.g.* with a lower salt or sugar content (Hawley et al., 2013). Additionally, when the food industry ensures the nutritional labelling accuracy, the competitiveness of the products increases (Kok & Radzi, 2017).

The awareness of consumers' healthy food choices and transparency in the food industry/consumers relationship led to the development of a comprehensive regulatory framework among EU countries (Kasapila & Shaarani, 2016). Outside EU, different label requirements were adopted. This is the case of United States, Canada, Mexico, Australia, China, Mercosur Member States (Argentina, Brazil, Chile, Colombia, Ecuador, Paraguay, and Uruguay) and New Zealand. Others follow Codex Standards (Kasapila & Shaarani, 2016).

Recently, in Europe, a more protective legislation, in what concerns to consumers, has been developed taking into account the need to harmonize the national legislations, allowing the free circulation of products and keep competition conditions within the internal market of the Member States (Edinger, 2016). The Regulation (EC) No. 178/2002 of the European Parliament and of the Council of 28 January 2002 as General Food Law (Edinger, 2016) establishes that a high level of protection of human life and health should be assured within EU countries, in all food manufacture system: production, processing and distribution (European Parliament and of the Council of the European Union, 2002).

The European Commission identified an irregular way on the information provided in the labels of prepacked foods between EU Member States. Therefore, on 25 October 2011, EU

has published the Regulation (EU) No. 1169/2011 on the provision of food information to consumers, in order to set common definitions, principles and procedures regarding this subject, that can ensure a balance between the protection of the internal market and the differences in the perception of consumers in the Member States (European Parliament and Council of the European Union, 2011). Above all, food information should not mislead consumers about the nature, characteristics, and food effects (European Parliament and Council of the European Union, 2011). This Regulation is directly applicable to all EU Member States and has completely entered into force on 13 December 2016. Figure 33 shows the mandatory information that must be provided on the label of a prepacked food and optional information in what concerns to the nutrition declaration.

Nutrition declaration labels supply information about the composition of foods allowing consumers to perform an informed and safe choice (European Parliament and Council of the European Union, 2011) (Himmelsbach, Allen, & Francas, 2014). In accordance with Regulation (EU) No. 1169/2011, article 31, point 4 *"The declared values shall be average values based on: the manufacturer's analysis of the food* or *a calculation from the known or actual average values of the ingredients used or (c) a calculation from generally established and accepted data"*. It is consensual that the most accurate method to determine the food nutritional composition is the analytical approach, by preferably an accredited laboratory or laboratories that successfully participate in proficiency testing schemes (Costa, Vasilopoulou, Trichopoulou, & Finglas, 2010; Machackova et al., 2018). However, the high number of "new" foods launched every day in the market and the cost and time consuming of the analyses are some of the appointed reasons, by the food industry, to justify the actual situation. Therefore, often, food producers choose to calculate or estimate the nutritional composition of their food products (Machackova et al., 2018).

Foods are naturally subjected to compositional changes due to many factors from production to consumption. Therefore, it is not possible to guarantee the same amount of a certain nutrient. Nonetheless, the differences between the declared values in the nutrition declaration should not deviate substantially from the nutrient content of foods in order to avoid misleading the consumer (European Commission, 2012). Therefore, in 2012, the European Commission released a guidance to Member States' control authorities and food business operators on the tolerances for nutrition labelling purposes (European Commission, 2012).

Figure 33. List of mandatory food information for prepacked foods and list of mandatory and optional nutrients for the nutrition declaration according to Regulation (EU) No. 1169/2011.

The aim of this work was to evaluate the compliance between the declared values and analysed values of prepacked processed foods, considering the permitted tolerance limits for salt, fat and saturated fatty acids (SFA). Moreover, an evaluation of the obtained results by categories of the selected processed foods was conducted and discussed.

Materials and methods

Overall design, sampling and data collection

Between 2015 and 2016, 209 prepacked food products available in Portugal were acquired in different stores (supermarkets, retail stores and take-away restaurants). Whenever possible, foods from different brands and different retailers were acquired. Upon reception of the food products at the laboratory, an Excel® form was filled out containing the following information: (i) food identification and description; (ii) place, date and time of collection; (iii) list of ingredients; and (iv) nutrition declaration. The selected food products included the major food categories of prepacked foods available in the market, namely savoury snacks (corn chips, sweet and salty popcorn); fast-food products (burgers, sandwiches, pizzas); nuts and oilseeds; cereal products (granola, muesli, cereal bars, breakfast cereals); ready-to-eat meals; sauces; potato and potato-products (potato chips, French fries); bakery products (croissants, sweet and salty breads, cakes); and pastry products (cookies, biscuits and wafers).

All the acquired items were stored in accordance with the label instructions. A composite sample of approximately 1 kg was prepared with at least three units of the same item. Afterwards, the selected food products were homogenised in a blender (GM200, RETSCH, Germany) at 5000 rpm during 1 to 3 min, depending on the food matrix. Then, samples were properly conditioned according to their perishability (*e.g.* high perishable foods were frozen at -20 ºC) until analysis.

The determined values were obtained by the analytical determination of salt, total fat and fatty acids content of the selected food products. For salt content, Charpentier-Volhard's titration was performed (Albuquerque, Sanches-Silva, Santos, & Costa, 2012); for total fat determination, an acid hydrolysis followed by Soxhlet extraction with petroleum ether was utilized (Albuquerque, Santos, et al., 2016); and for fatty acids composition, a cold transesterification followed by gas chromatography with flame ionization detection, was used (Albuquerque, Oliveira, Sanches-Silva, Bento, & Costa, 2016). Each sample was analysed in triplicate and chromatographic analyses performed in duplicate. The salt, fat and fatty acids content of the analysed food products are expressed as g/100 g of edible portion on a fresh weight basis.

Comparison of labelled and determined analytical values

The labelled concentration or declared values for salt, fat and SFA (in g/100 g) were obtained by the manufacturer's label declaration or in the manufacturer's website. For salt, if the concentrations were reported as sodium, they were multiplied by 2.5 to obtain the correspondent salt content (European Parliament and Council of the European Union, 2011). Considering the Guidance document with respect to the setting of tolerances for nutrient values declared on the label, the first step was to apply the rounding guidelines to the declared values in accordance with Table 3 of the above-mentioned document (European Commission, 2012). For example, if the declared value for fat is 12.6 g, the rounding rule is no decimals, therefore it should be declared as 13 g. The second step was to calculate the upper and lower bounds of the values that could be rounded to the declared value. For example, continuing with the previous value for fat (13 g), the lower and upper bounds of the rounding value should be 12.5 g and 13.4 g, respectively. The third step was to calculate the lower and upper tolerance, considering the established tolerances for foods (see Table 1 of European Commission, 2012) including the measurement uncertainty, since for all of the selected food products, the manufacturers did not provide an uncertainty range. For example, for a declared value of total fat between 10 g and 40 g per 100 g of food, the tolerance is ±20%. Therefore, the determined analytical value, in this case, should be within 13 ± 20% (10 g and 16 g) per 100 g of food. The fourth step was to compare the declared values and the determined analytical values, in order to evaluate which values were within or outside the tolerance limits previously calculated. Lastly, the declared values were analysed regarding their deviation. For example, if the declared value was higher than the analytical determined value, it was considered as an overestimated value.

Also, in the present study, samples were grouped into nine categories (snacks; fast-food; nuts and oilseeds; ready-to-eat meals; cereal products; sauces; potato and potato-products; bakery products; and pastry products), taking into account the main ingredients and features of the selected foodstuffs. A detailed analysis for the different established categories regarding all the aforementioned steps, concerning the compliance with EU tolerance limits, was also conducted.

Results and discussion

In our study, 209 food products were acquired and analysed, but it was not possible to use all of them to check for compliance with EU tolerance limits, since for some of these foods, depending on the nutrient, declared values were absent. For instance, for salt, 78.0% of the foods were checked, while for total fat and SFA it was 85.6% and 79.9%, respectively. From a legislation compliance perspective, we cannot point out that this fact was in disagreement,

since the nutrition declaration of foods only became mandatory after 13 December 2016 (European Parliament and Council of the European Union, 2011).

Currently, there has been a growing increase in nutritional policies to decrease salt intake. Indeed, Member States of the World Health Organization (WHO), like Portugal, agreed to a global target of a 30% reduction in salt intake by 2025 as a priority to reduce non-communicable diseases. Mandatory targets as the reduction of salt levels in bread have been introduced in several countries. This programme also comprises work partnerships with industries in order to reduce salt content in processed foods (Webster, Trieu, Dunford, & Hawkes, 2014). Monitoring salt levels by chemical analysis is the most accurate method to evaluate and monitor changes in food salt levels. The obtained information is very useful for different sectors involved in reducing salt intake, such as health professionals, governments, and food industry. It helps to proceed with improvements on the products' nutritional profile (Dunford et al., 2012) as well as the efforts of food industry engagement in such task. The major sources of salt in the diet are processed foods. In fact, 75-80% of the salt intake comes from processed food products rather than from the salt added to food during cooking (10-15%). The naturally occurring salt in food contributes with 5-10% of dietary salt intake (Dötsch-Klerk, Goossens, Meijer, & Van Het Hof, 2015). In what concerns salt content, 99 of the studied food products (60.7%) had a declared value lower than 1.25 g/100 g (Figure 34). In fact, WHO recommends 5 g of salt for adults (corresponding to 2 g of sodium) per day (Dötsch-Klerk et al., 2015). Regulation (EU) No. 1169/2011 establishes 6 g of salt as daily reference intake for an adult (European Parliament and Council of the European Union, 2011). Nevertheless, in many countries, the average daily salt consumption is estimated to vary between 9 and 12 g (Dötsch-Klerk et al., 2015).

With respect to fat content, to perform the compliance with EU tolerance limits, food products were divided into three groups according to their declared fat content: <10 g/100 g (*n* = 39, 21.8%); 10 – 40 g/100 g (*n* = 118; 65.9%); and >40 g/100 g (*n* = 22, 12.3%). For SFA declared values, 110 of the foods (65.9%) had a content ≥4 g/100 g. The overconsumption of foods with high amounts of fat and SFA is associated with low health outcomes (Kraak, Swinburn, Lawrence, & Harrison, 2014). In 2012, the EU Framework for National Initiatives on Selected Nutrients introduced saturated fat as a target for reduction initiatives. It was proposed to decrease the intake of saturated fat by at least 5% (in 4 years) and further 5% by 2020 (Fuster, 2016). The Commission White Paper highlighted the nutrients with negative impact on public health such as saturated fat, sugars or sodium and introduced a mandatory standardized presentation (European Parliament and Council of the European Union, 2011). However, with the entrance into force of Regulation (EU) No. 1169/2011, the term 'salt' was adopted instead of the corresponding term of the nutrient

'sodium', to ensure that the final consumer easily understands the information (European Parliament and Council of the European Union, 2011). Considering Regulation (EU) No. 1169/2011, for an average adult, the reference intake for SFA is 20 g and for total fat is 70 g (European Parliament and Council of the European Union, 2011). Overall, considering the studied products, a significant number presents a high content in both nutrients, particularly in SFA (Figure 34). For a healthy diet, it is recommended an intake of SFA as low as possible.

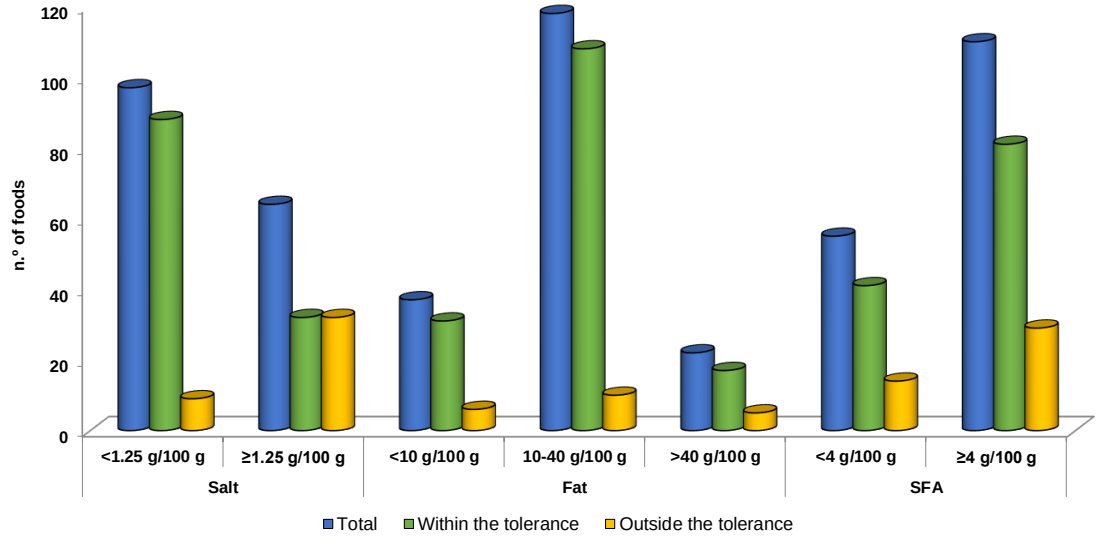

Figure 34. Prepacked processed foods for which the declared values for salt, fat and saturated fatty acids (SFA) are within or outside the EU tolerance limits.

Compliance with EU tolerance limits

Tolerances refer to the acceptable differences between the nutrient values declared on the label and those established in the course of official controls as comprised in Regulation (EU) No. 1169/2011 relative to the nutritional labelling (European Commission, 2012). Tolerance limits consider that the actual amount of a nutrient may not significantly vary compared to the value declared on the label. As a result, the European Commission has published guidance setting tolerances for declared nutrient values (European Commission, 2012).

The fixed EU tolerance limits vary according to the type of nutrient, but also with its concentration (European Commission, 2012). For salt content, the tolerance limits are ±0.375 g, if the food has a content <1.25 g/100 g, and ±20% if the food has a salt content ≥1.25 g/100 g (European Commission, 2012). With respect to the salt declared values, 25% of the selected food products were not in compliance with EU tolerance limits. The foods

that had a content ≥1.25 g of salt/100 g were the ones for which a higher number of foods (32 out of 64 foods) were outside of the tolerance limits (Figure 34).

For the selected nutrients, for which it was possible to perform the evaluation on the compliance with EU tolerance limits, fat was the one with a higher percentage of foods (88%) in compliance, in comparison with salt (75%) and SFA (74%) declared values. For fat content, three tolerance limits are fixed according to the declared values (European Commission, 2012). For instance, for a food with a fat content lower than 10 g/100 g, the tolerance limit is ±2 g, while for foods with a fat content of 10-40 g/100 g or >40 g/100 g, it is ±20% or ±8 g, respectively (European Commission, 2012). Taking into account our results, the highest number of foods (*n* = 92) with a declared value for total fat in agreement with the tolerance limits, have a fat content ranging from 10 to 40 g/100 g. For the other two groups, for which the declared fat value was <10 g or >40 g/100 g, the food percentage outside of the tolerance limit was equal (18%) (Figure 34). The tolerance limit for SFA is ±0.8 g if the declared value is lower than 4 g/100 g and is ±20% if the declared value is greater than or equal to 4 g/100 g. In both cases, in our study, the percentage of declared values within or outside the tolerance limits was very similar. For instance, 26% of the foods with declared SFA value <4 g/100 g were outside the tolerance limit, while for the foods with a declared value ≥4 g/100 g, 27% were also outside the tolerance limit (Figure 34).

In accordance to the Guidance document, "*for nutrients where consumers are generally interested in reducing their intakes (such as fats, sugars and salt/sodium), the declared values should not be established at the lower tolerance range*" (European Commission, 2012). Therefore, it is recommended that declared values for salt, fat and SFA should be above the analytically determined values and/or estimated values and/or calculated values. In our study, the evaluation regarding this recommendation was performed (Figure 35). It was observed that for salt content, 63% of the declared values were overestimated in relation to the analytically determined values, which means that these values are lower than the declared ones, and it is in agreement with the recommendation. On the other hand, for fat, 58% of the declared values were underestimated. It is noticeable that for fat declared values, in all groups (fat content <10 g; 10-40 g and >40 g), the highest percentage of foods had declared values higher than the analytically determined, especially for the foods with a fat declared value <10 g/100 g, for which the percentage of underestimated values was 74%. The opposite was observed for salt and SFA declared values of the prepacked foods in comparison with the determined analytical values (Figure 35). With respect to salt, the percentages of underestimated values were 59% and 70% for the foods with a declared salt content <1.25 g and ≥1.25 g/100 g, respectively.

For the foods with a declared value for SFA lower than 4 g/100 g, 61% of the values were overestimated, while for SFA content ≥4 g/100 g, this value increased to 72%.

The accuracy of nutritional labels is essential to consumers control the energy and certain nutrients intakes. For instance, individuals that are in a program of weight loss and/or maintenance, the energy and fat intake can be useful information (Jumpertz et al., 2013). Likewise, for salt, the

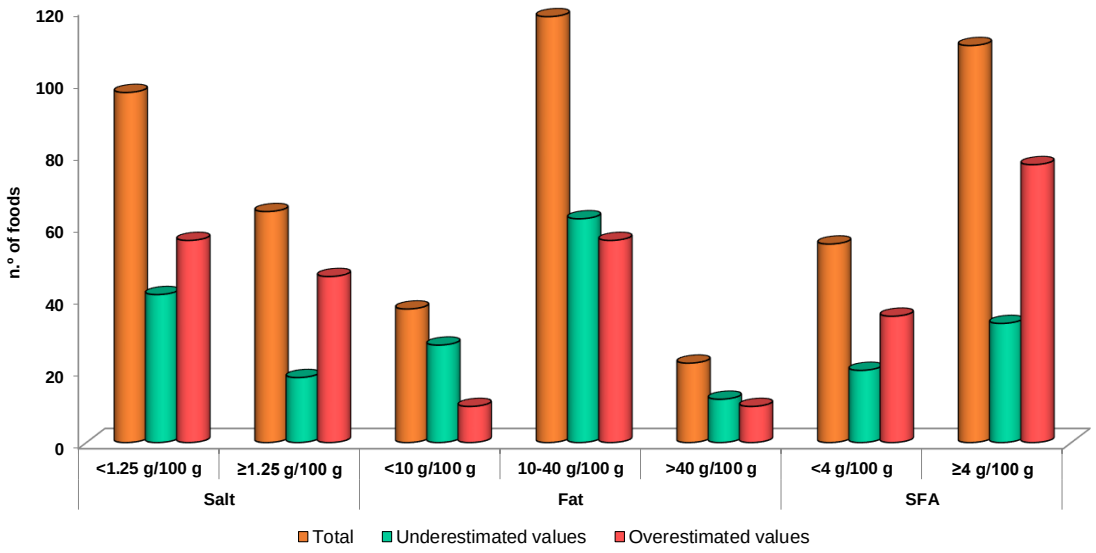

Figure 35. Prepacked processed foods that have underestimated or overestimated declared values for salt, fat and saturated fatty acids (SFA) in comparison with analytically determined values.

accurate label nutrition declaration is extremely important for individuals that control the salt intake due, for example, to chronic diseases that require low sodium diets. Fitzpatrick et al. (2014) analysed the accuracy of Canadian food labels for calories, *trans* fat, saturated fat, and sugar. These authors showed that the actual sodium content of food was higher (≈18%) when compared to the declared values meaning an underestimation trend of the declared values for that nutrient (Fitzpatrick et al., 2014). Also, the verification of the sodium label compliance of processed foods selected from Brazilian market was performed. That study showed non-compliance between the chemical analysis and the labelled value. Indeed, 13 of the 17 products analysed, had sodium amounts that exceeded by more than 20% on the nutrition declaration (Ribeiro, Ribeiro, Vasconcelos, Andrade, & Stamford, 2013).

Evaluation of the results by food categories

Most of the processed foods are recognized as a source of salt and fat, namely SFA and *trans* fatty acids. Therefore, in the last years, great attention has been devoted to this food

type and several actions have been performed by different countries to produce healthier foods, with lower salt and *trans* fatty acids contents (Mouratidou, Livaniou, Saborido, Wollgast, & Caldeira, 2014; World Health Organization, 2013). Therefore, the accuracy of the labelled nutrition declaration of prepacked foods is of utmost importance, for consumers to perform an informed choice. Considering the nutrients under analysis (salt, fat and SFA), for snacks, ready-to-eat meals, cereal products, and bakery products categories, the highest percentage of declared values outside the tolerance limits is for salt content (Figure 36). The snacks category has a higher percentage (54%) of declared values for SFA outside the tolerance limits than the food percentage (46%) that was in compliance with the tolerance limits. Similar results were obtained for fast-food and nuts and oilseeds categories, but for salt instead of SFA. The fat declared values of all cereal products and sauces were in agreement with the tolerance limits. For cereal products it was also observed that the salt declared values agree with the tolerance limits. This category of food has been under public scrutiny over the last years due to its high content in added sugars, saturated fat and salt (Williams, 2014). Therefore, food industries have been reducing these nutrients related to chronic diseases. For example, in bread, cheese, chips, soups and sauces, the reduction of the salt amount varied between 10 and 40% (Kloss, Meyer, Graeve, & Vetter, 2015).

The highest percentage of foods with fat declared values outside the tolerance limits were observed in fast-food and ready-to-eat meals categories, while for SFA it was snacks, fast-food and ready-to-eat meals categories with 54%, 50% and 38% of the declared values outside the limits, respectively.

With respect to the evaluation of the over- or underestimated declared values by food categories, it was possible to observe that for fast-food and potato and potato-products, all the declared values were overestimated, meaning that the declared values for all the nutrients under analysis were higher than the analytically determined values (Figure 37).

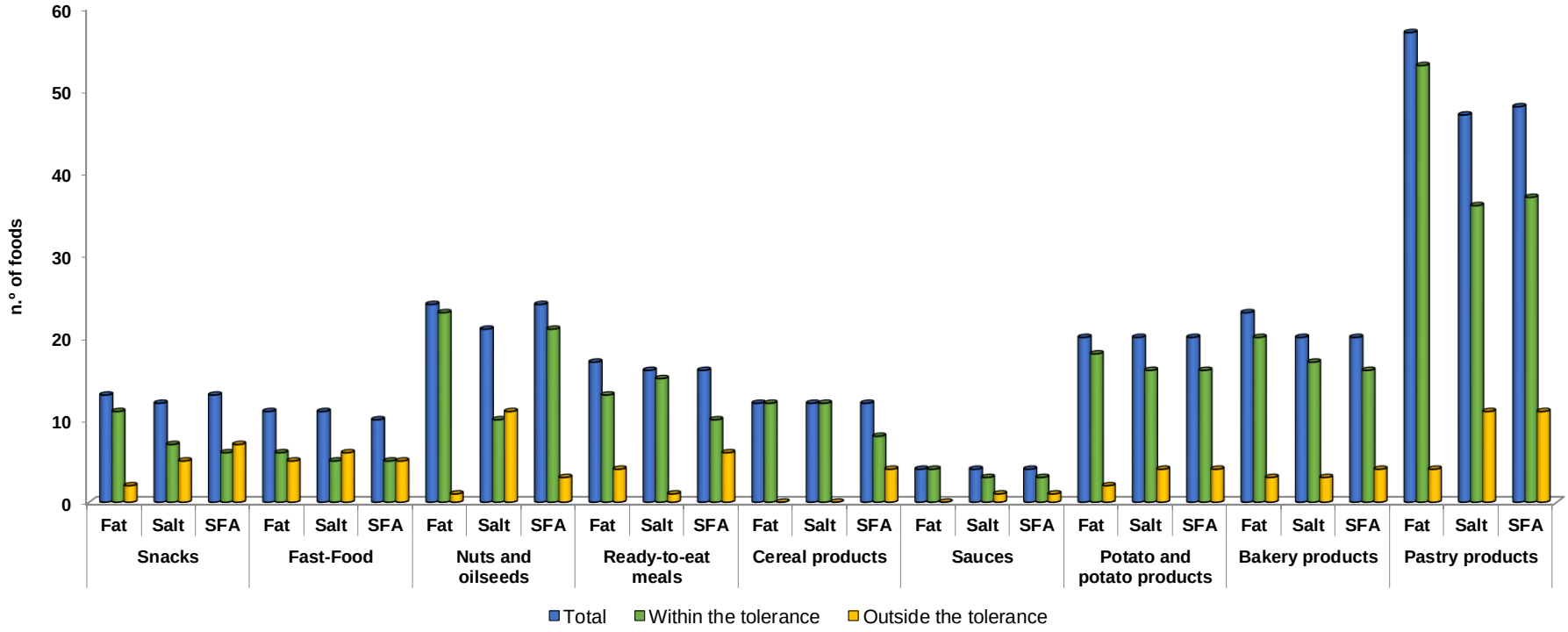

Figure 36. Prepacked processed foods by categories for which the declared values are within or outside the EU tolerance limits for salt, fat and saturated fatty acids (SFA).

For salt content, in 6 out of 9 food categories, the declared values were overestimated, while for SFA, for all the categories under study, the declared values were overestimated. Contrarily, for fat content in the selected prepacked foods, only fast-food, potato and potato-products, and bakery products categories had overestimated declared values, indicating that for the other food categories the analytically determined values were higher than the declared values (Figure 37). Sauces was the category with the highest percentage of underestimated declared values for fat (100%) and for salt (75%), while for SFA, it was the pastry products category (44%). According to the Guidance Document there are several factors to take in account when the measured value is outside the tolerance for the declared value, such as the natural variation of the nutrient (for example, by the influence of seasons), the nutrient lability, and the rate of degradation of particular nutrients in a specific food matrix (European Commission, 2012).

Other reasons to be considered are the analytical variability and sampling procedure (European Commission, 2012). In EU, calculation methods are a legally acceptable process for nutritional labelling (European Parliament and Council of the European Union, 2011). However, manufacturers can also analyse food products in external analytical laboratories and/or in their own laboratories (Kok & Radzi, 2017). Kok et al. (2017) presented some reasons that can cause non-compliance between determined and labelled nutrient amounts: (1) the methodologies applied in the determination of the nutrients content, despite following standard AOAC methods, can present some differences. For instance, for the determination of fat content, a Soxhlet extraction without or with acid hydrolysis can be used (as performed in this work). Therefore, the total fat content may vary in food products, for example containing nuts, where an acid hydrolysis is required; (2) samples preparation and homogeneity have a particular importance since it can produce false results. This is particularly important, for example, in canned foods where the solids may be or not be separated from the liquid in the nutritional analysis (Kok & Radzi, 2017).

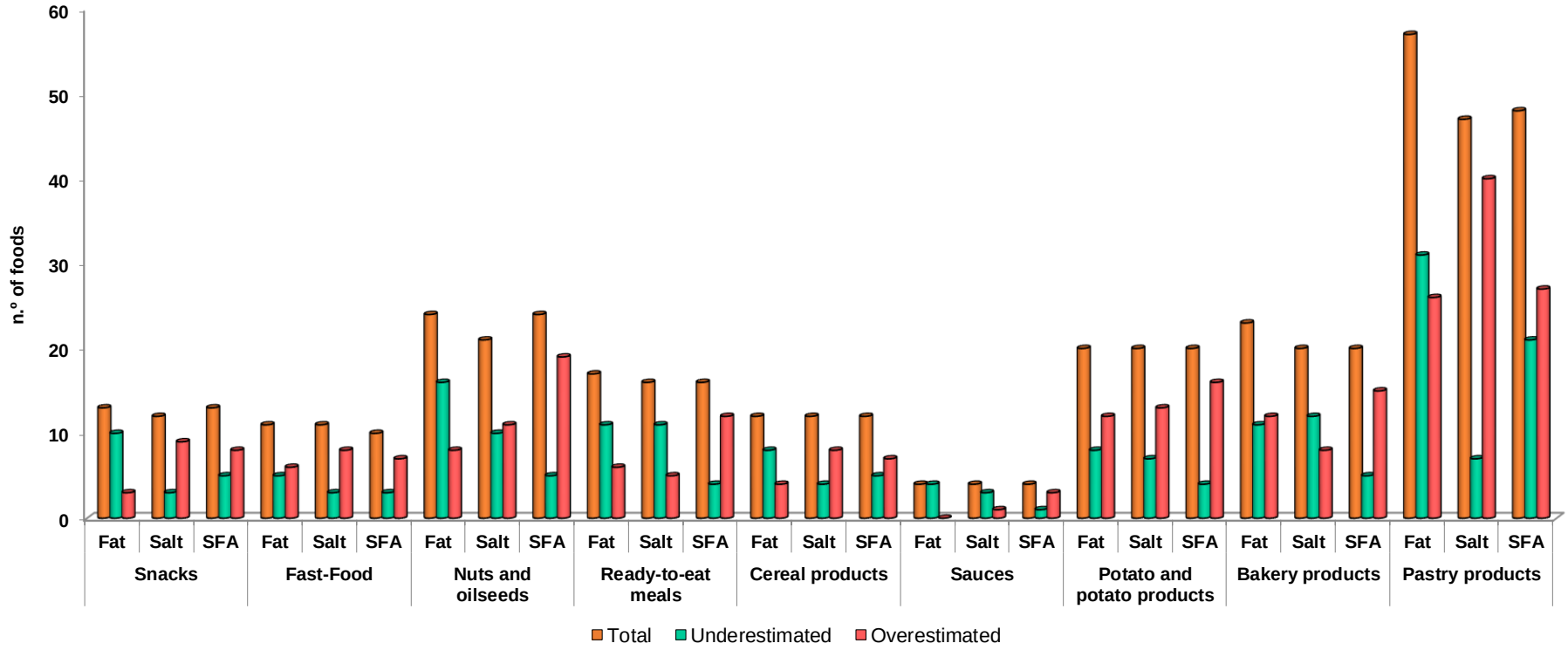

Figure 37. Prepacked processed foods by categories that have underestimated or overestimated declared values for salt, fat and saturated fatty acids (SFA) in comparison with analytical determined values.

Conclusions

The WHO recognized the product reformulation as a viable approach of food industries to participate, along with health organizations, on the reduction of salt, sugar, and fat. Nevertheless, it is a challenge to reduce for *e.g.* the product salt content, without compromising sensorial characteristics and/or shelf life. Also, consumer acceptance and technological alternatives to replace the nutrients are limitations to be considered. A snapshot of the actual fat, saturated fat, and salt contents as well as the declared values compliance with EU tolerance limits were presented in this work. Considering the results, additional efforts are still needed to allow better compliance on declared values mostly fast-food and snacks. Also, further monitoring of other food categories and through time are advisable. However, authors also recognize that the reported results can be noteworthy for consumers, public health organizations, and food industries.

References

Albert, J. (2010). *Innovations in food labelling*. *The Food and Agriculture Organization of the United Nations and Woodhead Publishing Limited* (1st ed.). Woodhead Publishing. https://doi.org/10.1016/B978-1-84569-754-9.50027-9

Albuquerque, T. G., Oliveira, M. B. P. P., Sanches-Silva, A., Bento, A. C., & Costa, H. S. (2016). The impact of cooking methods on the nutritional quality and safety of chicken breaded nuggets. *Food Funct.*, *7*(6), 2736–2746. https://doi.org/10.1039/C6FO00353B

Albuquerque, T. G., Sanches-Silva, A., Santos, L., & Costa, H. S. (2012). An update on potato crisps contents of moisture, fat, salt and fatty acids (including trans -fatty acids) with special emphasis on new oils/fats used for frying. *International Journal of Food Sciences and Nutrition*, *63*, 713–717. https://doi.org/10.3109/09637486.2011.644768

Albuquerque, T. G., Santos, F., Sanches-Silva, A., Beatriz Oliveira, M., Bento, A. C., & Costa, H. S. (2016). Nutritional and phytochemical composition of Annona cherimola Mill. fruits and by-products: Potential health benefits. *Food Chemistry*, *193*, 187–195. https://doi.org/10.1016/j.foodchem.2014.06.044

Cheftel, J. C. (2005). Food and nutrition labelling in the European Union. *Food Chemistry*, *93*(3), 531–550. https://doi.org/10.1016/j.foodchem.2004.11.041

Codex Alimentarius. (2013). Guidelines on Nutrition Labelling. *Cac/Gl 2-1985*, *53*(9), 1689–1699. https://doi.org/10.1017/CBO9781107415324.004

Costa, H. S., Vasilopoulou, E., Trichopoulou, A., & Finglas, P. (2010). New nutritional data on traditional foods for European food composition databases. *European Journal of Clinical Nutrition*, *64*, S73–S81. https://doi.org/10.1038/ejcn.2010.215

Dötsch-Klerk, M., Goossens, W., Meijer, G. W., & Van Het Hof, K. H. (2015). Reducing salt in food; Setting product-specific criteria aiming at a salt intake of 5 g per day. *European Journal of Clinical Nutrition*, *69*(7), 799–804. https://doi.org/10.1038/ejcn.2015.5

Dunford, E., Webster, J., Metzler, A. B., Czernichow, S., Mhurchu, C. N., Wolmarans, P., … Neal, B. (2012). International collaborative project to compare and monitor the nutritional composition of processed foods. *European Journal of Preventive Cardiology*, *19*(6), 1326–1332. https://doi.org/10.1177/1741826711425777

Edinger, W. H. (2016). Promoting Educated Consumer Choices. Has EU Food Information Legislation Finally Matured? *Journal of Consumer Policy*, *39*(1), 9–22. https://doi.org/10.1007/s10603-015-9307-3

European Commission. (2012). *Guidance document for competent authorities for the control of compliance with EU legislation on: Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers*.

European Parliament and Council of the European Union. (2011). *Regulation (EU) No. 1169/2011 of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers*.

European Parliament and of the Council of the European Union. (2002). Regulation (EC) No. 178/2002 of 28 January 2002 laying down the general principles and requirements of food law, establishing the European Food Safety Authority and laying down procedures in matters of food safety. *Official Journal of the European Communities*, *L31*, 1–24. https://doi.org/2004R0726 - v.7 of 05.06.2013

Fitzpatrick, L., Arcand, J. A., L'Abbe, M., Deng, M., Duhaney, T., & Campbell, N. (2014). Accuracy of Canadian food labels for sodium content of food. *Nutrients*, *6*(8), 3326–3335. https://doi.org/10.3390/nu6083326

Fuster, V. (2016). Unlikely Allies: Partnering With the Food Industry to Fight the Obesity Epidemic. *Journal of the American College of Cardiology*, *68*(8), 871–873. https://doi.org/10.1016/j.jacc.2016.07.712

Hawley, K. L., Roberto, C. A., Bragg, M. A., Liu, P. J., Schwartz, M. B., & Brownell, K. D. (2013). The science on front-of-package food labels. *Public Health Nutrition*, *16*(3), 430–439. https://doi.org/10.1017/S1368980012000754

Himmelsbach, E., Allen, A., & Francas, M. (2014). *Study on the Impact of Food Information on Consumers' Decision Making*. https://ec.europa.eu/food/sites/food/files/safety/docs/labelling_legislation_study_food-info-vs-cons-decision_2014.pdf

Jumpertz, R., Venti, C. A., Le, D. S., Michaels, J., Parrington, S., Krakoff, J., & Votruba, S. (2013). Food label accuracy of common snack foods. *Obesity*, *21*(1), 164–169. https://doi.org/10.1002/oby.20185

Kasapila, W., & Shaarani, S. M. (2016). Legislation - Impact and Trends in Nutrition Labeling: A Global Overview. *Critical Reviews in Food Science and Nutrition*, *56*(1), 56–64. https://doi.org/10.1080/10408398.2012.710277

Kleef, E. Van, & Dagevos, H. (2015). The Growing Role of Front-of-Pack Nutrition Profile Labeling: A Consumer Perspective on Key Issues and Controversies. *Critical Reviews in Food Science and Nutrition*, *55*(3), 291–303. https://doi.org/10.1080/10408398.2011.653018

Kloss, L., Meyer, J. D., Graeve, L., & Vetter, W. (2015). Sodium intake and its reduction by food reformulation in the European Union - A review. *NFS Journal*, *1*, 9–19. https://doi.org/10.1016/j.nfs.2015.03.001

Kok, S. C., & Radzi, C. W. J. M. (2017). Accuracy of nutrition labels of pre-packaged foods in Malaysia. *British Food Journal*, *119*(2), 230–241. https://doi.org/10.1108/JHOM-09-2016-0165

Machackova, M., Giertlova, A., Porubska, J., Roe, M., Ramos, C., & Finglas, P. (2018). EuroFIR Guideline on calculation of nutrient content of foods for food business operators. *Food Chemistry*, *238*, 35–41. https://doi.org/10.1016/j.foodchem.2017.03.103

Miller, L. M. S., & Cassady, D. L. (2015). The effects of nutrition knowledge on food label use. A review of the literature. *Appetite*, *92*, 207–216. https://doi.org/10.1016/j.appet.2015.05.029

Mouratidou, T., Livaniou, A., Saborido, C. M., Wollgast, J., & Caldeira, S. (2014). *Trans fatty acids in Europe: where do we stand? A synthesis of the evidence: 2003-2013*.

European Commission Joint Research Centre Science and Policy Report,. https://doi.org/10.2788/1070

Ribeiro, V. F., Ribeiro, M. de A., Vasconcelos, M. A. da S., Andrade, S. A. C., & Stamford, T. L. M. (2013). Processed foods aimed at children and adolescents: Sodium content, adequacy according to the dietary reference intakes and label compliance. *Revista de Nutrição*, *26*(4), 397–406. https://doi.org/10.1590/S1415-52732013000400002

Talati, Z., Pettigrew, S., Neal, B., Dixon, H., Hughes, C., Kelly, B., & Miller, C. (2017). Consumers' responses to health claims in the context of other on-pack nutrition information: A systematic review. *Nutrition Reviews*, *75*(4), 260–273. https://doi.org/10.1093/nutrit/nuw070

Webster, J., Trieu, K., Dunford, E., & Hawkes, C. (2014). Target salt 2025: A global overview of national programs to encourage the food industry to reduce salt in foods. *Nutrients*, *6*(8), 3274–3287. https://doi.org/10.3390/nu6083274

Williams, P. G. (2014). The Benefits of Breakfast Cereal Consumption : A Systematic Review of the Evidence Base 1 – 4. *American Society for Nutrition*, *5*, 636–673. https://doi.org/10.3945/an.114.006247.animal

World Health Organization. (2012). Action Plan for implementation of European Strategy for the Prevention and Control of Noncommunicable Diseases 2012-2016. *WHO Regional Office for Europe*, 33.

World Health Organization. (2013). *Mapping salt reduction initiatives in the WHO European Region*. *WHO publications*. Copenhagen.

CAPÍTULO 3. Ocorrência de 4-Hidroxi-2-alcenais em alimentos processados e óleos vegetais

No presente capítulo apresentam-se os resultados relativos à ocorrência de 4-hidroxi-2-alcenais em diferentes grupos de alimentos processados, bem como o potencial impacto na saúde e risco de exposição, metodologias analíticas para a sua determinação em matrizes alimentares, fatores que estão relacionados com a sua formação/ocorrência e estratégias de mitigação.

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3.1 4-Hydroxy-2-alkenals: A potential toxicological concern of vegetable oils?

Este sub-capítulo apresenta os resultados relativos à revisão bibliográfica sobre a ocorrência dos 4-hidroxi-2-alcenais nos diferentes óleos e gorduras, considerando as diferentes origens e condições de processamento. Verificou-se uma grande amplitude nos resultados reportados para o mesmo tipo de matriz. Confirmou-se a necessidade mais estudos, sobretudo no que diz respeito à avaliação do impacto de condições realísticas de fritura.

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Reference Module in Food Science
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4-Hydroxy-2-Alkenals: A Potential Toxicological Concern of Vegetable Oils?

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Abstract

4-hydroxy-2-alkenals, namely 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE) are secondary lipid oxidation products of n-6 and n-3 polyunsaturated fatty acids, respectively. An overview of the current knowledge about HNE and HHE in vegetable oils, including factors influencing their formation, health effects, analytical approaches, as well as mitigation strategies and future challenges are discussed. During the last years, an increased attention is being paid to these mutagenic, cytotoxic and genotoxic compounds. Due to their high reactivity and implication in numerous undesirable reactions, they can be linked with several diseases and medical conditions. Vegetable oils were identified as one of the major sources of these compounds, becoming a potential toxicological concern for public health. Up to now, the fatty acids composition, time, temperature and type of frying, as well as different processing conditions were evaluated in order to find a relationship with the presence of these hazardous compounds in vegetable oils. With respect to mitigation strategies to reduce the occurrence of HNE and HHE in vegetable oils, atmospheres with low oxygen content during frying, carbon dioxide blanketing as well as addition of phenolic compounds to the vegetable oils were studied. In the near future, it is important to exploit other conditions to explain the different amounts reported for the same type of vegetable oil from different origins, as well as to evaluate realistic conditions of frying, especially the presence of food, frying equipment and temperatures, among others.

Introduction

Lipid oxidation is still one of the major concerns in food processing, especially in vegetable oils. Fatty acids oxidation leads to primary and secondary oxidation products, such as 4-hydroxy-2-nonenal (HNE) and 4-hydroxy-2-hexenal (HHE). HNE is a secondary oxidation product from the n-6 polyunsaturated fatty acids (PUFA), while HHE formation derives from the oxidation of n-3 PUFA. In recent years, great attention has been devoted to these compounds identified as mutagenic, genotoxic and cytotoxic. Due to their high reactivity with proteins and DNA, they can induce structural damage and changes in the functionality of such molecules (Csala et al., 2015; Esterbauer, Schaur, & Zollner, 1991; Guéraud, 2017; Ma & Liu, 2017). Up to now different factors (e.g. fatty acids composition, effect of temperature, time and type of frying), were studied to understand the behaviour of these compounds, namely HNE, in different food matrices. One of the most researched were vegetable oils, due to their high consumption rates by consumers and food industry. An overview of the current knowledge about HNE and HHE occurrence in vegetable oils, including factors that influence their formation, health effects, analytical approaches, as well as mitigation strategies and challenges are discussed.

Oils and Fats

Worldwide production

Oils and fats are natural constituents of plants and animals. Up to now, around thirty vegetable oils and animal fats have been commercially exploited, however only twelve have worldwide importance (Food and Agriculture Organization of the United Nations, 2008). The world domestic supply of vegetable oils increased significantly (~50%) in the last 15 years, being palm oil, rape and mustard oil, and soybean oil the most supplied, increasing by 64.0%, 55.0% and 53.5%, respectively, during this period (FAOSTAT, 2017). Some of the reasons appointed for this fact are the population expansion and the *per capita* consumption (Food and Agriculture Organization of the United Nations, 2008). According to Food and Agriculture Organization of the United Nations (FAO), during 2001 to 2003, developing countries produced more vegetable oils than developed countries, 68.8% and 31.2%, respectively (Food and Agriculture Organization of the United Nations, 2008). During this period, Asia was the main producer of palm, rape and mustard, groundnut, coconut, and cottonseed oils. South America was the main producer of soybean oil and Europe the main producer of sunflower and olive oils (Food and Agriculture Organization of the United Nations, 2008).

Fatty acids profile of edible oils/fats

Table 19 presents the fatty acids profile (g/100 g of edible portion) of selected commercially available oils and fats. The different vegetable oils and fats available in the market for human consumption differ in fatty acid composition, depending on factors such as climate conditions, geographical origin, soil, plant varieties and state of ripeness. Moreover, in hydrogenated oils, fatty acids profile is strongly dependent on the hydrogenation conditions (Minihane & Harland, 2007).

Table 19. Fatty acids profile (g/100 g of edible portion) of selected commercially available oils and fats.[a]

Oils and fats	SFA	MUFA	PUFA
Coconut oil	86.5	5.80	1.80
Cottonseed oil	25.9	17.8	51.9
Peanut oil	16.9	46.2	32.0
Olive oil	13.8	73.0	10.5
Palm oil	49.3	37.0	9.30
Palm kernel	81.5	11.4	1.60
Rice bran oil	19.7	39.3	35.0
Sesame oil	14.2	39.7	41.7
Soybean oil	15.7	22.8	57.7
Soybean oil (partially hydrogenated)	14.9	43.0	37.6
Sunflower oil	10.3	19.5	65.7
Sunflower oil (high oleic >70%)	9.86	83.7	3.80
Lard	39.2	45.1	11.2
Margarine (soybean, hydrogenated)	16.7	39.3	20.9
Butter (salted)	51.4	21.0	3.00
Shortening (household, lard and vegetable oil)	40.3	44.4	10.9

SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids

[a] The values given in the table are adapted from US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory, 2016.

There is a common sense, that only oils and fats from animal origin have high contents of saturated fatty acids (SFA). However, according to Table 19, the major sources of SFA are coconut oil (86.5 g/100 g), palm kernel oil (81.5 g/100 g), butter (51.4 g/100 g) and palm oil

(49.3 g/100 g). With respect to the major SFA for the above oils and fats, there are also differences, being lauric acid (C12:0) the most abundant for coconut and palm kernel oil, and palmitic acid (C16:0) the major fatty acid in butter and palm oil. Scientific literature has indicated that the intake of SFA closely relates with the increase of low density lipoprotein cholesterol concentrations (European Food Safety Authority, 2010). Moreover, the replacement of SFA by PUFA decreases the number of cardiovascular events (European Food Safety Authority, 2010). According to this, the European Food Safety Authority (EFSA) Panel has concluded that limiting the intake of SFA should be considered when establishing nutrient goals and recommendations (European Food Safety Authority, 2010). For monounsaturated fatty acids (MUFA), the richest sources are high-oleic sunflower (>70%) and olive oil with 83.7 and 73.0 g/100 g, respectively (Table 19). Regarding PUFA, the richest sources are sunflower (65.7 g/100 g), soybean (57.7 g/100 g) and cottonseed (51.9 g/100 g) oils.

4-Hydroxy-2-alkenals

The presence of 4-hydroxy-2-alkenals in foods was early detected by Schauenstein in the 1960's (Spickett, 2013). 4-hydroxy-2-alkenals were the major products of low molecular weight resulting from lipid peroxidation, with HNE considered to be the most cytotoxic, as well as the most abundant (Spickett, 2013). These 4-hydroxy-2-alkenals belong to the class of α, β-unsaturated aldehydes, and are able to react in Michael addition and Schiff base formation (Spickett, 2013). HNE is a secondary lipid peroxidation product derived from the oxidation of n-6 PUFA, namely linoleic acid. In the literature, this compound is sometimes confused with HHE, which is also a product of lipid oxidation (Csala et al., 2015). However, HHE is formed by the oxidation of n-3 PUFA, namely docosahexaenoic and eicosapentaenoic fatty acids (Long & Picklo, 2010). Compounds such as HNE and HHE are formed as a consequence of fatty acids degradation in the presence of oxygen, and when vegetable oils are exposed to thermal treatment (Seppanen & Csallany, 2002).

Health effects

4-hydroxy-2-alkenals are a group of compounds derived from 2-alkenals, which are highly reactive due to the carbon-carbon double bond conjugated to an electron carbonyl group on carbon 1 (Guéraud, 2017). HNE was reported in several tissues and organs, namely in the human plasma at 0.074 μM concentrations (Gil et al., 2006), and in rat liver at 0.33 nmol/g (Esterbauer et al., 1991). HNE could also be formed within membranes after peroxidation of phospholipid bound fatty acids and under oxidative stress conditions, with the concentrations reported around 5 mM (Guéraud, 2017; Uchida, 2003). According to Esterbauer et al. (1991), HNE concentrations higher than 100 μM can be responsible for

the inhibition of several basic catabolic (e.g. mitochondrial respiration) and anabolic (DNA, RNA and protein synthesis) cell functions, leading to structural damage and functional changes (Esterbauer et al., 1991). Because of the high reactivity of these compounds, their presence was related to several human illnesses, namely myocardial diseases, cancer and neurodegenerative diseases, like Parkinson's, Alzheimer's and Huntington's diseases (Csala et al., 2015; Esterbauer et al., 1991). Concerning HHE, Long et al. (2010) have performed a comparison with HNE actions and concluded that HHE have some properties in common with HNE, but there are considerable differences namely regarding the adduction targets and detoxification pathways (Long & Picklo, 2010). Nonetheless, it was demonstrated that HHE is a cytotoxic and mutagenic compound as well, but further studies are needed to determine the biochemical and physiological roles of HHE and its related aldehyde, 4-oxo-2-hexenal (Long & Picklo, 2010). The median lethal dose (LD50) for mice and by intra-peritoneal intake has been reported as 0.44 mmol/kg for HNE and 0.98 mmol/kg of body weight for HHE (Esterbauer et al., 1991; Papastergiadis et al., 2014a).

Analytical determination

Due to the referred above, the analysis of 4-hydroxy-2-alkenals in foods and edible oils has been the focus of several research works. Most of the analytical methods reported in the literature involve several extraction steps, which sometimes can induce important losses, leading to an underestimation of the actual amount in the samples. For the analytical determination of 4-hydroxy-2-alkenals the following methodologies were reported: high performance liquid chromatography with ultraviolet detection (HPLC-UV) or mass spectrometry detection (HPLC-MS/MS), and gas chromatography (GC)-MS (Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2014). Liquid chromatography (LC)-MS has the advantage of not requiring derivatization, and thus samples can be analysed with less extraction intermediate steps (Spickett, 2013). For GC-MS analyses, it is required sample derivatization, e.g. using *O*-(2,3,4,5,6-pentafluorophenyl)methylhydroxylamine hydrochloride, followed by silylation. HNE may be detected with negative chemical ionization and quantified by comparison to the internal standard (Spickett, 2013).

Factors influencing the 4-hydroxy-2-alkenals occurrence in vegetable oils

In the last years, the occurrence of several toxic compounds in vegetable oils, especially those that are commonly associated with lipid oxidation, was a big concern. This is the case of 4-hydroxy-2-alkenals, compounds also linked to the thermal food processing, namely temperature and time of exposure. Therefore, up to now different factors were studied to evaluate whether they are responsible for the increase of these toxic compounds in

vegetable oils, such as their fatty acids composition, time, temperature and type of frying, processing conditions, among others. For example, HNE formation along different periods of time (2, 4, 6, 8 and 10 h) at the same frying conditions (185 ºC) was studied (Seppanen & Csallany, 2002). According to the reported findings, it was notable that after 6 h, HNE concentration reached the maximum value (42.5 µg/g of oil). Afterwards, in the following 4 h of heating, the amount of HNE decreased, probably due to its degradation. Another interesting aspect of this study was the relationship between HNE formation and the presence of tocopherols. Apparently, tocopherols from soybean oil restrict the formation of these aldehydes in the first heating hours (Seppanen & Csallany, 2002). Seppanen et al. (2006) investigated the effect of intermittent and continuous heating on the formation of HNE, HHE, 4-hydroxy-2-*trans*-octenal and 4-hydroxy-2-*trans*-decenal in soybean oil. For both treatments, over a total of 5 h, a similar increase was observed (Seppanen & Csallany, 2006). The total heating time and temperature were similar for intermittent (185 ± 5 ºC; 1 h/day; during 5 days) and continuous (185 ± 5 ºC; 6 h) heating, but the treatments were different concerning the equipment and the used volume of oil (Seppanen & Csallany, 2006). The same authors evaluated the influence of air bubbling into the oil under continuous heating. The reported results showed that when air was bubbled into the oil, lipid oxidation greatly increased and a higher amount of HNE was determined (Seppanen & Csallany, 2006). Han and Csallany (2008) also investigated the temperature dependence of HNE formation in vegetable oils and butter (Han & Csallany, 2008). Therefore, corn and soybean oils (higher contents of linoleic acid) and butter (lower contents of linoleic acid) were heated for different periods of time at 190 ºC and 218 ºC. HNE amounts after 30 min of heating at 218 ºC were 3.7, 4.9 and 8.7 times higher than at 190 ºC, for soybean, corn and butter oil, respectively (Han & Csallany, 2008).

Primary and secondary oxidation products of corn oil were studied at room temperature (between 20 a 25 ºC), under different air-oil volume ratios and different air-oil contact surfaces (Guillen & Goicoechea, 2009). The highest increase of 4-hydroxy-2-alkenals was observed for samples stored with higher air-oil volume ratios than for those with lower air-oil volume ratios (Guillen & Goicoechea, 2009).

Surh et al. (2010) have also evaluated the concentrations of HHE and HNE depending on types of perilla and sesame oils processing (Surh, Lee, & Kwon, 2010). In the traditional process, the oil is obtained simply by roasting and pressing; industrialized oil production includes degumming, alkali-refining, bleaching, or deodorizing. No significant differences were observed for the different manufacturing processes for perilla oils. However, in sesame oils, the amounts of HNE were significantly different ($p<0.05$), being higher for the traditional method than for the industrialized one (Surh et al., 2010). Concerning the HHE

levels in sesame oils, no significant differences were also observed for the two processing methods (Surh et al., 2010). Moreover, Surh et al. (2010) have investigated the correlation between the fatty acids composition and the amounts of HNE and HHE in 73 Korean foods, including sesame oils (n=20), perilla oils (n=18), olive oils (n=3), corn oils (n=3), soybean oils (n=3), pepper seed oils (n=2), sunflower oil (n=1), safflower oil (n=1) and rice bran oil (n=1). According to their results, HNE concentration strongly and significantly correlates with amounts of n-6 ($r=0.512$, $p<0.01$) and total PUFA ($r=0.462$, $p<0.01$) (Surh et al., 2010). On the other hand, weak correlations have been observed between the concentrations of HHE and n-3 PUFA ($r=0.086$, $p=0.607$) (Surh et al., 2010).

Petersen et al. (2013) have studied the effect of 32-hours deep-frying on the levels of HNE in different vegetable oils, including sunflower, high-oleic sunflower, rapeseed, high-oleic rapeseed and palm oils. HNE content was higher in sunflower oil than in the other tested oils. Moreover, after 27-hours of deep-frying, palm oil revealed the lowest content of HNE (Petersen, Jahreis, Busch-Stockfisch, & Fritsche, 2013). The authors explanation for the results was based on the differences in fatty acids composition of the different samples as well as the differences in the presence of antioxidant compounds (Petersen et al., 2013). Hua et al. (2016) studied the impact of refining process on the levels of HHE and HNE in soybean and rapeseed oils (Hua, Zhao, Wu, & Li, 2016). Crude oils had higher amounts of these toxic compounds than the refined oils. These compounds significantly decreased during neutralization, bleaching and deodorization steps (Hua et al., 2016). Recently, palm, corn, rapeseed, linseed and camellia oils were heated at different temperatures (60 and 180 ºC) for 30 days and 5h, respectively, to evaluate the influence on HNE and HHE contents (Ma & Liu, 2017). HHE was not detected in palm, corn and camellia oils, for all tested conditions, due to the low levels of HHE precursors, namely n-3 PUFA (Ma & Liu, 2017).

4-hydroxy-2-alkenals in vegetable oils

Table 20 shows data regarding the reported amounts for HNE and HHE in several vegetable oils. Only few data are reported for HHE (Table 20). Considerable differences in HNE and HHE contents are found for samples of the same oil, which seems to be dependent on the applied processing conditions, but also due to samples origin, pre- and post-harvest conditions, variety, soil, and climate conditions.

According to the literature and considering data for HNE and HHE, the highest amounts of HNE were determined in crude oils, namely soybean (644.2 mg/kg) and rapeseed (264.4 mg/kg) oils (Hua et al., 2016). The highest amount of HHE was reported for linseed oil after 24 days of storage at 60 ºC (42.4 mg/kg) (Douny et al., 2015). For corn oil and HNE

amounts, samples from United States of America (USA) (ranging from 5.5 to 15.5 mg/kg) presented higher levels than samples from Italy (between 2.0 and 2.7 mg/kg) and China (Gabbanini, Matera, Valvassori, & Valgimigli, 2015; Han & Csallany, 2008; Ma & Liu, 2017). Furthermore, after the thermal treatment (180 ºC, 90 min) the lowest amount of HNE was observed in the samples of corn oil obtained from a local industry in China, while in these samples HHE was not detected (Ma & Liu, 2017). In all the studied samples of corn oil, from different countries, it was possible to verify that fresh samples had lower HNE amounts than those subjected to different treatments.

Soybean oil represents one of the most studied vegetable oils regarding the occurrence of HNE and HHE, probably due to its high consumption and utilization (Gabbanini et al., 2015; Han & Csallany, 2008; Hua et al., 2016; Liu, Miao, & Toyo'oka, 1996; Seppanen & Csallany, 2001, 2002, 2004, 2006). For this oil, results on HNE and HHE content from four different countries, namely USA, China, Italy and Japan, are available. Significant differences can be seen between countries; for fresh samples from USA, HNE content is 5.8 ± 1.1 mg/kg; for samples from Japan it is much lower (5.6 and 4.1 ng/kg) , while in samples from Italy HNE is not detected (Gabbanini et al., 2015; Liu et al., 1996; Seppanen & Csallany, 2004).Concerning samples from the same country, but submitted to different processing conditions, there was temperature and time dependence, and the amounts of HNE and HHE substantially decreased from crude to refined soybean oil samples (Table 20). With respect to palm oil, as far as the authors are aware, only Ma et al. (2017) analysed the HNE content in this type of oil (Ma & Liu, 2017). The lack of interest for this oil can be related to its fatty acids composition. It is mostly composed of SFA, and HNE and HHE are oxidation products of PUFA. Nonetheless, palm oil is the most produced vegetable oil around the world and is widely used in food industry. Other less common vegetable oils were also analysed with respect to 4-hydroxy-2-alkenals, namely apricot kernel oil, avocado oil, blackcurrant oil, camellia oil, echium oil, kiwiseed oil, perilla oil and wheat germ oil (Gabbanini et al., 2015; Surh & Kwon, 2003; Surh et al., 2010; Viau, Genot, Ribourg, & Meynier, 2016). In general, low amounts of HNE were reported for the aforementioned oils, with the highest values, 0.55 ± 0.02 and 0.52 ± 0.02, reported for blackcurrant and kiwi seed fresh oils, respectively (Gabbanini et al., 2015; Viau et al., 2016).

Table 20. HNE and HHE occurrence (mg/kg) in vegetable oils.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Apricot kernel oil	As purchased	Italy; grocery stores	0.05	0.00	-	-	(Gabbanini et al., 2015)
Apricot kernel oil	90 min; 75 ºC	Italy; grocery stores	0.11	0.00	-	-	(Gabbanini et al., 2015)
Avocado oil	As purchased	Italy; grocery stores	0.14	0.00	-	-	(Gabbanini et al., 2015)
Avocado oil	90 min; 75 ºC	Italy; grocery stores	0.37	0.02	-	-	(Gabbanini et al., 2015)
Blackcurrant oil	As purchased	Italy; grocery stores	0.55	0.02	-	-	(Gabbanini et al., 2015)
Blackcurrant oil	90 min; 75 ºC	Italy; grocery stores	0.80	0.03	-	-	(Gabbanini et al., 2015)
Camellia oil	As purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Camellia oil	5h; 180 ºC	China; local industry	1.40E-03	1.10E-04	nd	-	(Ma & Liu, 2017)
Colza oil	As purchased	Belgium	0.11	-	0.15	-	(Papastergiadis et al., 2014a)
Corn oil	1h; 190 ºC	USA; retail stores	5.46	-	-	-	(Han & Csallany, 2008)
Corn oil	5 min; 218 ºC	USA; retail stores	5.93	-	-	-	(Han & Csallany, 2008)
Corn oil	30 min; 218 ºC	USA; retail stores	15.48	-	-	-	(Han & Csallany, 2008)
Corn oil	As purchased	Italy; grocery stores	nd	nd	-	-	(Gabbanini et al., 2015)
Corn oil	1h; 180 ºC	Italy; grocery stores	2.02	0.10	-	-	(Gabbanini et al., 2015)
Corn oil	7h; 180 ºC	Italy; grocery stores	2.59	0.23	-	-	(Gabbanini et al., 2015)
Corn oil	3 years, room temperature	Italy; grocery stores	2.68	0.04	-	-	(Gabbanini et al., 2015)
Corn oil	As purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Corn oil	5h; 180 ºC	China; local industry	4.78E-03	1.80E-04	nd	-	(Ma & Liu, 2017)
Echium oil	As purchased	Italy; grocery stores	0.18	0.01	-	-	(Gabbanini et al., 2015)
Echium oil	90 min; 75 ºC	Italy; grocery stores	0.18	0.01	-	-	(Gabbanini et al., 2015)
Extra virgin olive oil (A)	As purchased	Belgium	0.07	-	0.16	-	(Papastergiadis et al., 2014a)
Extra virgin olive oil (B)	As purchased	Belgium	0.12	-	0.14	-	(Papastergiadis et al., 2014a)
Extra virgin olive oil (C)	As purchased	Belgium	0.06	-	0.10	-	(Papastergiadis et al., 2014a)
Extra-virgin olive oil	As purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	1h; 180 ºC	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	7h; 180 ºC	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)

Frying oil	As purchased	Belgium	3.76	0.02	0.18	0.01	(Papastergiadis et al., 2014a)
Frying oil (A)	As purchased	Korea; local market	1.968E-07	3.437E-08	-	-	(Surh & Kwon, 2003)
Frying oil (B)	As purchased	Korea; local market	2.062E-07	3.437E-08	-	-	(Surh & Kwon, 2003)
Frying oil (C)	As purchased	Korea; local market	3.437E-07	6.562E-08	-	-	(Surh & Kwon, 2003)
Frying oil ω-3 enriched	5h; 170 ºC	Belgium	3.75	0.02	0.52	0.01	(Papastergiadis et al., 2014a)
Kiwiseed oil	As purchased	France	0.52	0.02	0.17	0.02	(Viau et al., 2016)
Linseed oil	60 ºC; 12 days of storage	Belgium	63.92	-	9.44	-	(Douny et al., 2015)
Linseed oil	60 ºC; 24 days of storage	Belgium	191.76	-	42.40	-	(Douny et al., 2015)
Linseed oil	As purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Linseed oil	5h; 180 ºC	China; local industry	2.80E-03	2.30E-04	5.96E-03	2.70E-04	(Ma & Liu, 2017)
Mixed seeds oil	As purchased	Italy; grocery stores	0.05	0.00	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	1h; 180 ºC	Italy; grocery stores	1.41	0.09	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	7h; 180 ºC	Italy; grocery stores	3.47	0.11	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Olive oil	As purchased	Korea; household	2.667E-06	7.218E-07	-	-	(Surh & Kwon, 2003)
Palm oil	As purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Palm oil	5h; 180 ºC	China; local industry	1.51E-03	1.50E-04	nd	-	(Ma & Liu, 2017)
Peanut oil	As purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Peanut oil	1h; 180 ºC	Italy; grocery stores	1.11	0.02	-	-	(Gabbanini et al., 2015)
Peanut oil	7h; 180 ºC	Italy; grocery stores	2.00	0.13	-	-	(Gabbanini et al., 2015)
Peanut oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Perilla oil	As purchased	Korea; household	3.343E-07	1.875E-08	-	-	(Surh & Kwon, 2003)
Perilla oil	Traditionally produced	Korea; local shops	0.07	0.03	0.25	0.19	(Surh et al., 2010)
Perilla oil	Industrially produced	Korea; industry	0.07	0.04	0.22	0.35	(Surh et al., 2010)
Plant oils	As purchased	Belgium; supermarket chains	0.04	-	0.02	-	(Papastergiadis et al., 2014a)
Rapeseed oil	As purchased	France; local supermarket	0.08	0.02	0.19	0.06	(Viau et al., 2016)
Rapeseed oil	Crude	China; local industry	264.4	-	21.5	-	(Hua et al., 2016)
Rapeseed oil	After refining	China; local industry	37.35	-	nd	-	(Hua et al., 2016)
Rapeseed oil	As purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Rapeseed oil	5h; 180 ºC	China; local industry	2.71E-03	1.30E-04	2.23E-03	1.50E-04	(Ma & Liu, 2017)

Sesame oil	Traditionally produced	Korea; local shops	0.28	0.15	0.06	0.02	(Surh et al., 2010)
Sesame oil	Industrially produced	Korea; industry	0.10	0.09	0.08	0.05	(Surh et al., 2010)
Sesame oil	As purchased	Italy; grocery stores	0.06	0.00	-	-	(Gabbanini et al., 2015)
Sesame oil	90 min; 75 ºC	Italy; grocery stores	0.09	0.00	-	-	(Gabbanini et al., 2015)
Sesame oil (A)	As purchased	Japan	9.33E-08	4.69E-10	-	-	(Liu et al., 1996)
Sesame oil (B)	As purchased	Japan	1.23E-07	8.44E-10	-	-	(Liu et al., 1996)
Soybean oil	8h; 185 ºC	USA	2.45	0.15	0.17	0.02	(Seppanen & Csallany, 2001)
Soybean oil	6h; 180 ºC	USA; Industry	42.50	-	-	-	(Seppanen & Csallany, 2002)
Soybean oil	As purchased	USA; local grocery store	5.76	1.10	-	-	(Seppanen & Csallany, 2004)
Soybean oil	5h; 185 ºC	USA; local grocery store	4.39	0.41	-	-	(Seppanen & Csallany, 2004)
Soybean oil	1h; 185ºC	USA; local stores	2.27	0.22	0.40	0.08	(Seppanen & Csallany, 2006)
Soybean oil	1h; 185 ºC; storage 1 day	USA; local stores	2.85	0.65	0.14	0.01	(Seppanen & Csallany, 2006)
Soybean oil	2h; 185ºC	USA; local stores	2.01	0.28	1.23	0.46	(Seppanen & Csallany, 2006)
Soybean oil	2h; 185 ºC; storage 1 day	USA; local stores	1.91	0.36	0.26	0.03	(Seppanen & Csallany, 2006)
Soybean oil	3h; 185ºC	USA; local stores	2.62	0.13	0.30	0.07	(Seppanen & Csallany, 2006)
Soybean oil	3h; 185 ºC; storage 1 day	USA; local stores	3.22	0.17	0.44	0.07	(Seppanen & Csallany, 2006)
Soybean oil	4h; 185ºC	USA; local stores	3.33	0.15	0.94	0.14	(Seppanen & Csallany, 2006)
Soybean oil	4h; 185 ºC; storage 1 day	USA; local stores	3.58	0.56	0.37	0.09	(Seppanen & Csallany, 2006)
Soybean oil	1h; 190 ºC	USA; retail stores	3.73	-	-	-	(Han & Csallany, 2008)
Soybean oil	5 min; 218 ºC	USA; retail stores	8.08	-	-	-	(Han & Csallany, 2008)
Soybean oil	30 min; 218 ºC	USA; retail stores	10.72	-	-	-	(Han & Csallany, 2008)
Soybean oil	As purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Soybean oil	1h; 180 ºC	Italy; grocery stores	1.43	0.06	-	-	(Gabbanini et al., 2015)
Soybean oil	7h; 180 ºC	Italy; grocery stores	2.87	0.08	-	-	(Gabbanini et al., 2015)
Soybean oil	3 years; room temperature	Italy; grocery stores	13.32	0.09	-	-	(Gabbanini et al., 2015)
Soybean oil	Crude	China; local industry	644.2	-	16.3	-	(Hua et al., 2016)
Soybean oil	After refining	China; local industry	260.0	-	5.65	-	(Hua et al., 2016)
Soybean oil (A)	As purchased	Japan	4.06E-09	1.27E-09	-	-	(Liu et al., 1996)
Soybean oil (B)	As purchased	Japan	5.62E-09	5.47E-10	-	-	(Liu et al., 1996)
Sunflower oil	As purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Sunflower oil	1h; 180 ºC	Italy; grocery stores	1.35	0.04	-	-	(Gabbanini et al., 2015)

Sunflower oil	7h; 180 ºC	Italy; grocery stores	3.30	0.08	-	-	(Gabbanini et al., 2015)
Sunflower oil	3 years, room temperature	Italy; grocery stores	19.05	0.18	-	-	(Gabbanini et al., 2015)
Sunflower oil	As purchased	France; local supermarket	0.02	0.00	0.16	0.00	(Viau et al., 2016)
Wheat germ oil	As purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Wheat germ oil	90 min; 75 ºC	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)

nd, not detected; different letters for the same type of oil indicate that those oils were obtained from different producers/stores or brands

Mitigation strategies

Besides the study on the optimal conditions of frying temperature to reduce the occurrence of 4-hydroxy-2-alkenals, few studies reported other alternatives to inhibit the formation of these compounds in vegetable oils (Aladedunye, Matthäus, & Przybylski, 2011; Aladedunye & Przybylski, 2011; Fujisaki, Endo, & Fujimoto, 2002; Zamora, Aguilar, Granvogl, & Hidalgo, 2016; Zhu et al., 2009). Fujisaki et al. (2002) have evaluated the generation of volatile aldehydes using refined, bleached and deodorized high-oleic safflower oil (heated at 180 ºC for 30 h) by spraying water into the atmospheres with four levels of oxygen concentrations (2, 4, 10 and 20%) (Fujisaki et al., 2002). The achieved results indicated that heating oil in atmospheres with low oxygen concentrations significantly reduces the generation of volatiles (Fujisaki et al., 2002). On the other hand, Aladedunye et al. (2011) investigated whether carbon dioxide blanketing could reduce the formation and accumulation of HNE (Aladedunye et al., 2011). Therefore, canola oil was used to fry French fries for 7 days (185 ± 5 ºC, 7h/day) without and with carbon dioxide. Authors found that a reduction of 62% on HNE formation could be achieved with the use of carbon dioxide (Aladedunye et al., 2011).

Another interesting approach is to use phenolic compounds to reduce the formation of undesirable compounds during frying because these compounds increase the antioxidant capacity of the oil and retard the degradation, increasing its shelf-life and safety. Therefore, Aladedunye et al. (2011) have evaluated the ability of selected phenolic acids (ferulic, caffeic, dihydrocaffeic, gallic and vanillic acids) to improve the frying performance of commercially refined, bleached and deodorized canola oil (Aladedunye & Przybylski, 2011). According to the reported results, the HNE levels were significantly reduced by 45% when canola oil was supplemented with phenolic acids. Recently, Zamora et al. (2016) have studied the lipid-derived carbonyl trapping ability of phenolics under common food processing conditions by determining the presence of carbonyl-phenol adducts in onions fried at the laboratory, but in commercially crispy fried onions as well (Zamora et al., 2016). Authors pointed out that food polyphenols can have a possible protective role on the removal of this toxicological aldehydes produced during deep-frying.

Summary points and future trends

4-Hydroxy-2-alkenals formation is strongly dependent on temperature and time of frying, as well as directly correlated with the fatty acids composition of vegetable oils, with the most polyunsaturated fatty acids mostly susceptible to the formation of these toxic compounds;

A lack of data regarding HHE was verified in this review, possibly because vegetable oils generally have low content of n-3 polyunsaturated fatty acids, which are the precursors of HHE;

In general, crude oils present higher amounts of HNE than refined vegetable oils. This is a very important point, because one of the objectives of processing is to reduce the presence of undesirable compounds;

Significant differences in HNE and HHE amounts were observed for the same oil types of different origins, suggesting further research for other countries as well, but also the evaluation of the main causes for the differences;

Regarding mitigation strategies, up to now, few studies have focused on this matter. Therefore, it is important to develop research in this area, considering that the industry application and consumption of vegetable oils or foodstuffs containing them will increase in the future.

References

Aladedunye, F. A., Matthäus, B., & Przybylski, R. (2011). Carbon dioxide blanketing impedes the formation of 4-hydroxynonenal and acrylamide during frying. A novel procedure for HNE quantification. *European Journal of Lipid Science and Technology*, *113*(7), 916–923. https://doi.org/10.1002/ejlt.201100021

Aladedunye, F. A., & Przybylski, R. (2011). Antioxidative properties of phenolic acids and interaction with endogenous minor components during frying. *European Journal of Lipid Science and Technology*, *113*(12), 1465–1473. https://doi.org/10.1002/ejlt.201100142

Csala, M., Kardon, T., Legeza, B., Lizák, B., Mandl, J., Margittai, É., … Bánhegyi, G. (2015). On the role of 4-hydroxynonenal in health and disease. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1852*(5), 826–838. https://doi.org/10.1016/j.bbadis.2015.01.015

Douny, C., Tihon, A., Bayonnet, P., Brose, F., Degand, G., Rozet, E., … Scippo, M. L. (2015). Validation of the analytical procedure for the determination of malondialdehyde and three other aldehydes in vegetable oil using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and application to linseed oil. *Food Analytical Methods*, *8*(6), 1425–1435. https://doi.org/10.1007/s12161-014-0028-z

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, *11*(1), 81–128. https://doi.org/10.1016/0891-5849(91)90192-6

European Food Safety Authority. (2010). Scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. *EFSA Journal*, *8*(3), 1461. https://doi.org/10.2903/j.efsa.2010.1461.

FAOSTAT. (2017). Food and Agriculture Organization of the United Nations, Statistics Division, URL http://faostat3.fao.org/home/E. Accessed 07.01.18.

Food and Agriculture Organization of the United Nations. (2008). *Fats and fatty acids in human nutrition, Report of an expert consultation*. *FAO, Food and Nutrition Paper*. Rome. Retrieved from http://foris.fao.org/preview/25553-0ece4cb94ac52f9a25af77ca5cfba7a8c.pdf

Fujisaki, M., Endo, Y., & Fujimoto, K. (2002). Retardation of volatile aldehyde formation in the exhaust of frying oil by heating under low oxygen atmospheres. *JAOCS, Journal of*

the American Oil Chemists' Society, *79*(9), 909–914. https://doi.org/10.1007/s11746-002-0578-3

Gabbanini, S., Matera, R., Valvassori, A., & Valgimigli, L. (2015). Rapid liquid chromatography-tandem mass spectrometry analysis of 4-hydroxynonenal for the assessment of oxidative degradation and safety of vegetable oils. *Analytica Chimica Acta*, *869*, 50–58. https://doi.org/10.1016/j.aca.2015.02.046

Gil, L., Siems, W., Mazurek, B., Gross, J., Schroeder, P., Voss, P., & Grune, T. (2006). Age-associated analysis of oxidative stress parameters in human plasma and erythrocytes. *Free Radical Research*, *40*(5), 495–505. https://doi.org/10.1080/10715760600592962

Guéraud, F. (2017). 4-Hydroxynonenal metabolites and adducts in pre-carcinogenic conditions and cancer. *Free Radical Biology and Medicine*, *111*(January), 196–208. https://doi.org/10.1016/j.freeradbiomed.2016.12.025

Guillen, M. D., & Goicoechea, E. (2009). Oxidation of corn oil at room temperature: Primary and secondary oxidation products and determination of their concentration in the oil liquid matrix from 1H nuclear magnetic resonance data. *Food Chemistry*, *116*(1), 183–192. https://doi.org/10.1016/j.foodchem.2009.02.029

Han, I. H., & Csallany, A. S. (2008). Temperature dependence of HNE formation in vegetable oils and butter oil. *Journal of the American Oil Chemists' Society*, *85*(8), 777–782. https://doi.org/10.1007/s11746-008-1250-x

Hua, H., Zhao, X., Wu, S., & Li, G. (2016). Impact of refining on the levels of 4-hydroxy-trans-alkenals, parent and oxygenated polycyclic aromatic hydrocarbons in soybean and rapeseed oils. *Food Control*, *67*, 82–89. https://doi.org/10.1016/j.foodcont.2016.02.028

Liu, Y. M., Miao, J. R., & Toyo'oka, T. (1996). Determination of 4-hydroxy-2-nonenal by precolumn derivatization and liquid chromatography with laser fluorescence detection. *Journal of Chromatography A*, *719*(2), 450–456. https://doi.org/10.1016/0021-9673(95)00733-4

Long, E. K., & Picklo, M. J. (2010). Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: Make some room HNE... *Free Radical Biology and Medicine*, *49*(1), 1–8. https://doi.org/10.1016/j.freeradbiomed.2010.03.015

Ma, L., & Liu, G. (2017). Simultaneous Analysis of Malondialdehyde, 4-Hydroxy-2-hexenal, and 4-Hydroxy-2-nonenal in Vegetable Oil by Reversed-Phase High-Performance Liquid Chromatography. *Journal of Agricultural and Food Chemistry*, *65*(51), 11320–11328. https://doi.org/10.1021/acs.jafc.7b04566

Minihane, A. M., & Harland, J. I. (2007). Impact of oil used by the frying industry on population fat intake. *Critical Reviews in Food Science and Nutrition*, *47*(3), 287–297. https://doi.org/10.1080/10408390600737821

Papastergiadis, A., Fatouh, A., Jacxsens, L., Lachat, C., Shrestha, K., Daelman, J., … De Meulenaer, B. (2014). Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. *Food and Chemical Toxicology*, *73*, 51–58. https://doi.org/10.1016/j.fct.2014.06.030

Papastergiadis, A., Mubiru, E., Van Langenhove, H., & De Meulenaer, B. (2014). Development of a sensitive and accurate stable isotope dilution assay for the simultaneous determination of free 4-hydroxy-2-(E)-nonenal and 4-hydroxy-2-(E)-hexenal in various food matrices by gas chromatography-mass spectrometry. *Food Analytical Methods*, *7*(4), 836–843. https://doi.org/10.1007/s12161-013-9689-2

Petersen, K. D., Jahreis, G., Busch-Stockfisch, M., & Fritsche, J. (2013). Chemical and sensory assessment of deep-frying oil alternatives for the processing of French fries. *European Journal of Lipid Science and Technology*, *115*(8), 935–945. https://doi.org/10.1002/ejlt.201200375

Seppanen, C. M., & Csallany, A. S. (2001). Simultaneous determination of lipophilic aldehydes by high-performance liquid chromatography in vegetable oil. *JAOCS, Journal of the American Oil Chemists' Society*, *78*(12), 1253–1260. https://doi.org/10.1007/s11745-001-0422-9

Seppanen, C. M., & Csallany, A. S. (2002). Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature. *JAOCS, Journal of the American Oil Chemists' Society*, *79*(10), 1033–1038. https://doi.org/10.1007/s11746-002-0598-z

Seppanen, C. M., & Csallany, A. S. (2004). Incorporation of the toxic aldehyde 4-hydroxy-2-trans-nonenal into food fried in thermally oxidized soybean oil. *JAOCS, Journal of the American Oil Chemists' Society*, *81*(12), 1137–1141. https://doi.org/10.1007/s11746-004-1031-3

Seppanen, C. M., & Csallany, A. S. (2006). The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of 4-hydroxy-2-trans-nonenal and other α-, β-unsaturated hydroxyaldehydes. *Journal of the American Oil Chemists' Society*, *83*(2), 121–127. https://doi.org/10.1007/s11746-006-1184-0

Spickett, C. M. (2013). The lipid peroxidation product 4-hydroxy-2-nonenal: Advances in chemistry and analysis. *Redox Biology*, *1*(1), 145–152. https://doi.org/10.1016/j.redox.2013.01.007

Surh, J., & Kwon, H. (2003). Simultaneous determination of 4-hydroxy-2-alkenals, lipid peroxidation toxic products. *Food Additives and Contaminants*, *20*(4), 325–330. https://doi.org/10.1080/0265203031000122012

Surh, J., Lee, B. Y., & Kwon, H. (2010). Influence of fatty acids compositions and manufacturing type on the formation of 4-hydroxy-2-alkenals in food lipids. *Food Science and Biotechnology*, *19*(2), 297–303. https://doi.org/10.1007/s10068-010-0043-9

Uchida, K. (2003). 4-Hydroxy-2-nonenal: A product and mediator of oxidative stress. *Progress in Lipid Research*, *42*(4), 318–343. https://doi.org/10.1016/S0163-7827(03)00014-6

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. (2016). USDA National Nutrient Database for Standard Reference, Release 28. Nutrient Data Laboratory. URL: http://www.ars.usda.gov/ba/bhnrc/ndl. Accessed 10.01.18.

Viau, M., Genot, C., Ribourg, L., & Meynier, A. (2016). Amounts of the reactive aldehydes, malonaldehyde, 4-hydroxy-2-hexenal, and 4-hydroxy-2-nonenal in fresh and oxidized edible oils do not necessary reflect their peroxide and anisidine values. *European Journal of Lipid Science and Technology*, *118*(3), 435–444. https://doi.org/10.1002/ejlt.201500103

Zamora, R., Aguilar, I., Granvogl, M., & Hidalgo, F. J. (2016). Toxicologically Relevant Aldehydes Produced during the Frying Process Are Trapped by Food Phenolics. *Journal of Agricultural and Food Chemistry*, *64*(27), 5583–5589. https://doi.org/10.1021/acs.jafc.6b02165

Zhu, Q., Zheng, Z. P., Cheng, K. W., Wu, J. J., Zhang, S., Yun, S. T., … Wang, M. (2009). Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chemical Research in Toxicology*, *22*(10), 1721–1727. https://doi.org/10.1021/tx900221s

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3.2 4-Hydroxy-2-alkenals in foods: State-of-the-art and challenges for the future

Este sub-capítulo apresenta os resultados relativos à revisão bibliográfica sobre a ocorrência de 4-hidroxi-2-alcenais em diferentes grupos de alimentos, nomeadamente óleos e gorduras, lacticínios, carnes, peixes e outros alimentos processados. Analisa-se a influência de alguns fatores na formação destes compostos, bem como estratégias de mitigação. Verificou-se que os alimentos naturalmente ricos em ácidos gordos polinsaturados (ómega-3- e ómega-6) podem carecer de especial atenção. No entanto, o grupo de alimentos mais relevante é aquele em que estes ácidos gordos são adicionados. É de referir que os resultados indicam que, mesmo para os alimentos com os teores mais elevados, o risco de exposição é relativamente baixo.

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4-hydroxy-2-alkenals in foods: State-of-the-art and challenges for the future

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Abstract

A comprehensive review is provided on 4-hydroxy-2-alkenals in foods, namely: (i) health concerns, exposure and risk assessment; (ii) analytical methodologies, including extraction conditions and chromatographic details; (iii) factors linked with the formation and mitigation of these compounds; (iv) occurrence in oils and fats, infant formulas, milk and dairy products, fish and fish products, meat and meat products, and other foods; and (v) achievements and needs.

Undoubtedly, significant advances were performed concerning 4-hydroxy-2-alkenals research on foods, and their formation by double oxidation of polyunsaturated fatty acids. But further studies are still needed, especially on their occurrence in foods enriched with n-3 and n-6 fatty acids, as well as in foods for infants and processed foods. Major factors concerning the formation of 4-hydroxy-2-alkenals were discussed, namely the influence of fatty acids composition, time/temperature, processing conditions, salt, among others. Regarding mitigation, the most effective strategies are adding phenolic extracts to foods matrices, as well as other antioxidants, such as vitamin E. Exposure assessment studies revealed 4-hydroxy-2-alkenals values that could not be considered a risk for human health. However, these toxic compounds remain unaltered after digestion and can easily reach the systemic circulation. Therefore, it is crucial to develop *in vivo* research, with the inclusion of the colon phase, as well as, cell membranes of the intestinal epithelium. In conclusion, according to our review it is possible to eliminate or effectively decrease 4-hydroxy-2-alkenals in foods using simple and economic practices.

Introduction

Lipid oxidation is responsible for important losses across the food chain, with impact on important characteristics of foods, namely odor, flavor and texture (Guéraud et al., 2010; Vieira, Zhang, & Decker, 2017). Several compounds are formed during lipid oxidation of polyunsaturated fatty acids (PUFA), namely alkanes, ketones, alcohols, furans and

aldehydes. One of the most reactive group of lipid peroxidation products are α,β-unsaturated aldehydes (Esterbauer, Schaur, & Zollner, 1991; Guéraud et al., 2010; Guillén & Goicoechea, 2008b; Vieira et al., 2017).

4-hydroxy-2-alkenals are highly reactive compounds formed by at least three functional groups, aldehyde (CHO), alkene (C2=C3 double bound), and a group that could be a hydroxyl (4-hydroxy-2-alkenals), hydroperoxyl (-OOH), or oxo group (OH at the chiral center C4) (Guéraud et al., 2010; Guillén & Goicoechea, 2008b; Sousa, Pitt, & Spickett, 2017).

The presence of 4-hydroxy-2-alkenals in foods was early detected by Schauenstein in the 1960's (Esterbauer et al., 1991). 4-hydroxy-2-alkenals were considered as the major products from lipid peroxidation, and of these, 4-hydroxy-2-nonenal (HNE) is the most studied, followed by 4-hydroxy-2-hexenal (HHE). HNE and HHE are secondary lipid peroxidation of n-6 and n-3 PUFA, respectively (Esterbauer et al., 1991; Guéraud et al., 2010; Spickett, 2013). These fatty acids are widely and naturally present in several foods, but in the last years, due to the link of PUFA to health benefits, an increasing trend to enrich foods with these fatty acids or a high intake of food sources rich in PUFA is being observed. This behavior can increase the potential exposure to these toxic compounds. Furthermore, 4-hydroxy-2-alkenals were also found in several body tissues, organs and fluids at different concentrations (Guéraud, 2017).

In recent years, HNE and HHE were linked to several diseases, like cancer, atherogenesis, diabetes, chronic inflammation, and neurodegenerative diseases (Alzheimer's or Parkinson's diseases), among others (Csala et al., 2015; Esterbauer et al., 1991; Guéraud, 2017; Pillon et al., 2012; Sousa et al., 2017). Due to the high reactivity and biological activity of these compounds, research on their occurrence in foods has increased, especially on edible oils and fats (Guillén & Uriarte, 2012; Han & Csallany, 2008; Ma & Liu, 2017; Papastergiadis et al., 2014a; Seppanen & Csallany, 2001, 2002, 2006), as well as in foods enriched with PUFA (Meynier et al., 2014; Surh & Kwon, 2005; Surh, Lee, & Kwon, 2007). The findings indicate that there are other food matrices of interest, namely fish and fish products, and milk, infant formulas and dairy products. Other aspects that are increasing the attention of researchers are the formation mechanisms of 4-hydroxy-2-alkenals, their analytical determination (Gabbanini, Matera, Valvassori, & Valgimigli, 2015; Papastergiadis, Mubiru, Van Langenhove, & De Meulenaer, 2014b; Uchida, Gotoh, & Wada, 2002) and strategies to reduce their occurrence (Aladedunye, Matthäus, & Przybylski, 2011; Fujisaki, Endo, & Fujimoto, 2002; Zamora, Aguilar, Granvogl, & Hidalgo, 2016; Zhu et al., 2009).

Our review aims to bring an overview of the recent advances on 4-hydroxy-2-alkenals research in several food matrices, mainly focusing on the factors linked with their formation in foods, analytical methods, occurrence and mitigation strategies. In addition, health effects, exposure and risk assessment (including bioavailability and bioaccessibility data), gaps and future challenges are also discussed.

4-hydroxy-2-alkenals

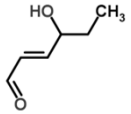
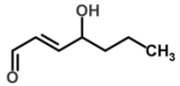
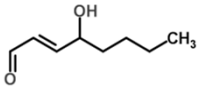
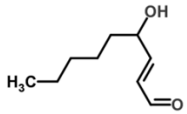
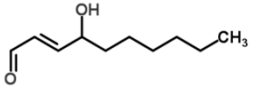
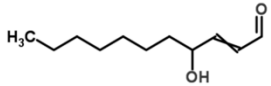
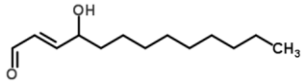
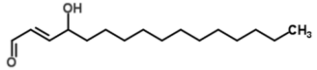
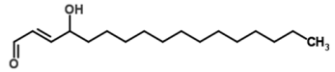
4-hydroxy-2-alkenals are formed by the double oxidation of PUFA, being the first step, the decomposition of a hydroperoxide to an alcohol, followed by the formation of a second hydroperoxide that will further undergo β-scission to fragment the fatty acids (Vieira et al., 2017). HHE is a secondary lipid peroxidation product resulting from the oxidation of docosahexaenoic (C22:6, n-3) and eicosapentaenoic acids (C22:5, n-3), while HNE is generated in the degradation process of n-6 PUFA, such as linoleic (C18:2, n-6) or arachidonic (C20:4, n-6) acids. In addition to the formation of HNE, the aforementioned fatty acids were also identified as precursors of 4-oxo-nonenal (4-HOE) which is a γ-keto aldehyde (Ayala, Muñoz, & Argüelles, 2014; Long & Picklo, 2010; Spickett, 2013). The chemical and physical properties of 4-hydroxy-2-alkenals is shown in Table 21.

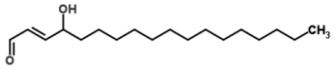
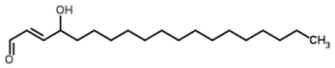
Among the referred 4-hydroxy-2-alkenals, HNE is the most studied because it easily modifies and causes crosslinking of proteins, resulting in toxic effects. Schiff base formation and Michael addition are the most common chemical reactions leading to the formation of protein adducts. These protein adducts are associated with several cytotoxic consequences, including disruption of cell signaling, inhibition of enzyme activity, change in tertiary structure, altered gene regulation, mitochondrial dysfunction and loss of cytoskeletal formation (Guéraud et al., 2010).

Health concerns, exposure and risk assessment

Under oxidative stress conditions 4-hydroxy-2-alkenals can be endogenously formed. As mentioned before, these compounds are highly reactive, being able to produce adducts and instigating dysfunctions, as well as to inhibit enzymatic activities and protein synthesis, among others (Guillén & Goicoechea, 2008b). Cancer, Alzheimer, Parkinson's disease, chronic inflammation, atherogenesis, and diabetes, are examples of diseases that were

Table 21. Chemical and physical properties of 4-hydroxy-2-alkenals.

Compound	Molecular Formula	Molecular weight	Chemical structure	CAS registry number	Density	Boiling point	Vapor pressure
4-hydroxy-2-hexenal	$C_6H_{10}O_2$	114.143		17427-08-6	1.0 ± 0.1 g/cm^3	233.5 ± 23.0 ºC at 760 mmHg	0.0 ± 1.0 mmHg at 25 ºC
4-hydroxy-2-heptenal	$C_7H_{12}O_2$	128.17		17427-09-7	1.0 ± 0.1 g/cm^3	243.3 ± 23.0 ºC at 760 mmHg	0.0 ± 1.0 mmHg at 25 ºC
4-hydroxy-2-octenal	$C_8H_{14}O_2$	142.197		17449-15-9	1.0 ± 0.1 g/cm^3	259.4 ± 23.0 ºC at 760 mmHg	0.0 ± 1.2 mmHg at 25 ºC
4-hydroxy-2-nonenal	$C_9H_{16}O_2$	156.223		29343-52-0	0.9 ± 0.1 g/cm^3	275.6 ± 23.0 ºC at 760 mmHg	0.0 ± 1.3 mmHg at 25 ºC
4-hydroxy-2-decenal	$C_{10}H_{18}O_2$	170.25		29389-17-1	0.9 ± 0.1 g/cm^3	291.8 ± 23.0 ºC at 760 mmHg	0.0 ± 1.4 mmHg at 25 ºC
4-hydroxy-2-undecenal	$C_{11}H_{20}O_2$	184.277		29343-58-6	0.9 ± 0.1 g/cm^3	307.7 ± 25.0 ºC at 760 mmHg	0.0 ± 1.5 mmHg at 25 ºC
4-hydroxy-2-tridecenal	$C_{13}H_{24}O_2$	212.331		29343-62-2	0.9 ± 0.1 g/cm^3	338.9 ± 25.0 ºC at 760 mmHg	0.0 ± 1.7 mmHg at 25 ºC
4-hydroxy-2-hexadecenal	$C_{16}H_{30}O_2$	254.411		142449-98-7	0.9 ± 0.1 g/cm^3	383.5 ± 25.0 ºC at 760 mmHg	0.0 ± 2.0 mmHg at 25 ºC
4-hydroxy-2-heptadecenal	$C_{17}H_{32}O_2$	268.438		142449-99-8	0.9 ± 0.1 g/cm^3	397.8 ± 25.0 ºC at 760 mmHg	0.0 ± 2.1 mmHg at 25 ºC

4-hydroxy-2-octadecenal	$C_{18}H_{34}O_2$	282.465		142450-00-8	0.9 ± 0.1 g/cm^3	411.8 ± 28.0 ºC at 760 mmHg / 0.0 ± 2.2 mmHg at 25 ºC
4-hydroxy-2-nonadecenal	$C_{19}H_{36}O_2$	296.491		142450-01-9	0.9 ± 0.1 g/cm^3	425.5 ± 28.0 ºC at 760 mmHg / 0.0 ± 2.3 mmHg at 25 ºC

related with the presence of these hazardous compounds (Csala et al., 2015; Esterbauer et al., 1991; Guéraud et al., 2010; Guéraud, 2017; Guillén & Goicoechea, 2008b; Pillon et al., 2012; Spickett, 2013; Vieira et al., 2017; Zarkovic, 2003; Zhong & Yin, 2015). HNE has a higher genotoxic potential than HHE and other similar compounds (Eckl & Bresgen, 2017). Different concentrations of HNE have been linked with several effects, namely irreversible damage in Ehrlich ascites tumor cells (10-20 μM); DNA fragmentation (≥60 μM); and reduction of CHO and V79 cells survival (≥10 μM) (Csala et al., 2015; Eckl & Bresgen, 2017).

The hypothesis that these compounds can be absorbed from the diet was not clear, until studies with animals confirmed that after HNE oral administration, it is absorbed and detected in rat tissues (Kanazawa & Ashida, 1998; Oarada, Miyazawa, & Kaneda, 1986). Concerning humans, it is known that these compounds remained unaltered after digestion, being available to be absorbed and reach the systemic circulation. Nevertheless, there is a lack of studies concerning this subject, especially with respect to the inclusion of the colon phase, as well

as, cell membranes of the intestinal epithelium (Steppeler, Haugen, Rødbotten, & Kirkhus, 2016). So far, to the best of our knowledge, a safe dose for these aldehydes has not been established. Although, following the recommendations of the European Food Safety Authority, Papastergiadis et al. (2014a) have established a Threshold of Toxicological Concern (TTC) for HNE and HHE of 1.5 µg/kg of body weight/day (European Food Safety Authority, 2012). According to European Food Safety Authority, "*The TTC approach is applicable to substances for which the chemical structure is known but for which there are few or no relevant toxicity data; and for application of the TTC approach it is essential to have exposure assessments that take account of high exposure scenarios, and, where possible, take account of exposure from all routes and sources*" (European Food Safety Authority, 2012).

To estimate the exposure and/or risk assessment of hazard compounds, it is necessary to have reliable data on the occurrence of these compounds and their consumption (Alexander et al., 2012). Although, nowadays, it is also assumed that it is necessary to go a step forward and have data on the bioaccessibility and if possible bioavailability, which in general are obtained after *in vitro* digestion of foods. This approach aims to simulate physiological conditions *in vivo* and normally include oral, gastric and small intestine phases, being less frequent to include large intestinal fermentation (Minekus et al., 2014). As said before, 4-hydroxy-2-alkenals are highly reactive compounds and it is of utmost importance to evaluate their presence in foods, but also to increase knowledge on the concentration that will be available after digestion to be released to the intestinal lumen. Most of the time, compound bioaccessibility from its matrix is used to estimate its maximum oral bioavailability because it is difficult to evaluate the bioavailability of compounds (Encarnación Goicoechea et al., 2008).

Exposure assessment studies regarding HNE and HHE, for Korean and Belgium foods, were carried out by Surh and Kwon (2005) and Papastergiadis et al. (2014a). According to Surh and Kwon, the total exposure of 4-hydroxy-2-alkenals from vegetable oils, fish, shellfish and fried foods could be 16.1 µg/day, which corresponds to 0.3 µg/kg of body weight/day for an adult with approximately 60 kg of body weight (Surh & Kwon, 2005). Consequently, authors have concluded that the value found could not be considered a risk for human health. The results reported by Papastergiadis et al. are in agreement with Surh and Know, except for cured minced meat products, for which consumers can be exposed to HNE levels above 1.5 µg/kg of body weight/day, meaning that the exposure risk may occur if consumers frequently eat this specific category of food (Papastergiadis et al., 2014a).

Goicoechea et al. (2008) have submitted sunflower oil and thermally oxidized sunflower oil to an *in vitro* digestion model to evaluate if α,β-unsaturated aldehydes, like HNE and HHE, could reach the systemic circulation after ingestion or if these compounds react with other components of food and enzymes. After *in vitro* digestion, author findings indicate that a significant amount of these toxic compounds persists after digestion being available to be absorbed in the gastrointestinal tract, and therefore, they can easily reach the systemic circulation. Later, the same group of researchers have submitted an infant formula with thermodegraded vegetable oil to *in vitro* digestion and similar results were obtained. After the digestion of foods, the highest percentage of 4-hydroxy-2-alkenals was in the lipidic phase (82.2 and 96.6% for HNE and HHE, respectively) (Goicoechea, Brandon, Blokland, & Guillen, 2011).

Besides this type of matrices, other studies have applied *in vitro* digestion to cooked beef, pork, chicken, salmon and herring to evaluate the amounts of 4-hydroxy-2-alkenals (Larsson, Harrysson, Havenaar, Alminger, & Undeland, 2016; Steppeler et al., 2016). Accordingly with the findings reported by Steppeler et al. higher amounts of HHE were found for salmon than for minced beef after digestion, especially in the intestinal phase. Nevertheless, the amounts of HNE and HHE before *in vitro* digestion were higher for minced beef than for salmon. Furthermore, the levels of HNE and HHE for salmon were similar in the gastric phase, but were significantly different in the intestinal phase, being the content of HHE approximately three times higher in the intestinal phase than in the gastric. Some reasons were appointed for the different results, namely the content of pro- and antioxidants, salt, muscle type, fatty acids composition and oxygen availability. Furthermore, in what concerns the differences between the digestion phases, authors indicate that the high amounts of HHE in the intestinal phase, are probably due to the emulsifying nature of bile that leads to an increased lipid droplet surface area susceptible to lipid oxidation (Steppeler et al., 2016).

Baltic herring (17% and 4% fat) and farmed salmon with high levels of astaxanthin were investigated regarding the potential health risks of the formed oxidation products during digestion, before and after oven baking (Larsson et al., 2016). After digestion HHE content was higher for raw herring than for raw salmon (3.8-fold and 33-times, in the gastric and intestinal phases, respectively). This is possible due to the higher portion of dark muscle and larger quantity of n-3 PUFA in herring in comparison with salmon. In what concerns the effects of oven baking, higher concentrations of HHE were reported for baked salmon than for raw. On the other hand, for herring (4% fat), the amounts were reduced by approximately 45% after cooking. After gastric digestion, HHE levels were higher for both cooked products than for raw (Larsson et al., 2016). Concerning the intestinal lumen, higher quantities of

HHE were reported for baked salmon than for raw, while for herring the amounts remain similar. Although there are some inconsistency within this study and in comparison with other studies, these results herein show that higher quantities of HHE will be available after digestion, especially in the intestines, for cooked salmon than for herring.

Analytical determination

For several years, great attention has been devoted to the analysis of 4-hydroxy-2-alkenals. In the early years, the studies focused on the development of analytical methods to evaluate 4-hydroxy-2-alkenals in biological samples, being HNE the mostly determined compound. Therefore, up to now there is not a standardized methodology for the simultaneous separation, identification and quantification of 4-hydroxy-2-alkenals in foods, especially in complex food matrices, allowing a good recovery. The selection of the method depends on the study objective, sample nature and the compounds under evaluation. A literature review on the applied sample preparation and extraction conditions, as well as the chromatographic settings are shown in Table 22.

Extraction conditions

Most of the analytical methods reported in the literature involve several extraction steps, which sometimes can induce important losses, leading to an underestimation of the actual amount present in the sample (Papastergiadis et al., 2014b). Moreover, most of the extraction techniques are time consuming, including several extraction steps, and involve the use of large amounts of organic solvents, which is not acceptable especially for routine laboratories since it increases the analysis costs and the results are needed in due time (Gabbanini et al., 2015). Furthermore, nowadays there is an increase in the search for environmentally friendly methodologies.

Matera et al. (2012) have studied different derivatization reagents because according to their findings, most of the time, the conversion of HNE into the desired imine, oxime, or hydrazine is generally incomplete, mainly due to the formation of other compounds. 2-(trifluoromethyl)-phenylhydrazine and 2,3,4,5,6-pentafluorophenylhydrazine were considered the best derivatization reagents due to their stability and favorable UV absorbance (Matera, Gabbanini, Valvassori, Triquigneaux, & Valgimigli, 2012).

Table 22. Sample preparation, extraction and chromatographic conditions applied for the determination of 4-hydroy-2-alkenals in several food matrices.

Food matrices	Samples preparation/ Extraction	Compounds	Chromatographic conditions	Validation data	References
Minced pork and beef; Fish; smoked fish	5 g of sample was mixed with 25 mg of BHT; add 50 mL of 1 M HCl containing 2.5 mmol DNPH to the sample (keep it in the dark for 2 h at 4 ºC); add 150 mL of dichloromethane; evaporated and redissolved in 0.2 mL of chloroform; DNPH derivatives were separated into a silica gel column by washing with n-hexane/chloroform (2:1 v/v) and eluted with chloroform; then, chloroform was evaporated, and the residue was redissolved in 0.5 mL of methanol	HNE	System: HPLC-UV-vis Column: Ultrasphere C18 (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase (gradient elution): (A) sodium citrate (30 mM); (B) acetate buffer (pH 4.75) Flow rate: 1 mL/min Injection volume: - Column temperature: 40 ºC Detection (λ): 365 nm	-	(Sakai et al., 1995, 1997, 2000, 2004, 2006; Sakai & Kuwazuru, 1995)
Soybean and sesame oils	0.25 - 1.0 g of oil samples and 1.0 mL of the freshly prepared solution (0.25%, w/v) trichloroacetic acid, 150 pg/mL BHT and 0.5 mM 7-nitro-2,1,3-benzoxadiazole in MeOH-H_2O (8:2, v/v)); vortex for 1 min and then heated at 60 °C for 10 min; cooled on ice and centrifuged at 3000 *g* for 10 min at 5°C; inject the supernatant	HNE	System: HPLC-Fluorescence Column: Inertsil ODS-80A (150 x 4.6 mm i.d., 5.0 µm particle size) Run time: 40 min Mobile phase (gradient elution): (A) MeOH; (B) CH_3CN Flow rate: 1 mL/min Injection volume: 5 µL Column temperature: Emission (λ): 540 nm Excitation (λ): 490 nm	Range: 0-250 nM; LOD: 10 fmol	(Liu et al., 1996)
Soybean seeds	-	HNE	System: GC-MS Column: SPB-1 (30 m x 0.32 mm i.d. x 0.25 µm film thickness) Run time: - Mobile phase: 160 to 200 ºC at 4 ºC/min followed by 200 to 300 ºC at 10 ºC/min Flow rate: 2mL/min Injection mode: - Injection volume: - Injector temperature: 260ºC Detector temperature: 310 ºC Ion source temperature: - Ionization energy: -	-	(Takamura & Gardner, 1996)
Eggs	1 g of egg yolk with 1 mL of DNPH solution (1.8 mM in 1 M HCl) in presence of BHT in final concentration of 10 mM; keep it for 2 h in the dark and for 1 h in an	HNE	System: HPLC-UV-vis Column: Supelcosil (150 x 4.0 mm i.d., 5.0 µm particle size)	-	(Grune et al., 2001)

	ice bath; extract three times with 4 mL of dichloromethane; centrifuge at 900 ×*g*, and evaporated at 35°C; transfer for vials with 1.0 mL of dichloromethane into small vials; extract three times with 5 mL each of methanol and evaporate to dryness; dissolve the residue with 1 mL of methanol		Run time: - Mobile phase: MeOH/H_2O (80:20, v/v) Flow rate: 1 mL/min Injection volume: - Column temperature: - Detection (λ): -		
Beef	Samples were immediately reacted with an equal volume of DNPH solution (50 mL of DNPH in 100 mL of HCl (1 N)); keep it in the dark for 2h at room temperature; extract three times with dichlorometane; evaporate and redissolve in 200 µl of chloroform; sample was applied to a TLC plate; elute three times with methanol; evaporate and redissolve in 200 µl of methanol	HNE	System: HPLC-UV-vis Column: Spherical C18 (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase (gradient elution): (A) H_2O/ACN/THF (60/30/10, v/v/v); (B) H_2O/ACN (20/80, v/v) Flow rate: 1 mL/min Injection volume: 35 µL Column temperature: - Detection (λ): 378 nm	-	(Lynch et al., 2001)
Soybean oil	3 g of sample reacted with 6 mL of DNPH solution; incubate overnight at room temperature in the dark with shaking at ~120 rpm; extract three times with 10 mL HPLC-grade methanol/water (75:25, v/v); separate by centrifugation at 1360 × *g* for 10 min; extract three times with 10 mL dichloromethane and separated by centrifugation; dichloromethane was evaporated until the sample size was ~1 mL; aliquots (500 µL) of the DNPH derivatives were applied to silica gel TLC plates ; combined methanol extracts were centrifuged at 1360 × *g* for 15 min; supernatant fractions were concentrated to 2.0 mL	HNE; HHE; HOE	System: HPLC-UV-vis Column: - Run time: 40 min Mobile phase: MeOH/H_2O (75:25, v/v) Flow rate: 0.8 mL/min Injection volume: - Column temperature: - Detection (λ): 378 nm	LOD: 1 ng	(Seppanen & Csallany, 2001)
Soybean oil	2 g of sample reacted overnight at room temperature with 5 mL of DNPH reagent (10 mg recrystallized DNPH with 20 mL 1 N HCl); extract first with methanol/water (75:25 v/v) (3 × 10 mL) and then dichloromethane (3 × 10 mL); elute from the TLC plates with methanol (3 × 10 mL); and evaporate to 1 mL	HNE; HHE; HOE	System: HPLC-UV-vis Column: Ultrasphere ODS C18 (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: 45 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: 0.8 mL/min Injection volume: 100 µL Column temperature: - Detection (λ): 378 nm	-	(Seppanen & Csallany, 2002, 2004, 2006)
		HNE	System: HPLC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness)		

			Run time: 10 min Oven ramp: 100 to 230 ºC at a rate of 35 ºC/min, followed by 10 min hold at 230 ºC; then increase 50 ºC/min until 300 ºC Carrier gas: Helium Flow rate: 1 mL/min Injection mode: splitless Injector temperature: - Detector temperature: - Ion source temperature: - Ionization energy: - Range m/z: -		
Soybean oil and porcine liver	100 mg of sample was added to an HNE-methanol solution (2.91 nmol and 0.29 nmol) in 1% volume; methanol was removed from the oil sample under a nitrogen stream, and the sample was then extracted twice with 2 mL of distilled water containing 0.1% BHT; extracts were combined and centrifuged at 3,000 × *g* for 10 min; extracts from oil samples were combined and immediately reacted with an equal volume of DNPH reagent (3.5 mg of DNPH dissolved in 10 mL of 1 M HCl); store in the dark at room temperature for 2 h; after the reaction, the pH of the reaction mixture was adjusted to 7.0 with 1 N NaOH and made to 10 mL with NaCl solution; 1 g of porcine liver sample was added to an HNE-methanol solution (2.91 nmol and 0.29 nmol); sample was homogenized with 2 mL of methanol containing 0.1% BHT for 2 min; the homogenate was centrifuged at 3000 × *g* for 10 min, and the supernatant was collected; the residue was re-extracted by the same procedure, and the supernatant was collected; the supernatants were combined and made to 10 mL with NaCl solution	HNE	System: HPLC-UV-vis Column: ODS (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase: ACN/H_2O (40 :60, v/v) Flow rate: - Injection volume: - Column temperature: - Detection (λ): 223 nm	Range: 0-0.8 nmol/mL; r^2: 0.9938	(Uchida et al., 2002)
Pork products	10 g of sample were homogenized in 20 mL of distilled water by an Ultra-Turrax ; homogenate was centrifuged for 10 min at 5000 rpm and 4 °C; upper liquid phase was filtered by a filter paper, and the residue was further homogenized in 20 mL of distilled water; centrifugation at 5000 rpm and 4 °C for 10 min; the joint upper filtered phases (≈35 mL) were loaded on an SPE C18 end-capped cartridge	HNE	System: HPLC-MS Column: LiCrospher (250 x 4.0 mm i.d., 5.0 µm particle size) Run time: - Mobile phase: MeOH (0.1% formic acid) Flow rate: 0.8 mL/min Injection volume: - Column temperature: -	Range: 0.1-10 mg/kg; LOD: 0.005 mg/L	(Zanardi et al., 2002)

	previously equilibrated with 3 mL of methanol and 3 mL of distilled water; residual lipid substances were eluted by 15 mL of petroleum ether; residual water was eliminated by anhydrous sodium sulphate; after filtration and evaporation to dryness by a steam of nitrogen, the sample was dissolved in 1 mL of methanol acidified by 0.1% formic acid and filtered through a 0.45 ím PTFE filter		Ionization mode: - Detection mode: - Range m/z: - Source temperature: -		
Ham, bacon and sausage	Ground meat (5 g) was mixed with 25 mg BHT; HCl (50 mL of 1 mol/L) containing 2.5 mmol DNPH was added to the BHT mixture; reaction was carried out in the dark for 2 h at room temperature; DNPH derivatives were extracted three times with 150 mL dichloromethane; dichloromethane extract was evaporated and the residue was redissolved in 0.2 mL chloroform; the solution was applied to a disposable silica gel extraction column, which had been pre-equilibrated with n-hexane/chloroform (2:1 v/v); the resulting chloroform eluate was evaporated in vacuum and the residue was redissolved in 0.5 mL methanol	HNE	System: HPLC-UV-vis Column: Ultrasphere C18 (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase (gradient elution): (A) sodium citrate (30 mM); (B) acetate buffer (27.7 mM, pH 4.75) Flow rate: 1 mL/min Injection volume: - Column temperature: 40 ºC Detection (λ): 365 nm	-	(Munasinghe et al., 2003)
Olive and perilla oils	The oil sample (1 g) was extracted twice with 10 mL distilled water containing 0.1% of BHT; each extraction was performed on a horizontal shaker at 150 rpm for 20 min; the combined aqueous phases were centrifuged at 3000 g for 20 min; the aqueous phase was subjected to further extraction with 20 mL chloroform three times; the chloroform extract was concentrated to 1 mL in a rotary evaporator below 30 ºC at reduced pressure and concentrated again using a gentle stream of nitrogen.	HOE, HNE and HHE	System: GC-MS Column: FFAP (30 m x 0.20 mm i.d. x 0.33 µm film thickness) Run time: 25 min Oven ramp: 120 ºC (hold 10 min), then increased to 220 ºC at a rate of 5 ºC/min Carrier gas: Helium Flow rate: 0.7 mL/min Injection mode: - Injection volume: - Injector temperature: - Detector temperature: - Ion source temperature: 230 ºC Ionization energy: 70 eV Range m/z: -	-	(Surh & Kwon, 2003)
Vegetable oils, fish, bivalve molluscs, infant formulas, raw cereal,	BHT (0.01 g per sample) was added to 5 g of the homogenized sample, and then 10 mL distilled water were added; the homogenate was centrifuged at 5000 x *g* (4 ºC for 10 min); the aqueous phase was filtered; the residue was further centrifuged in 10 mL distilled water; after the second centrifugation, the combined aqueous filtrates were applied to SPE C18	HHE; HNE	System: GC-MS Column: SPB-5 (50 m x 0.20 mm i.d. x 0.33 µm film thickness) Run time: 20 min Oven ramp: 120 ºC (hold 5 min), increased at a rate of 5 ºC/min to 160 ºC and then 15 ºC/min to 200 ºC (hold 5 min)	**HHE**: LOD (5 µg/kg); LOQ (9 µg/kg) **HNE**: LOD (3 µg/kg); LOQ (5 µg/kg)	(Surh & Kwon, 2005; Surh et al., 2010, 2007)

vegetable powder, snacks, fish sticks, tuna cans, and baby foods	cartridge pre-equilibrated with 3 mL methanol followed by 3 mL distilled water; after loading the sample to the SPE cartridge, residual lipid substances were washed off by 15 mL petroleum ether; 4-hydroxy-2-alkenals were eluted using 2 mL of methanol; evaporation to dryness and redissolved in 500 mL acetonitrile; BSTFA (200 mL) was added and the mixture was heated at 70 ºC for 15 min for derivatization		Carrier gas: Helium Flow rate: 0.7 mL/min Injection mode: split Injection volume: 2 µL Injector temperature: 210 ºC Detector temperature: 220 ºC Ion source temperature: 230 ºC Ionization energy: 70 eV Range m/z: -		
Chicken meat, beef meat, liver pâté, blood sausage	Samples (1–2 g) were homogenized in 30 ml water with an Ultra-Turrax for 2 min and then centrifuged (10,000 rpm/10 min); the residue was homogenized and centrifuged again; the supernatants were pooled and lipophilic compounds were extracted twice with 60 ml of dichloromethane; organic phases were evaporated under reduced pressure; the extract was defatted using a partition between 16 ml isooctane saturated acetonitrile and 4 ml acetonitrile saturated isooctane; acetonitrile was evaporated under reduced pressure and the dry extract containing HNE was dissolved in 500 µL acetonitrile/water (50:50, v/v)	HNE	System: HPLC-UV-vis Column: ODS2 Spherisorb (100 x 4.6 mm i.d., 5.0 µm particle size) Run time: - Mobile phase: ACN/H_2O (50:50, v/v) Flow rate: 1 mL/min Injection volume: - Column temperature: - Detection (λ): 221 nm	-	(Gasc et al., 2007)
Butter, corn and soybean oils	1 g of sample was reacted overnight at room temperature with 5 ml freshly prepared DNPH reagent; the DNPH derivatives were extracted with 10 ml of methanol/water (75:25, v/v) from the oil repeat two more times and the methanol: water extracts were combined; re-extracted with 10 ml of dichloromethane three times from the combined methanol/water extracts and concentrate to about 1 ml; apply to two TLC plates; extract three times with 10 ml of methanol; evaporate to the exact volume of 1 ml	HNE	System: HPLC-UV-vis Column: Ultrasphere ODS (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: 35 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: - Injection volume: - Column temperature: - Detection (λ): 378 nm	-	(Han & Csallany, 2008)
Mackerel	1 g of sample was shaken with 20 ml of deionised water containing 1 mL/L of BHT; repeat three times; 30 ml of sample was collected and centrifuged at 5000 × *g* for 5 min; aqueous layer was applied onto a disposable ODS column conditioned and equilibrated with 3 mL of methanol and 3 ml of deionized water; petroleum ether (15 mL) was added; 4-HNE was eluted with 2 mL of methanol; evaporate off the petroleum ether and redissolve the dry sample in 1 mL of methanol acidified	HNE	System: HPLC-UV-vis-MS Column: Spherisorb S5ODS2 (250 x 4.6 mm i.d., 5 µm particle size) Run time: 10 min Mobile phase: ACN/H_2O (40:60, v/v) Flow rate: 1.0 mL/min Injection volume: 50 µL Column temperature: - Detection (λ): 220 nm Ionization mode: electrospray	Range: 0.1-5.0 mg/kg; $r^{2:}$ 0.9868	(Alghazeer & Howell, 2008)

			Detection mode: positive Range m/z:- Source temperature: -		
Deep-frying fat, sunflower oil	Extraction of the volatile and semivolatile components of the headspace of the oil and fat samples (5 g in a 20 mL vial) was accomplished automatically using a CombiPAL autosampler; the fiber used was coated with DVB/CAR/PDMS (50/30 µm film thickness, 1 cm long), which was inserted into the headspace of the sample and was maintained for 60 min (50 °C, 250 rpm)	HNE	System: GC-MS Column: VF-1MS (60 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: - Oven ramp: 50 ºC (5 min hold), increased to 290 ºC at 4 ºC/min (hold 30 min) Carrier gas: Helium Injection mode: splitless Injector temperature: - Detector temperature: - Ion source temperature: -	-	(Encarnación Goicoechea et al., 2008)
Infant formulas and human milk	500 µl of milk or 200 mg of powders was spiked with 15–20 ng of both internal deuterated standards; 50 µl citric acid (0.15 M) and 1 mL of O-pentafluorobenzyl hydroxylamine (50% in PIPES buffer, pH 6.5) were added; after vortexing, the reaction took place under nitrogen for 30 min at room temperature; the following reagents were then successively added, with vortexing for 1 min between each addition: methanol (2.5 mL), hexane (5 mL), and 98% H_2SO_4 (60 µl); after centrifugation at 1800 rpm for 5 min, upper phases were transferred into 4 mL conical glass tubes; after evaporation under nitrogen, 100 µl of BSTFA was added; the reaction took place at 60 ºC for 30 min, after which samples were transferred into vials for injection	HNE; HHE	System: GC-MS Column: HP-1MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: - Oven ramp: 2 min at 57 ºC, then increased to 180 ºC at 20 ºC/min, followed by an increase to 280 ºC at 4 ºC/min Carrier gas: Helium Flow rate: 1 mL/min Injection mode: splitless Injector temperature: 260 ºC Detector temperature: - Ion source temperature: 130 ºC Ionization energy: 70 eV Range m/z: 50-800	-	(Michalski et al., 2008)
Cod liver oil	Vials containing 1 g of oil were introduced into a water bath (50 ºC); sample equilibration (15 min); a fiber coated with DVB/CAR/PDMS (50/30 µm film thickness), was inserted into the headspace of the sample and was maintained for 60 min	HHE	System: GC-MS Column: HP-5MS (60 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: - Oven ramp: 50 ºC (5 min hold), increased to 280 ºC at 4 ºC/min (hold 2 min) Carrier gas: Helium Injection mode: splitless Injector temperature: 220 ºC Detector temperature: 280 ºC Ion source temperature: 130 ºC	-	(Guillén & Goicoechea, 2009)

Fatty acids methyl esters; Mozzarella cheeses	1 g of FAMEs was reacted overnight at room temperature with 5 ml DNPH; DNPH derivatives were extracted with 10 ml of methanol/water (75:25, v/v); repeat two more times and combine the methanol/water extracts; DNPH derivatives were re-extracted with 10 ml of dichloromethane three times from the combined methanol/water extracts; concentrate to about 1 ml; apply on two TLC plates and developed in dichloromethane; extract three times with 10 mL of methanol; combine methanol extracts and centrifuge at 1,360 x *g* for 15 min; supernatant fractions were concentrated to the exact volume of 1 mL; 100 g of cheese and 200 ml of hexane; blend for 10 min; filtration; 10 ml of hexane extracts were reacted overnight with 5 mL DNPH at room temperature; .DNPH derivatives were extracted with 10 mL of methanol:/water (75:25, v/v); repeat two more times and combine the extracts; extract four times with 10 mL of dichloromethane; evaporate to about 1 mL the combined dichloromethane extracts	HNE; HHE; HDE; HOE	System: HPLC-UV-vis Column: Ultrasphere ODS (250 x 4.6 mm i.d., 5.0 µm particle size) Run time: 40 min Mobile phase (gradient elution): (A) MeOH; (B) H_2O Flow rate: - Injection volume: - Column temperature: - Detection (λ): 378 nm	-	(Han & Csallany, 2009, 2012)
Corn-soy oil blend	1 g of oil with 5.0 µl of a 0.606 µg/µL solution of $^2H_2$-HNE in methanol (*3 ppm in the oil) was added; vortex mixed for 1 min; HNE was extracted two times (10 mL of distilled water; shaken for 20 min; and centrifuge for 15 min at 3000 x *g*; pool the aqueous layers and mix with 1 mL of a methanolic solution containing 25 mg/mL PFBHA and 0.1% (w/v) BHT, and sonicate for 1 h; HNE-oxime was then extracted into pentane (2 x 10 mL); pentane extract was evaporated to dryness at 40 ºC; 200 µl of 10% TMCS in BSTFA was added; mixture was heated at 90 ºC for 1 h; 1.0 ml of dichloromethane was added	HNE	System: GC-MS Column: Sac-5 (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: 35 min Oven ramp: 40 ºC (5 min); increase to 300 ºC at a rate of 10 ºC/min (hold 20 min) Carrier gas: Helium Flow rate: 1 mL/min Injection mode: - Injector temperature: - Detector temperature: - Ion source temperature: 250 ºC Ionization energy: 70 eV	-	(LaFond et al., 2011)
Canola oil	Novel procedure: 200 mg of oil in isooctane was loaded into column with conditioned to 5% water silica gel; non-polar fraction was eluted with 10 mL of 15% diisopropyl ether in hexane; polar fraction was eluted with four 5 mL portions of methanol; combined polar fractions were subsequently concentrated to the volume of 5 mL; then centrifuged	HNE	System: HPLC-UV-Vis Column: C18 Novapak (300 x 3.9 mm, i.d., 4 µm particle size) Run time: 35 min Mobile phase: ACN/H_2O (30:70, v/v) Flow rate: 0.75 mL/min Injection volume: 20 µL Column temperature: -	-	(Aladedunye et al., 2011; Aladedunye & Przybylski, 2011, 2012)

	at 2300 rpm for 5 min, and the supernatant analyzed for HNE DNPH method: 1 g of oil was mixed with 2 mL of DNPH and incubated overnight at room temperature in the dark; the oil was extracted three times with 5 mL methanol/water (75:25, v/v); centrifugation at 2500 rpm for 10 min; extract three times with dichloromethane (5 mL each); centrifuge and evaporate the dichloromethane extract until the sample size 1 mL; the sample was separated on a silica gel TLC plate using dichloromethane; the band corresponding to HNE–DNPH was extracted three times with methanol (10 mL each); the combined methanol extract was centrifuged at 2500 rpm for 10 min; the supernatant was concentrated to 2.0 mL Solvent extraction: 200 mg of sample dissolved in 2 mL isooctane; homogenized with 5 mL of methanol/water (8:2, v/v); extraction was repeated three times and the combined extract was centrifuged at 2500 rpm for 5 min; sample volume was reduced to 5 mL; solution was centrifuged, and the supernatant was analyzed		Detection (λ): 223 nm		
Sunflower, high-oleic sunflower, rapeseed, and high-oleic rapeseed oils; palm olein and French fries	5 g of oil in 10 mL glass headspace vials; fully automated extraction using a GERSTEL MPS-2 autosampler configured for auto-DHS injection; after each oil had incubated in the sample bottle for 5 min at 120 °C, dynamic sampling was performed by connecting a tube to the outlet of the sample bottle and applying a flow of nitrogen at 50 mL/min for 30 min; the desorption of the trapped volatile compounds was carried out for 15 min using a Gerstel thermal desorption unit-2 thermal desorption unit mounted on top of the CIS-3 PTV injector; for all experiments, desorption was in split mode (split ratio 1:10) using helium at a flow rate of 15 ml/min; the thermal desorption unit-2 was programmed from 40 to 300 °C at 720 °C/min with a final time of 5 min	HNE	System: GC-MS Column: VF-624MS (30 m x 0.18 mm i.d. x 0.32 µm film thickness) Run time: - Oven ramp: 60 ºC (1 min), then 5º C/min to 90 ºC and 30 ºC/min to 240 ºC Carrier gas: Helium Flow rate: 1.5 mL/min Injection mode: split Injector temperature: - Detector temperature: - Ion source temperature: 230 ºC Ionization energy: - Range m/z: -	Range: 1-65 µg/mL	(Petersen et al., 2013)

Potato crisps, beef, vegetable oils, infant formula, cookies, soya based products, meat based products, cheese, walnuts, and fish	Oils: 500 mg sample were mixed with 0.5 ml of hexane; vortex for 1 min; add 2 ml of water/methanol (60:40, v/v) and vortex; centrifuge at 2,000 × *g* for 2 min; 1 ml of the aqueous portion was subjected to derivatization Infant formulas: QuEChERS were applied; 500 mg sample was mixed with 2 ml of water; vortex for 1 min; 3 ml of acetonitrile was added; vortex 1 min; add 0.3 g of NaCl and 1 g of MgSO4 and mix 1 min; centrifuge at 3,600 × *g* for 5 min; 2 mL of the supernatant was collected and evaporated prior to derivatization Potato crisps: 500 mg of sample mixed with 5 ml of 1.66 M H2SO4/methanol (60:40, v/v); vortex 2 min; centrifuge at 3,600 × *g* for 5 min; the aqueous phase was filtered; 2.5 mL of the filtrate was subjected to derivatization; Extracts were mixed with 1 mL of methanolic solution of 4 mg/ml PFBHA; samples were incubated for 1 h at 40 °C; extract two times with 2 mL of pentane and dried over sodium sulphate prior to collection; rotary evaporator at 30 °C; the remaining oximes were transferred to a glass vial with 200 µL of pentane; evaporate; for silylation, 20 µL of 10 % TMCS in BSTFA and 80 µL of pyridine were added; the mixture was vortexed for 1 min	HNE; HHE	System: GC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: 30 min Oven ramp: 50 ºC (held 1 min), increased to 150 ºC at a rate of 10 ºC/min, from 150 to 200 ºC at a rate of 3 ºC/min and finally up to 250 ºC at a rate of 40 ºC/min Carrier gas: Helium Flow rate: 0.8 mL/min Injection mode: splitless Injection volume: 1 µL Injector temperature: 200 ºC Detector temperature: - Ion source temperature: - Ionization energy: 70 eV Range m/z: -	**HNE**: r^2 (0.9625-0.9995); LOD (4.2-32 ng/g); LOQ (12.8-97.3 ng/g) **HHE**: r^2 (0.9966 – 0.9994); LOD (4.2-10.4 ng/g); LOQ (12.7-31.1 ng/g)	(Papastergiadis et al., 2014a; 2014b)
French fries, vegetable oils	1 g of the oil extracted were mixed with 5 mL DNPH overnight at room temperature; DNPH derivatives were extracted from the oil with methanol/water (75:25, v/v) and with dichloromethane; DNPH derivatives were then separated by TLC with dichloromethane; elute from the TLC plates with methanol and evaporate to 1 mL	HNE	System: HPLC-UV-Vis Column: Ultrasphere ODS (250 x 4.6 i.d., 5 µm particle size) Run time: 40 min Mobile phase: MeOH/ H_2O (50:50, v/v) Flow rate: 0.8 mL/min Injection volume: 10 µL Column temperature: - Detection (λ): 378 nm System: HPLC-MS Column: Ultrasphere ODS (250 x 4.6 i.d., 5 µm particle size) Mobile phase (gradient elution): (A) H_2O; (B) ACN with 0.1% formic acid	-	(Csallany et al., 2015)

			Ionization mode: electrospray Detection mode: negative Range m/z:50-1000 Source temperature:120 ºC		
Linseed oil	2 g of oil was mixed with 200 µL BHT (1 mg/ml in chloroform), 100 µL MeMDA (10 ng/µL) and 1,900 µL water/ethanol (50:50, v/v); vortex 15 s; centrifuge at 3,700 x *g* for 5 min; repeat the extraction with 2 ml water/ethanol (50:50, v/v); filtrate the supernatant; DNPH prepared by 250 µL DNPH solution (0.05 M in ethanol/HCl 12 M 9:1 (v/v)) was added to 750 µL of extract in an injection vial and reaction took place for 2 h at 60 °C	HNE; HHE	System: HPLC-MS Column: Atlantis T3 C18 (150 x 2.1 mm i.d., 3 µm particle size) Run time: - Mobile phase (gradient elution): (A) ACN; (B) CH_3COOH (pH 3.55) Flow rate: 0.25 mL/min Injection volume: 20 µL Column temperature: 40 ºC Detection (λ): - Ionization mode: electrospray Detection mode: negative Range m/z:249-293 (HNE) ; 293-335 (HHE) Source temperature: -	**HNE**: range (0.06-3.0 mg/kg); r^2 (0.9938); LOD (0.14 mg/kg); LOQ (0.7-3.0 mg/kg) **HHE**: range (0.06-3.0 mg/kg); r^2 (0.9953); LOD (0.02 mg/kg); LOQ (0.06-2.34 mg/kg)	(Douny et al., 2015)
Oils of avocado, blackcurrant, apricot kernel, echium, sesame, and wheat germ	3 ml of oil were mixed with 3 ml of acetonitrile; vortex 30 s; centrifuge at 2500 x *g* for 2 min; 1 ml of upper phase were mixed with 100 ml of 20mM pentafluorophenylhydrazine solution and 10 ml of 22mM TFA in acetonitrile, then shake; after 10 min the supernatant was withdrawn and analyzed	HNE	System: UHPLC-MS-MS Column: Hypersil Gold (50 x 2.1 mm i.d., 1.9 µm particle size) Run time: 8 min Mobile phase (gradient elution): (A) H_2O (0.1% formic acid); (B) CH_3CN (0.1% formic acid) Flow rate: 0.25 mL/min Injection volume: 2 µL Column temperature: 25 ºC Detection (λ): - Ionization mode: electrospray Detection mode: positive Range m/z:315-336 Source temperature: -	Range: 11-21362 ng/g; r^2: 0.9974; LOD: 3.4 ng/g; LOQ: 11 ng/g	(Gabbanini et al., 2015)
Rapeseed, sunflower, kiwiseed and tuna oils	15 – 100 mg of oil were acidified with H2SO4 and extracted with hexane, using SPE silica cartridge (500 mg; 6 ml) previously equilibrated with 5 ml ethanol and 5 ml hexane; lipids were eluted by 5 ml hexane, and the oxime derivatives with 5 ml hexane/diethyl ether (50/50; v/v); the hydroxyl group was converted into trimethylsilylether during a 30 min treatment at 60°C with BSTFA	HNE; HHE	System: GC-MS Column: HP-5MS (30 m x 0.25 mm i.d. x 0.25 µm film thickness) Run time: - Oven ramp: 57 ºC, increased to 180 ºC at 20 ºC/min, and to 280 ºC at 4 ºC/min Carrier gas: Helium Flow rate: - Injection mode: splitless	**HNE**: range (0.1-1.5 µg/g); LOQ (0.5 nmoles/g) **HHE**: range (0.1-1.5 µg/g); LOQ (0.7 nmoles/g)	(Viau et al., 2016)

Injection volume: -
Injector temperature: 260 ºC
Detector temperature: -
Ion source temperature: 130 ºC
Ionization energy: 70 eV
Range m/z: -

For most of the reported liquid chromatographic methods, usually butylated hydroxytoluene (BHT) is added directly to the sample or to the extract to reduce or inhibit the oxidation process (Table 22). This step is of utmost importance for the extraction of matrices with a high content in PUFA (Ma & Liu, 2017). Afterwards, 2,4-dinitrophenylhydrazine (DNPH) is added for the derivatization step, which is commonly carried out overnight and at room temperature. Although, concerning the preparation of DNPH, it is necessary to be freshly prepared and usually hydrochloric acid (1 M) is applied. Then, several other steps can be used, namely centrifugation, filtration, separation with thin layer chromatography (TLC), evaporation and reconstitution of residues with different organic solvents, being methanol the most used (Aladedunye, Matthäus, & Przybylski, 2011; Aladedunye & Przybylski, 2011, 2012; Csallany et al., 2015; Grune, Krämer, Hoppe, & Siems, 2001; Lynch, Faustman, Silbart, Rood, & Furr, 2001; T. Sakai et al., 2004, 2006; Sakai & Kuwazuru, 1995; Sakai, Kuwazuru, Yamauchi, & Uchida, 1995; Sakai, Kazuhiro, & Eto, 2000; Tadashi Sakai, Matsushita, Sugamoto, & Uchida, 1997; Seppanen & Csallany, 2001, 2002, 2004, 2006; Uchida, Gotoh, & Wada, 2002; Zanardi, Jagersma, Ghidini, & Chizzolini, 2002). Methods like liquid chromatography coupled to mass spectrometry (LC-MS) have the advantage of not requiring derivatization, and thus the sample may be analyzed with less extraction intermediate steps (Sousa et al., 2017; Spickett, 2013).

On the other hand, for gas chromatography coupled to mass spectrometry (GC-MS), the sample requires derivatization, which can be considered a limiting and crucial step. Regarding gas chromatographic methods, solid phase extraction cartridges (Surh & Kwon, 2005; Surh, Lee, & Kwon, 2010; Surh et al., 2007; Viau, Genot, Ribourg, & Meynier, 2016) are frequently used, as well as centrifugation, evaporation and reconstitution of the residue (Table 22). Other authors, applied solid phase micro extraction, using a fiber coated with

divinylbenzene/carboxen/polydimethylsiloxane (Encarnación Goicoechea et al., 2008; María D. Guillén & Goicoechea, 2009). For derivatization *N,O*-bis(trimethylsilyl)trifluoroacetamide (BSTFA) is the most applied (LaFond, Jerrell, Cadwallader, & Artz, 2011; Michalski, Calzada, Makino, Michaud, & Guichardant, 2008; Papastergiadis, Fatouh, et al., 2014; Papastergiadis, Mubiru, et al., 2014; Surh & Kwon, 2005; Surh et al., 2010, 2007; Viau et al., 2016). Derivatization of samples is an important step for GC-MS analysis because it contributes to increase the volatility and thermal stability of the molecules (LaFond et al., 2011).

Chromatographic methods

The most frequent methodologies reported in the literature for food analysis of 4-hydroxy-2-alkenals (Table 22) are: high-performance liquid chromatography (HPLC) coupled to ultraviolet (UV) (Aladedunye et al., 2011; Aladedunye & Przybylski, 2011, 2012; Alghazeer & Howell, 2008; Csallany et al., 2015; Gasc et al., 2007; Grune et al., 2001; Han & Csallany, 2008, 2009; Lynch et al., 2001; Munasinghe, Ichimaru, Matsui, Sugamoto, & Sakai, 2003; T. Sakai et al., 2004, 2006; T Sakai & Kuwazuru, 1995; T Sakai et al., 1995; Tadashi Sakai et al., 2000, 1997, Seppanen & Csallany, 2001, 2002, 2004, 2006; Uchida et al., 2002); fluorescence (FL) (Liu, Miao, & Toyo'oka, 1996) or mass spectrometry (MS) detection (Alghazeer & Howell, 2008; Csallany et al., 2015; Douny et al., 2015; Gabbanini et al., 2015; Zanardi et al., 2002) and gas chromatography mass-spectrometry (GC-MS) (Encarnación Goicoechea et al., 2008; Guillén & Goicoechea, 2009; LaFond et al., 2011; Michalski et al., 2008; Papastergiadis, Fatouh, et al., 2014; Papastergiadis et al., 2014b; Petersen, Jahreis, Busch-Stockfisch, & Fritsche, 2013; Surh & Kwon, 2003, 2005, Surh et al., 2007, 2010; Takamura & Gardner, 1996; Viau et al., 2016). Other techniques have also been reported, such as: liquid chromatography with electrochemical detection (Goldring, Casini, Maellaro, del Bello, & Comporti, 1993), gas chromatographic analysis with electron capture detection (GC-ECD) (Santaniello, Repetto, Chiesa, & Biondi, 2007), ^{1}H-NMR spectroscopy (Guillén & Goicoechea, 2008a; Guillén & Ruiz, 2008b); headspace with solid phase extraction and gas chromatography coupled to mass spectrometry detection (HS-SPME-GC-MS) (Guillén & Uriarte, 2012).

Regarding the choice of analytical columns in HPLC-UV or HPLC-MS, silica-based reversed-phase C18 columns have been widely used (Table 22). The combination of different proportions of organic solvents, namely acetonitrile (Aladedunye et al., 2011; Aladedunye & Przybylski, 2011, 2012; Alghazeer & Howell, 2008; Csallany et al., 2015; Douny et al., 2015; Gabbanini et al., 2015; Gasc et al., 2007; Liu et al., 1996; Lynch et al., 2001; Uchida et al., 2002) or methanol (Csallany et al., 2015; Grune et al., 2001; Han & Csallany, 2008, 2009, Seppanen & Csallany, 2001, 2002, 2004, 2006; Zanardi et al., 2002)

with ultrapure water (aqueous phase) are the most used composition for the mobile phases. For UV detection wavelength, the values reported range from 221 to 378 nm, but the most used is 378 nm (Csallany et al., 2015; Han & Csallany, 2008, 2009; Lynch et al., 2001; Seppanen & Csallany, 2001, 2002, 2004, 2006). Generally, the column is kept at room temperature, the reported flow rate varies between 0.25 and 1 mL/min, while the injection volume ranges from 2 to 100 µL.

In the GC-MS methods for 4-hydroxy-2-alkenals separation and quantification in food matrices, the most frequently applied columns are fused silica capillary columns coated with (5%-phenyl)-methylpolysiloxane (Guillén & Goicoechea, 2009; LaFond et al., 2011; Papastergiadis et al., 2014a; Papastergiadis et al., 2014b; Surh & Kwon, 2005; Surh et al., 2010, 2007; Viau et al., 2016), being 30 m and 0.25 µm the most used length and film thickness of the columns, respectively. In Table 22, detailed information concerning the applied oven temperature programme, as well as detection and injection temperatures, for the separation of 4-hydroxy-2-alkenals in food matrices is provided. The carrier gas used in GC-MS analysis of the aforementioned compounds is helium and the flow rate varies between 0.7 mL/min and 2.0 mL/min; while splitless is usually used as injection mode (Goicoechea et al., 2008; Guillén & Goicoechea, 2009; Michalski et al., 2008; Papastergiadis et al., 2014a; Papastergiadis et al., 2014b).

Conditions linked with the formation and mitigation of 4-hydroxy-2-alkenals in foods

Up to now, different authors have dedicated their research to the influence of several factors on the formation of 4hydroxy-2-alkenals in foods, namely the importance of fatty acids composition of foodstuffs; effect of air, temperature and/or time; and processing technology for vegetable oils manufacture, among others. However, other mitigation approaches have also been studied, such as the addition of phenolic acids extracts, vitamin E and modified atmospheres (Aladedunye et al., 2011; Alghazeer & Howell, 2008; Fujisaki et al., 2002; Zamora et al., 2016; Zhu et al., 2009). In the following sections, some of the findings of these studies are shown and discussed.

Fatty acids composition

Surh et al. (2007) have studied the occurrence of HHE and HNE in 56 commercially available polyunsaturated fortified foods, including infant formulas (n=12), baby foods (n=7), children-friendly soymilks (n=5), cereals/vegetable powders (n=5), yoghurts (n=5), children-friendly milks (n=7), fruit juices (n=2), fish sticks (n=4), tuna cans (n=3) and snacks (n=6). Moreover, authors have studied the time dependence of 4-hydroxy-2-alkenals levels at 10 and 30 days after opening infant formulas and baby foods (Surh et al., 2007). It was verified

that for infant formulas and baby foods low correlations were observed between PUFA and 4-hydroxy-2-alkenals occurrence, while for the other foods there was no correlation, which were attributed to the presence of antioxidant/prooxidant constituents in the analyzed foods (Surh et al., 2007).

Later, this study was extended to 73 Korean foods, namely sesame oils (n=20), perilla oils (n=18), olive oils (n=3), corn oils (n=3), soybean oils (n=3), pepper seed oils (n=2), sunflower oil (n=1), safflower oil (n=1), rice bran oil (n=1), fish (n=11) and shellfish (n=10) (Surh et al., 2010). However, the results were quite different from the previous ones because HNE concentration was strongly and significantly correlated with n-6 PUFA ($r=0.512$, $p<0.01$) and with PUFA ($r=0.462$, $p<0.01$). On the other hand, weak correlations were observed between HHE and n-3 PUFA ($r=0.086$, $p=0.607$) (Surh et al., 2010).

In 2009, Han et al. (2009) have studied fatty acid methyl esters (methyl stearate, methyl oleate and methyl linoleate) thermally treated (0 to 6h at 185 ºC) to follow the formation of 4-hydroxy-2-octenal (HOE), HNE, HHE and 4-hydroxy-2-decenal (HDE), as well as 2,4-decadienal which is a precursor of HNE (Han & Csallany, 2009). This study has contributed to investigate how the level of unsaturation of fatty acids are involved in lipophilic aldehydes formation during induced lipid peroxidation processes. Therefore, according to their results, methyl stearate and methyl oleate did not produce any lipophilic aldehyde under study. However, HHE was found in higher concentrations especially in methyl linolenate, while HNE was detected in higher amounts in methyl linoleate. On the other hand, authors have concluded that 2,4-decadienal was not found to be a precursor of lipophilic aldehydes in thermally oxidized fatty acids (Han & Csallany, 2009).

In 2013, Petersen et al. (2013) have evaluated volatile compounds in different frying oils, being the highest amount of HNE reported for sunflower oil in comparison with other oils (high-oleic sunflower oil, rapeseed oil, high-oleic rapeseed oil and palm olein), which was probably due to differences in the composition of the oils, not only regarding their fatty acids profile, but also due to the presence of antioxidants (Petersen et al., 2013).

Recently, Csallany et al. (2015) have analyzed the HNE content of French fries acquired in six different fast-food chains from Twin Cities, Minnesota and have correlated the observed HNE amounts with the fatty acids profile of these samples (Csallany et al., 2015). Linoleic acid content in the fat extracted from French fries varied between 24.7 and 54.9 % of total fatty acids and the samples with a higher content of linoleic acid also contained a higher amount of HNE. Nonetheless, one exception was reported, since for two of the samples of French fries with higher amounts of linoleic acid (52.3 and 54.9 % of total fatty acids), the content of HNE was 2.35 and 0.68 µg/g of fat, respectively. This was probably due to

differences in the length of heating time and temperature of the oil used to fry (Csallany et al., 2015).

Temperature/Time

HNE formation along different periods of time (2, 4, 6, 8 and 10 h) at the same frying conditions (185 ºC) was studied (Seppanen & Csallany, 2002). According to the reported findings, it was notable that after 6h HNE concentration reached the maximum value (42.5 µg/g of oil). Then, in the following 4h of heating the amount of HNE decreased, which was probably due to the degradation of HNE in other compounds. Another interesting aspect of this study was that authors have investigated the relationship between HNE formation and the presence of tocopherols. Apparently, the presence of tocopherols in soybean oil restricts the formation of these aldehydes in the first hours of heating (Seppanen & Csallany, 2002).

Seppanen & Csallany (2006) have investigated the effect of intermittent and continuous heating on the formation of HNE, HHE, HOE and HDE in soybean oil, and for both treatments, over a total of 5 h, a similar increase was observed. With respect to the applied methods, it is very important to take into account that the total heating time and temperature was similar for intermittent (185 ± 5 ºC; 1 h/day; during 5 days) and continuous (185 ± 5 ºC; 6 h) heating, however the treatments were different concerning the equipment and the volume of oil used (Seppanen & Csallany, 2006). Also, the same group of researchers have evaluated the influence of air bubbled into the oil under continuous heating. The reported results showed that when air was bubbled into the oil, lipid oxidation greatly increased and a higher amount of HNE was determined (Seppanen & Csallany, 2006).

Han & Csallany (2008) investigated the temperature dependence of HNE formation in vegetable oils and butter. Samples of corn and soybean oils (higher contents of linoleic acid) and butter (lower contents of linoleic acid) were heated for different periods at 190 ºC (recommended frying temperature) and 218 ºC. It was found that HNE amounts after 30 min of heating at 218 ºC were 3.7, 4.9 and 8.7 times higher than at 190 ºC, for soybean, corn and butter oil, respectively (Han & Csallany, 2008). It is important to enhance, that 218 ºC is not a real frying temperature, being these results correlated with extreme frying conditions. Furthermore, these experiments were conducted in the absence of food, which highly influences the frying temperature, since after food immersion the frying temperature decreases.

Primary and secondary oxidation products of corn oil were studied in samples stored (up to 121 months) at room temperature (between 20 a 25 ºC), under different air-oil volume ratios and different air-oil contact surfaces (Guillén & Goicoechea, 2009). For samples of corn oil stored for longer periods of time (103 and 121 months) under the same air-oil contact

surfaces conditions, but with different air-oil volume ratios (0.3; 1.321; 6.230; 7.090), it was found that the increase of 4-hydroxy-2-alkenals amounts was correlated with a higher air-oil volume ratio (Guillén & Goicoechea, 2009).

In 2012, the effects of heat treatment on the formation of HNE in natural low-moisture part-skim and imitation Mozzarella cheeses have been studied (Han & Csallany, 2012). The samples of cheese were heated at 204 ºC (30 and 60 min) and at 232 ºC (15 and 30 min), since these temperatures are commonly applied at home and in commercial applications. Regarding the comparison between natural and imitation Mozzarella cheeses, Han & Csallany (2012) concluded that the HNE formations were much higher in imitation cheeses than in natural cheeses, which was expected since the imitation cheeses are manufactured with vegetable oils usually rich in linoleic acid that is a precursor of HNE. With respect to the temperature and periods of time applied for both types of samples, it was observed that HNE amounts increased greatly when higher temperatures or long periods of time are used, since in imitation cheeses it increases from 0.11 µg/g to 0.88 µg/g of HNE, for 204 ºC and 232 ºC, respectively, while for natural cheeses it increases from 0.013 µg/g to 0.18 µg/g, for 204 ºC and 232 ºC, respectively (Han & Csallany, 2012).

Besides the temperature during frying, another important aspect is the presence or absence of foods, since it can significantly contribute to the changes that occur in the oil/fat. Therefore, Lafond et al. (2011) have evaluated the formation of HNE in a corn-soy oil blend submitted to frying conditions with or without food products. Authors have reported that the frying oil (without food) have a higher concentration of HNE than the frying oil (with food), which is in disagreement with other studies (LaFond et al., 2011; Seppanen & Csallany, 2002, 2004). Lafond et al. (2011) justify their results based on the type of experiment carried out (volume-to-surface ratios), since there are differences between a small-scale bench top experiment and a pilot plant scale experiment.

Concerning the time of frying and the occurrence of HNE, Petersen et al. (2013) have subjected sunflower oil, high-oleic sunflower oil, rapeseed oil, high-oleic rapeseed oil, and palm olein to frying (170 ± 2 ºC) up to 36h. It was possible to observe that until 20h of deep-frying, sunflower oil contained significantly more 4-HNE compared to high-oleic sunflower oil. Moreover, after 27h of deep-frying palm olein showed the lowest amount of HNE (Petersen et al., 2013).

Recently, Ma et al. (2017) have performed accelerated oxidation tests (60 ºC for 30 days and 180 ºC for 5h) on palm, corn, rapeseed, camellia and linseed oils, to study the effect on HNE and HHE occurrence. Higher amounts of HNE and HHE occur when oils were subjected to 60 ºC for 30 days than for 180 ºC for 5h, except for corn oil, suggesting that

the relationship between temperature and time could highly influence lipid oxidation reactions.

Palm, corn, rapeseed, linseed and camellia oils were treated at different temperatures (60 and 180 ºC) for 30 days and 5h/day, respectively, to evaluate the influence of the heat treatment on HNE and HHE formation (Ma & Liu, 2017). Author's findings indicate that for all the tested conditions HHE was not detected in palm, corn and camellia oils because these have low contents of HHE precursors, namely n-3 PUFA.

Sodium chloride

Sodium chloride (NaCl), commonly known as salt, is frequently added to foods for sensorial purposes, as well as to reduce microbial activity. Nevertheless, salt may promote lipid oxidation, namely because: (i) it is able to disrupt cell membrane integrity facilitating the access of oxidizing agents to lipid substrates; (ii) it contributes to the liberation of iron ions; and (iii) it inhibits the activity of antioxidant enzymes such as catalase, glutathione peroxidase and superoxide dismutase (Hernández, Park, & Rhee, 2002; Lee, Mei, & Decker, 1997; Mariutti & Bragagnolo, 2017). Therefore, the relationship between salt concentration and the formation of 4-hydroxy-2-alkenals was investigated but to a lesser extent than other factors.

In 2004, Sakai et al. have performed a study on the effects of 1% and 2% NaCl on HNE formation in minced pork and beef, during 10 days of storage at 0 ºC (Sakai, Munasinghe, Kashimura, Sugamoto, & Kawahara, 2004). Authors have justified their research scope based on other previous works that reported uncertainty of NaCl effect on lipid oxidation. For pork, HNE content increased only after 7 and 10 days of storage when 2% of NaCl was used, while for beef, HNE content increased in all samples during storage (Sakai et al., 2004). Later, the effect of boiling of pork meat without (control sample) and with 1% or 2% of NaCl on HNE formation was studied, and researchers concluded that neither boiling nor NaCl have effect on the concentration of HNE. Moreover, they also evaluated the effect of storage time and observed that the HNE content of the control sample increased significantly after 3-days and was much higher than in the samples that contained NaCl (Sakai, Shimizu, & Kawahara, 2006). The results reported herein are controversial and it is not possible to evaluate whether the content of NaCl can be linked with 4-hydroxy-2-alkenals increase in this type of foods.

Phenolic acids

The most common approach is to assess the effect of phenolic compounds on the lipid oxidation reduction, particularly in oils. Phenolic compounds, also known as polyphenols,

are a group of compounds constituted by one or more aromatic rings bearing hydroxyl substituent(s), that are derived from the secondary metabolism of plants (Maqsood, Benjakul, Abushelaibi, & Alam, 2014). For several years, synthetic antioxidants have been widely utilized to retard lipid oxidation in foods. Nevertheless, several concerns have arisen due to their possible toxicity. Therefore, the demand for natural antioxidants has increased, and consequently the research concerning the potential of several plant foods as natural sources of antioxidants has also emerged (Costa et al., 2015). Phenolic compounds are naturally present in different foods, namely aromatic plants, fruits, vegetables, coffee, tea, wine and chocolate, among others. To retard the oxidation reactions, it is common to prepare extracts from these matrices to be further added to other food products and increase their antioxidant properties (Maqsood et al., 2014).

So far, few studies were conducted aiming to evaluate mitigation strategies for HNE and HHE reduction. For instance, Zhu et al. have studied the effectiveness of 21 natural polyphenols as scavenging agents of HNE and eight of these compounds effectively scavenged HNE (13.4 – 90.1%). The most effective was phloretin, followed by theaflavin-3,3'-digallate, epigallocatechin-3-gallate, epicatechin-3-gallate and epigallocatechin (Zhu et al., 2009).

The capacity of phenolic acids (ferulic, caffeic, dihydrocaffeic, gallic and vanillic acids) to improve frying performance of canola oil was evaluated (Aladedunye & Przybylski, 2011). Frying experiments were conducted at 180 ºC during 2h and canola oil was fortified with 500 mg/kg of each phenolic acid. All the phenolic compounds significantly contributed to HNE decrease, because the control sample after 2h of frying reached 11.2 mg/kg of HNE, while for the samples enriched with phenolic acids the HNE amount varied between 4.9 and 7.1 mg/kg (Aladedunye & Przybylski, 2011). In this research study, phenolic acids were also combined with the sterol and the tocopherol fractions of canola oil. The reported results support a positive interaction between phenolic acids and endogenous canola sterols, probably due to the formation of steryl ferulates (γ-oryzanol) during frying (Aladedunye & Przybylski, 2011).

To mitigate the formation of HNE in mackerel, green tea extracts were used (Alghazeer & Howell, 2008). Green tea is a source of phenolic compounds, namely catechins that represent 85% of the total polyphenol content (Nikoo, Regenstein, & Ahmadi Gavlighi, 2018). The major phenolic compounds in green tea are epigallocatechin-3-gallate (7 to 74 mg/g), epigallocatechin (0 to 55 mg/g), epicatechin gallate (1 to 41 mg/g) and epicatechin (0.1 to 17 mg/g) (Nikoo et al., 2018). Mackerel is rich in linoleic, linolenic, eicosapentaenoic (EPA) and docosahexaenoic (DHA) acids, which are precursors of HNE and HHE.

Therefore, green tea was added to mackerel at 250 and 500 mg/kg and the occurrence of HNE was studied during 26 weeks at -10 ºC against control samples. The results indicated that HNE amounts were higher in the control samples (0.45 mg/kg) than for the samples of mackerel with green tea. Nonetheless, the levels of HNE were higher for frozen mackerel samples with 500 mg/kg of green tea (HNE, 0.25 mg/kg) than for samples with 250 mg/kg (0.15 mg/kg) (Alghazeer & Howell, 2008).

Vitamin E

Vitamin E is a fat-soluble vitamin that comprises eight enantiomers, namely α-, β-, γ-, δ-tocopherols, and α-, β-, γ-, δ-tocotrienols. It is an important antioxidant with capacity to deactivate photosynthesis-derived reactive oxygen species, as well as to prevent lipid peroxidation by scavenging lipid peroxyl radicals (Brewer, 2011). It is recognized that dietary supplementation of vitamin E (α-tocopherol) enhances the inclusion of the antioxidant into the phospholipid membrane where PUFA are located, and therefore decreases lipid peroxidation and increases the stability (Brewer, 2011). Therefore, Grune et al. have studied the influence of supplementation of the diet of Leghorn laying hens with different concentrations of vitamin E (0 to 160 IU/kg) on the occurrence of cytotoxic lipid peroxidation products (malondialdehyde, HNE and HHE) during the production and storage of n-3 PUFA enriched eggs (Grune et al., 2001). After 4 weeks of storage, it was notable that a diet supplemented with vitamin E (≥ 80 IU/kg) was effective and no difference in the content of HNE and HHE was observed. Furthermore, these findings enhance that with these doses of vitamin E, it is possible to protect against oxidation, high contents of n-3 and n-6 PUFA in eggs (Grune et al., 2001).

Aladedunye and Przybylski have tested several combinations of tocopherol isomers during frying with canola oil triacylglycerols on the mitigation of HNE (Aladedunye & Przybylski, 2012). The different combinations of tocopherols were obtained by isolation from rice bran and canola oils. The vitamin E fraction of rice bran oil was composed by 822.2 ± 57 mg/g of vitamin E, being γ-tocopherol the most abundant isomer (505.1 mg/g). On the other hand, the other fraction, that was obtained from canola oil, had a lower total vitamin E content (791.1 ± 40 mg/g), but seven enantiomers of vitamin E were quantified and the most abundant was γ-tocotrienol (317.4 mg/g). Results indicated that the HNE content significantly decreased in the presence of tocopherols (from 11.7 to 6.2 mg/kg). Nevertheless, concerning the different vitamin E fractions no significant differences were observed in the amounts of HNE formed (Aladedunye & Przybylski, 2012).

Modified atmospheres

One other feasible approach to reduce lipid oxidation is the use of modified atmospheres, namely by the reduction of oxygen or by adding carbon dioxide (Hotchkiss, Werner, & Lee, 2006). Aladedunye et al. have evaluated the effect of carbon dioxide blanketing on the formation of HNE in French fries in comparison with common frying procedures (Aladedunye et al., 2011). To perform this test, authors have used a fryer with 8L capacity, that was heated at 185 ºC, 7h/day for 7 days, and every day eight batches (400 g) of frozen French fries were prepared using canola oil. The carbon dioxide was delivered into the fryer at a flow rate of 2.5 L/min, 10 min prior to frying, and after finishing each frying day, the flow of carbon dioxide was kept until the frying oil reached 100 ºC. The reported results indicate that HNE was reduced by about 62%, when frying under carbon dioxide was applied (Aladedunye et al., 2011). This is a very interesting approach, although questionable if it is feasible at a large-scale industry, namely concerning cost effectiveness.

Another proposed approach, by Fujisaki et al. was to use a low oxygen atmosphere to reduce the formation of volatile aldehydes (Fujisaki et al., 2002). Nevertheless, these experiments were carried out at a laboratory scale and did not use equipment usually applied under realistic frying conditions. Although authors concluded that if the atmosphere concentration of oxygen is kept below 2%, it is possible to significantly decrease the presence of these hazardous compounds, namely HNE. Once more, it was not evaluated if this technique is reasonable for an industrial scale, as well as the economic viability and impact.

4-hydroxy-2-alkenals occurrence in foods

During the last forty years, the number of studies concerning the development and application of new analytical methodologies for the detection and quantification of 4-hydroxy-2-alkenals in several foodstuffs has increased (Tables 23-27). Up to now, several types of foodstuffs were studied regarding the occurrence of these toxic compounds, but one of the most studied groups is vegetable oils and fats. In the following sections, data from the literature on the amounts of HNE and HHE for different food groups, namely vegetable oils and fats; infant formulas, milk and dairy products; fish and fish products; meat and meat products; and other foods, are presented. This makes sense in this literature review because the occurrence of these compounds are linked with several factors, but especially with the fatty acids composition, and all of these groups have specific features. Some difficulties were encountered during this review, namely because of the presentation of data in graphics (Aladedunye & Przybylski, 2012; Grune et al., 2001; LaFond et al., 2011; Michalski et al., 2008; Petersen et al., 2013; Uchida et al., 2002); impossibility to convert

results in order to perform comparisons with other reported results; and the presentation of the sum of HNE and HHE, instead of individual values.

Oils and fats

Oils and fats processing usually aims to improve their quality, stability and safety (Albuquerque, Oliveira, & Costa, 2018). Despite the removal of a large amount of impurities from the oil, processing can often originate new contaminants that can cause additional health hazards to those who consume these foods (Choe & Min, 2006). In general, vegetable oils have several similar features, for example low moisture content and are mainly composed by triacylglycerides (Dubois, Breton, Linder, Fanni, & Parmentier, 2007; Foster, Williamson, & Lunn, 2009). However, they also have differences which are linked with raw materials and factors such as climate, soil, plant varieties, geography and state of ripeness (Minihane & Harland, 2007). These differences are most of the time responsible for their behavior and functionality, namely in what concerns fatty acids composition; and the presence of minor compounds, such as vitamin E, sterols and phenolics.

Edible oils and fats are very susceptible to oxidation reactions which affect their quality, safety and shelf-life. Many of these reactions take place during processing and storage and are influenced by the presence of oxygen and exposure to temperature/light (Choe & Min, 2006).

In Table 23, an overview of the occurrence data on 4-hydroxy-2-alkenals in vegetable oils is provided. It is possible to confirm that the most analyzed compound is undoubtedly HNE and the most studied is soybean oil (Gabbanini et al., 2015; Han & Csallany, 2008; Hua, Zhao, Wu, & Li, 2016; Liu et al., 1996; Seppanen & Csallany, 2004, 2006). In what concerns the reported amounts, very different values were found for the different origins of soybean oil. For instance, the determined HNE amounts varied between not detected and 644.2 mg/kg, for sunflower oil acquired in Italy and crude sunflower oils from China, respectively (Gabbanini et al., 2015; Hua et al., 2016). Nevertheless, it should be highlighted that crude oils are not edible oils; therefore, this value could be considered less important. Although, after refining, the same sample still contained high amounts of HNE (260 mg/kg), which may be of concern (Hua et al., 2016).

Table 23. Occurrence of 4-hydroxy-2-alkenals in edible oils.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Soybean oil A	as purchased	Japan	4.06E-09	1.27E-09	-	-	(Liu et al., 1996)
Soybean oil B	as purchased	Japan	5.62E-09	5.47E-10	-	-	(Liu et al., 1996)
Sesame oil A	as purchased	Japan	9.33E-08	4.69E-10	-	-	(Liu et al., 1996)
Sesame oil B	as purchased	Japan	1.23E-07	8.44E-10	-	-	(Liu et al., 1996)
Soybean oil	8h; 185 ºC	United States	2.45	0.15	0.17	0.02	(Seppanen & Csallany, 2001)
Soybean oil	6h; 180 ºC	United States; Industry	42.50	-	-	-	(Seppanen & Csallany, 2002)
Olive oil	as purchased	Korea; household	2.667E-06	7.218E-07	-	-	(Surh & Kwon, 2003)
Perilla oil	as purchased	Korea; household	3.343E-07	1.875E-08	-	-	(Surh & Kwon, 2003)
Frying oil A	as purchased	Korea; local market	1.968E-07	3.437E-08	-	-	(Surh & Kwon, 2003)
Frying oil B	as purchased	Korea; local market	2.062E-07	3.437E-08	-	-	(Surh & Kwon, 2003)
Frying oil C	as purchased	Korea; local market	3.437E-07	6.562E-08	-	-	(Surh & Kwon, 2003)
Soybean oil	as purchased	United States; local grocery store	5.76	1.10	-	-	(Seppanen & Csallany, 2004)
Soybean oil	5h; 185 ºC	United States; local grocery store	4.39	0.41	-	-	(Seppanen & Csallany, 2004)
Soybean oil	1h; 185ºC	United States; local stores	2.27	0.22	0.40	0.08	(Seppanen & Csallany, 2006)
Soybean oil	1h; 185 ºC; storage 1 day	United States; local stores	2.85	0.65	0.14	0.01	(Seppanen & Csallany, 2006)
Soybean oil	2h; 185ºC	United States; local stores	2.01	0.28	1.23	0.46	(Seppanen & Csallany, 2006)
Soybean oil	2h; 185 ºC; storage 1 day	United States; local stores	1.91	0.36	0.26	0.03	(Seppanen & Csallany, 2006)
Soybean oil	3h; 185ºC	United States; local stores	2.62	0.13	0.30	0.07	(Seppanen & Csallany, 2006)
Soybean oil	3h; 185 ºC; storage 1 day	United States; local stores	3.22	0.17	0.44	0.07	(Seppanen & Csallany, 2006)
Soybean oil	4h; 185ºC	United States; local stores	3.33	0.15	0.94	0.14	(Seppanen & Csallany, 2006)
Soybean oil	4h; 185 ºC; storage 1 day	United States; local stores	3.58	0.56	0.37	0.09	(Seppanen & Csallany, 2006)
Butter oil	3h; 190 ºC	United States; retail stores	1.85	-	-	-	(Han & Csallany, 2008)
Butter oil	5 min; 218 ºC	United States; retail stores	2.13	-	-	-	(Han & Csallany, 2008)
Butter oil	30 min; 218 ºC	United States; retail stores	6.71	-	-	-	(Han & Csallany, 2008)
Corn oil	1h; 190 ºC	United States; retail stores	5.46	-	-	-	(Han & Csallany, 2008)
Corn oil	5 min; 218 ºC	United States; retail stores	5.93	-	-	-	(Han & Csallany, 2008)
Corn oil	30 min; 218 ºC	United States; retail stores	15.48	-	-	-	(Han & Csallany, 2008)
Soybean oil	1h; 190 ºC	United States; retail stores	3.73	-	-	-	(Han & Csallany, 2008)

Soybean oil	5 min; 218 ºC	United States; retail stores	8.08	-	-	-	(Han & Csallany, 2008)
Soybean oil	30 min; 218 ºC	United States; retail stores	10.72	-	-	-	(Han & Csallany, 2008)
Perilla oil	Traditional produced	Korea; local shops	0.07	0.03	0.25	0.19	(Surh et al., 2010)
Perilla oil	Industrial produced	Korea; industry	0.07	0.04	0.22	0.35	(Surh et al., 2010)
Sesame oil	Traditional produced	Korea; local shops	0.28	0.15	0.06	0.02	(Surh et al., 2010)
Sesame oil	Industrial produced	Korea; industry	0.10	0.09	0.08	0.05	(Surh et al., 2010)
Frying oil ω-3 enriched	5h; 170 ºC	Belgium	3.75	0.02	0.52	0.01	(Papastergiadis et al., 2014a)
Frying oil		Belgium	3.76	0.02	0.18	0.01	(Papastergiadis et al., 2014a)
Colza oil		Belgium	0.11	-	0.15	-	(Papastergiadis et al., 2014a)
Extra virgin olive oil A		Belgium	0.07	-	0.16	-	(Papastergiadis et al., 2014a)
Extra virgin olive oil B		Belgium	0.12	-	0.14	-	(Papastergiadis et al., 2014a)
Extra virgin olive oil C		Belgium	0.06	-	0.10	-	(Papastergiadis et al., 2014a)
Plant oils	as purchased	Belgium; supermarket chains	0.04	-	0.02	-	(Papastergiadis et al., 2014a)
Linseed oil	60 ºC; 12 days of storage	Belgium	63.92	-	9.44	-	(Douny et al., 2015)
Linseed oil	60 ºC; 24 days of storage	Belgium	191.76	-	42.40	-	(Douny et al., 2015)
Mixed seeds oil	as purchased	Italy; grocery stores	0.05	0.00	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	1h; 180 ºC	Italy; grocery stores	1.41	0.09	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	7h; 180 ºC	Italy; grocery stores	3.47	0.11	-	-	(Gabbanini et al., 2015)
Mixed seeds oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Corn oil	as purchased	Italy; grocery stores	nd	nd	-	-	(Gabbanini et al., 2015)
Corn oil	1h; 180 ºC	Italy; grocery stores	2.02	0.10	-	-	(Gabbanini et al., 2015)
Corn oil	7h; 180 ºC	Italy; grocery stores	2.59	0.23	-	-	(Gabbanini et al., 2015)
Corn oil	3 years, room temperature	Italy; grocery stores	2.68	0.04	-	-	(Gabbanini et al., 2015)
Soybean oil	as purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Soybean oil	1h; 180 ºC	Italy; grocery stores	1.43	0.06	-	-	(Gabbanini et al., 2015)
Soybean oil	7h; 180 ºC	Italy; grocery stores	2.87	0.08	-	-	(Gabbanini et al., 2015)
Soybean oil	3 years; room temperature	Italy; grocery stores	13.32	0.09	-	-	(Gabbanini et al., 2015)
Peanut oil	as purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Peanut oil	1h; 180 ºC	Italy; grocery stores	1.11	0.02	-	-	(Gabbanini et al., 2015)
Peanut oil	7h; 180 ºC	Italy; grocery stores	2.00	0.13	-	-	(Gabbanini et al., 2015)
Peanut oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Sunflower oil	as purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)

Sunflower oil	1h; 180 ºC	Italy; grocery stores	1.35	0.04	-	-	(Gabbanini et al., 2015)
Sunflower oil	7h; 180 ºC	Italy; grocery stores	3.30	0.08	-	-	(Gabbanini et al., 2015)
Sunflower oil	3 years, room temperature	Italy; grocery stores	19.05	0.18	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	as purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	1h; 180 ºC	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	7h; 180 ºC	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Extra-virgin olive oil	3 years, room temperature	Italy; grocery stores	-	-	-	-	(Gabbanini et al., 2015)
Avocado oil	as purchased	Italy; grocery stores	0.14	0.00	-	-	(Gabbanini et al., 2015)
Avocado oil	90min; 75 ºC	Italy; grocery stores	0.37	0.02	-	-	(Gabbanini et al., 2015)
Blackcurrant oil	as purchased	Italy; grocery stores	0.55	0.02	-	-	(Gabbanini et al., 2015)
Blackcurrant oil	90min; 75 ºC	Italy; grocery stores	0.80	0.03	-	-	(Gabbanini et al., 2015)
Apricot kernel oil	as purchased	Italy; grocery stores	0.05	0.00	-	-	(Gabbanini et al., 2015)
Apricot kernel oil	90min; 75 ºC	Italy; grocery stores	0.11	0.00	-	-	(Gabbanini et al., 2015)
Echium oil	as purchased	Italy; grocery stores	0.18	0.01	-	-	(Gabbanini et al., 2015)
Echium oil	90min; 75 ºC	Italy; grocery stores	0.18	0.01	-	-	(Gabbanini et al., 2015)
Sesame oil	as purchased	Italy; grocery stores	0.06	0.00	-	-	(Gabbanini et al., 2015)
Sesame oil	90min; 75 ºC	Italy; grocery stores	0.09	0.00	-	-	(Gabbanini et al., 2015)
Wheat germ oil	as purchased	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Wheat germ oil	90min; 75 ºC	Italy; grocery stores	nd	-	-	-	(Gabbanini et al., 2015)
Kiwiseed oil	as purchased	France	0.52	0.02	0.17	0.02	(Viau et al., 2016)
Tuna oil	as purchased	France	0.20	0.06	0.16	0.02	(Viau et al., 2016)
Sunflower oil	as purchased	France; local supermarket	0.02	0.00	0.16	0.00	(Viau et al., 2016)
Rapeseed oil	as purchased	France; local supermarket	0.08	0.02	0.19	0.06	(Viau et al., 2016)
Soybean oil	Crude	China; local industry	644.2	-	16.3	-	(Hua et al., 2016)
Rapeseed oil	Crude	China; local industry	264.4	-	21.5	-	(Hua et al., 2016)
Soybean oil	After refining	China; local industry	260.0	-	5.65	-	(Hua et al., 2016)
Rapeseed oil	After refining	China; local industry	37.35	-	nd	-	(Hua et al., 2016)
Palm oil	as purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Palm oil	5h; 180 ºC	China; local industry	1.51E-03	1.50E-04	nd	-	(Ma & Liu, 2017)
Corn oil	as purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Corn oil	5h; 180 ºC	China; local industry	4.78E-03	1.80E-04	nd	-	(Ma & Liu, 2017)
Rapeseed oil	as purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)

Rapeseed oil	5h; 180 ºC	China; local industry	2.71E-03	1.30E-04	2.23E-03	1.50E-04	(Ma & Liu, 2017)
Camellia oil	as purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Camellia oil	5h; 180 ºC	China; local industry	1.40E-03	1.10E-04	nd	-	(Ma & Liu, 2017)
Linseed oil	as purchased	China; local industry	nd	-	nd	-	(Ma & Liu, 2017)
Linseed oil	5h; 180 ºC	China; local industry	2.80E-03	2.30E-04	5.96E-03	2.70E-04	(Ma & Liu, 2017)

nd, not detected

Palm oil, which is the most consumed worldwide, is the less studied regarding the presence of these compounds. To our knowledge, only Ma et al. (2017) have evaluated palm oil, and HNE was not detected in the sample as acquired, and a very low amount (1.51 µg/kg) was detected when the sample was subjected to 180 ºC for 5h. The lack of interest in this type of food by researchers is probably due to its fatty acids composition because it is mainly composed by saturated and monounsaturated fatty acids, which are not considered precursors of these compounds.

Regarding HHE, the highest amounts were reported for linseed oil (42.4 mg/kg), rapeseed oil (21.5 mg/kg) and soybean oil (16.3 mg/kg) (Douny et al., 2015; Hua et al., 2016). However, two of these samples were crude oils from China and the amounts of HHE significantly decreased after processing, being not detected for rapeseed oil and 5.65 mg/kg for soybean oil. Another interesting aspect concerning the origin of samples is for example the values reported by Hua et al. (2016) and Ma et al. (2017), for oils acquired from local industries in China. For instance, both authors analyzed rapeseed oil, but Hua et al. (2016) reported that after refining, it still had 37.4 mg/kg of HNE and HHE was not detected, while Ma et al. (2017) have not detected both compounds. This is probably linked with different processing conditions applied or even with the initial composition of the matrix (fatty acids and antioxidants) and oxidation *status*, among others.

In what concerns the samples analyzed as purchased, very low amounts of HNE were reported (Table 23). Furthermore, for almost 35% of the results reported for oils, HNE was not detected and the highest result (5.76 mg/kg) was reported for sunflower oil. This is a crucial information, because it allows us to clearly understand that most of the oils analyzed so far are safe when they are purchased by consumers, independently on the type of oil and the local where it is sold (Gabbanini et al., 2015; Liu et al., 1996; Ma & Liu, 2017; Papastergiadis et al., 2014a; Surh & Kwon, 2003; Viau et al., 2016).

Infant formulas, milk and dairy products

In Table 24, an overview of the reported results for HNE and HHE occurrence in infant formulas, milk and dairy products is provided. Infant formulas and milk are especially focused because children and young people, which have a lower body weight than adults, consume a high quantity of these products; therefore, their exposure risk is higher.

It is recommended that breastfeeding should be exclusive until six months of age, but sometimes it is not possible to follow this recommendation and infant formulas are needed to assure a normal growth development and a good health status (Silva, Albuquerque, Oliveira, & Costa, 2017).

One of the quality features of human breast milk is the presence of n-3 and n-6 PUFA. It is intended that infant formulas be as similar as possible to human milk; therefore, these products are enriched in PUFA to fulfil the nutritional needs of infants (Michalski et al., 2008).

The amounts reported for HNE content in infant formulas varied between 0.041 and 0.291 mg/kg, while for HHE it ranged from not detected to 0.188 mg/kg (Papastergiadis et al., 2014; Surh et al., 2007). For instance, Surh et al. (2007) have analyzed 12 samples of different infant formulas at the moment of purchase and a great variability of results for HNE was observed, being in some cases 3 times higher. Some of the possible reasons appointed for the results found are: different techniques for pulverizing fats and oils in the manufacture of infant formulas; differences in the drying methods applied during manufacture; and differences between their composition, namely fatty acids, antioxidants (ascorbic acid and lecithin) and pro-oxidant metals (iron, zinc, copper and manganese) (Surh et al., 2007).

Table 24. Occurrence of 4-hydroxy-2-alkenals in infant formulas, milk and dairy products.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Infant formula A	as purchased	Korea; local markets	0.05	0.01	0.04	0.00	(Surh et al., 2007)
Infant formula A	10 days storage, room temperature	Korea; local markets	0.17	0.02	0.07	0.01	(Surh et al., 2007)
Infant formula B	as purchased	Korea; local markets	0.05	0.02	0.04	0.00	(Surh et al., 2007)
Infant formula B	10 days storage, room temperature	Korea; local markets	0.21	0.01	0.11	0.02	(Surh et al., 2007)
Infant formula C	as purchased	Korea; local markets	0.07	0.03	0.05	0.00	(Surh et al., 2007)
Infant formula C	10 days storage, room temperature	Korea; local markets	0.29	0.03	0.11	0.00	(Surh et al., 2007)
Infant formula D	as purchased	Korea; local markets	0.07	0.01	0.08	0.00	(Surh et al., 2007)
Infant formula D	10 days storage, room temperature	Korea; local markets	0.28	0.09	0.12	0.06	(Surh et al., 2007)
Infant formula E	as purchased	Korea; local markets	0.06	0.01	0.07	0.02	(Surh et al., 2007)
Infant formula E	10 days storage, room temperature	Korea; local markets	0.42	0.11	0.12	0.02	(Surh et al., 2007)
Infant formula F	as purchased	Korea; local markets	0.13	0.05	0.06	0.01	(Surh et al., 2007)
Infant formula F	10 days storage, room temperature	Korea; local markets	0.50	0.04	0.19	0.03	(Surh et al., 2007)
Infant formula G	as purchased	Korea; local markets	0.06	0.01	0.04	0.01	(Surh et al., 2007)
Infant formula G	10 days storage, room temperature	Korea; local markets	0.24	0.06	0.09	0.01	(Surh et al., 2007)
Infant formula H	as purchased	Korea; local markets	0.09	0.03	0.05	0.01	(Surh et al., 2007)
Infant formula H	10 days storage, room temperature	Korea; local markets	0.49	0.05	0.12	0.01	(Surh et al., 2007)
Infant formula I	as purchased	Korea; local markets	0.12	0.01	0.08	0.01	(Surh et al., 2007)
Infant formula I	10 days storage, room temperature	Korea; local markets	0.79	0.03	0.22	0.03	(Surh et al., 2007)
Infant formula J	as purchased	Korea; local markets	0.10	0.03	0.06	0.01	(Surh et al., 2007)
Infant formula J	10 days storage, room temperature	Korea; local markets	0.65	0.23	0.16	0.03	(Surh et al., 2007)
Infant formula K	as purchased	Korea; local markets	0.05	0.04	nd	-	(Surh et al., 2007)
Infant formula K	10 days storage, room temperature	Korea; local markets	0.08	0.07	nd	-	(Surh et al., 2007)
Infant formula L	as purchased	Korea; local markets	0.04	0.01	nd	-	(Surh et al., 2007)

Infant formula L	11 days storage, room temperature	Korea; local markets	0.29	0.13	0.05	0.01	(Surh et al., 2007)
Yoghurt	as purchased	Korea; local markets	1.10E-03	4.00E-04	nd	-	(Surh et al., 2007)
Yoghurt PUFA fortified A	as purchased	Korea; local markets	1.70E-03	6.00E-04	nd	-	(Surh et al., 2007)
Yoghurt PUFA fortified B	as purchased	Korea; local markets	1.00E-03	2.00E-04	nd	-	(Surh et al., 2007)
Yoghurt PUFA fortified C	as purchased	Korea; local markets	1.33E-02	8.00E-03	nd	-	(Surh et al., 2007)
Yoghurt PUFA fortified D	as purchased	Korea; local markets	6.10E-03	1.40E-03	nd	-	(Surh et al., 2007)
Soymilk A	as purchased	Korea; local markets	1.19E-02	7.00E-04	1.20E-03	1.10E-03	(Surh et al., 2007)
Soymilk B	as purchased	Korea; local markets	1.42E-02	6.30E-03	2.30E-03	2.20E-03	(Surh et al., 2007)
Soymilk C	as purchased	Korea; local markets	1.81E-02	3.10E-03	3.90E-03	6.00E-04	(Surh et al., 2007)
Soymilk D	as purchased	Korea; local markets	3.00E-02	4.80E-03	7.40E-03	1.70E-03	(Surh et al., 2007)
Soymilk E	as purchased	Korea; local markets	1.42E-02	4.00E-03	3.30E-03	3.00E-03	(Surh et al., 2007)
Milk A	as purchased	Korea; local markets	4.90E-03	4.00E-03	nd	-	(Surh et al., 2007)
Milk B	as purchased	Korea; local markets	9.00E-03	6.70E-03	nd	-	(Surh et al., 2007)
Milk C	as purchased	Korea; local markets	1.01E-02	7.70E-03	1.10E-03	9.00E-04	(Surh et al., 2007)
Milk D	as purchased	Korea; local markets	1.00E-02	6.00E-03	5.00E-04	5.00E-04	(Surh et al., 2007)
Milk E	as purchased	Korea; local markets	5.70E-03	1.20E-03	nd	-	(Surh et al., 2007)
Milk F	as purchased	Korea; local markets	1.76E-02	5.40E-03	nd	-	(Surh et al., 2007)
Milk G	as purchased	Korea; local markets	4.40E-03	1.10E-03	nd	-	(Surh et al., 2007)
Natural mozzarella cheese A	30 min, 204 ºC	United States; local store	0.01	-	-	-	(Han & Csallany, 2012)
Natural mozzarella cheese B	30 min, 204 ºC	United States; local store	0.01	-	-	-	(Han & Csallany, 2012)
Natural mozzarella cheeses	30 min, 204 ºC	United States; local store	0.01	-	-	-	(Han & Csallany, 2012)
Natural mozzarella cheeses	30 min, 232 ºC	United States; local store	0.18	-	-	-	(Han & Csallany, 2012)
Imitation mozzarella cheese A	30 min, 204 ºC	United States	0.09	-	-	-	(Han & Csallany, 2012)

Imitation mozzarella cheese B	30 min, 204 ºC	United States	0.13	-	-	-	(Han & Csallany, 2012)
Imitation mozzarella cheese A	60 min, 204 ºC	United States	1.12	-	-	-	(Han & Csallany, 2012)
Imitation mozzarella cheese B	60 min, 204 ºC	United States	0.78	-	-	-	(Han & Csallany, 2012)
Imitation mozzarella cheeses	30 min, 204 ºC	United States	0.11	-	-	-	(Han & Csallany, 2012)
Imitation mozzarella cheeses	30 min, 232 ºC	United States	0.88	-	-	-	(Han & Csallany, 2012)
Cheese	as purchased	Belgium; supermarket chains	0.03	-	0.01	-	(Papastergiadis et al., 2014a)
Infant formula	as purchased	Belgium	0.21	0.01	0.02	0.003	(Papastergiadis et al., 2014a)
Full fat milk	as purchased	Belgium; supermarket chains	0.03	-	0.005	-	(Papastergiadis et al., 2014a)

nd, not detected; PUFA, polyunsaturated fatty acids

Comparing infant formulas, soymilk and milk, it is possible to observe that at the moment of purchase, in general, the values for HNE content are higher for infant formulas than for the other. HNE content varied between 0.004 and 0.032 mg/kg for milk and from 0.012 to 0.030 mg/kg in soymilk, while HHE ranged from not detected to 0.0011 and from 0.0012 to 0.0074 mg/kg, for milk and soymilk, respectively (Papastergiadis et al., 2014; Surh et al., 2007). Concerning the other evaluated dairy products, namely cheeses and yoghurt with and without fortification with PUFA, the amounts for HNE are low for both products, except for cheese when it is thermally treated (1.12 mg/kg, when it is exposed to 204 ºC for 60 min) (Han & Csallany, 2012).

Fish and fish products

Fish and fish products are recognized as an essential part of a balanced diet due to its nutritional and bioactive composition (Hosomi, Yoshida, & Fukunaga, 2012). These products are a source of high-quality proteins, n-3 fatty acids, as well as minerals and vitamins, which are linked with many potential health benefits (Food and Agriculture Organization of the United Nations/World Health Organization, 2010).

The several beneficial health effects are mainly associated with the presence of n-3 PUFA, such as EPA and DHA fatty acids, which are characteristic of this group of foods. An overview on the lipid and fatty acid composition of 159 fish, mollusc and crustacean samples, concluded that salmon, swordfish, halibut, mackerel and sardines are the ones with the highest total fat content, which varied from 5.7 to 20.4 g/100 g (Sirot, Oseredczuk, Bemrah-Aouachria, Volatier, & Leblanc, 2008). Regarding the fatty acids composition, for cod, whiting and pollack, n-3 PUFA are 55%, 52% and 51% of the total fatty acids (Sirot et al., 2008).

This is an interesting food group to investigate the presence of 4-hydroxy-2-alkenals, especially HHE because it is formed by the oxidation of n-3 PUFA, namely DHA and EPA fatty acids (Long & Picklo, 2010).

One of the most studied matrices regarding the presence of HNE and HHE is salmon, which is commonly consumed around the world. Nevertheless, total fat significantly varies accordingly to the species, origin, feeding and production type, among other factors. For instance, the United Stated Department of Agriculture National Nutrient Database for Standard Reference, reports that the fat content in salmon varied between 4.40 and 6.34 g/100 g, for pink salmon and Atlantic salmon, respectively (USDA, 2017). Furthermore, the amounts of EPA and DHA are also very different among the same type of fish. Atlantic salmon and Coho salmon, that were both wild, presented higher amounts of DHA than EPA, while for the other two (Pink salmon and Sockeye salmon) the opposite was observed

(USDA, 2017). According to our literature review, HHE content reported for smoked salmon (Table 25) varied between not detected and 1.24 mg/kg, while HNE amounts were lower than 0.015 mg/kg (Munasinghe et al., 2003; Papastergiadis et al., 2014a; Sakai & Kuwazuru, 1995).

The information on the abundance of HNE and HHE occurrence in fish and fish products is scarce (Table 25). The highest concentrations of HHE were reported for yellowtail (6.94 mg/kg) and smoked salmon (1.24 mg/kg). Although, it is worthy of mention that the result reported for yellowtail is after 28 weeks of storage at -20 ºC, being the HHE content much lower (0.37 mg/kg) at the moment of purchase (Sakai et al., 2000). This fact confirms that HNE is a good marker of food decomposition, namely concerning oxidation reactions (Alghazeer & Howell, 2008). For the other types of fish, the HHE amounts were lower than for example for the other food groups discussed previously.

In what concerns HNE occurrence in fish and fish products, the reported amounts varied between not detected and 0.06 mg/kg, except for mackerel after 26 weeks of storage at -10 ºC (0.45 mg/kg) (Alghazeer & Howell, 2008). More studies are needed about the presence of these hazardous compounds in this type of matrices, because it undoubtedly affects the safety of fish and fish products, as well as its quality and nutritional value.

Meat and meat products

Over the last decades, trends in the consumption of meat and meat products have significantly changed. Consumers are aware of the potential health risks associated with a high intake of red meat, therefore, the demand for white meat has increased (Lucarini, Durazzo, Sánchez del Pulgar, Gabrielli, & Lombardi-Boccia, 2018). Although one of the main quality and safety concerns is lipid oxidation of meat, several efforts are being developed to retard or decrease it, namely by applying antioxidants (Kumar, Yadav, Ahmad, & Narsaiah, 2015). Lipid oxidation contributes to the development of undesired aromatic compounds that are linked with changes on meat taste and flavor. Among the compounds formed during lipid oxidation of meat, the most reactive are α,β-unsaturated aldehydes, such as HNE and HHE (Guyon, Meynier, & de Lamballerie, 2016).

Table 25. Occurrence of 4-hydroxy-2-alkenals in fish and fish products.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Horse mackerel	as purchased	Japan; commercial markets	nd	-	-	-	(Sakai et al., 1995)
Sardine	as purchased	Japan; commercial markets	1,22E-07 - 7,72E-07	-	-	-	(Sakai et al., 1995)
Yellowfin tuna	as purchased	Japan; commercial markets	nd	-	-	-	(Sakai et al., 1995)
Skipjack	as purchased	Japan; commercial markets	nd	-	-	-	(Sakai et al., 1995)
Flying fish	as purchased	Japan; commercial markets	nd	-	-	-	(Sakai et al., 1995)
Yellowtail	as purchased	Japan; commercial markets	5,00E-08 - 2,95E-06	-	-	-	(Sakai et al., 1995)
Coho salmon	as purchased	Japan; commercial markets	3,41E-07 - 1,47E-06	-	-	-	(Sakai et al., 1995)
Yellowtail	as purchased	Japan; commercial markets	-	-	4.79E-07	-	(Sakai et al., 1997)
Horse mackerel	as purchased	Japan; commercial markets	-	-	1.94E-07	-	(Sakai et al., 1997)
Mackerel	as purchased	Japan; commercial markets	-	-	4.34E-07	-	(Sakai et al., 1997)
Carp	as purchased	Japan; commercial markets	-	-	1.05E-06	-	(Sakai et al., 1997)
Yellowtail sample 1	as purchased	Japan; commercial markets	5.47E-08	-	8.48E-07	-	(Sakai et al., 1997)
Yellowtail sample 2	as purchased	Japan; commercial markets	nd	-	7.98E-07	-	(Sakai et al., 1997)
Yellowtail sample 3	as purchased	Japan; commercial markets	nd	-	6.36E-07	-	(Sakai et al., 1997)
Yellowtail	as purchased	Japan; commercial markets	-	-	0.37	0.22	(Sakai et al., 2000)
Yellowtail	after 28 weeks of storage; -20ºC	Japan; commercial markets	-	-	6.94	0.50	(Sakai et al., 2000)
Smoked salmon I	as purchased	Japan; commercial markets	-	-	1.24	0.76	(Munasinghe et al., 2003)
Smoked salmon II	as purchased	Japan; commercial markets	-	-	nd	-	(Munasinghe et al., 2003)
Smoked salmon III	as purchased	Japan; commercial markets	-	-	0.01	0.00	(Munasinghe et al., 2003)
Fish meat sausage	as purchased	Japan; commercial markets	-	-	0.02	0.00	(Munasinghe et al., 2003)
Yellow croaker	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	(Surh & Kwon, 2005)
Mackerel raw	as purchased	Korea; local markets	0.01	0.00	0.01	0.01	(Surh & Kwon, 2005)
Mackerel salted	as purchased	Korea; local markets	0.02	0.00	0.08	0.00	(Surh & Kwon, 2005)
Tuna canned	as purchased	Korea; local markets	0.01	0.00	0.03	0.01	(Surh & Kwon, 2005)
Spanish mackerel	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	(Surh & Kwon, 2005)
Hair tail	as purchased	Korea; local markets	nd	-	nd	-	(Surh & Kwon, 2005)
Pacific saury raw	as purchased	Korea; local markets	0.02	0.01	0.06	0.02	(Surh & Kwon, 2005)
Pacific saury canned	as purchased	Korea; local markets	0.01	0.00	0.04	0.00	(Surh & Kwon, 2005)

Flat fish	as purchased	Korea; local markets	nd	-	nd	-	(Surh & Kwon, 2005)
Alaska pollak	as purchased	Korea; local markets	0.01	0.00	0.02	0.00	(Surh & Kwon, 2005)
Alabesque greenling	as purchased	Korea; local markets	0.01	0.00	0.05	0.01	(Surh & Kwon, 2005)
Murex shell	as purchased	Korea; local markets	0.01	0.00	0.04	0.01	(Surh & Kwon, 2005)
Granulated ark shell	as purchased	Korea; local markets	nd	-	nd	-	(Surh & Kwon, 2005)
Hard-shelled mussel	as purchased	Korea; local markets	nd	-	nd	-	(Surh & Kwon, 2005)
Pen shell	as purchased	Korea; local markets	nd	-	nd	-	(Surh & Kwon, 2005)
Oyster	as purchased	Korea; local markets	0.04	0.01	0.14	0.00	(Surh & Kwon, 2005)
Surf clam	as purchased	Korea; local markets	0.01	0.00	0.04	0.01	(Surh & Kwon, 2005)
Jack-knife clam	as purchased	Korea; local markets	0.01	0.00	0.03	0.00	(Surh & Kwon, 2005)
Venus clam	as purchased	Korea; local markets	0.02	0.01	0.07	0.02	(Surh & Kwon, 2005)
Orient hard clam	as purchased	Korea; local markets	0.05	0.01	0.16	0.05	(Surh & Kwon, 2005)
Little neck clam	as purchased	Korea; local markets	0.06	0.00	0.18	0.01	(Surh & Kwon, 2005)
Fish sticks A	as purchased	Korea; local markets	0.01	0.01	nd	-	(Surh et al., 2007)
Fish sticks B	as purchased	Korea; local markets	0.03	0.01	nd	-	(Surh et al., 2007)
Fish sticks C	as purchased	Korea; local markets	0.04	0.02	0.01	0.01	(Surh et al., 2007)
Fish sticks D	as purchased	Korea; local markets	0.04	0.02	0.01	0.01	(Surh et al., 2007)
Tuna canned A	as purchased	Korea; local markets	0.02	0.02	nd	nd	(Surh et al., 2007)
Tuna canned B	as purchased	Korea; local markets	0.03	0.01	nd	-	(Surh et al., 2007)
Tuna canned C	as purchased	Korea; local markets	nd	-	nd	-	(Surh et al., 2007)
Mackerel	26 weeks of storage; -10 ºC	United Kingdom; Industry	0.45	0.02	-	-	(Alghazeer & Howell, 2008)
Sardines canned	as purchased	Belgium	nd	-	0.03	0.00	(Papastergiadis et al., 2014a)
Frozen and fresh salmon	as purchased	Belgium; supermarket chains	0.01	-	0.03	-	(Papastergiadis et al., 2014a)
Smoked salmon	as purchased	Belgium; supermarket chains	0.02	-	0.00	-	(Papastergiadis et al., 2014a)

nd, not detected

Sakai et al. have extensively evaluated the HNE content in pork meat under different conditions of storage (Sakai et al., 1995, 2004, 2006; Sakai, Yamauchi, Kuwazuru, & Gotoh, 1998). However, the reported values for HNE content are very low in comparison with other food matrices such as fish and edible oils (Table 26), which is probably linked with the low content of PUFA in pork meat. Although, Munasinghe et al. have also studied this type of food and HNE content ranged from not detected to 10.6 mg/kg (Munasinghe et al., 2003). However, this value was for pork meat stored at 0 ºC for 10 days, which clearly exceeds the recommended period of storage at this temperature.

Regarding meat products, up to now different types of products were evaluated mainly concerning HNE, such as sausages, ham, bacon and mortadella (Munasinghe et al., 2003; Papastergiadis et al., 2014a; Zanardi et al., 2002). The HNE amounts were slightly higher in these type of processed products than for pork meat, being the highest amount (14.9 mg/kg) reported for ham (Munasinghe et al., 2003). Among the different types of sausage evaluated, the highest reported HNE contents were for blood sausage (12.5 mg/kg), followed by Wiener sausage (6.98 mg/kg) (Gasc et al., 2007; Munasinghe et al., 2003).

Undoubtedly, there is a lack of data on 4-hydroxy-2-alkenals occurrence in meat and meat products. Nevertheless, considering the reported results, if appropriate conditions of storage are applied, HNE amounts are probably not a risk for human health. However, because these compounds are very reactive, it is important in further studies to include other types of meat and meat products, widely available in the market.

Other foods

In Table 27, data on the occurrence of 4-hydroxy-2-alkenals in other foods such as nuts, bakery products, snacks, potato products and algae are shown. In fact, this is a miscellaneous group of foods because there are few data to allow performing other types of comparisons.

Nuts consumption is associated with potential health benefits, mainly due to the presence of PUFA, but also of other bioactive compounds, such as tocopherols and phytosterols. Globisch et al. and Papastergiadis et al. have evaluated the presence of 4-hydroxy-2-alkenals in peanuts, although Globish et al. reported the results per kg of oil, while Papastergiadis et al. reported the results per kg of sample, which are not comparable (Table 27). The HNE content ranged from 0.12 to 0.60 mg/kg for peanuts, while for walnuts it varied between 0.14 and 0.41 mg/kg (Papastergiadis et al., 2014a). Nevertheless, HHE was not detected in peanuts, and for walnuts low values were reported (0.02 and 0.03 mg/kg).

Table 26. Occurrence of 4-hydroxy-2-alkenals in meat and meat products.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Beef meat	as purchased	Japan; commercial markets	2.19E-06 – 21.9E-06	-	-	-	(Sakai et al., 1995)
Pork meat	as purchased	Japan; commercial markets	1.56E-07 – 23.7E-06	-	-	-	(Sakai et al., 1995)
Pork meat	as purchased	Japan; commercial markets	9.42E-07	2.17E-07	-	-	(Sakai et al., 1998)
Pork meat	3 days of storage; 0 ºC	Japan; commercial markets	1.10E-06	3.19E-07	-	-	(Sakai et al., 1998)
Pork meat	7 days of storage; 0 ºC	Japan; commercial markets	1.16E-06	2.94E-07	-	-	(Sakai et al., 1998)
Pork meat	12 days of storage; 0 ºC	Japan; commercial markets	4.37E-06	8.73E-07	-	-	(Sakai et al., 1998)
Pork meat	2 months of storage; -20 ºC	Japan; commercial markets	2.97E-08	1.56E-08	-	-	(Sakai et al., 1998)
Pork meat	4 months of storage; -20 ºC	Japan; commercial markets	7.98E-07	3.44E-07	-	-	(Sakai et al., 1998)
Pork meat	6 months of storage; -20 ºC	Japan; commercial markets	7.97E-07	7.50E-07	-	-	(Sakai et al., 1998)
Pork meat	8 months of storage; -20 ºC	Japan; commercial markets	2.34E-06	1.08E-06	-	-	(Sakai et al., 1998)
Pork meat	11 months of storage; -20 ºC	Japan; commercial markets	2.19E-06	8.75E-07	-	-	(Sakai et al., 1998)
Pork meat	2 months of storage; -80 ºC	Japan; commercial markets	1.33E-07	1.06E-07	-	-	(Sakai et al., 1998)
Pork meat	4 months of storage; -80 ºC	Japan; commercial markets	4.64E-07	3.06E-07	-	-	(Sakai et al., 1998)
Pork meat	6 months of storage; -80 ºC	Japan; commercial markets	1.37E-06	8.59E-07	-	-	(Sakai et al., 1998)
Pork meat	8 months of storage; -80 ºC	Japan; commercial markets	8.59E-07	6.72E-07	-	-	(Sakai et al., 1998)
Pork meat	11 months of storage; -80 ºC	Japan; commercial markets	1.72E-07	1.41E-07	-	-	(Sakai et al., 1998)
Milano sausage	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Cacciatore sausage	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Smoked sausage A	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Smoked sausage B	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Smoked sausage C	as purchased	Italy; local supermarkets	0.41	0.04			(Zanardi et al., 2002)
Smoked sausage D	as purchased	Italy; local supermarkets	0.28	0.14			(Zanardi et al., 2002)
Parma ham	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Cooked ham	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Zampone A	as purchased	Italy; local supermarkets	nd				(Zanardi et al., 2002)
Zampone B	as purchased	Italy; local supermarkets	0.34	0.09			(Zanardi et al., 2002)
Zampone C	as purchased	Italy; local supermarkets	0.13	0.09			(Zanardi et al., 2002)
Zampone D	as purchased	Italy; local supermarkets	0.15	0.06			(Zanardi et al., 2002)

Frankfurter A	as purchased	Italy; local supermarkets	0.71	0.14	(Zanardi et al., 2002)
Frankfurter B	as purchased	Italy; local supermarkets	0.62	0.05	(Zanardi et al., 2002)
Frankfurter C	as purchased	Italy; local supermarkets	0.27	0.05	(Zanardi et al., 2002)
Frankfurter D	as purchased	Italy; local supermarkets	0.46	0.04	(Zanardi et al., 2002)
Mortadella A	as purchased	Italy; local supermarkets	0.06	0.01	(Zanardi et al., 2002)
Mortadella B	as purchased	Italy; local supermarkets	0.16	0.04	(Zanardi et al., 2002)
Mortadella C	as purchased	Italy; local supermarkets	0.32	0.05	(Zanardi et al., 2002)
Mortadella D	as purchased	Italy; local supermarkets	0.08	0.02	(Zanardi et al., 2002)
Ham A	as purchased	Japan; local market	14.9	2.35	(Munasinghe et al., 2003)
Ham B	as purchased	Japan; local market	0.59	0.53	(Munasinghe et al., 2003)
Ham C	as purchased	Japan; local market	3.19	2.86	(Munasinghe et al., 2003)
Bacon A	as purchased	Japan; local market	1.28	0.40	(Munasinghe et al., 2003)
Bacon B	as purchased	Japan; local market	1.39	0.81	(Munasinghe et al., 2003)
Wiener sausage A	as purchased	Japan; local market	6.98	1.32	(Munasinghe et al., 2003)
Wiener sausage B	as purchased	Japan; local market	0.59	0.59	(Munasinghe et al., 2003)
Frankfurter sausage	as purchased	Japan; local market	2.69	2.40	(Munasinghe et al., 2003)
Pork meat	as purchased	Japan; local market	nd	nd	(Munasinghe et al., 2003)
Pork meat	3 days storage in 1% Sugi wood vinegar, 0 ºC	Japan; local market	0.03	0.03	(Munasinghe et al., 2003)
Pork meat	7 days storage in 1% Sugi wood vinegar, 0 ºC	Japan; local market	10.6	1.89	(Munasinghe et al., 2003)
Pork meat	10 days storage, 0 ºC	Japan; local market	nd	nd	(Sakai et al., 2004)
Pork meat	10 days storage with 1% NaCl, 0 ºC	Japan; local market	3.44E-03	3.12E-04	(Sakai et al., 2004)
Pork meat	10 days storage with 2% NaCl, 0 ºC	Japan; local market	4.31E-02	3.44E-03	(Sakai et al., 2004)
Beef meat	10 days storage, 0 ºC	Japan; local market	0.04	0.01	(Sakai et al., 2004)
Beef meat	10 days storage with 1% NaCl, 0 ºC	Japan; local market	0.15	0.03	(Sakai et al., 2004)
Beef meat	10 days storage with 2% NaCl, 0 ºC	Japan; local market	0.11	0.04	(Sakai et al., 2004)
Pork meat	raw; after 3 days of storage	Japan; local market	1.56E-08	6.25E-09	(Sakai et al., 2006)
Pork meat	cooked 100 ºC with 1% NaCl; after 3 days of storage	Japan; local market	1.56E-09	1.56E-09	(Sakai et al., 2006)
Pork meat	cooked 100 ºC with 2% NaCl; after 3 days of storage	Japan; local market	1.56E-09	1.56E-09	(Sakai et al., 2006)
Pork meat	5 min; cooked 100 ºC	Japan; local market	8.28E-08	4.22E-08	(Sakai et al., 2006)
Pork meat	10 min; cooked 100 ºC	Japan; local market	1.11E-07	2.19E-08	(Sakai et al., 2006)

Pork meat	15 min; cooked 100 ºC	Japan; local market	2.39E-07	4.53E-08		(Sakai et al., 2006)
Pork meat	5 min; cooked 100 ºC; after 3 days storage	Japan; local market	2.44E-07	8.44E-08		(Sakai et al., 2006)
Pork meat	10 min; cooked 100 ºC; after 3 days storage	Japan; local market	4.64E-07	1.48E-07		(Sakai et al., 2006)
Pork meat	15 min; cooked 100 ºC; after 3 days storage	Japan; local market	3.14E-07	1.09E-07		(Sakai et al., 2006)
Chicken meat	as purchased	France; local supermarket	nd			(Gasc et al., 2007)
Beef meat	as purchased	France; local supermarket	nd			(Gasc et al., 2007)
Liver paté	as purchased	France; local supermarket	0.11			(Gasc et al., 2007)
Blood sausage	as purchased	France; local supermarket	12.5			(Gasc et al., 2007)
Bacon	as purchased	Belgium; supermarket chains	0.01		0.00	(Papastergiadis et al., 2014a)
Cured ham	as purchased	Belgium; supermarket chains	0.08		0.00	(Papastergiadis et al., 2014a)
Cured and cooked meat products	as purchased	Belgium; supermarket chains	0.11		0.01	(Papastergiadis et al., 2014a)
Cured minced meat products	as purchased	Belgium; supermarket chains	0.55		0.05	(Papastergiadis et al., 2014a)
Chilled cooked meals	as purchased	Belgium; supermarket chains	0.10		0.01	(Papastergiadis et al., 2014a)

nd, not detected

Potato crisps and French fries are palatable foods, highly appreciated by people of all ages. In addition, the supply of this type of products has increased a lot in the last years, existing nowadays a wide range of products. There has also been a change in the type of oil used in frying, and there is a tendency to use oils rich in unsaturated fatty acids, making this an interesting group to be studied regarding the presence of 4-hydroxy-2-alkenals (Albuquerque, Sanches-Silva, Santos, & Costa, 2012).

Table 27. Occurrence of 4-hydroxy-2-alkenals in other food products.

Food matrices	Conditions	Country of origin	HNE (mg/kg)		HHE (mg/kg)		Reference
			Mean	SD	Mean	SD	
Peanuts A	packed under modified atmosphere	Belgium	0.60	-	nd	-	(Papastergiadis et al., 2014a)
Peanuts B	packed under modified atmosphere	Belgium	0.12	-	nd	-	(Papastergiadis et al., 2014a)
Walnuts A	packed under modified atmosphere	Belgium	0.14	-	0.02	-	(Papastergiadis et al., 2014a)
Walnuts B	packed under modified atmosphere	Belgium	0.41	-	0.03	-	(Papastergiadis et al., 2014a)
Dry nuts	as purchased	Belgium; supermarket chains	0.31	-	0.01	-	(Papastergiadis et al., 2014a)
Peanuts	as purchased; raw	Germany; local market	1.56E-07[a)]	-		-	Globisch et al., 2015
Peanuts	20min; 170 ºC	Germany; local market	6.25E-07[a)]	-		-	Globisch et al., 2015
Peanuts	40 min; 170 ºC	Germany; local market	9.37E-07[a)]	-		-	Globisch et al., 2015
Cookies A	as purchased	Belgium	0.13	-	2.50E-02	-	(Papastergiadis et al., 2014a)
Cookies B	as purchased	Belgium	0.10	-	2.10E-02	-	(Papastergiadis et al., 2014a)
Cookies C	as purchased	Belgium	0.18	-	2.10E-02	-	(Papastergiadis et al., 2014a)
Potato crisps	as purchased	Belgium; supermarket chains	0.12	-	8.00E-03	-	(Papastergiadis et al., 2014a)
Cookies	as purchased	Belgium; supermarket chains	0.09	-	4.00E-03	-	(Papastergiadis et al., 2014a)
Soya based products	as purchased	Belgium; supermarket chains	0.06	-	2.00E-03	-	(Papastergiadis et al., 2014a)
Fried snacks	as purchased	Belgium; fast-food shops	0.62	-	2.00E-03	-	(Papastergiadis et al., 2014a)
Fruit juice A	as purchased	Korea; local markets	0.00	0.00	nd	nd	(Surh et al., 2007)
Fruit juice B	as purchased	Korea; local markets	0.00	0.00	nd	nd	(Surh et al., 2007)
Raw cereal/vegetable powder A	as purchased	Korea; local markets	0.14	0.07	0.03	0.01	(Surh et al., 2007)
Raw cereal/vegetable powder B	as purchased	Korea; local markets	0.06	0.01	0.02	0.01	(Surh et al., 2007)
Raw cereal/vegetable powder C	as purchased	Korea; local markets	0.31	0.07	0.02	0.00	(Surh et al., 2007)
Raw cereal/vegetable powder D	as purchased	Korea; local markets	0.19	0.06	0.03	0.01	(Surh et al., 2007)

Raw cereal/vegetable powder E	as purchased	Korea; local markets	0.13	0.04	0.03	0.02	(Surh et al., 2007)
Snacks A	as purchased	Korea; local markets	0.06	0.05	0.01	0.00	(Surh et al., 2007)
Snacks B	as purchased	Korea; local markets	0.02	0.01	nd	nd	(Surh et al., 2007)
Snacks C	as purchased	Korea; local markets	0.07	0.02	nd	nd	(Surh et al., 2007)
Snacks D	as purchased	Korea; local markets	0.08	0.02	0.01	0.01	(Surh et al., 2007)
Snacks E	as purchased	Korea; local markets	0.04	0.03	nd	nd	(Surh et al., 2007)
Snacks F	as purchased	Korea; local markets	0.03	0.00	nd	nd	(Surh et al., 2007)
French fries	5h; 185 ºC	United States; local grocery store	0.49	0.05	-	-	Seppanen & Csallany, 2004
French fries	as purchased	Belgium; fast-food shops	0.07	-	0.00		(Papastergiadis et al., 2014a)
French fries A	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
French fries B	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
French fries C	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
French fries D	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
French fries E	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
French fries F	as purchased	United states; fast-food restaurants	0.00	0.00	-	-	(Csallany et al., 2015)
Egg yolk powder	Enriched with n-3 fatty acids; pasteurized and dried	France	6,85E-05 - 1,48E-04	-	5.47E-05	-	(Meynier et al., 2014)
Porphyra spp.	Grade A; dried	Japan; Industry	0.04[b)]	0.07[b)]	1.83[b)]	1.66[b)]	(Tanaka et al., 2016)
Porphyra spp.	Grade B; dried	Japan; Industry	0.05[b)]	0.05[b)]	7.11[b)]	4.80[b)]	(Tanaka et al., 2016)
Porphyra spp.	Grade C; dried	Japan; Industry	0.04[b)]	0.04[b)]	6.71[b)]	4.03[b)]	(Tanaka et al., 2016)
Porphyra spp.	Grade D; dried	Japan; Industry	0.06[b)]	0.03[b)]	6.79[b)]	4.41[b)]	(Tanaka et al., 2016)

nd, not detected; [a)] mg/kg of oil; [b)] mg/kg of dry weight

Concerning potato products, namely French fries and potato crisps, the highest HNE value (0.49 mg/kg) was reported by Seppanen and Csallany for French fries, but the frying procedure was conducted under extreme conditions since thermal oxidized soybean oil was used

(Seppanen & Csallany, 2004). On the other hand, Csallany et al. evaluated the HNE contents in six types of French fries, being the values for all the samples lower than 0.001 mg/kg (Csallany et al., 2015).

Algae are increasingly being consumed for functional benefits linked with their nutritional and bioactive composition (Wells et al., 2017). In what concerns lipids, algae have a low content of fat, but it is mainly composed by PUFA, namely EPA. As far as authors know, only Tanaka et al. have evaluated the occurrence of 4-hydroxy-2-alkenals in dried laver *Porphyra* spp. that is consumed not only in Japan but also in other regions, such as Europe and America (Tanaka, Ishimaru, Hatate, Sugiura, & Matsushita, 2016). HHE content in dried laver *Porphyra* spp. varied between 1.83 and 6.79 mg/kg, while HNE content was much lower, ranging from 0.04 to 0.06 mg/kg (Table 27).

Final remarks

Avoiding lipid oxidation in foods is of utmost importance and crucial to assure the safety and quality of foods, because it is responsible for the formation of hazardous compounds such as 4-hydroxy-2-alkenals. Figure 38 summarizes the achievements and the needs in the near future concerning 4-hydroxy-alkenals in foods.

With this study, it was possible to understand that these compounds occur all over the food groups, but essentially in oils and fats and foods with a high content in n-3 and n-6 PUFA that are precursors of HHE and HNE, respectively. This is worthy of special attention, mainly because there is a trend to increase the consumption of these foods, but also to enrich other foods that do not naturally contain this type of fatty acids, which possibly will represent an increase in the intake of 4-hydroxy-2-alkenals. However, the literature also evidenced that there are few studies showing that it is possible to mitigate or retard the formation of these compounds in foods using antioxidants, like vitamin E and phenolics.

As far as authors know, up to now, there is not a standard analytical methodology to determine 4-hydroxy-2-alkenals in foods, which is a difficult task taking into account the complexity of the different food matrices. Furthermore, some of the existent methods lack sensitivity and do not have acceptable recoveries. Regarding exposure and risk assessment, up to now, a low number of studies have dedicated their research to this subject, which is critical because this data is needed to establish safe doses for these aldehydes.

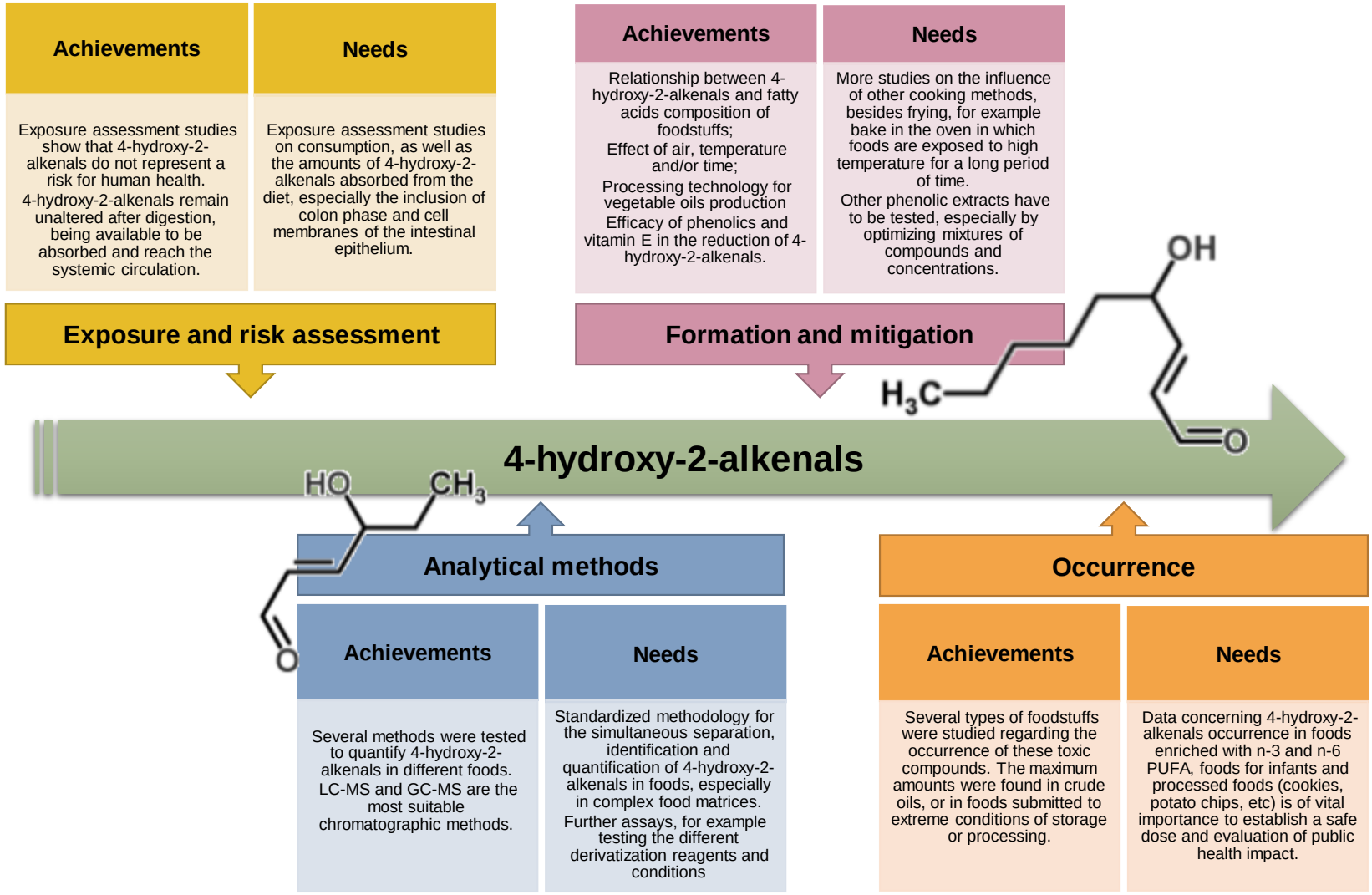


Figure 38. Summary concerning the achievements and the needs for 4-hydroxy-2-alkenals in foods, namely on exposure and risk assessment, analytical methods, formation and mitigation, and occurrence.

Nevertheless, the reported results already indicate that a considerable amount of these compounds remain unaltered after digestion, which increases its availability to reach the systemic circulation.

In summary, apart from what the future holds, plentiful research should be done soon, especially concerning the occurrence of these toxic compounds in foods enriched with n-3 and n-6 PUFA. Additionally, it will only be possible to realize the desired goals, if joint efforts, that must encompass food industry, governing bodies, research and academia, are taken. Nevertheless, we believe that it is possible to eliminate or effectively reduce 4-hydroxy-2-alkenals in foods using simple and economic practices.

References

Aladedunye, F. A., Matthäus, B., & Przybylski, R. (2011). Carbon dioxide blanketing impedes the formation of 4-hydroxynonenal and acrylamide during frying. A novel procedure for HNE quantification. *European Journal of Lipid Science and Technology*, *113*(7), 916–923. https://doi.org/10.1002/ejlt.201100021

Aladedunye, F. A., & Przybylski, R. (2011). Antioxidative properties of phenolic acids and interaction with endogenous minor components during frying. *European Journal of Lipid Science and Technology*, *113*(12), 1465–1473. https://doi.org/10.1002/ejlt.201100142

Aladedunye, F. A., & Przybylski, R. (2012). Frying performance of canola oil triacylglycerides as affected by vegetable oils minor components. *JAOCS, Journal of the American Oil Chemists' Society*, *89*(1), 41–53. https://doi.org/10.1007/s11746-011-1887-8

Albuquerque, T. G., Oliveira, M. B. P. P., & Costa, H. S. (2018). 4-Hydroxy-2-Alkenals : A Potential Toxicological Concern of Vegetable Oils ? In G. W. Smithers (Ed.), *Reference Module in Food Science* (pp. 1–8). Academic Press. https://doi.org/10.1016/B978-0-08-100596-5.21014-7

Albuquerque, T. G., Sanches-Silva, A., Santos, L., & Costa, H. S. (2012). An update on potato crisps contents of moisture, fat, salt and fatty acids (including trans -fatty acids) with special emphasis on new oils/fats used for frying. *International Journal of Food Sciences and Nutrition*, *63*, 713–717. https://doi.org/10.3109/09637486.2011.644768

Alexander, J., Benford, D., Boobis, A., Eskola, M., Fink-gremmels, J., Fürst, P., … Leeuwen, R. Van. (2012). Risk assessment of contaminants in food and feed. *EFSA Journal*, *10*(10), 1–12. https://doi.org/10.2903/j.efsa.2012.s1004.

Alghazeer, R., & Howell, N. K. (2008). Formation of 4-hydroxynonenal (4-HNE) in frozen mackerel (Scomber scombrus) in the presence and absence of green tea. *Journal of the Science of Food and Agriculture*, *88*, 1128–1134. https://doi.org/10.1002/jsfa.3117

Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, *2014*. https://doi.org/10.1155/2014/360438

Brewer, M. S. (2011). Natural Antioxidants: Sources, Compounds, Mechanisms of Action, and Potential Applications. *Comprehensive Reviews in Food Science and Food Safety*, *10*(4), 221–247. https://doi.org/10.1111/j.1541-4337.2011.00156.x

Choe, E., & Min, D. B. (2006). Mechanisms and factors for edible oil oxidation. *Comprehensive Reviews in Food Science and Food Safety*, *5*(4), 169–186. https://doi.org/10.1111/j.1541-4337.2006.00009.x

Costa, D. C., Costa, H. S., Albuquerque, T. G., Ramos, F., Castilho, M. C., & Sanches-Silva, A. (2015). Advances in phenolic compounds analysis of aromatic plants and their potential applications. *Trends in Food Science and Technology*, *45*(2), 336–354. https://doi.org/10.1016/j.tifs.2015.06.009

Csala, M., Kardon, T., Legeza, B., Lizák, B., Mandl, J., Margittai, É., … Bánhegyi, G. (2015). On the role of 4-hydroxynonenal in health and disease. *Biochimica et Biophysica Acta - Molecular Basis of Disease*, *1852*(5), 826–838. https://doi.org/10.1016/j.bbadis.2015.01.015

Csallany, A. S., Han, I., Shoeman, D. W., Chen, C., & Yuan, J. (2015). 4-Hydroxynonenal (HNE), a Toxic Aldehyde in French Fries from Fast Food Restaurants. *JAOCS, Journal of the American Oil Chemists' Society*, *92*(10), 1413–1419. https://doi.org/10.1007/s11746-015-2699-z

Douny, C., Tihon, A., Bayonnet, P., Brose, F., Degand, G., Rozet, E., … Scippo, M. L. (2015). Validation of the analytical procedure for the determination of malondialdehyde and three other aldehydes in vegetable oil using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) and application to linseed oil. *Food Analytical Methods*, *8*(6), 1425–1435. https://doi.org/10.1007/s12161-014-0028-z

Dubois, V., Breton, S., Linder, M., Fanni, J., & Parmentier, M. (2007). Fatty acid profiles of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology*, *109*(7), 710–732. https://doi.org/10.1002/ejlt.200700040

Eckl, P. M., & Bresgen, N. (2017). Genotoxicity of lipid oxidation compounds. *Free Radical Biology and Medicine*, *111*(December 2016), 244–252. https://doi.org/10.1016/j.freeradbiomed.2017.02.002

Esterbauer, H., Schaur, R. J., & Zollner, H. (1991). Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. *Free Radical Biology and Medicine*, *11*(1), 81–128. https://doi.org/10.1016/0891-5849(91)90192-6

European Food Safety Authority. (2012). *Scientific Opinion on Exploring options for providing advice about possible human health risks based on the concept of Threshold of Toxicological Concern (TTC)*. *EFSA Journal* (Vol. 10). https://doi.org/10.2903/j.efsa.2012.2750

Food and Agriculture Organization of the United Nations/World Health Organization. (2010). *Report of the joint FAO/WHO expert consultation on the risks and benefits of fish consumption*. *FAO Fisheries and Acquaculture report n.º 978*. Retrieved from http://www.fao.org/docrep/014/ba0136e/ba0136e00.pdf

Foster, R., Williamson, C. S., & Lunn, J. (2009). Culinary oils and their health effects. *Nutrition Bulletin*, *34*(1), 4–47. https://doi.org/10.1111/j.1467-3010.2008.01738.x

Fujisaki, M., Endo, Y., & Fujimoto, K. (2002). Retardation of volatile aldehyde formation in the exhaust of frying oil by heating under low oxygen atmospheres. *JAOCS, Journal of the American Oil Chemists' Society*, *79*(9), 909–914. https://doi.org/10.1007/s11746-002-0578-3

Gabbanini, S., Matera, R., Valvassori, A., & Valgimigli, L. (2015). Rapid liquid chromatography-tandem mass spectrometry analysis of 4-hydroxynonenal for the assessment of oxidative degradation and safety of vegetable oils. *Analytica Chimica Acta*, *869*, 50–58. https://doi.org/10.1016/j.aca.2015.02.046

Gasc, N., Taché, S., Rathahao, E., Bertrand-Michel, J., Roques, V., & Guéraud, F. (2007). 4-Hydroxynonenal in Foodstuffs: Heme Concentration, Fatty Acid Composition and Freeze-Drying Are Determining Factors. *Redox Report : Communications in Free Radical Research*, *12*(1), 40–44. https://doi.org/10.1179/135100007X162257

Goicoechea, E., Brandon, E. F. A., Blokland, M. H., & Guillen, M. D. (2011). Fate in digestion in vitro of several food components, including some toxic compounds coming from omega-3 and omega-6 lipids. *Food and Chemical Toxicology*, *49*(1), 115–124. https://doi.org/10.1016/j.fct.2010.10.005

Goicoechea, E., Van Twillert, K., Duits, M., Brandon, E. D. F. A., Kootstra, P. R., Blokland, M. H., & Guillén, M. D. (2008). Use of an in vitro digestion model to study the bioaccessibility of 4-hydroxy-2-nonenal and related aldehydes present in oxidized oils rich in omega-6 acyl groups. *Journal of Agricultural and Food Chemistry*, *56*(18), 8475–8483. https://doi.org/10.1021/jf801212k

Goldring, C., Casini, A. F., Maellaro, E., del Bello, B., & Comporti, M. (1993). Determination of 4-Hydroxynonenal by High.Performance Liquid Chromatography with Electrochemical Detection. *Lipids*, *28*, 141–145. https://doi.org/10.1007/BF02535778

Grune, T., Krämer, K., Hoppe, P. P., & Siems, W. (2001). Enrichment of eggs with n-3 polyunsaturated fatty acids: effects of vitamin E supplementation. *Lipids*, *36*(8), 833–8. https://doi.org/10.1007/s11745-001-0792-7

Guéraud, F. (2017). 4-Hydroxynonenal metabolites and adducts in pre-carcinogenic conditions and cancer. *Free Radical Biology and Medicine*, *111*(January), 196–208. https://doi.org/10.1016/j.freeradbiomed.2016.12.025

Guéraud, F., Atalay, M., Bresgen, N., Cipak, A., Eckl, P. M., Huc, L., … Uchida, K. (2010). Chemistry and biochemistry of lipid peroxidation products. *Free Radical Research*, *44*(10), 1098–1124. https://doi.org/10.3109/10715762.2010.498477

Guillén, M. D., & Goicoechea, E. (2008a). Formation of oxygenated α,β-unsaturated aldehydes and other toxic compounds in sunflower oil oxidation at room temperature in closed receptacles. *Food Chemistry*, *111*(1), 157–164. https://doi.org/10.1016/j.foodchem.2008.03.052

Guillén, M. D., & Goicoechea, E. (2008b). Toxic oxygenated α,β-unsaturated aldehydes and their study in foods: A review. *Critical Reviews in Food Science and Nutrition*, *48*(2), 119–136. https://doi.org/10.1080/10408390601177613

Guillén, M. D., & Goicoechea, E. (2009). Oxidation of corn oil at room temperature: Primary and secondary oxidation products and determination of their concentration in the oil liquid matrix from 1H nuclear magnetic resonance data. *Food Chemistry*, *116*(1), 183–192. https://doi.org/10.1016/j.foodchem.2009.02.029

Guillén, M. D., & Ruiz, A. (2008). Monitoring of heat-induced degradation of edible oils by proton NMR. *European Journal of Lipid Science and Technology*, *110*(1), 52–60. https://doi.org/10.1002/ejlt.200600299

Guillén, M. D., & Uriarte, P. S. (2012). Aldehydes contained in edible oils of a very different nature after prolonged heating at frying temperature: Presence of toxic oxygenated α,β

unsaturated aldehydes. *Food Chemistry*, *131*(3), 915–926. https://doi.org/10.1016/j.foodchem.2011.09.079

Guyon, C., Meynier, A., & de Lamballerie, M. (2016). Protein and lipid oxidation in meat: A review with emphasis on high-pressure treatments. *Trends in Food Science and Technology*, *50*, 131–143. https://doi.org/10.1016/j.tifs.2016.01.026

Han, I. H., & Csallany, A. S. (2008). Temperature dependence of HNE formation in vegetable oils and butter oil. *Journal of the American Oil Chemists' Society*, *85*(8), 777–782. https://doi.org/10.1007/s11746-008-1250-x

Han, I. H., & Csallany, A. S. (2009). Formation of toxic α,β-unsaturated 4-hydroxy-aldehydes in thermally oxidized fatty acid methyl esters. *JAOCS, Journal of the American Oil Chemists' Society*, *86*(3), 253–260. https://doi.org/10.1007/s11746-008-1343-6

Han, I. H., & Csallany, A. S. (2012). The toxic aldehyde, 4-hydroxy-2-trans-nonenal (HNE) formation in natural and imitation mozzarella cheeses: Heat treatment effects. *JAOCS, Journal of the American Oil Chemists' Society*, *89*(10), 1801–1805. https://doi.org/10.1007/s11746-012-2084-0

Hernández, P., Park, D., & Rhee, K. S. (2002). Chloride salt type/ionic strength, muscle site and refrigeration effects on antioxidant enzymes and lipid oxidation in pork. *Meat Science*, *61*(4), 405–410. https://doi.org/10.1016/S0309-1740(01)00212-1

Hosomi, R., Yoshida, M., & Fukunaga, K. (2012). Seafood Consumption and Components for Health. *Global Journal of Health Science*, *4*(3), 72–86. https://doi.org/10.5539/gjhs.v4n3p72

Hotchkiss, J. H., Werner, B. G., & Lee, E. Y. C. (2006). Addition of carbon dioxide to dairy products to improve quality: A comprehensive review. *Comprehensive Reviews in Food Science and Food Safety*, *5*(4), 158–168. https://doi.org/10.1111/j.1541-4337.2006.00008.x

Hua, H., Zhao, X., Wu, S., & Li, G. (2016). Impact of refining on the levels of 4-hydroxy-trans-alkenals, parent and oxygenated polycyclic aromatic hydrocarbons in soybean and rapeseed oils. *Food Control*, *67*, 82–89. https://doi.org/10.1016/j.foodcont.2016.02.028

Kanazawa, K., & Ashida, H. (1998). Dietary hydroperoxides of linoleic acid decompose to aldehydes in stomach before being absorbed into the body. *Biochimica et Biophysica*

Acta - Lipids and Lipid Metabolism, *1393*(2–3), 349–361. https://doi.org/10.1016/S0005-2760(98)00089-7

Kumar, Y., Yadav, D. N., Ahmad, T., & Narsaiah, K. (2015). Recent Trends in the Use of Natural Antioxidants for Meat and Meat Products. *Comprehensive Reviews in Food Science and Food Safety*, *14*(6), 796–812. https://doi.org/10.1111/1541-4337.12156

LaFond, S. I., Jerrell, J. P., Cadwallader, K. R., & Artz, W. E. (2011). Formation of 4-hydroxy-2-(E)-nonenal in a corn-soy oil blend: A controlled heating study using a French fried potato model. *JAOCS, Journal of the American Oil Chemists' Society*, *88*(6), 763–772. https://doi.org/10.1007/s11746-010-1738-z

Larsson, K., Harrysson, H., Havenaar, R., Alminger, M., & Undeland, I. (2016). Formation of malondialdehyde (MDA), 4-hydroxy-2-hexenal (HHE) and 4-hydroxy-2-nonenal (HNE) in fish and fish oil during dynamic gastrointestinal in vitro digestion. *Food and Function*, *7*(2), 1176–1187. https://doi.org/10.1039/c5fo01401h

Lee, S. K., Mei, L., & Decker, E. a. (1997). Influence of sodium chloride on antioxidant enzyme activity and lipid oxidation in frozen ground pork. *Meat Science*, *46*(4), 349–55. https://doi.org/10.1016/S0309-1740(97)00029-6

Liu, Y. M., Miao, J. R., & Toyo'oka, T. (1996). Determination of 4-hydroxy-2-nonenal by precolumn derivatization and liquid chromatography with laser fluorescence detection. *Journal of Chromatography A*, *719*(2), 450–456. https://doi.org/10.1016/0021-9673(95)00733-4

Long, E. K., & Picklo, M. J. (2010). Trans-4-hydroxy-2-hexenal, a product of n-3 fatty acid peroxidation: Make some room HNE... *Free Radical Biology and Medicine*, *49*(1), 1–8. https://doi.org/10.1016/j.freeradbiomed.2010.03.015

Lucarini, M., Durazzo, A., Sánchez del Pulgar, J., Gabrielli, P., & Lombardi-Boccia, G. (2018). Determination of fatty acid content in meat and meat products: The FTIR-ATR approach. *Food Chemistry*, *267*, 223–230. https://doi.org/10.1016/j.foodchem.2017.11.042

Lynch, M. P., Faustman, C., Silbart, L. K., Rood, D., & Furr, H. C. (2001). Detection of lipid-derived aldehydes and aldehyde: Protein adducts in vitro and in beef. *Journal of Food Science*, *66*(8), 1093–1099. https://doi.org/10.1111/j.1365-2621.2001.tb16087.x

Ma, L., & Liu, G. (2017). Simultaneous Analysis of Malondialdehyde, 4-Hydroxy-2-hexenal, and 4-Hydroxy-2-nonenal in Vegetable Oil by Reversed-Phase High-Performance

Liquid Chromatography. *Journal of Agricultural and Food Chemistry*, *65*(51), 11320–11328. https://doi.org/10.1021/acs.jafc.7b04566

Maqsood, S., Benjakul, S., Abushelaibi, A., & Alam, A. (2014). Phenolic Compounds and Plant Phenolic Extracts as Natural Antioxidants in Prevention of Lipid Oxidation in Seafood: A Detailed Review. *Comprehensive Reviews in Food Science and Food Safety*, *13*(6), 1125–1140. https://doi.org/10.1111/1541-4337.12106

Mariutti, L. R. B., & Bragagnolo, N. (2017). Influence of salt on lipid oxidation in meat and seafood products: A review. *Food Research International*, *94*, 90–100. https://doi.org/10.1016/j.foodres.2017.02.003

Matera, R., Gabbanini, S., Valvassori, A., Triquigneaux, M., & Valgimigli, L. (2012). Reactivity of (E)-4-hydroxy-2-nonenal with fluorinated phenylhydrazines: Towards the efficient derivatization of an elusive key biomarker of lipid peroxidation. *European Journal of Organic Chemistry*, (20), 3841–3851. https://doi.org/10.1002/ejoc.201200346

Meynier, A., Leborgne, C., Viau, M., Schuck, P., Guichardant, M., Rannou, C., & Anton, M. (2014). N-3 fatty acid enriched eggs and production of egg yolk powders: An increased risk of lipid oxidation? *Food Chemistry*, *153*, 94–100. https://doi.org/10.1016/j.foodchem.2013.12.028

Michalski, M.-C., Calzada, C., Makino, A., Michaud, S., & Guichardant, M. (2008). Oxidation products of polyunsaturated fatty acids in infant formulas compared to human milk - A preliminary study. *Molecular Nutrition & Food Research*, *52*(12), 1478–1485. https://doi.org/10.1002/mnfr.200700451

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food-an international consensus. *Food and Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Minihane, A. M., & Harland, J. I. (2007). Impact of oil used by the frying industry on population fat intake. *Critical Reviews in Food Science and Nutrition*, *47*(3), 287–297. https://doi.org/10.1080/10408390600737821

Munasinghe, D. M. S., Ichimaru, K., Matsui, T., Sugamoto, K., & Sakai, T. (2003). Lipid peroxidation-derived cytotoxic aldehyde, 4-hydroxy-2-nonenal in smoked pork. *Meat Science*, *63*(3), 377–380. https://doi.org/10.1016/S0309-1740(02)00096-7

Nikoo, M., Regenstein, J. M., & Ahmadi Gavlighi, H. (2018). Antioxidant and Antimicrobial Activities of (-)-Epigallocatechin-3-gallate (EGCG) and its Potential to Preserve the

Quality and Safety of Foods. *Comprehensive Reviews in Food Science and Food Safety*, *17*(3), 732–753. https://doi.org/10.1111/1541-4337.12346

Oarada, M., Miyazawa, T., & Kaneda, T. (1986). Methyl Linoleate Hgdroperoxides and Their Secondary. *Lipids*, *21*(2), 150–154

Papastergiadis, A., Fatouh, A., Jacxsens, L., Lachat, C., Shrestha, K., Daelman, J., … De Meulenaer, B. (2014). Exposure assessment of Malondialdehyde, 4-Hydroxy-2-(E)-Nonenal and 4-Hydroxy-2-(E)-Hexenal through specific foods available in Belgium. *Food and Chemical Toxicology*, *73*, 51–58. https://doi.org/10.1016/j.fct.2014.06.030

Papastergiadis, A., Mubiru, E., Van Langenhove, H., & De Meulenaer, B. (2014). Development of a sensitive and accurate stable isotope dilution assay for the simultaneous determination of free 4-hydroxy-2-(E)-nonenal and 4-hydroxy-2-(E)-hexenal in various food matrices by gas chromatography-mass spectrometry. *Food Analytical Methods*, *7*(4), 836–843. https://doi.org/10.1007/s12161-013-9689-2

Petersen, K. D., Jahreis, G., Busch-Stockfisch, M., & Fritsche, J. (2013). Chemical and sensory assessment of deep-frying oil alternatives for the processing of French fries. *European Journal of Lipid Science and Technology*, *115*(8), 935–945. https://doi.org/10.1002/ejlt.201200375

Pillon, N. J., Croze, M. L., Vella, R. E., Soul??re, L., Lagarde, M., & Soulage, C. O. (2012). The lipid peroxidation by-product 4-hydroxy-2-nonenal (4-HNE) induces insulin resistance in skeletal muscle through both carbonyl and oxidative stress. *Endocrinology*, *153*(5), 2099–2111. https://doi.org/10.1210/en.2011-1957

Sakai, T., Kazuhiro, S., & Eto, N. (2000). Cytotoxicity of 4-Hydroxy-2E-hexenal, a Lipid Peroxidation-derived Aldehyde, and Changes of Its Content in Frozen Yellowtail Meat. *Journal of the Food Hygienic Society of Japan*, *41*(6), 368–370. https://doi.org/10.3358/shokueishi.41.368

Sakai, T., & Kuwazuru, S. (1995). a Lipid Peroxidation-Derived Aldehyde, 4-Hydroxy-2-Nonenal, Contents in Several Fish Meats. *Fisheries Science*, *61*(3), 527–528. https://doi.org/10.2331/fishsci.61.527

Sakai, T., Kuwazuru, S., Yamauchi, K., & Uchida, K. (1995). A lipid peroxidation-derived aldehyde, 4-hydroxy-2-nonenal and omega 6 fatty acids contents in meats. *Bioscience, Biotechnology, and Biochemistry*, *59*(7), 1379–1380. https://doi.org/10.1271/bbb.59.1379

Sakai, T., Matsushita, Y., Sugamoto, K., & Uchida, K. (1997). Lipid Peroxidation-derived Hepatotoxic Aldehyde, 4-Hydroxy-2-hexenal, in Fish. *Bioscience, Biotechnology, and Biochemistry*, *61*(8), 1399–1400. https://doi.org/10.1271/bbb.61.1399

Sakai, T., Munasinghe, D. M. S., Kashimura, M., Sugamoto, K., & Kawahara, S. (2004). Effect of NaCl on lipid peroxidation-derived aldehyde, 4-hydroxy-2-nonenal formation in minced pork and beef. *Meat Science*, *66*(4), 789–792. https://doi.org/10.1016/S0309-1740(03)00138-4

Sakai, T., Shimizu, Y., & Kawahara, S. (2006). Effect of NaCl on the Lipid Peroxidation-Derived Aldehyde, 4-Hydroxy-2-nonenal, Formation in Boiled Pork. *Bioscience, Biotechnology, and Biochemistry*, *70*(4), 815–820. https://doi.org/10.1271/bbb.70.815

Sakai, T., Yamauchi, K., Kuwazuru, S., & Gotoh, N. (1998). Relationships between 4-hydroxy-2-nonenal, 2-thiobarbituric acid reactive substances and n-6 polyunsaturated fatty acids in refrigerated and frozen pork. *Bioscience Biotechnology and Biochemistry*, *62*(10), 2028–2029. https://doi.org/10.1271/bbb.62.2028

Santaniello, E., Repetto, A., Chiesa, L. M., & Biondi, P. A. (2007). Synthesis and characterization of 4-hydroxy-2-nonenal derivatives for gas chromatographic analysis with electron capture detection (GC-ECD). *Redox Report*, *12*(1–2), 55–58. https://doi.org/10.1179/135100007X162293

Seppanen, C. M., & Csallany, A. S. (2001). Simultaneous determination of lipophilic aldehydes by high-performance liquid chromatography in vegetable oil. *JAOCS, Journal of the American Oil Chemists' Society*, *78*(12), 1253–1260. https://doi.org/10.1007/s11745-001-0422-9

Seppanen, C. M., & Csallany, A. S. (2002). Formation of 4-hydroxynonenal, a toxic aldehyde, in soybean oil at frying temperature. *JAOCS, Journal of the American Oil Chemists' Society*, *79*(10), 1033–1038. https://doi.org/10.1007/s11746-002-0598-z

Seppanen, C. M., & Csallany, A. S. (2004). Incorporation of the toxic aldehyde 4-hydroxy-2-trans-nonenal into food fried in thermally oxidized soybean oil. *JAOCS, Journal of the American Oil Chemists' Society*, *81*(12), 1137–1141. https://doi.org/10.1007/s11746-004-1031-3

Seppanen, C. M., & Csallany, A. S. (2006). The effect of intermittent and continuous heating of soybean oil at frying temperature on the formation of 4-hydroxy-2-trans-nonenal and other α-, β-unsaturated hydroxyaldehydes. *Journal of the American Oil Chemists' Society*, *83*(2), 121–127. https://doi.org/10.1007/s11746-006-1184-0

Silva, M. A., Albuquerque, T. G., Oliveira, M. B. P. P., & Costa, H. S. (2017). Vitamin C evaluation in foods for infants and young children by a rapid and accurate analytical method. *Food Chemistry*, *267*(January 2017), 83–90. https://doi.org/10.1016/j.foodchem.2017.11.046

Sirot, V., Oseredczuk, M., Bemrah-Aouachria, N., Volatier, J. L., & Leblanc, J. C. (2008). Lipid and fatty acid composition of fish and seafood consumed in France: CALIPSO study. *Journal of Food Composition and Analysis*, *21*(1), 8–16. https://doi.org/10.1016/j.jfca.2007.05.006

Sousa, B. C., Pitt, A. R., & Spickett, C. M. (2017). Chemistry and analysis of HNE and other prominent carbonyl-containing lipid oxidation compounds. *Free Radical Biology and Medicine*, *111*(February), 294–308. https://doi.org/10.1016/j.freeradbiomed.2017.02.003

Spickett, C. M. (2013). The lipid peroxidation product 4-hydroxy-2-nonenal: Advances in chemistry and analysis. *Redox Biology*, *1*(1), 145–152. https://doi.org/10.1016/j.redox.2013.01.007

Steppeler, C., Haugen, J. E., Rødbotten, R., & Kirkhus, B. (2016). Formation of Malondialdehyde, 4-Hydroxynonenal, and 4-Hydroxyhexenal during in Vitro Digestion of Cooked Beef, Pork, Chicken, and Salmon. *Journal of Agricultural and Food Chemistry*, *64*(2), 487–496. https://doi.org/10.1021/acs.jafc.5b04201

Surh, J., & Kwon, H. (2003). Simultaneous determination of 4-hydroxy-2-alkenals, lipid peroxidation toxic products. *Food Additives and Contaminants*, *20*(4), 325–330. https://doi.org/10.1080/0265203031000122012

Surh, J., & Kwon, H. (2005). Estimation of daily exposure to 4-hydroxy-2-alkenals in Korean foods containing n-3 and n-6 polyunsaturated fatty acids. *Food Additives and Contaminants*, *22*(8), 701–708. https://doi.org/10.1080/02652030500164359

Surh, J., Lee, B. Y., & Kwon, H. (2010). Influence of fatty acids compositions and manufacturing type on the formation of 4-hydroxy-2-alkenals in food lipids. *Food Science and Biotechnology*, *19*(2), 297–303. https://doi.org/10.1007/s10068-010-0043-9

Surh, J., Lee, S., & Kwon, H. (2007). 4-Hydroxy-2-alkenals in polyunsaturated fatty acids-fortified infant formulas and other commercial food products. *Food Additives and Contaminants*, *24*(11), 1209–1218. https://doi.org/10.1080/02652030701422465

Takamura, H., & Gardner, H. W. (1996). Oxygenation of (3Z)-alkenal to (2E)-4-hydroxy-2-alkenal in soybean seed (Glycine max L.). *Biochimica et Biophysica Acta - Lipids and Lipid Metabolism*, *1303*(2), 83–91. https://doi.org/10.1016/0005-2760(96)00076-8

Tanaka, R., Ishimaru, M., Hatate, H., Sugiura, Y., & Matsushita, T. (2016). Relationship between 4-hydroxy-2-hexenal contents and commercial grade by organoleptic judgement in Japanese dried laver Porphyra spp. *Food Chemistry*, *212*, 104–109. https://doi.org/10.1016/j.foodchem.2016.05.166

Uchida, T., Gotoh, N., & Wada, S. (2002). Method for analysis of 4-hydroxy-2-(E)-nonenal with solid-phase microextraction. *Lipids*, *37*(6), 621–626. https://doi.org/10.1007/s11745-002-0941-z

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. (2017). USDA National Nutrient Database for Standard Reference, Release 28. Nutrient Data Laboratory. URL: http://www.ars.usda.gov/ba/bhnrc/ndl. Accessed 10.07.18

Viau, M., Genot, C., Ribourg, L., & Meynier, A. (2016). Amounts of the reactive aldehydes, malonaldehyde, 4-hydroxy-2-hexenal, and 4-hydroxy-2-nonenal in fresh and oxidized edible oils do not necessary reflect their peroxide and anisidine values. *European Journal of Lipid Science and Technology*, *118*(3), 435–444. https://doi.org/10.1002/ejlt.201500103

Vieira, S. A., Zhang, G., & Decker, E. A. (2017). Biological Implications of Lipid Oxidation Products. *Journal of the American Oil Chemists' Society*, *94*(3), 339–351. https://doi.org/10.1007/s11746-017-2958-2

Wells, M. L., Potin, P., Craigie, J. S., Raven, J. A., Merchant, S. S., Helliwell, K. E., … Brawley, S. H. (2017). Algae as nutritional and functional food sources: revisiting our understanding. *Journal of Applied Phycology*, *29*(2), 949–982. https://doi.org/10.1007/s10811-016-0974-5

Zamora, R., Aguilar, I., Granvogl, M., & Hidalgo, F. J. (2016). Toxicologically Relevant Aldehydes Produced during the Frying Process Are Trapped by Food Phenolics. *Journal of Agricultural and Food Chemistry*, *64*(27), 5583–5589. https://doi.org/10.1021/acs.jafc.6b02165

Zanardi, E., Jagersma, C. G., Ghidini, S., & Chizzolini, R. (2002). Solid Phase Extraction and Liquid Chromatography − Tandem Mass Spectrometry for the Evaluation of 4-

Hydroxy-2-nonenal in Pork Products. *Journal of Agricultural and Food Chemistry*, *50*(14), 5268–5272. https://doi.org/10.1021/jf020201h

Zarkovic, N. (2003). 4-Hydroxynonenal as a bioactive marker of pathophysiological processes. *Molecular Aspects of Medicine*, *24*(4–5), 281–291. https://doi.org/10.1016/S0098-2997(03)00023-2

Zhong, H., & Yin, H. (2015). Role of lipid peroxidation derived 4-hydroxynonenal (4-HNE) in cancer: Focusing on mitochondria. *Redox Biology*, *4*, 193–199. https://doi.org/10.1016/j.redox.2014.12.011

Zhu, Q., Zheng, Z. P., Cheng, K. W., Wu, J. J., Zhang, S., Yun, S. T., … Wang, M. (2009). Natural polyphenols as direct trapping agents of lipid peroxidation-derived acrolein and 4-hydroxy-trans-2-nonenal. *Chemical Research in Toxicology*, *22*(10), 1721–1727. https://doi.org/10.1021/tx900221s

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CAPÍTULO 4. Cloropropanóis e ésteres glicidílicos de ácidos gordos em óleos vegetais e alimentos processados

No presente capítulo apresentam-se os resultados relativos à ocorrência de cloropropanóis e de ésteres glicidílicos de ácidos gordos em óleos e gorduras. Também são abordadas questões relacionadas com o potencial impacto na saúde, metodologias analíticas, precursores e estratégias de mitigação destes compostos.

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4.1 3-MCPD Occurrence in vegetable oils: Impact on human nutrition and future challenges

Este sub-capítulo apresenta os resultados relativos à revisão bibliográfica sobre a ocorrência de 3-monocloropropano-1,2-diol nos diferentes óleos e gorduras. Verificou-se que existem diferenças consideráveis entre os diferentes tipos de óleos avaliados, mas também para o mesmo tipo de óleo. Considera-se que as condições de processamento, nomeadamente refinação e desodorização, podem ter um impacto substancial na presença destes compostos. São necessários mais estudos, sobretudo no que diz respeito à avaliação do impacto de condições realísticas de fritura.

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3-MCPD Occurrence in Vegetable Oils: Impact on Human Nutrition and Future Challenges

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Abstract

Over the last years, the global production of vegetable oils increased, and palm oil is still the most produced vegetable oil, followed by soybean, rapeseed and sunflower oils. Processing of vegetable oils is essential to remove impurities from the oil and to assure their quality and safety. Nonetheless, some of the applied conditions, namely during deodorization, can lead to the formation of contaminants, such as chloropropanols. In this review, an overview of the occurrence of 3-monochloropropane-1,2-diol in vegetable oils, as well as its potential impact on human nutrition, based on exposure assessment to this contaminant, and future challenges are discussed. According to this literature review, notable differences are found for the occurrence of 3-monochloropropane-1,2-diol in the different vegetable oils, but also among the same type of oil, which is possibly due to the geographical origin of samples, their composition, but also due to the processing conditions applied. It has been observed that unprocessed oils/fats have non-detectable or very low amounts of 3-monochloropropane-1,2-diol, while the refined oils have high amounts. Amongst the reviewed data, the highest values reported were for rice bran oil (1449 – 2564 mg/kg) and edible blending oil (1367 mg/kg). For instance, for palm oil, which is the most widely consumed vegetable oil, the values for 3-monochloropropane-1,2-diol ranged from not detected to 540 mg/kg. With respect to mitigation strategies, the use of radical scavengers, such as phenolic compounds, is efficient, but there is still a lot of work to be done in this area of research. Regarding exposure assessment to this contaminant, few studies have focused on this subject, but the reported results indicate that the mean exposure value is lower than the tolerable daily intake (2 µg/kg of body weight/day). Nonetheless, among the different food groups evaluated for exposure assessment, vegetable oils/fats were identified as the major contributor for children. In the near future, it

is crucial to evaluate other processing conditions, namely cooking methods (e.g. frying and baking), since it can have a significant impact on the occurrence of this hazardous compound. Also, it is necessary to monitor the occurrence of 3-monochloropropane-1,2-diol in other foodstuffs, to accurately estimate the exposure assessment.

Introduction

Plant foods are the main sources of edible oils/fats, namely seeds (e.g. sunflower and rapeseed), legumes (e.g. soybean), nuts (e.g. walnut and almond) and fruits (e.g. palm and olive). In the last years, alternative sources of vegetable oils are being studied, namely food industry by-products, such as apricot kernel, grape seeds and melon seeds.

Over the last years, the global production of vegetable oils increased, but palm oil is still the most produced vegetable oil, followed by soybean, rapeseed and sunflower oils (1). Generally, vegetable oils are distinguished by their fatty acids composition, although the presence of other minor compounds (tocopherols, phytosterols and carotenoids) is very important, especially for their oxidative stability. In fact, this is one of the major concerns, since it decreases the shelf life of the oil itself, but also because it has a major impact on the quality and safety of foodstuffs.

From a nutrition and public health perspective, the inappropriate production and use of vegetable oils is linked to the pathogenesis of several diseases, namely cardiovascular diseases and cancer. Therefore, oils and fats are processed to improve their quality, stability and safety. Despite the removal of a large amount of impurities from the oil, processing can often originate new contaminants that can cause additional health hazards to those who consume these foods (2).

Besides industrial processing of vegetable oils, it is of utmost importance to consider the effect of cooking methods on the quality of vegetable oils, namely frying, because it is largely used. The most frequent chemical reactions taking place during frying are: hydrolysis, oxidation, isomerisation and polymerization, which lead to the formation of several degradation products, namely, free fatty acids, aldehydes, ketones, diglycerides and monoglycerides, trans isomers, hydrocarbons, triacylglycerols, conjugated fatty acids, and cyclic fatty acids (3-5).

3-monochloropropane-1,2-diol (3-MCPD) is a food processing contaminant included in the group of compounds known as chloropropanols (6). In recent years, high quantities of 3-MCPD esters were reported in edible oils/fats and other foods. Therefore, in this manuscript an overview of the occurrence of 3-MCPD in vegetable oils, as well as its potential impact

on human nutrition based on exposure assessment to 3-MCPD and future challenges are discussed.

Methodology

In this literature review electronic databases were used, namely Science Direct, PubMed, and Google Scholar. Moreover, the following keywords were used: 'chloropropanols', 'edible oils and toxic compounds', 'vegetable oils and chloropropanols', '3-MCPD', 'chloropropanols precursors', '3-MCPD legislation', 'chloropropanols and mitigation', and 'analytical methods for chloropropanols determination'. One of the exclusion criterion was the language of the manuscripts, being only English papers considered.

Afterwards, a detailed review concerning sample preparation procedures, food matrices analysed, as well as geographical origin of samples, available data concerning the occurrence in edible oils and fats, mitigation strategies and impact on human nutrition, were compiled.

3-monochloropropane-1,2-diol

The 3-MCPD is a food contaminant, member of the chemical group of chloropropanols, which are a group of alcohols comprised of a 3-carbon backbone substituted with one or two chlorine atoms (7). Chemically, 3-MCPD is a glycerol chlorohydrin, formed when one hydroxyl group is replaced by a chlorine atom (8). Despite the structural similarity of 3-MCPD and 2-monochloropropane-1,3-diol (2-MCPD) compounds, they can have different and specific metabolic and toxicological profiles. Depending on the type of food, 3-MCPD may occur as a free substance, as ester with fatty acids or in both forms (9).

In 1978, chloropropanols were first described in acid-hydrolysed vegetable proteins, leading to intensive scientific research concerning this subject. Later, the presence of chloropropanols was also described in soy sauces. After some years, these compounds started to receive wider scientific and regulatory attention, since significant amounts were detected in several heat-processed foods, as well as in vegetable oils (9-11).

In 2001, the European Community has set a regulatory limit of 0.02 mg/kg for 3-MCPD in hydrolysed vegetable protein and soy sauce, with the maximum level given for the liquid product containing 40% of dry matter, corresponding to a maximum level of 0.05 mg/kg in the dry matter (12). These levels of 3-MCPD in foods were established after the recommendation from the Scientific Committee on Food that has set a tolerable daily intake of 2 µg/kg of body weight and has concluded that 3-MCPD was a non-genotoxic carcinogen for humans (13).

According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), kidney is the main target organ for 3-MCPD toxicity, with chronic oral exposure resulting in nephropathy and tubular hyperplasia and adenomas (14). The International Agency for Research on Cancer (15) has classified 3-MCPD as a possible human carcinogen (group 2B).

Therefore, the European Commission has established two first priorities: (1) to reduce the levels of 3-MCPD esters by mitigation measures to be applied by the food business operators; and (2) to consider possible maximum levels of 3-MCPD esters in foods once more information is available on the pathways of formation and on what levels are achievable (16).

In 2013, the European Food Safety Authority (EFSA) published a report that highlighted the food groups that mainly contribute to the exposure to these contaminants. Margarine and vegetable oils/fats were identified as the foods/ingredients with high quantities of these hazardous compounds (6).

Recently, EFSA published an update of the tolerable daily intake from 0.8 to 2 µg/kg of body weight/day for 3-MCPD and its fatty acid esters (17).

3-MCPD in oils/fats: precursors, occurrence and mitigation

It is assumed that 3-MCPD is formed when fat and salt containing foods are processed at high temperatures (16). Moreover, other studies showed that these compounds are mainly formed during the high-temperature deodorization step of the refining process of oils, which is an essential step to reduce the amount of undesirable compounds that can negatively impact the taste, appearance, shelf life, safety and consumer acceptance (18). Other studies appointed that 3-MCPD formation is linked with mono- and diacylglycerols.

Table 28 presents 3-MCPD content in oils and fats available in the literature. Large variations for 3-MCPD content have been observed between oils of different origin, but also amongst the oils of the same kind. Amongst the reviewed data, the highest values were reported by Zhou et al. (2014) for rice bran oil (1449 – 2564 mg/kg) and edible blending oil (1367 mg/kg) (19).

For refined seed oils, Zelinková et al. (2006) have reported values for 3-MCPD ranging from <0.3 to 1.23 mg/kg, while Kuhlmann (2011) has presented results varying between 0.2 and 19 mg/kg (11, 20). With respect to olive oil, the content of 3-MCPD in refined olive oils was approximately eight times higher than for virgin olive oils (11). Recently, Li et al. (2015) have evaluated 102 refined edible oils and 41 crude edible oils. It was possible to observe that for sunflower, peanut, rapeseed, sesame, soybean and camellia oils, the reported values

for 3-MCPD content were significantly higher for refined (1.044 – 2.586 mg/kg) than for crude oils (0.083 – 0.555 mg/kg) (21).

Around forty vegetable oils from the Canadian market were analysed in 2011 and 2013, but it was not possible to establish a trend concerning the decrease of 3-MCPD amounts, because for some types of oil, the values were lower in 2011 than in 2013, but the opposite was also observed (22). Nonetheless, the high amount of 3-MCPD in 2011 was determined for rice bran oil (8.340 mg/kg), while in 2013 it was for palm oil shortening (8.420 mg/kg). Also, authors reported that for unprocessed oils, 3-MCPD was only detected in trace amounts or not detected (22).

As mentioned before, palm oil is the most used vegetable oil. Therefore, several authors have reported 3-MCPD values for palm oil, varying between not detected and 540 mg/kg (10, 19, 22-27).

Regarding the mitigation of 3-MCPD, different strategies have been already evaluated, such as: (a) removal of potential precursors; (b) modifications of processing parameters; and (c) degradation or removal of the compounds in the final product (28). Concerning the first one, washing the crude oil before refining to remove water-soluble chloride is appointed as feasible and effective (29). Concerning the modifications of processing parameters, one of the main difficulties to decrease the formation of these compounds is the fact that MCPD esters begin forming at 180 – 200 °C, which are the temperatures applied in deodorisation step (24). Nonetheless, for the last strategy, the use of radical scavengers is efficient, namely phenolic compounds from other foods (like lipophilic tea polyphenols and rosemary extract) (30).

Exposure assessment to 3-MCPD

Food processing induces changes in foods and these modifications can result in harmful, as well as beneficial effects on the food quality, and therefore on human nutrition. As previously described, 3-MCPD is a processing food contaminant, but its mitigation is not

Table 28. 3-MCPD content (mg/kg) for oils and fats.

Analytical method		Oils and fats	3-MCPD content (mg/kg)	Reference
Derivatization	**Chromatographic conditions**			
Acid cleavage	Gas chromatography Detector: MS Column: EquityTM-1 (30 m x 0.25 mm I.D., 1 µm film thickness) Injection mode: Splitless Injector temperature: 250 °C Oven ramp: 80 °C (1 min) to 300 °C (37 min) at a rate of 10 °C/min Carrier gas: Helium (0.8 mL/min) Injection volume: 1 µL	Virgin seed oils (n=9) Almond Soybean Rapeseed Sunflower Sesame (unroasted seed) Sesame (roasted seed) Hazelnut Peanut Pumpkin Refined seed oils (n=5) Soybean Rapeseed (n=2) Sunflower Maize Virgin olive oils (n=4) Extra virgin olive oil (n=2) Virgin olive oil (n=2) Refined olive oils (n=5) Olive oil (n=3) Olive pomace oil (n=2)	<0.1 - 0.34 <0.1 <0.1 <0.1 <0.1 <0.3 0.34 <0.1 <0.1 <0.1 <0.3 – 1.23 1.23 0.38 – 0.48 <0.3 0.37 <0.1 - <0.3 <0.1 - <0.3 <0.1 <0.3 - 2.46 <0.3 – 2.46 1.05 – 2.33	(11)
Acid cleavage	Gas chromatography Detector: FID Column: SP-2560 (100 m x 0.25 mm, I.D.; 0.2 µm film thickness) Injection mode: Split (75:1) Injector temperature: 240 °C Oven ramp: 175 °C to 240 °C at the rate of 4 °C/min or 90 °C to 200 °C at the rate of 6.9 °C/min, and from 200 °C to 240 °C at the rate of 2 °C/min Carrier gas: Helium (0.8 mL/min) Injection volume: 1 µL	Virgin oils (n=13) Virgin seed oil (n=9) Virgin olive oils (n=4) Refined oils (n=19) Refined seed oils (n=5) Refined palm kernel oils (n=5) Refined olive oils (n=3) Refined coconut oils (n=2) Refined palm oils (n=4)	0.06 – 0.08 0.06[a] 0.08[a] 0.52 – 2.82 0.52[a] 1.17[a] 1.46[a] 1.56[a] 2.82[a]	(23)
Acid cleavage	Gas chromatography Detector: MS	Fat mixes (n=11) Fat (palm olein, 46%) Fat (palm olein, 46%)	0.90 – 2.44 0.14 0.16	(10)

	Column: HP-1MS (60 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: - Injector temperature: - Oven ramp: 80 °C (1.5 min), increase of 30 °C/min until 300 °C and held for 10 min Carrier gas: Helium (1.0 mL/min) Injection volume: -	Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 46%) Fat (palm olein, 55%) Fat (palm olein, 55%) Salmon oil in dietary supplement capsules Evening primrose oil Borage oil Rose hip oil Shea butter Wheat germ oil Palm kernel oil Palm oil degummed and bleached	0.90 1.04 0.97 1.33 1.53 1.40 2.04 2.44 2.22 0.7 – 13 0.8 – 5.2 <0.1 – 0.2 0.8 0.2 0.2 0.2 – 0.9 1.0	
DGF C-III 18(09)	Gas chromatography Detector: FID Column: DB-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: splitless Injector temperature: 250 °C Oven ramp: 60 °C (5 min) raised to 280 °C, with a heating rate of 10 °C/min, held by 20 min Carrier gas: Helium (1.2 mL/min) Injection volume: 1 µL	Refined olive oil (n=9) Cold-pressed safflower oil (n=8) Refined safflower oil (n=3)	0.14 – 0.16 <0.1 – 2.46 2.34 – 3.22	(33)
DGF C-III 18(09)	Gas chromatography Detector: FID Column: DB-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: splitless Injector temperature: 250 °C Oven ramp: 60 °C (5 min) raised to 280 °C, with a heating rate of 10 °C/min, held by 20 min Carrier gas: Helium (1.2 mL/min) Injection volume: 1 µL	Refined vegetable oils (n=57) Palm kernel oil (n=3) Coconut oil (n=4) Olive oil (n=6) Sunflower oil (n=15) Rapeseed oil (n=10) Soybean oil (n=6) Safflower oil (n=8) Corn oil (n=5)	0.4 – 1.7 1.7[a] 0.6[a] 1.2[a] 1.0[a] 0.4[a] 0.9[a] 1.4[a] 1.7[a]	(34)

-	Liquid chromatography Detector: TOF/MS Column: Phenomenex Luna C18 (50 mm x 3 mm, I.D.; 3 µm film thickness) Mobile phase: A (methanol/acetonitrile/methanol-sodium acetate solution (0.26 mM), 8:1:1, v/v/v) and B (methanol-sodium acetate solution (0.26 mM)/methylene chloride/acetonitrile, 1:8:1, v/v/v)	Cocoa butter (n=2) Palm shortening/olein (n=6) Vegetable oils (n=10) Corn oil Canola oil Soybean oil Sesame oil Walnut oil	<0.5 0.4 – 0.6 <0.5 <0.5 <0.5 <0.5 <0.5 <0.5	(35)
Alkali/Br-	Gas chromatography Detector: MS Column: Resteck Rxi®-17 GC column (30 m x 0.25 mm, I.D., 0.25 µm film thickness); HP-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: Split/Splitless Injector temperature: - Oven ramp: 90 °C, isothermal 0.1 min, with 78 °C/min to 175 °C, isothermal 1.0 min with 20 °C/min to 290 °C, isothermal 8.3 min Carrier gas: Helium (1.2 mL/min) Injection volume: 2 µL	Refined seed oils (n=8) Apricot kernel oil Coconut oil Corn oil Hazelnut oil Grape seed oil Peanut oil Safflower oil Walnut oil Olive oil	0.2 – 19 0.4 0.2 – 0.4 0.2 19 0.8 – 4.2 0.1 – 0.9 0.6 – 1.0 1.2 – 19 0.3 – 1.2	(20)
DGF C-III 18(09)	Gas chromatography Detector: FID Column: DB-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: splitless Injector temperature: 250 °C Oven ramp: 60 °C (5 min) raised to 280 °C, with a heating rate of 10 °C/min, held by 20 min Carrier gas: Helium (1.2 mL/min) Injection volume: 1 µL	Native or cold-pressed vegetable oils (n=57) Refined vegetable oils (n=144) Margarine (n=37) Frying fat, used and unused (n=38)	<0.1 – 0.4 0.2 – 14.7 0.4 – 4.5 0.5 – 5.2	(36)
-	Liquid chromatography Detector: MS/MS Column: Pursuit XRs C18 (150 mm x 2 mm i.d., 3 µm particle size)	Almond oil, unrefined (n=1) Almond oil (n=1) Butter unrefined (n=4) Canola oil (n=7)	<LOQ 2.11 <LOQ – 0.045 <LOQ – 0.33	(24)

	Injection volume: 5 µL Flow: 0.2 – 0.25 mL/min Mobile phase: (A) 2 mM ammonium formate/0.05% formic acid in methanol/water (92:8, v/v); (B) 2 mM ammonium formate/0.05% formic acid in isopropanol/water (98:2, v/v)	Coconut oil, unrefined (n=2) Coconut oil (n=7) Corn oil (n=9) Cottonseed oil (n=2) Extra virgin olive oils, unrefined (n=5) Flaxseed oil (n=1) Grape seed oil (n=3) Hemp oil, unrefined (n=2) Macadamia nut oil (n=1) Mixed oils (n=5) Olive oil (n=5) Palm oil, unrefined (n=1) Palm oil (n=14) Palm kernel oil (n=2) Palm olein (n=5) Palm stearin (n=1) Peanut oil, unrefined (n=2) Peanut oil (n=3) Pumpkin seed oil (n=1) Safflower oil (n=5) Sesame oil, unrefined (n=3) Shortening oil (n=5) Soybean oil (n=6) Sunflower oil, unrefined (n=1) Sunflower oil (n=4) Vegetable edible fats (n=6) Walnut oil (n=1)	<LOQ 0.025 – 0.38 0.06 – 0.42 0.14 – 0.72 <LOQ – 0.025 <LOQ 0.24 – 3.91 <LOQ – 0.039 <LOQ 0.035 – 1.88 0.15 – 0.73 <LOQ 1.51 – 7.23 0.038 – 0.20 1.40 – 8.43 3.24 <LOQ 0.14 – 0.69 <LOQ 0.28 – 1.77 0.16 – 0.45 0.35 – 0.46 0.041 – 0.24 <LOQ 0.19 – 0.93 0.009 – 1.10 0.63	
-	Liquid chromatography Detector: MS/MS Column: Luna-3u C18 (50 mm x 2.1 mm i.d., 1.7 µm particle size) Flow: 0.2 mL/min Injection volume: 10 µL Mobile phase: (A) water; (B) 0.01 mol/L ammonium acetate in methanol; (C) methanol; (D) 2-propanol	Soybean oil Rapeseed oil Rice oil Safflower oil Sesame oil Olive oil Grape seed oil Perilla oil Palm oil Lard, refined	0.58 0.50 1.78 0.83 0.58 4.34 25.35 1.43 14.4 0.76	(25)

-	Gas chromatography Detector: MS Column: VF-1MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: - Injector temperature: - Oven ramp: 60 °C (1 min), 6 °C/min to 190 °C, 20 °C/min to 280 °C (held for 30 min) Carrier gas: Helium (1.2 mL/min) Injection volume: -	Soybean oil (A)	0.19 ± 0.02 – 1.19 ± 0.01	(26)
		Soybean oil (B)	nd – <0.10	
		Soybean oil (C)	<0.10 – 0.16 ± 0.04	
		Soybean oil (D)	0.34 ± 0.01 – 0.37 ± 0.04	
		Soybean oil (E)	1.11 ± 0.03	
		Soybean oil (F)	0.21 ± 0.05 – 0.23 ± 0.00	
		Corn oil (A)	< 0.10 – 0.12 ± 0.01	
		Corn oil (B)	<0.10	
		Corn oil (C)	nd	
		Corn oil (D)	0.20 ± 0.00	
		Corn oil (E)	<0.10	
		Corn oil (F)	1.04 ± 0.01 – 1.12 ± 0.05	
		Sunflower oil (A)	0.14 ± 0.02 – 0.16 ± 0.01	
		Sunflower oil (B)	0.13 ± 0.01 – 0.15 ± 0.02	
		Sunflower oil (C)	0.10 ± 0.00 – 0.20 ± 0.02	
		Sunflower oil (D)	0.22 ± 0.00 – 0.21 ± 0.03	
		Sunflower oil (E)	0.25 ± 0.01 – 0.27 ± 0.06	
		Sunflower oil (F)	0.25 ± 0.07	
		Canola oil (A)	0.13 ± 0.00 – 0.16 ± 0.02	
		Canola oil (B)	<0.10 – 0.10 ± 0.02	
		Canola oil (C)	0.11 ± 0.02 – 0.30 ± 0.04	
		Canola oil (D)	<0.10 – 0.14 ± 0.03	
		Canola oil (E)	0.14 ± 0.00 – 0.25 ± 0.07	
		Canola oil (F)	0.14 ± 0.03 – 0.24 ± 0.03	
		Canola oil (G)	0.23 ± 0.03	
		Maize, sunflower, canola oils (A)	<0.10 – 0.12 ± 0.02	
		Maize, sunflower, canola oils (B)	0.39 ± 0.02	
		Olive extra virgin oil (A)	nd	
		Olive extra virgin oil (B)	0.10 ± 0.03 – 1.29 ± 0.01	
		Olive oil (virgin + refined)	0.14 ± 0.03 – 0.33 ± 0.08	
		Olive + pomace oil (A)	1.46 ± 0.03	
		Olive + pomace oil (B)	5.09 ± 0.02	
		Palm oil (A)	0.25 ± 0.01 – 0.32 ± 0.02	
		Palm oil (B)	0.30 ± 0.05 – 0.33 ± 0.02	
		Palm oil (C)	nd	
		Palm kernel oil	0.17 ± 0.05	
		Palm fat (A)	1.64 ± 0.01 – 3.31 ± 0.03	
		Palm fat (B)	2.20 ± 0.02 – 2.56 ± 0.03	
		Palm fat (C)	2.29 ± 0.05 – 2.60 ± 0.02	

		Palm fat (D)	1.47 ± 0.03 – 2.45 ± 0.08	
		Peanut oil (A)	0.13 ± 0.00 – 0.29 ± 0.03	
		Sesame oil (A)	0.48 ± 0.00 – 0.58 ± 0.05	
		Hydrogenated vegetable fat (A)	0.29 ± 0.06 – 0.45 ± 0.08	
		Mix of fats	0.40 ± 0.00 – 0.66 ± 0.04	
		Shortening	3.14 ± 0.02 – 3.87 ± 0.03	
-	Liquid chromatography Detector: TOF/MS Column: Acquity C18 (50 mm x 2.1 mm, 1.7 µm particle size) Flow: 0.3 mL/min Injection volume: 1 µL Mobile phase: (A) Methanol; (B) 10 mM aqueous sodium acetate solution containing 0.1% formic acid	Edible vegetable blending oil	nd	(19)
		Natura cereal blending oil	81 ± 3.0	
		Edible blending oil	1367 ± 0.03	
		Sunflower oil	nd	
		Rice bran oil	65 ± 3.7	
		Rice bran oil	122 ± 1.1	
		Camellia seed oil	nd	
		Rice oil	1449 ± 6.2 – 2564 ± 4.4	
		Camellia blending oil	nd	
		Peanut oil	738 ± 1.4	
		Soybean oil	58 ± 2.7	
		Palm oil	336 ± 3.6 – 540 ± 1.9	
		Corn oil	132 ± 1.5 – 143 ± 3.1	
		Canola oil	685 ± 1.0	
		Corn oil (n=1)	0.019	
		Crude palm oil (n=1)	nd	
		Edible blend oil (n=5)	0.026 – 0.301	
		Extra virgin olive oil (n=1)	nd	
		Maize germ oil (n=1)	0.102	
		Peanut oil (n=7)	0.080 – 1.046	
		Peanut sesame blend oil (n=1)	0.164	
		Rapeseed oil (n=1)	0.427	
		Sesame blend oil (n=1)	0.227	
		Soybean crude oil (n=1)	nd	
		Soybean oil (n=6)	0.322 – 1.167	
		Sunflower oil (n=2)	0.164 – 0.313	
		Virgin rapeseed oil (n=1)	0.007	
		Tea seed oil (n=1)	0.052	
-	-	Edible oils and fats sampled in 2011 (n=43)	nd – 8.340	(22)
		Extra virgin olive oil (n=6)	nd	

Light olive oil (n=2)	0.584 – 1.560
Vegetable oil spray (n=5)	nd – 0.481
Mix vegetable oil (n=3)	nd – 1.220
Coconut oil (n=1)	0.315
Coconut oil, unrefined (n=1)	nd
Canola oil, unrefined (n=1)	nd
Canola oil (n=2)	0.191 – 0.218
Canola and extra virgin olive oil (n=1)	0.299
Canola and sunflower oil (n=1)	0.165
Rice bran oil (n=1)	8.340
Corn oil (n=1)	0.239
Sunflower oil, unrefined (n=1)	nd
Sunflower oil (n=2)	0.150 – 0.245
Grapeseed oil (n=2)	1.380 – 3.190
Walnut oil (n=1)	11.6
Almond oil (n=1)	0.515
Peanut oil, unrefined (n=1)	nd
Avocado oil, unrefined (n=1)	nd
Avocado oil (n=1)	0.912
Toasted sesame oil (n=1)	0.757
Toasted sesame oil, unrefined (n=1)	0.700
Margarine (n=4)	0.092 – 0.434
Vegetable shortening (n=1)	0.502
Lard (n=1)	0.432
Edible oils and fats sampled in 2013 (n=44)	nd – 8.420
Extra virgin olive oil (n=6)	nd
Light olive oil (n=2)	0.739 – 0.921
Vegetable oil spray (n=2)	nd – 0.130
Mix vegetable oil (n=2)	nd – <0.1
Coconut oil (n=1)	0.333
Coconut oil, unrefined (n=1)	nd
Canola oil, unrefined (n=1)	nd
Canola oil (n=2)	0.210 – 0.304
Canola and extra virgin olive oil (n=1)	0.235
Canola and sunflower oil (n=1)	0.077
Rice bran oil (n=1)	0.368
Corn oil (n=1)	0.121
Sunflower oil, unrefined (n=1)	nd
Sunflower oil (n=2)	0.090 – 2.54

		Grapeseed oil (n=2)	2.14 – 3.60	
		Walnut oil (n=1)	2.87	
		Almond oil (n=1)	1.04	
		Peanut oil, unrefined (n=1)	nd	
		Avocado oil, unrefined (n=1)	0.062	
		Avocado oil (n=1)	0.435	
		Sesame oil (n=1)	1.290	
		Toasted sesame oil, unrefined (n=1)	0.588	
		Margarine (n=4)	0.164 – 0.441	
		Vegetable shortening (n=1)	0.551	
		Lard (n=1)	0.412	
		Peanut oil (n=1)	0.384	
		Palm oil shortening (n=1)	8.420	
		Palm oil unrefined (n=3)	0.087 – 0.558	
DGF method C-VI 18 (10)	Gas chromatography Detector: MS Column: HP-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness) Injection mode: Splitless Injector temperature: 280 °C Oven ramp: 85 °C (0.5 min), 6 °C/min to 150 °C, 12 °C/min to 180 °C, 25 °C/min to 280 °C (held for 7.16 min) Carrier gas: Helium (1.0 mL/min) Injection volume: -	Refined edible oils (n=102)	0.219 – 2.586	(21)
		Sunflower seed oil (n=6)	0.504 – 1.044	
		Peanut oil (n=15)	0.450 – 1.187	
		Rapeseed oil (n=18)	0.226 – 1.069	
		Sesame oil (n=4)	0.651 – 1.344	
		Soybean oil (n=18)	0.224 – 1.090	
		Corn germ oil (n=12)	0.219 – 1.826	
		Blend oil (n=11)	0.246 – 0.806	
		Palm oil (n=3)	1.294 – 1.646	
		Lard (n=5)	0.225 – 0.310	
		Camellia oil (n=5)	0.988 – 2.586	
		Margarine (n=5)	0.789 – 1.602	
		Crude edible oils (n=41)	0.025 – 0.555	
		Sunflower seed oil (n=8)	0.025 – 0.098	
		Peanut oil (n=6)	0.025 – 0.083	
		Rapeseed oil (n=9)	0.025 – 0.438	
		Sesame oil (n=6)	0.025 – 0.356	
		Soybean oil (n=7)	0.025 – 0.109	
		Camellia oil (n=5)	0.025 – 0.555	
DGF method C-VI 18 (10)	Gas chromatography Detector: MS Column: HP-5MS (30 m x 0.25 mm, I.D.; 0.25 µm film thickness)	Sunflower oil	0.765 ± 0.033	(37)
		Soybean oil	0.479 ± 0.036	
		Lard	0.302 ± 0.021	
		Rapeseed oil	0.622 ± 0.026	

	Injection mode: Splitless Injector temperature: 280 °C Oven ramp: 85 °C (0.5 min), 6 °C/min to 150 °C, 12 °C/min to 180 °C, 25 °C/min to 280 °C (held for 7.16 min) Carrier gas: Helium (1.0 mL/min) Injection volume: -	Sesame oil Camellia oil Peanut oil Blend oil Peanut oil (crude, hot squeezed) Rapeseed oil (crude, hot squeezed) Sesame oil (crude, cold squeezed) Rapeseed oil (crude, cold squeezed)	0.734 ± 0.028 1.156 ± 0.048 0.699 ± 0.039 0.566 ± 0.030 0.101 ± 0.023 0.112 ± 0.020 nd nd	
AOCS Cd 29b-13	Gas chromatography Detector: MS Column: J&W (60 m x 0.25 mm, I.D.; 1 µm film thickness) Injection mode: Split Injector temperature: 150 °C Oven ramp: 40 °C (6 min), 5 °C/min to 140 °C, 20 °C/min to 230 °C (held for 3 min) Carrier gas: Helium (0.9 mL/min) Injection volume: 25 µL	Refined sunflower seed oils (n=11) Refined palm oils, palm stearin, palm mid fraction (n=6) Refined rapeseed oils (n=5) Crude and refined oils and fats (n=13)	0.08 – 0.96 0.18 – 2.48 0.03 – 0.51 <0.005 – 7.55	(27)

[a] Results are expressed as mean value; LOQ, limit of quantification, nd, not detected

always easy to achieve. Furthermore, literature data shows (Table 28) that some vegetable oils contain high amounts of this cytotoxic and mutagenic compound. On the other hand, vegetable oils are largely used in different procedures, for example for frying, but are also ingredients of several foodstuffs, which enlarges the probability of exposure to these hazardous compounds. Nonetheless, few studies have focused on risk assessment of these compounds (21, 26, 31). Another difficulty in this assessment is the fact that most of the times it is not possible to accurately evaluate the effect of vegetable oils, because they are ingredients of the other foods, and the food itself can also contain 3-MCPD. Arisseto et al. (2014) evaluated the exposure assessment to 3-MCPD for 17 food groups, but did not include edible oils and fats, while Arisseto et al. (2017) evaluated the exposure assessment to this hazardous compound only of infant formulas (26, 31). Therefore, as far as we know, only Li et al. (2015) have specifically evaluated the exposure assessment to 3-MCPD of edible oils and fats (21).

Li et al. (2015) evaluated the exposure assessment of Chinese population to 3-MCPD esters, using the determined values for the concentrations of 3-MCPD of 143 edible oils and fats from Chinese market. The mean exposure values for children aged between 7 and 10 years old was 1.29 and 1.31 µg/kg body weight/day for males and females, respectively. For adolescents (14 – 17 years old) it was 0.72 µg/kg body weight/day (males) and 0.82 µg/kg body weight/day (females), which is very similar to the values obtained for adults (0.71 µg/kg body weight/day). Also, it is notable that the mean exposure values decreased when age increased, being almost the double for children than for adults (>50 years old) (21). Nonetheless, these values are lower than the recently revised tolerable daily intake (2 µg/kg of body weight/day) (17).

In 2016, EFSA published a report on “Risks for human health related to the presence of 3- and 2-MCPD, and their fatty acid esters, and glycidyl fatty acid esters in food” (32). In this report, 7175 occurrence data were used, and these were distributed in three groups: soy sauce, hydrolysed vegetable protein and related products; oils and fats; and other food groups. According to the reported results, the mean exposure to 3-MCPD was 0.5–1.5 µg/kg body weight/day for infants, toddlers and other children; while for adolescents and adult population it ranged from 0.2 to 0.7 µg/kg body weight/day (32).

Also, the major food groups that contribute to 3-MCPD exposure by age groups were evaluated. It was concluded that vegetable fats and oils were one of the major contributors for infants and other children, while for adults and elderly, it was the group of margarines and similar products (32).

Summary points and future challenges

In general, there is a great variation of 3-MCPD content between the different vegetable oils, but also in the same type of oils, which makes it difficult to establish a relationship between the source of the oil and the occurrence of this contaminant;

Higher levels of 3-MCPD are reported for refined vegetable oils/fats than for unprocessed oils, which strongly supports that processing conditions are related to the increase of these compounds. Nonetheless, it is of utmost importance to consider that processing is essential to assure the quality and safety of vegetable oils. Therefore, more studies should accurately evaluate realistic processing conditions that mitigate the formation of 3-MCPD, but do not compromise the safety of these oils;

Concerning exposure assessment, it is urgent to monitor the occurrence of 3-MCPD in edible oils, but also in other food matrices, to increase the number of studies regarding this subject. Nonetheless, it is possible to conclude that children are the most vulnerable group, and among the different food groups studied, vegetable oils and fats were described as the major contributors;

In the near future, it is necessary to involve academy, researchers, industry to obtain more analytical data, as well as health professionals to perform dietary surveys to accurately estimate exposure assessment and potential impact for public health. Also, sharing knowledge from different research areas and from experts with different skills, will certainly contribute to efficiently diminish the presence of this cytotoxic and mutagenic compound in vegetable oils.

References

1. Foster, R., et al. “Culinary oils and their health effects”. Nutrition Bulletin (2009): 4-47. https://www.nutrition.org.uk/attachments/113_Culinary%20oils%20and%20their%20health%20effects.pdf
2. Albuquerque, T.G., et al. “4-Hydroxynonenal: A parameter of quality and safety of vegetable oils”. EC Nutrition ECO.01 (2017): 27-29. https://www.ecronicon.com/eco16/pdf/ECNU-01-ECO-14.pdf
3. Choe, E., & Min, D. B. “Chemistry of Deep-Fat Frying Oils”. Journal of Food Science 72 (2007): 77-86. https://onlinelibrary.wiley.com/doi/epdf/10.1111/j.1750-3841.2007.00352.x

4. Zhang, Q., et al. “Chemical alterations taken place during deep-fat frying based on certain reaction products: a review”. Chemistry and Physics of Lipids 165 (2012):662-681. https://www.sciencedirect.com/science/article/pii/S0009308412000813
5. Brühl, L. “Fatty acid alterations in oils and fats during heating and frying”. European Journal of Lipid Science and Technology, 116 (2014): 707-715
6. EFSA, European Food Safety Authority. “Analysis of occurrence of 3-monochloropropane-1,2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment”. EFSA Journal 11 (2013): 3381. https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2013.3381
7. Stadler, R. H. “Monochloropropane-1,2-diol esters (MCPDEs) and glycidyl esters (GEs): and update”. Current Opinion in Food Science 6 (2015): 12-18. https://www.sciencedirect.com/science/article/pii/S221479931500137X
8. Jędrkiewicz, R., et al. “3-MCPD: A Worldwide Problem of Food Chemistry”. Critical Reviews in Food Science and Nutrition, 56 (2016): 2268–2277. https://www.tandfonline.com/doi/abs/10.1080/10408398.2013.829414?journalCode=bfsn20
9. Svejkovská, B., et al. “Esters of 3-chloropropane-1,2-diol in foodstuffs”. Czech Journal of Food Sciences 22 (2004): 190-196. http://www.agriculturejournals.cz/publicFiles/50671.pdf
10. Seefelder, W., et al. “Esters of 3-chloro-1,2-propanediol (3-MCPD) in vegetable oils: significance in the formation of 3-MCPD”. Food Additives & Contaminants: Part A 25 (2008) 391-400. https://doi.org/10.1080/02652030701385241
11. Zelinková, Z., et al. “Fatty acid esters of 3-chloropropane-1,2-diol in edible oils”. Food Additives & Contaminants 23 (2006): 1290-1298. https://www.tandfonline.com/doi/abs/10.1080/02652030600887628?journalCode=tfac19
12. European Commission. “Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs”. Official Journal of the European Communities L77 (2001): 1-13. https://publications.europa.eu/en/publication-detail/-/publication/52b2484d-39e0-4aa9-ba19-4b13a887bb1c/language-en
13. SCF (Scientific Committee on Food). “Opinion of the scientific committee on food on 3-monochloro-propane-1,2-diol (3-MCPD)”. Updating the SCF opinion of 1994. (2001) Brussels, Belgium: European Commission. http://ec.europa.eu/food/fs/sc/scf/out91_en.pdf
14. JECFA (Joint FAO/WHO Expert Committee on Food Additives). “3-Chloro-1,2-propanediol. Safety evaluation of certain food additives and contaminants”. Prepared

by the fifty-seventh meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), (2002) Geneva WHO Food Additives Series, 48. http://www.who.int/ipcs/publications/jecfa/reports/trs940.pdf

15. IARC (International Agency for Research on Cancer). “3-Monochloro-1,2-propanediol”. In: IARC Monographs Volume 101. Some Chemicals Present in Industrial and Consumer Products, Food and Drinking-water. Lyon, France, (2012): 349-374. https://monographs.iarc.fr/ENG/Monographs/vol101/mono101-010.pdf
16. Larsen, J. C. “3-MCPD esters in food products”. (2009) ILSI Europe. http://www.ilsi.org/Europe/Publications/Final%20version%203%20MCPD%20esters.pdf
17. EFSA, European Food Safety Authority. “Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters”. EFSA Journal 16 (2018): 1-48. https://doi.org/10.2903/j.efsa.2018.5083
18. Hori, K., et al. “Simultaneous determination of 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils using liquid chromatography time-of-flight mass spectrometry”. LWT – Food Science and Technology 48 (2012): 204-208. https://doi.org/10.1016/j.chroma.2012.06.017
19. Zhou, H., et al. “Direct measurement of 3-chloropropane-1,2-diol fatty acid esters in oils and fats by HPLC method”. Food Control 36 (2014): 111-118. https://www.sciencedirect.com/science/article/pii/S0956713513003897
20. Kuhlmann, J. “Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils”. European Journal of Lipid Science and Technology 113 (2011): 335–344. https://doi.org/10.1002/ejlt.201000313
21. Li, C., et al. “Exposure assessment of 3-monochloropropane-1, 2-diol esters from edible oils and fats in China”. Food and Chemical Toxicology, 75 (2015) 8-13. https://doi.org/10.1016/j.fct.2014.10.003
22. Becalski, A., et al. “A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in foods on the Canadian market 2011-2013”. Journal of Food Composition and Analysis 37 (2015) 58–66. https://doi.org/10.1016/j.jfca.2014.09.002
23. Karšulínová, L., et al. “Analysis of the lipid fractions of coffee creamers, cream aerosols, and bouillon cubes for their health risk associated constituents”. Czech Journal of Food Sciences 25 (2007) 257-264. http://www.agriculturejournals.cz/publicFiles/00365.pdf
24. MacMahon, S., et al. 2Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States”. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 30 (2013) 2081-2092. https://doi.org/10.1080/19440049.2013.840805

25. Yamazaki, K., et al. “A new, direct analytical method using LC-MS/MS for fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD esters) in edible oils”. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 30 (2013) 52-68. https://doi.org/10.1080/19440049.2012.713031
26. Arisseto, A. P., et al. “Determination of 3-monochloropropane-1,2-diol fatty acid esters in Brazilian vegetable oils and fats by an in-house validated method”. Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment 31 (2014): 1385-1392. https://doi.org/10.1080/19440049.2014.926400
27. Kuhlmann, J. “Analysis and occurrence of dichloropropanol fatty acid esters and related process-induced contaminants in edible oils and fats”. European Journal of Lipid Science and Technology 118 (2016): 382-395. https://doi.org/10.1002/ejlt.201400518
28. Arisseto, A., et al. “Ésteres de cloropropanóis e de glicidol em alimentos”. Quimica Nova 36 (2013): 1406–1415. https://doi.org/10.1590/S0100-40422013000900022
29. Matthäus, B., et al. “Strategies for the reduction of 3-MCPD esters and related compounds in vegetable oils”. European Journal of Lipid Science and Technology 113 (2011): 380-386. https://doi.org/10.1002/ejlt.201000300
30. Zhang, H., et al. “Mitigation of 3-Monochloro-1,2-propanediol ester formation by radical scavengers”. Journal of Agricultural and Food Chemistry 64 (2016): 2-7. https://doi.org/10.1021/acs.jafc.6b02016
31. Arisseto, A. P., et al. “3-MCPD and glycidyl esters in infant formulas from the Brazilian market: Occurrence and risk assessment”. Food Control 77 (2017): 76-81. https://www.sciencedirect.com/science/article/pii/S0956713517300385
32. EFSA, European Food Safety Authority. “Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food”. EFSA Journal, 14 (2016). http://doi.wiley.com/10.2903/j.efsa.2016.4426
33. Weißhaar, R. “Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide”. European Journal of Lipid Science and Technology 110 (2008): 183-186. https://doi.org/10.1002/ejlt.200700197
34. Weißhaar, R., & Perz, R. “Fatty acid esters of glycidol in refined fats and oils”. European Journal of Lipid Science and Technology 112 (2010): 158–165. https://doi.org/10.1002/ejlt.200900137
35. Haines, T. D., et al. “Direct determination of MCPD fatty acid esters and glycidyl fatty acid esters in vegetable oils by LC-TOFMS”. Journal of the American Oil Chemists’ Society 88 (2011) 1–14. https://doi.org/10.1007/s11746-010-1732-5

36. Weiβhaar, R. “Fatty acid esters of 3-MCPD: Overview of occurrence and exposure estimates”. European Journal of Lipid Science and Technology 113 (2011): 304-308. https://onlinelibrary.wiley.com/doi/abs/10.1002/ejlt.201000312
37. Li, C., et al. “Formation and reduction of 3-monochloropropane-1,2-diol esters in peanut oil during physical refining”. Food Chemistry 199 (2016): 605–611. https://doi.org/10.1016/j.foodchem.2015.12.015

4.2 Are chloropropanols and glycidyl fatty acid esters a matter of concern in palm oil?

Este sub-capítulo apresenta os resultados relativos à revisão bibliográfica sobre a ocorrência de cloropropanóis e ésteres glicidílicos de ácidos gordos em óleos de palma. Foi incluída uma análise das opiniões científicas e legislação relativa a estes compostos, metodologias analíticas, condições relacionadas com a sua formação e mitigação. Verificou-se ser extramente importante desenvolver mais trabalhos, sobretudo relativos aos ésteres glicidílicos de ácidos gordos.

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Are chloropropanols and glycidyl fatty acid esters a matter of concern in palm oil?

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Abstract

Background: Palm oil is the most consumed worldwide, being evident its importance due to several applications by the food industry. The presence of toxic and mutagenic compounds, such as chloropropanols and glycidyl fatty acid esters has increased the attention on this oil. Very recently, there have been several alerts about the safety of certain foods containing palm oil in their composition. Consequently, the European Commission has demanded the evaluation of these hazardous compounds in several food matrices, including palm oil, turning this issue into a public health concern.

Scope and Approach: This review focuses on chloropropanols and glycidyl fatty acid esters in palm oil, their precursors, occurrence and mitigation strategies, as well as scientific opinions, current legislation and analytical approaches.

Key Findings and Conclusions: Liquid chromatography coupled to mass spectrometry detection is preferred in the analysis of chloropropanols and glycidyl esters in palm oil. Concerning the precursors, the presence of chlorides and mono- and diglycerides is related to high amounts of these compounds, especially glycidyl esters. Several differences in the occurrence of these compounds in palm oil were reported, namely in what concerns to applied processing (refining/deodorization conditions; temperature/time of frying in the presence or absence of food). There is still much work to be done to implement efficient mitigation strategies without compromise the safety of palm oil. Furthermore, regarding the maximum levels in foodstuffs, European Commission is finalising the new EU legislation, which will include values for the maximum levels of glycidyl fatty acid esters in vegetable oils.

Introduction

Palm oil (PO) is the most consumed worldwide and its importance is evidenced by its significant production levels, but also due to its vast applications by food industry. In recent years, some health concerns were pointed out due to the presence of toxic and mutagenic compounds in PO, such as chloropropanols, namely 3-monochloro-2-propanediol (3-MCPD) and glycidyl fatty acid esters (GEs).

In 1978, chloropropanols were described for the first time in acid-hydrolysed vegetable proteins, leading to intensive scientific research concerning this subject. Later, the presence of chloropropanols was also described in soy sauces, heat-processed foods, as well as in vegetable oils. Monochlopropanols are chlorinated analogues of glycerol that have a chlorine atom in positions 1 or 2, and they can occur in several forms, namely as mono- or diesters (Crews et al., 2013). Food and Agriculture Organization of the United Nations, World Health Organization, European Commission and Expert Committee on Food Additives have established a maximum tolerable daily intake for 3-MCPD because of its potential carcinogenicity and genotoxicity.

GEs are fatty acid esters of glycidol (2-oxiranemethanol) and were reported for the first time by Weißhaar & Perz (2010), as a result of an overestimation of 3-MCPD analysed by indirect methods in edible oils. Therefore, the interest on GEs research mainly arose from the inconsistency reported between the literature data observed for 3-MCPD. Nowadays, these compounds are considered the main contaminants in processed oils, being formed especially during refining and deodorization steps of edible oils processing (Cheng, Liu, Wang, & Liu, 2017; Weißhaar, 2008a).

The interest on chloropropanols and GEs occurrence in foods, especially on vegetable oils, has emerged and several studies have evaluated the occurrence of these hazardous compounds. Therefore, this review focuses on chloropropanols and GEs in PO and related products (palm olein, stearin and palm kernel), by describing their precursors, occurrence and mitigation strategies, as well as scientific opinions, current legislation and analytical approaches.

Palm oil

Origin and production

In 1763, Nicholas Joseph Jacquin introduced for the first time the botanical classification of *Elaeis* genus and *Arecaceae* family (Barcelos et al., 2015). According to this taxonomic classification, the *Elaeis* genus comprises two species, namely *Elaeis guineensis* Jacq. (African PO) and *Elaeis oleifera* (Kunth) Cortés (American PO) (Barcelos et al., 2015). The

PO (*E. guineensis* Jacq.) originally from South Africa was later introduced in East Asia, namely in Indonesia and Malaysia (Barcelos et al., 2015; Lin, 2011). In what concerns to *E. oleifera* (Kunth) Cortés, it is originated from Central and South America.

PO is the major vegetable oil produced in the World followed by soybean, rapeseed and sunflower oils, representing in 2014, 34%, 27%, 15% and 9% of the World production, respectively (FAOSTAT, 2017). Two different types of oils are produced from palm fruit, namely PO that is obtained from the fruit and palm kernel oil (PKO) that is extracted from the seed (non-edible part of the fruit). In 2014, the World production of PO reached 57 million tonnes and the production of PKO was around 6 million tonnes (FAOSTAT, 2017). It is expected that the production of PO continues to increase because of its low-cost production, high yield of oil per unit of fruit and its several applications in industry (Corley, 2009). In 2016, 21,087,444 hectares of palm tree were planted worldwide, and the world production of palm fruit was around 300,252,193 tonnes (FAOSTAT, 2017). The top leading countries in the production of PO and PKO in 2014 were Indonesia, Malaysia, Thailand and Colombia, accounting for 51%, 34%, 3% and 2% of the World production for PO, respectively. Between 1994 and 2004, Malaysia was the country with the highest production amounts, but after 2004 Indonesia is the leading country regarding the production of PO and PKO.

Nutritional and bioactive properties

With respect to the nutritive value of PO, different aspects can contribute to the differences reported on the literature, namely the soil and climate conditions, species and cultivar, as well as pre- and post-harvest conditions, processing type and part of the fruit used. For example, regarding the species, according to the data reported by Lin (2011) in *E. guineensis*, the major fatty acid is palmitic acid (C16:0), a saturated fatty acid, while for *E. oleifera* the major fatty acid is oleic acid (C18:1), a monounsaturated fatty acid. Moreover, the comparison of the carotenoids profile of both species is similar, but *E. guineensis* contains a higher amount of lycopene.

In what concerns to the processing, the crude PO is rich in minor compounds such as carotenoids, tocopherols and tocotrienols, and sterols, but during the refining steps these valuable compounds have some losses, like in other vegetable oils (Gee, 2007; Lin, 2011). For example, tocopherols and tocotrienols amounts are reduced from 600-1000 µg/g to 350-630 µg/g, for crude PO and refined PO, respectively (Lin, 2011).

As aforementioned, PO can be extracted from the palm fruit itself or from seed, since it contains around 50% of oil. In general, the PKO is a light-yellow oil, while PO is orange-red (Edem, 2002). Regarding their fatty acids composition, PKO contains higher amounts (81.5

g/100 g) of saturated fatty acids (SFA) than PO that contains around 49.3 g/100 g (USDA, 2017). Moreover, regarding the type of SFA significant differences are found, being the major SFA for PKO lauric acid (C12:0) while for PO it is palmitic acid (C16:0) (Dubois, Breton, Linder, Fanni, & Parmentier, 2007; USDA, 2017). These features will be crucial for their industrial applications. Regarding the presence of other minor compounds, such as vitamin E (particularly, α-tocopherol), PO has a content (15.94 mg/100 g) four times higher than PKO (3.81 mg/100 g) (USDA, 2017). On the other hand, vitamin K (phylloquinone) is more abundant in PKO with a content of 24.7 µg/100 g than in PO (8.0 µg/100 g) (USDA, 2017). PO, which is a semi-solid fat at room temperature, can be separated into two major fractions, namely palm olein (liquid fraction, with a melting point of 18-20 ºC) and palm stearin (solid fraction, with a melting point of 48-50 ºC) (Edem, 2002). Nowadays, other fractions from palm olein (super olein and double fractioned olein) with different characteristics can be obtained by using other processing techniques, which are adapted to the industry needs (Lin, 2011). Concerning the fatty acids composition of these two fractions and PO, they share the same fatty acids profile, but palm olein has a higher amount of oleic acid (C18:1) than palm stearin (Edem, 2002; Mancini et al., 2015).

Food industry applications

The European PO Alliance identified some benefits related with the use of PO: (i) stability to high temperatures; (ii) long shelf life; (iii) physical and chemical properties adequate for several industrial applications; and (iv) alternative to *trans* fats (www.palmoilandfood.eu). Throughout the world only 10% of PO is used for other applications (soap, oleochemical manufacturing, and cosmetics) instead of edible purposes (Edem, 2002). PO is widely used by food industry, namely to produce edible fats, confectioneries and bakery products, as well as for ice-creams, chocolate and other foods (Lin, 2011). Sometimes, in food formulation, industry prefers to use different combinations of PO fractions or the mixture of PO and PKO, or even the mixture of PO with other vegetable oils, depending on the desired features for the final product. Food industry has a particular interest in PO and PKO due to their economic value, high availability during all seasons and their low content of polyunsaturated fatty acids coupled to the presence of tocopherols and tocotrienols, making it very stable against oxidation reactions (Aini & Miskandar, 2007). PO and PKO are more used at industrial level than at households. Moreover, like other vegetable oils, the most used form of this oil is the refined one, instead of crude PO. However, in Southeast Asia, Africa and Brazil crude PO is widely used for domestic cooking (www.palmoilandfood.eu).

To produce margarines and shortenings, PO is considered a very suitable product because it provides oxidative stability, consistency, texture and structure to the final products (Aini & Miskandar, 2007). Besides the application on the formulation of margarines and

shortenings, these products are widely used by bakery and pastry industry as an ingredient for cakes, cookies, cream fillings and icings, bread, among others. Also, PO and PKO are used as frying oils, due to its good stability regarding oxidation and polymerization. Since, PO has a low content of polyunsaturated fatty acids, besides the presence of other minor compounds that act as antioxidants and prevent degradation during exposure to external factors, such as oxygen, temperature and water; it fulfils the requirements for frying (Aini & Miskandar, 2007; Edem, 2002).

Chloropropanols and glycidyl fatty acid esters

Chloropropanols are chlorinated analogues of glycerol, which have a chlorine atom in positions 1 or 2. These compounds can be also present in oils and fats as fatty acid esters (Crews et al., 2013; Jędrkiewicz, Kupska, Głowacz, Gromadzka, & Namieśnik, 2016b). Monochloropropanediols, such as 2-MCPD and 3-MCPD, can occur as mono- or diesters, while dichloropropanols (1,3-DCP and 2,3-DCP) can only occur as monoesters (Seefelder, Scholz, & Schilter, 2011). Despite the fact that all these compounds have similar structures, they have different metabolic and toxicological properties.

Scientific opinions and legislation

In 2001, after consulting the Scientific Committee for Food (SCF), the Commission for European Communities has set maximum levels for 3-MCPD. In previous reports from SCF, 3-MCPD was recognized as a potential genotoxic carcinogen, generated along the food processing, namely in hydrolysed vegetable protein, by acid hydrolysis (Commission of the European Communities, 2001). Before the entry into force Commission Regulation (EC) No. 466/2001, some Member States have also reported high levels of 3-MCPD in soy sauces. Therefore, the maximum level of 0.02 mg/kg for 3-MCPD present in hydrolysed vegetable protein and soy sauce was set. It is to take into account that this maximum level is given for the liquid product containing 40% of dry matter, corresponding to a maximum level of 0.05 mg/kg in the dry matter. So it is necessary to adjust the value accordingly to the dry matter content of the products (Commission of the European Communities, 2001). In 2001, SCF had set a tolerable daily intake of 2 µg/kg of body weight and had concluded that 3-MCPD was a non-genotoxic carcinogen for humans (Scientific Committee on Food (SCF), 2001). According to the Joint FAO/WHO Expert Committee on Food Additives (JECFA), the main target organ for 3-MCPD toxicity is the kidney, with chronic oral exposure resulting in nephropathy and tubular hyperplasia and adenomas (Food and Agriculture Organization of the United Nations and World Health Organization, 2002).

Later, the Commission Regulation (EC) No. 466/2001 that sets the maximum levels of certain contaminants in foodstuffs was amended by Commission Regulation (EC) No.

1881/2006, which indicates that the main contributors for 3-MCPD dietary intake are still soy sauce and soy-sauce based products (Commission of the European Communities, 2006). However, other potential food sources also emerged as a concern, namely bread and noodles because they are usually eaten in larger quantities. Therefore, the Commission Regulation (EC) No. 1881/2006 maintained the maximum values set for 3-MCPD occurrence in hydrolysed vegetable protein and soy sauce but sets that "*Member States were requested to examine other foodstuffs for the occurrence of 3-MCPD in order to consider the need to set maximum levels for additional foodstuffs*" (Commission of the European Communities, 2006). Therefore, in 2013, European Food Safety Authority (EFSA) has published the Scientific Report on the analysis of 3-MCPD occurrence in food, in Europe, from 2009-2011 and a preliminary exposure assessment (European Food Safety Authority, 2013). This document reports that 1235 analytical results were collected in European Member States and the higher 3-MCPD values were found in animal and vegetable fats and oils (European Food safety Authority, 2013). Also, the food groups that mainly contribute to the exposure to these contaminants were margarine and similar products, and vegetable fats and oils (European Food safety Authority, 2013). With these relevant data, the European Commission has published a recommendation on the monitoring of the presence of 2-MCPD and 3-MCPD, their esters and GEs in food, particularly in: (i) vegetable oils and fats, and derived products such as margarine and similar products; (ii) foods for particular nutritional uses, foods intended for infants and young children and dietary foods for special medical purposes; (iii) fine bakery wares, bread and rolls; (iv) canned meat and fish (smoked); (v) potato- or cereal based snacks and other fried potato; and (vi) vegetable oil containing foods and foods prepared/produced with vegetable oils (European Commission, 2014). Taking into account this recommendation and after several reports by the different Member States, EFSA Panel on Contaminants in the Food Chain (CONTAM) evaluated 7175 occurrence data regarding these compounds (European Food Safety Authority, 2016). The conducted analysis by CONTAM Panel indicates that 3-MCPD, 2-MCPD and GEs were found at higher levels in PO/fat, but other vegetable oils contain also substantial amounts (European Food Safety Authority, 2016). Moreover, the tolerable daily intake was reviewed based on the existent data and 0.8 µg/kg of body weight for 3-MCPD was set (European Food Safety Authority, 2016). Also, in 2016, JECFA, established a provisional maximum tolerable daily intake of 4 µg/kg of body weight for 3-MCPD and their esters singly or in combination (expressed as 3-MCPD equivalents) (Food and Agriculture Organization of the United Nations and World Health Organization, 2017). Recently, CONTAM Panel from EFSA published a risk assessment update on 3-MCPD and its esters, indicating an increase of the tolerable daily intake from 0.8 to 2 µg/kg of body weight per day (European Food Safety Authority, 2018). Furthermore, regarding the

maximum levels in foodstuffs, European Commission is finalising the new EU legislation, which is expected to include values for the maximum levels of GEs in vegetable oils and infants' formulas, besides revising the established limits for 3-MCPD occurrence in soy sauce and hydrolysed vegetable protein.

Analytical methods

For the reliable analysis of chloropropanols and GEs in different foodstuffs, and to obtain comparable analytical results, validated analytical methods, including sample preparation are crucial. In Tables 29 and 30, a literature review on gas chromatography and liquid chromatography methods applied for the analysis of chloropropanols and GEs in PO products is presented. Crews et al. (2013) reviewed the analytical approaches in several food matrices for chloropropanols and GEs determination. According to their point of view, the analysis of these contaminants is extremely complex, with two types of approach, direct and indirect methods.

Indirect methods are based in the conversion of individual MCPD esters into a single compound, 3- or 2-MCPD, that is quantified (Crews et al., 2013). These methods comprise several steps, namely addition of internal standard to the sample, transesterification, neutralisation, derivatization, and chromatographic analysis. Since it involves numerous steps, small differences in each step may have a significant impact in the specificity, repeatability, reproducibility and trueness of the analytical method. Samples transesterification used for 3-MCPD analyses include methanolysis catalysed under acidic or alkaline conditions, and enzymatic hydrolysis (lipase). However, alkaline transesterification may be more convenient due to its short duration, less than 10 min, compared with acid transesterification which could take up to 16 h (Weißhaar, 2008a). Neutralisation is an important step to remove lipophilic compounds from the mixture, being sodium chloride, sulphate salts and sodium bromide the most used (Kuhlmann, 2011; Weißhaar, 2008a). Indirect methods require the derivatization of free 3-MCPD prior to quantification, due to the low volatility and high polarity of this compound. Phenylboronic acid and heptafluorobutyrylimidazole are generally used as derivatizing reagents (Hamlet, Sadd, & Crews, 2002).

Table 29. Gas chromatography-mass spectrometry methods to evaluate chloropropanols and glycidyl fatty acid esters in palm oil.

Sample preparation	Analytes	Internal standard	Chromatographic conditions	Reference
Acid cleavage (sulphuric acid/methanol); derivatisation with phenylboronic acid	3-MCPD	PP-3-MCPD-d_5	Column: SPB™-1 (30 m × 0.25 mm i.d.; 1 µm film thickness) Carrier gas: Helium Flow (mL/min): 0.8 Oven ramp: 80°C (1 min) to 300°C (37 min) at the rate of 10°C/min. Injector temp (º C): 250 ºC Injection volume: 1 µL Injection mode: -	(Divinová, Svejkovská, Doležal, 2004; Karšulínová et al., 2007)
Accelerated solvent extraction; derivatisation with heptafluorobutyrylimidazole	3-MCPD	3-MCPD-d_5	Column: HP-1MS (60 m x 0.25 mm, i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.0 Oven ramp: 80 ºC (1.5 min), increase of 30 ºC/min until 300 ºC and held for 10 min Injector temp (º C): - Injection volume: - Injection mode: -	(Seefelder et al., 2008)
DGF method C-III 18; sulphuric acid/propanol	3-MCPD Glycidyl palmitate Glycidyl stearate Glycidyl oleate	-	Column: DB-5MS (30 m x 0.25 mm, i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: 60 ºC (5 min isotime) raised to 280 ºC with a heating rate of 10 ºC/min and 20 min final isotime Injector temp (º C): 230 Injection volume: 1 µL Injection mode: Splitless	(Weißhaar & Perz, 2010)
Alkaline hydrolysis and derivatisation with phenylboronic acid	3-MCPD Glycidyl esters 2-MCPD	3-MCPD-1,2bis-palmitoyl ester-d_5 3-MCPD-d_5 3-MCBD-d_5	Column: Resteck Rxi®-17 (30 m x 0.25 mm i.d.; 0.25 µm film thickness); HP-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness); HP-5MS (30 m x 0.32 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: 90 ºC, isothermal 0.1 min, with 78 ºC/min to 175 ºC, isothermal 1.0 min with 20 ºC/min to 290 ºC, isothermal 8.3 min Injector temp (º C): - Injection volume: 2 µL Injection mode: Splitless	(Kuhlmann, 2011)

BfR Method 008 (alkaline hydrolysis and derivatisation with heptafluorobutyric anhydride)	3-MCPD	Free d_5-3-MCPD	Column: HP-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: 60 ºC (1 min) to 190 ºC (1 min) at the rate of 6 ºC/min, and was then accelerated to 280 ºC at a rate of 30 ºC/min (held 5 min) Injector temp (º C): 180 Injection volume: 1 µL Injection mode: Splitless	(Razak et al., 2012)
Normal phase liquid chromatography (hexane/methyl tert-butyl ether/chloroform)	Glycidyl laurate Glycidyl myristate Glycidyl palmitate Glycidyl stearate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	Glycidyl palmitate-d_5 Glycidyl palmitate-d_{31}	Column: InertCap Pure WAX Carbowax (15 m x 0.25 mm i.d.; 0.20 µm film thickness) Carrier gas: Helium Flow (mL/min): - Oven ramp: 110 ºC for 1 min; increase to 160 ºC at 25 ºC/min and to 260 ºC at 10 ºC/min; hold 2 min Injector temp (º C): 250 Injection volume: 1 µL Injection mode: Splitless	(Steenbergen et al., 2013)
Acid-catalysed methanolysis; sulphuric acid/methanol	3-MCPD	PP-3-MCPD-d_5 1-O-3-MCPD-d_5	Column: VF-1MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: 60 °C (held for 1 min), 6 °C/min to 190 °C, 20 °C/min to 280°C (held for 30 min) Injector temp (º C): - Injection volume: - Injection mode: -	(Arisseto et al., 2014)
DGF method C-VI 18 (10); derivatisation with phenylboronic acid	3-MCPD	1,2-Bis-palmitoyl-3-MCPD-d_5	Column: HP-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.0 Oven ramp: Initial temperature of 85 ºC (isothermal 0.5 min), with 6 ºC/min to 150 ºC, with 12 ºC/min to 180 ºC, with 25 ºC to 280 ºC, isothermal 7.16 min Injector temp (º C): 280 Injection volume: - Injection mode: Splitless	(Li et al., 2016, 2015a)

AOCS Cd29c-13; phenylboronic acid for derivatisation	Glycidyl esters estimated by the amounts of 3-MCPD esters	Free d_5-3-MCPD	Column: TG-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.18 Oven ramp: 80 °C with an increase of 5 °C/min to obtain 155 °C, increase by 60 °C/min to 300 °C, held for 5 min Injector temp (º C): - Injection volume: 1 µL Injection mode: Splitless	(Cheng et al., 2016)
SGS "3-in-1" (alkaline transesterification and derivatisation with phenylboronic acid)	2-MCPD 3-MCPD	1,2-bis-palmitoyl-3-MCPD-d_5 1,3-distearoyl-2-MCPD-d_5	Column: DB-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: 60 ºC; increase of 6 ºC/min to 190 ºC; increase by 30 ºC/min to 280 ºC; held 10 min. Injector temp (º C): - Injection volume: 1 µL Injection mode: Splitless	(Jedrkiewicz et al., 2016a)
Derivatisation with phenylboronic acid	2-MCPD 3-MCPD Glycidyl esters	2-MCPD-d_5 3-MCPD-d_5 3-MCBD-d_5	Column: VF-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness); VF-7MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 1.2 Oven ramp: Held at 60 ºC for 1 min; increased at 10 ºC/min to 60-150 ºC, followed by 3 ºC/min to 150-180 ºC and 30 ºC/min to 180-300 ºC; held 8 min Injector temp (º C): 250 Injection volume: - Injection mode: Splitless	(Koyama et al., 2016)
AOCS Cd29c-13; alkaline; phenylboronic acid derivatisation	2,3-DCP 1,3-DCP 2-MCPD 3-MCPD	1,3-DCP-d_5 2,3-DCP-d_5 1,3-DCP-2-palmitate-d_5 2,3-DCP-1-palmitate-d_5 2-MCPD-d_5 3-MCPD-d_5 2-MCPD-1,3-bis-stearate-d_5 3-MCPD-1,2-bis-oleate-glycidyloleate-d_5	Column: Agilent J&W (60 m x 0.25 mm i.d.; 1 µm film thickness) Carrier gas: Helium Flow (mL/min): 0.9 Oven ramp: 40 ºC, 6 min isothermal, with 5 ºC/min to 140 ºC, with 20 ºC/min to 230 ºC, isothermal 3 min Injector temp (º C): 150 Injection volume: 25 µL Injection mode: Split	(Kuhlmann, 2016)
BfR Method 008 (purification by ammonium sulphate extraction and phenylboronic	3-MCPD	3-MCPD-d_5	Column: HP-1MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: - Flow (mL/min): -	(Zulkurnain et al., 2016)

acid for derivatisation)			Oven ramp: 50 °C (1 min hold); increased 10 °C/min up to 210 °C and then at 30 °C/min up to 300 °C; hold 20 min Injector temp (º C): - Injection volume: 1 µL Injection mode: -	
AOCS Cd 29a-13; sulphuric acid/methanol; derivatisation with phenylboronic acid	1,2-dipalmitoyl-3-MCPD 1,3-distearoyl-2-MCPD	1,2-dipalmitoyl-3- MCPD-d_5 1,3-distearoyl-2-MCPD-d_5	Column: DB-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: - Flow (mL/min): 1.2 Oven ramp: 60 ºC held for 1 min, 6 ºC/min till 150 ºC (held for 2 min), 30 ºC/min till 300 ºC held for 10 min Injector temp (º C): 250 Injection volume: 1 µL Injection mode: Splitless	(Zelinkova et al., 2017)
-	1,2-bis-palmitoyl-3-MCPD Glycidyl palmitate	1,2-bis-palmitoyl-3- MCPD-d_5 Glycidyl palmitate-d_5	Column: HP-5MS (30 m x 0.25 mm i.d.; 0.25 µm film thickness) Carrier gas: Helium Flow (mL/min): 0.8 Oven ramp: 80 ºC (1 min) to 170 ºC at a rate of 10 ºC/min, increased to 200 ºC at a rate of 3 ºC/min, and lastly elevated to 300 ºC (15 min) at a rate 15 ºC/min Injector temp (º C): 250 Injection volume: 1 µL Injection mode: Splitless	(Wong et al., 2017)

The quantification of chloropropanols in PO products is usually carried out by gas chromatography coupled with mass spectrometry (GC-MS) (Arisseto, Marcolino, & Vicente, 2014; Becalski, Feng, Lau, & Zhao, 2015; Cheng, Liu, & Liu, 2016; Divinová, Svejkovská, Doležal, 2004; Jedrkiewicz, Głowacz, Gromadzka, & Namieśnik, 2016a; Karšulínová, Folprechtová, Doležal, Dostálová, & Velíšek, 2007; Koyama et al., 2016; Kuhlmann, 2011, 2016; Li, Nie, Zhou, & Xie, 2015a; Razak et al., 2012; Seefelder et al., 2008; Steenbergen, Hrnčiřík, Ermacora, de Koning, & Janssen, 2013; Weißhaar & Perz, 2010; Wong et al., 2017; Zelinkova, Giri, & Wenzl, 2017; Zulkurnain et al., 2012; Lai, Tan, Latip, & Tan, 2016).

Some of the appointed disadvantages of indirect methods are related with the possible degradation of compounds during hydrolysis and formation of additional compounds, which can occur if chloride salts are used in the neutralisation step (Weißhaar, 2008a).

Concerning direct methods for chloropropanols and GEs, Liquid Chromatography/Time-of-Flight Mass Spectrometry *(*LC-TOF-MS) (Craft & Nagy, 2012; Destaillats, Craft, Sandoz, & Nagy, 2012; Haines et al., 2011; Hori et al., 2012a; Zhou, Jin, Wang, & Xu, 2014), Liquid Chromatography Tandem Mass Spectrometry (LC-MS-MS) (Aniołowska & Kita, 2015; Becalski et al., 2015; Li et al., 2015b; Macmahon, Begley, & Diachenko, 2013; MacMahon, Begley, & Diachenko, 2013a; MacMahon, Mazzola, Begley, & Diachenko, 2013b; Yamazaki et al., 2013; Zelinkova et al., 2017) and Liquid Chromatography coupled to Mass Spectrometry (LC-MS) (Blumhorst et al., 2013; Shiro, Kondo, Kibune, & Masukawa, 2011) are the most used techniques and allowed to quantify of up to fourteen chloropropanols (mono- and diesters) and seven GEs. Most of the analytical methods referred in the literature include the addition of deuterated internal standards. With respect to the analytical column, columns with a particle size lower than 2 µm are usually selected (Craft & Nagy, 2012; Destaillats et al., 2012; Hori et al., 2012a; Li et al., 2015b; Yamazaki et al., 2013; Zhou et al., 2014), and analysis performed under high pressure conditions (15,000 psi) to improve parameters such as sensitivity and resolution of the analytical method. The mobile phase composition includes different proportions of methanol; isopropranol or acetonitrile, and water acidified or not (Table 30). Direct methods for chloropropanols and GEs quantification do not require as many intermediate steps as indirect methods. Therefore, in general, direct methods are more suitable for the analysis of these compounds. However, the challenge of direct determination is the large number of compounds to be identified. Moreover, compared with indirect methods, a higher investment in deuterated internal standards and reference materials is needed (Crews et al., 2013).

Table 30. Liquid chromatography methods applied to evaluate chloropropanols and glycidyl fatty acid esters in palm oil.

Detector	Sample preparation	Analytes	Internal standard	Chromatographic conditions	Reference
TOF-MS	Dilution of samples with methyl tert-butyl ether and ethyl acetate (8:2, v/v); derivatisation with phenylboronic acid; extraction with n-hexane	3-MCPD monopalmitate 3-MCPD monostearate 3-MCPD dipalmitate Glycidyl stearate Glycidyl linolenate Glycidyl linoleate Glycidyl oleate Glycidyl palmitate	3-MCPD-d_5 Glycidyl palmitate-d_5	Column: Luna C18 (50 mm x 3 mm, i.d.; 3 µm particle size) Mobile phase: Gradient; (A) ethanol/acetonitrile/methanol-sodium acetate solution (0.26 mM), 8:1:1, v/v/v); (B) methanol-sodium acetate solution (0.26 mM)/methylene chloride/acetonitrile, 1:8:1, v/v/v) Flow (mL/min): 0.25 Column temp (ºC): - Injection volume: 5 µL	(Haines et al., 2011)
MS	Dilution of samples with methyl tert-butyl ether and ethyl acetate (4:1, v/v); SPE (Sep-Pak Vac RC C18 cartridge 500 mg); SPE (Sep-Pak Vac RC Silica cartridge 500 mg); residues were dissolved using methanol/isopropanol (1:1, v/v)	Glycidyl stearate Glycidyl palmitate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	Glycidyl heptadecanoate	Column: ODS (150 mm x 4.6 mm i.d., 5 µm particle size) Mobile phase: Gradient; (A) methanol; (B) 2-propanol Flow (mL/min): 1.0 Column temp (ºC): 40 Injection volume: 20 µL	(Shiro et al., 2011)
TOF-MS	-	Glycidyl palmitate Glycidyl stearate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	$^{13}C_3$-Sn1-palmitoyl-Sn2-stearyl-MCPD	Column: HSS C18 (150 mm x 2.1 mm i.d., 1.7 µm particle size) Mobile phase: Gradient; (A) 1mM ammonium formate in methanol; (B) 100 µM ammonium formate in isopropanol Flow (mL/min): 0.15-0.4 Column temp (ºC): - Injection volume: 1 µL	(Craft & Nagy, 2012; Destaillats et al., 2012)
TOF-MS	Dilution of samples with hexane saturated with acetonitrile; SPE (Sep-Pak Plus C18 cartridges, 500 mg); residues were dissolved with acetonitrile	Glycidyl palmitate; Glycidyl stearate Glycidyl oleate; Glycidyl linoleate Glycidyl linolenate; rac 1-linoleoyl-3-MCPD; rac 1-palmitoyl-3-MCPD; rac 1-oleoyl-	Glycidil stearate-d_{35} rac 1-palmitoyl-3-MCPD-d_5 rac 1,2-bis-linoleoyl-3-MCPD-d_5 rac 1,2-bis-palmitoyl-3-MCPD-d_5	Column: Acquity UPLC BEH C18 (50 mm x 2.1 mm i.d., 1.7 µm particle size) Mobile phase: Gradient; (A) water/MeOH (85/15, v/v); (B) MeOH/water (97.5/2.5, v/v)	(Hori et al., 2012b)

		3-MCPD; rac 1,2-bis-linoleoyl-3-MCPD; rac 1-palmitoyl-2-linoleoyl-3-MCPD; rac 1- oleoyl-2-linoleoyl-3-MCPD; rac 1,2-bis-palmitoyl-3-MCPD; rac 1,2-bis-palmitoyl-2-oleoyl-3-MCPD; rac 1,2-bis-oleoyl-3-MCPD		Flow (mL/min): 0.2-0.25 Column temp (ºC): - Injection volume: 10 µL	
MS-MS	Dilution of samples with methyl tert-butyl ether:ethyl acetate; Double SPE; residues were dissolved with isopropanol	**Monoesters:** 1-lauroyl-3-MCPD; 1-myristoyl-3-MCPD; 1-palmitoyl-3-MCPD; 1-linolenoyl-3-MCPD; 1-linoleoyl-3-MCPD; 1-oleoyl-3-MCPD; 1-stearoyl-3-MCPD; 2-oleoyl-3-MCPD; 2-palmitoyl-3-MCPD **Diesters:** 1,2-Bis-lauroyl-3-MCPD; Lauroyl-linolenoyl-3-MCPD; Lauroyl-myristoyl-3-MCPD; Lauroyl-linoleoyl-3-MCPD; 1,2-Bis-linolenoyl-3-MCPD; Myristoyl-linolenoyl-3-MCPD; 1,2-Bis-myristoyl-3-MCPD; Lauroyl-Palmitoyl-3-MCPD; Lauroyl-Oleoyl-3-MCPD; Linoleoyl-linolenoyl-3-MCPD; Myristoyl-linoleoyl-3-MCPD; 1,2-Bis-linoleoyl-3-MCPD; Palmitoyl-linoleoyl-3-MCPD; Oleoyl-linolenoyl-3-MCPD; Myristoyl-palmitoyl-3-MCPD; Lauroyl-stearoyl-3-MCPD; Myristoyl-oleoyl-3-MCPD; Palmitoyl-linoleoyl-3-MCPD; Oleoyl-linoleoyl-3-MCPD; Stearoyl-linoleoyl-3-MCPD; Myristoyl-stearoyl-3-MCPD; 1,2-Bis-palmitoyl-3-MCPD; Palmitoyl-oleoyl-3-MCPD; 1,2-Bis-oleoyl-3-MCPD; Stearoyl-linoleoyl-3-MCPD Palmitoyl-stearoyl-3-MCPD; Oleoyl-stearoyl-3-MCPD; 1,2-Bis-stearoyl-3-MCPD	Glycidyl laurate-d_5 Glycidyl myristate-d_5 Glycidyl palmitate-d_5 Glycidyl linolenate-d_5 Glycidyl linoleate-d_5 Glycidyl oleate-d_5 Glycidyl stearate-d_5 1-oleoyl-3-MCPD-d_5 1-palmitoyl-3-MCPD-d_5 1,2-dimyristoyl-3-MCPD-d_5 1,2-dioleoyl-3-MCPD-d_5 1,2-lilinolenoyl-3-MCPD-d_5 1,2-dilinoleoyl-3-MCPD-d_5 1-oleoyl-2-linolenoyl-3-MCPD-d_5 Linoleoyl-linolenoyl-3-MCPD-d_5 Palmitoyl-stearoyl-3-MCPD-d_5 1,2-distearoyl-3-MCPD-d_5	Column: Pursuit XRs C18 (150 mm x 2 mm i.d., 3 µm particle size) Mobile phase: Gradient; (A) 2 mM ammonium formate/0.05% formic acid in Methanol/Water (92:8, v/v); (B) 2 mM ammonium formate/0.05% formic acid in isopropanol/water (98:2, v/v) Flow (mL/min): 0.2-0.25 Column temp (ºC): - Injection volume: 5 µL	(Macmahon et al., 2013; MacMahon et al., 2013a; MacMahon et al., 2013b)

		Glycidyl esters; Glycidyl laurate; Glycidyl myristate; Glycidyl palmitate; Glycidyl linolenate; Glycidyl linoleate; Glycidyl oleate; Glycidyl stearate			
MS-MS	Dilution of samples with methyl tert-butyl ether and ethyl acetate; SPE (Sep-pak™ Env. C18); SPE (Sep-pak™ vac silica cartridge); re-dissolved in isopropanol	**Monoesters:** 1-Palmitoyl-3-MCPD; 1-Stearoyl-3-MCPD; 1-Oleoyl-3-MCPD; 1-Linoleoyl-3-MCPD; 1-Linolenoyl-3-MCPD **Homo-diesters:** 1,2-Dipalmitoyl-3-MCPD; 1,2-Distearoyl-3-MCPD; 1,2-Dioleoyl-3-MCPD; 1,2-Dilinoleoyl-3-MCPD; 1,2-Dilinolenoyl-3-MCPD **Hetero-diesters:** 1-Palmitoyl-2-stearoyl-3-MCPD; 1-Palmitoyl-2-oleoyl-3-MCPD; 1-Palmitoyl-2-linoleyl-3-MCPD; 1-palmitoyl-2-linolenoyl-3-MCPD; 1-Stearoyl-2-palmitoyl-3-MCPD; 1-Stearoyl-2-oleoyl-3-MCPD; 1-Stearoyl-2-linoleoyl-3-MCPD; 1-Stearoyl-2-linolenoyl-3-MCPD; 1-Oleoyl-2-palmitoyl-3-MCPD; 1-Oleoyl-2-stearoyl-3-MCPD; 1-Oleoyl-2-linoleoyl-3-MCPD; 1-Oleoyl-2-linolenoyl-3-MCPD; 1-Linoleoyl-2-palmitoyl-3-MCPD; 1-Linoleoyl-2-stearoyl-3-MCPD; 1-Linoleoyl-2-oleoyl-3-MCPD; 1-Linoleoyl-2-linolenoyl-3-MCPD; 1-Linolenoyl-2-palmitoyl-3-MCPD; 1-Linolenoyl-2-stearoyl-3-MCPD; 1-Linolenoyl-2-oleoyl-3-MCPD; 1-Linolenoyl-2-linoleoyl-3-MCPD	d_5-1-Palmitoyl-3-MCPD (d_5-3-MCPD-P); d_5-1,2-Dipalmitpyl-3-MCPD (d_5-3-MCPD-PP); d_5-1,2-Distearoyl-3-MCPD (d_5-3-MCPD-SS); d_5-1,2-Dilinoleoyl-3-MCPD (d_5-3-MCPD-LL); 3-MCPD (3-MCPD)	Column: Luna 3 u C18 (50 mm x 2.1 mm i.d., 1.7 µm particle size) Mobile phase: Gradient; (A) water; (B) 0.01 mol/L ammonium acetate in methanol; (C) methanol; (D) 2-propanol Flow (mL/min): 0.2 Column temp (ºC): 40 Injection volume: 10 µL	(Yamazaki et al., 2013)

TOF-MS	Dilution of samples in acetonitrile; centrifugation; solvent was removed, and the residue was reconstituted in n-hexane; isolation by HPLC-ELSD (SunFire™ C18 column) using acetonitrile/isopropanol (51:49, v/v); solvents were removed, and samples were reconstituted using acetonitrile	1-Linoleoyl-3-MCPD; 1-Oleoyl-3-MCPD; 1,2-Dipalmitoleoyl-3-MCPD; 1-Palmitoyl-2-palmitoleoyl-3-MCPD; 1,2-Dipalmitoyl-3-MCPD; 1-Palmitoyl-2-linoleoyl-3-MCPD; 1,2-Dilinolenoyl-3-MCPD; 1-Linoleoyl-2-linolenoyl-3-MCPD; 1,2-Dilinoleoyl-3-MCPD; 1-Oleoyl-2-linoleoyl-3-MCPD; 1,2-Dioleoyl-3-MCPD; 1-Stearoyl-2-oleoyl-3-MCPD	-	Column: Acquity C18 (50 mm x 2.1 mm, 1.7 µm particle size) Mobile phase: Gradient; (A) methanol; (B) 10 mM aqueous sodium acetate solution containing 0.1% formic acid Flow (mL/min): 0.3 Column temp (ºC): - Injection volume: 1 µL	(Zhou et al., 2014)
MS-MS	Dilution of samples with chloroform and acetone; SPE cartridge (Waters Sep-Pak C18); Normal-phase SPE cartridge (Waters Sep-Pak Silica); dry residue was reconstituted using methanol/isopropanol (1:1, v/v)	Glycidyl palmitate Glycidyl stearate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	Glycidyl palmitate-d_5 Glycidyl stearate-d_5 Glycidyl oleate-d_5 Glycidyl linoleate-d_5 Glycidyl linolenate-d_5	Column: Luna PFP (150 mm x 2 mm i.d., 3 µm particle size) Mobile phase: Gradient; (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile Flow (mL/min): 0.2 Column temp (ºC): 25 Injection volume: 10 µL	(Aniołowska & Kita, 2015, 2016a, 2016b, 2016c)
MS-MS	SPE (normal silica solid-phase C18 extraction cartridges); residue was reconstituted using methanol/isopropanol (1:1, v/v)	Glycidyl laureate Glycidyl myristate Glycidyl palmitate Glycidyl stearate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	Glycidyl laureate-d_5 Glycidyl myristate-d_5 Glycidyl palmitate-d_5 Glycidyl stearate-d_5 Glycidyl oleate-d_5 Glycidyl linoleate-d_5 Glycidyl linolenate-d_5		(Becalski et al., 2015)
MS-MS	SPE; acetonitrile/isopropanol (1:1, v/v)	**Monoesters:** 1-lauroyl-3-MCPD; 3-MCPD 1-linolenate; 1-myristoyl-3-MCPD; 3-MCPD 1-linoleate; 3-MCPD 1-palmitate; 3-MCPD 1-oleate; 3-MCPD 1-stearate **Diesters:** 3-MCPD dilinolenate; 3-MCPD dilinoleate; 1-oleoyl-2-linolenoyl-3-MCPD; 1-palmitoyl-2-linoleoyl-3-MCPD; 1-oleoyl-2-linoleoyl-3-MCPD; 3-MCPD dipalmitate; 3-MCPD dioleate; 1-linoleoyl-2-stearoyl-3-MCPD; 1-palmitoyl-2-stearoyl-3-MCPD; 1-oleoyl-2-stearoyl-3-MCPD; 3-MCPD distearate	3-MCPD 1-linolenate-d_5 3-MCPD 1-linoleate-d_5 3-MCPD 1-palmitate-d_5 3-MCPD 1-oleate-d_5 3-MCPD dilinolenate-d_5 3-MCPD dilinoleate-d_5 1-oleoyl-2-linolenoyl-4-MCPD-d_5 1-palmitoyl-2-linoleoyl-3-MCPD-d_5 1-oleoyl-2-linoleoyl-3-MCPD-d_5 3-MCPD dioleate-d_5 1-linoleoyl-2-stearoyl-3-MCPD-d_5 1-oleoyl-2-stearoyl-3-MCPD-d_5 3-MCPD distearate-d_5	Column: Acquity UHPLC BEH C18 (150 mm x 2.1 mm i.d., 1.7 µm particle size) Mobile phase: Gradient; (A) 0.05% of formic acid and 2 mmol/L ammonium formate in 2-propanol/water (98:2, v/v); (B) methanol/water with 0.05% formic acid and 2 mmol/L ammonium formate (92:8, v/v) Flow (mL/min): 0.2 Column temp (ºC): - Injection volume: 5 µL	(Li et al., 2015b)

MS-MS	Dilution of samples in cyclohexane:ethyl acetate (1:1, v/v); GPC column; residue was reconstituted using methanol/isopropanol (1:1, v/v)	Glycidyl laurate Glycidyl palmitate Glycidyl stearate Glycidyl oleate Glycidyl linoleate Glycidyl linolenate	Glycidyl oleate-d_5 Glycidyl palmitate-d_5	Column: Eclipse XDB-C18 (150 mm x 4.6 mm i.d., 5 µm particle size) Mobile phase: Gradient; (A) methanol:water (92:8, v/v); (B) 2-propanol Flow (mL/min): 1.0 Column temp (ºC): 40 Injection volume: 20 µL	(Zelinkova et al., 2017)

Precursors

Up to now, there are some precursors potentially associated with the formation of chloropropanols and GEs, namely monoglycerides, diglycerides, chlorides and total chlorine. Furthermore, Craft et al. (2012) performed a root-cause analysis of the factors linked with the formation of chloropropanol esters along the PO production chain: "*(1) chlorine input into oil palm production, (2) accumulation of inorganic chloride in the plant, (3) bioconversion of inorganic chlorides to organochlorines in the fruits, (4) formation of liposoluble organochlorines during fruit bunch sterilisation and (5) reaction of liposoluble organochlorines with palm oil triacylglycerols during oil refining and resulting in MCPD diesters*" (Craft, Nagy, Sandoz, & Destaillats, 2012). However, there is some controversy between the results reported in the literature. Therefore, some of these achievements are presented and discussed.

Franke et al. (2009) have evaluated the influence of monoglycerides, diglycerides, chlorides and total chlorine on 3-MCPD occurrence in crude and pre-refined POs, as well as in rapeseed oil, before and after refining (Franke, Strijowski, Fleck, & Pudel, 2009). According to the authors findings, the amount of monoglycerides was lower than 0.1 g/100 g in all the analysed vegetable oils and during all steps of processing, while the amount of diglycerides was much higher in POs (varying between 5.15 and 6.41 g/100 g) than in rapeseed oil (ranging from 1.81 to 2.49 g/100 g). Chlorides were not detected (< 1 mg/kg), while total chlorine was only present in refined PO (3 mg/kg) (Franke et al., 2009). Therefore, authors conclude that one of the possible reasons for the low amounts of 3-MCPD in rapeseed oil was the lower content of potential precursors, namely chlorine and diglycerides (Franke et al., 2009).

Three different strategies were proposed by Matthäus, Pudel, Fehling, Vosmann, & Freudenstein, 2011 in crude POs and other crude oils (avocado, olive, rapeseed, corn, soybean, sunflower and coconut): (i) removal of critical reactants from the raw material; (ii) changing of the refining process; and (iii) removal of 3-MCPD esters and related compounds from the refined product. Furthermore, authors correlated diacylglycerols and chlorides presence with the occurrence of 3-MCPD esters in the selected oils. A very weak correlation ($r = 0.4$) was found for diacylglycerols and 3-MCPD, indicating that it is not a monocausal dependence, but an influence of multicausal factors. Nonetheless, authors suggest that a threshold of 4% diacylglycerols in crude oils before deodorization can be one of the reasons for an enhanced formation of the esters (Matthäus et al., 2011). One of the main reasons for the presence of diacylglycerols before PO processing is the enzymatic degradation of triacylglycerols after fruit maturity. Therefore, Mathäus et al. (2011) have pointed out two approaches: a faster inactivation of lipase and/or intermittent treatment in the oil mill, but both processes are difficult; they also suggest washing crude oils, before processing, to reduce the amounts of precursors such as chloride. Facing this challenge, crude PO was washed with water and ethanol (75%) and a significant lower amount of 3-MCPD esters was determined in comparison with non-washed oils (Matthäus et al., 2011). Regarding chlorides, the addition of 0.1% tetra-n-butylammoniumchloride led to an increase of 3-MCPD in rapeseed oil, although for PO it is not known whether chlorides are responsible for the formation of 3-MCPD (Matthäus et al., 2011). Another approach was chloride precipitation with silver nitrate, but with worst results comparing with oil washing. In fact, further research studies, to clarify the inconsistency between the reported results are crucial.

Hrncirik et al. (2011) evaluated the correlation among monoacylglycerols, diacylglycerols and chlorides in neutralized/bleached PO, bleached PO and rapeseed oil before and during deodorization step. These compounds are appointed as possible precursors of 3-MCPD and GEs (Hrncirik & van Duijn, 2011). No correlation was observed between the aforementioned compounds and formed 3-MCPD esters, but the authors also suggested that other chlorinated compounds could be involved in the formation of 3-MCPD esters (Hrncirik & van Duijn, 2011).

3-MCPD esters in PO were assessed for a possible relationship with minor compounds, as their precursors, namely acylglycerols (mono- and diacylglycerols) and phospholipids (Zulkurnain et al., 2012). Reported findings indicate that diacylglycerols content ranging between 2.11% and 3.38%, result in a 3-MCPD ester level higher than 10 mg/kg. Nonetheless, the correlation between diacylglycerols and monoacylglycerols and 3-MCPD esters was weak ($r=-0.024$ for diacylglycerols and $r=0.297$ for monoacylglycerols). So it is

not possible to establish a direct relation between the above-mentioned compounds (Zulkurnain et al., 2012).

A study on the impact of various factors on the formation of 3-MCPD esters in edible oil models with sodium chloride as chlorine donator during heat processing (70 to 250 ºC, 0.25 to 24 h) was conducted (Li et al., 2016). In addition, authors have also evaluated the impact of sodium chloride concentration, pH value of the oil, heating method and metal ions (Li et al., 2016). Unfortunately, the reported results are only for rapeseed oil. According to Šmidrkal et al. (2016), 3-MCPD esters form approximately 2-5 times faster from dyacylglycerols than from monoacylglycerols.

Chloropropanols occurrence

In Table 31 data concerning the occurrence of chloropropanols in PO and PO products is presented.Karšulínová et al. (2007) analysed the 3-MCPD content in four virgin olive oils, nine virgin seed oils and nineteen types of refined vegetable oils, including five PKOs and four POs. The achieved results indicate that the highest 3-MCPD content was observed for PO samples (2.82 mg/kg) and the lowest amounts were found for virgin oils (0.06 – 0.08 mg/kg) (Karšulínová et al., 2007). A total of 57 samples of vegetable oils were evaluated concerning 3-MCPD content and considerable differences were found depending on the type of oil (Weißhaar & Perz, 2010). For instance, a batch of 12 samples of refined PO and PO-based fats was analysed and the total 3-MCPD esters varied between 2.16 and 15.2 mg/kg, while refined PKO had a much lower content (1.7 mg/kg). In comparison with the other vegetable oils, corn oil has a content similar to PKO, and the lowest content was determined for rapeseed oil (0.4 mg/kg).

Crude POs and other crude edible oils were analysed regarding their 3-MCPD esters content (Matthäus et al., 2011). PO from different origins (Malaysia, Indonesia, Ghana and Columbia) was analysed and great variations for 3-MCPD content were observed. For instance, the highest content of 3-MCPD esters and related compounds was observed for crude PO from Indonesia, followed by Malaysia (Matthäus et al., 2011). According to the authors, feasible reasons for the differences are: different soil and climate conditions, use of different fertilizers, post-harvest conditions, as well as the time period between fruits harvest and processing. In addition, other vegetable oils, such as coconut and corn, have a high content of 3-MCPD esters and related compounds (Matthäus et al., 2011).

Khulmann et al. (2011) evaluated the 3-MCPD and estimated the 2-MCPD content among different vegetable oils, including PO samples (PO, PKO and PO degummed and bleached). According to the results, 3-MCPD varied greatly in PO that were obtained from German and international suppliers (Kuhlmann, 2011). Comparing PKO and PO degummed

and bleached 3-MCPD amounts were very similar. Furthermore, other vegetable oils analysed, namely hazelnut and walnut oils have much higher concentrations of 3-MCPD (around 19 mg/kg) than the analysed POs (maximum value 10 mg/kg) (Kuhlmann, 2011). The authors have also evaluated the presence of 1,3-dichloropopanol-2-palmitoylester (1,3-DCP-2P) and 2,3-dichloropropanol-1-palmitoylester (2,3-DCP-1P) besides 3-MCPD and 2-MCPD. Regarding 1,3-DCP-2P and 2,3-DCP-1P they were not detected (<0.005 mg/kg) in all the analysed samples (palm, sunflower and rapeseed oils) (Kuhlmann, 2016). On the other hand, the mean value determined in refined POs, palm stearin and palm mid fraction for 3-MCPD and 2-MCPD was higher (0.65 and 1.33, for 2-MCPD and 3-MCPD, respectively) than for refined sunflower oils (0.11 and 0.23, for 2-MCPD and 3-MCPD, respectively) and refined rapeseed oils (0.21 and 0.44, for 2-MCPD and 3-MCPD, respectively) (Kuhlmann, 2016).

Soybean, rapeseed, corn, crude PO and three refined POs were analysed regarding the three species of 3-MCPD monoesters and six species of 3-MCPD diesters (Hori et al., 2012a). These compounds were only detected in refined PO samples, being absent in the other analysed vegetable oils. Furthermore, the values of 3-MCPD monoesters and diesters significantly varied between samples. For instance, the most abundant compound was rac-1,2-bispalmitoyl-2-oleolyl-3-MCPD ranging from 6.19 to 13.59 mg/kg for sample (A) and (C) of refined PO, respectively (Hori et al., 2012a).

Razak et al. (2012) have evaluated the 3-MCPD content in different products of PO from Malaysia refineries and compared the values with 10 other vegetable oils (olive pomace; corn; canola; sunflower; soybean; rice bran; palm olein; olein/peanut/sesame; olive oils) obtained from local supermarkets (Razak et al., 2012). According to the reported results, 3-MCPD content was low (not detected – 0.9 mg/kg) in most of the crude POs analysed, and the highest levels were determined for deodorized or fully refined products, especially for refined POs (56% of samples showed ester levels ranging from 0.25 to 2.0 mg/kg; and for the other samples the maximum value for 3-MCPD was 5.8 mg/kg). Regarding the other vegetable oils analysed, the highest 3-MCPD values varied in the following order: olein/peanut/sesame oil > palm olein > pomace olive oil; while soybean oil had the lowest amount (<0.25 mg/kg) (Razak et al., 2012).

Information about the 3-MCPD monoesters and diesters occurrence for 116 retail and/or industrial edible oils and fats available in the United States was reported by MacMahon, et al., 2013a. Crude PO (n=2), PO (n=14), PKO (n=2), palm olein (n=5) and palm stearin (n=1) were evaluated regarding the presence of these compounds, being found considerable differences. For instance, in crude PO 3-MCPD mono- and diesters were below the limit of

quantification, while the highest content of 3-MCPD monoesters was reported for palm olein (8.43 mg/kg) and for 3-MCPD diesters it was PO (6.17 mg/kg) (MacMahon et al., 2013a). The other analysed vegetable oils have lower contents than the different PO fractions.

Refined lard and edible oils (soybean, rapeseed, rice, safflower, sesame, olive, grape seed, perilla and palm) were analysed regarding the occurrence of five 3-MCPD monoesters, five 3-MCPD homo-diesters (compounds that have a pair of identical fatty acid groups) and twenty 3-MCPD hetero-diesters (compounds that have a pair of different fatty acid groups) (Yamazaki et al., 2013). The highest contents of total 3-MCPD esters were reported for grape seed oil (25.35 mg/kg) and PO (14.4 mg/kg), being the values for the other oils lower than 4 mg/kg. Furthermore, the distribution of monoesters, homo- and hetero-diesters was different among the analysed samples. For PO, hetero-diesters were present in higher amounts than the other esters, but for grape seed oil the most abundant were homo-diesters (Yamazaki et al., 2013).

Arisseto et al. (2014) have studied 97 vegetable oils and fats marketed in Brazil, concerning their 3-MCPD content. Authors have included samples from different producers/brands, and for the same producer different lots were selected to assure representativeness. Considering the presented results for PO samples and other products obtained from palm, huge differences were observed between the different lots from the same brand, being the maximum levels quantified for palm fat samples that consist in a mixture of PO and palm stearin (Arisseto et al., 2014). In comparison with the other vegetable oils analysed, only olive pomace oil presented higher 3-MCPD values (5.09 mg/kg) than PO.

Eighteen types of edible oils acquired in China supermarkets were analysed regarding the presence of 3-MCPD mono- and diesters (Zhou et al., 2014). Monoesters were always below the limit of detection in the analysed samples. In addition, 3-MCPD diesters content varied between not detected and 2564 mg/kg (sample of rice oil). With respect to the POs analysed the values ranged from 336 to 540 mg/kg (Zhou et al., 2014).

A total of 30 edible oils, including crude and refined, belonging to 11 different types of vegetable oils were evaluated, regarding the presence of six 3-MCPD monoesters and eleven 3-MCPD diesters (Li et al., 2015b). Furthermore, the authors have compared two different methods, namely a direct method (LC-MS-MS) and an indirect method (GC-MS), and in general the indirect method gave higher results. The highest value of 3-MCPD esters (1.17 mg/kg) was reported for one sample of soybean oil. Concerning PO, 3-MCPD esters were not detected in the crude oil, and in refined PO it was 0.42 mg/kg, which is much lower than the abovementioned value for soybean oil (Li et al., 2015b).

Becalski et al. (2015) evaluated the occurrence of 2- and 3-MCPD esters and GEs in several oils, fats and products containing fats/oils (cookies and cooking sprays), collected in Canada. Furthermore, authors have considered two sampling periods, 2011 and 2013, allowing to study temporal trends (Becalski et al., 2015). Concerning the reported results, for PO high variability was observed with 3-MCPD content varying between <0.1 and 8.42 mg/kg. In comparison with the other vegetable oils analysed, undoubtedly palm shortening has the highest content of glycidol equivalent (10.6 mg/kg), but walnut oil sampled in 2011 had the highest amount of MCPDs equivalents (17.1 mg/kg). However, in 2013, walnut oil presented a significant reduction of MCPDs equivalents (3.11 mg/kg) (Becalski et al., 2015). A total of 102 refined vegetable oils from 11 varieties, including PO, and 41 crude oils from six varieties were collected in local markets from China and analysed regarding their content in 3-MCPD (Li et al., 2015a). The reported findings indicated that the highest content of 3-MCPD was found for refined oils, namely camellia oil (mean: 1.61 mg/kg, range: 0.99-2.59 mg/kg), PO (mean:1.52 mg/kg, range: 1.29-1.65 mg/kg), and margarine (mean: 1.24 mg/kg, range: 0.79-1.60 mg/kg) (Li et al., 2015a).

Different stages/conditions of oils processing

In 2009, Franke et al. published results on the formation of 3-MCPD esters in POs (crude and pre-refined) and rapeseed oil (Franke et al., 2009). The reported amounts of 3-MCPD in both POs before refining indicate that the content in pre-refined PO (5.15 ± 0.16 mg/kg) was much higher than for crude PO (0.98 ± 0.15 mg/kg). Interestingly after all the steps included in the processing of the oil, namely hydration, degumming, neutralization, washing, drying, bleaching and deodorization, the amount of 3-MCPD was similar for both POs (4.56 and 4.39 mg/kg, for pre-refined and crude PO, respectively). Comparing the analysed POs and rapeseed oil, before refining, the lowest content of 3-MCPD was reported for rapeseed with values lower than 4 mg/kg. After refining the levels of these compounds in POs decreased, while in rapeseed oil they increased to 1.04 mg/kg (Franke et al., 2009). Also, this research group evaluated the monoycerides, diglycerides and 3-MCPD during the several steps of oil processing, after deodorization at different temperatures (180, 210, 240 and 270 ºC) and times (20, 40 and 60 minutes) but only for rapeseed oil. Nonetheless, authors reported that the formation of 3-MCPD was lower when the temperature was 180 ºC (<0.4 mg/kg) than for 270 ºC (1.94 mg/kg) during the same period (20 min). Furthermore, 3-MCPD content increased from 1.07 mg/kg (240 ºC, 20 min) to 1.43 mg/kg (240 ºC, 60 min), while the diglycerides amount decreased from 2.03 to 1.85 g/100 g in the same conditions (Franke et al., 2009). Weißhaar and Perz (2010) studied the effect of different temperatures/times (210 to 250 ºC, for 3 h or 6 h) during deodorization on the formation of 3-MCPD esters in POs. The achieved results were higher than the ones reported by Franke

et al. (2009), but also support the fact that higher temperatures (250 ºC, 3 h) lead to higher amounts of 3-MCPD (4.4 mg/kg) (Weißhaar & Perz, 2010).

Ramli et al. (2011) studied the role of bleaching (six different types of bleaching clays, mainly natural or acid activated), degumming (phosphoric acid at 0.02-0.1%) and water degumming (2.0%) and deodorization (260 ºC for 90 min) on the formation of 3-MCPD esters in crude PO. 3-MCPD esters were not detected, while the highest amounts were observed in refined PO when 0.1% phosphoric acid was used in degumming in combination with acid activated clays were used (Ramli et al., 2011). Authors concluded that natural bleaching clays and acid activated clays with more neutral pH are a good option to reduce the formation of these compounds. However, the harvesting in plantations, as well as the post-harvest conditions before sterilization process of fruits are crucial factors to be considered (Ramli et al., 2011).

The impact of deodorization conditions (180 or 230 ºC/up to 5 h) on the occurrence of 3-MCPD and GEs were assessed in bleached PO, neutralized/bleached PO and rapeseed oil samples obtained in refineries from Europe (Hrncirik & van Duijn, 2011). For the same period of time, the 3-MCPD esters in neutralized/bleached PO was higher when lower temperatures (180 ºC) were applied. Regarding the bleached PO, a temperature and time dependence was observed for 3-MCPD, with higher values (4.0 mg/kg) achieved when deodorization was carried out for 5 h at 230 ºC (Hrncirik & van Duijn, 2011). In fact, these results are much higher than the 3-MCPD esters content (2.7 mg/kg) reported by Weißhaar et al. (2010) when PO was deodorized for 6 h at 230 ºC. Zulkurnain et al. (2012) have also evaluated the effect of different deodorization temperatures (180 to 270 ºC) on the occurrence of 3-MCPD in four crude POs with different “grades” of quality. The formation of 3-MCPD significantly increased as the deodorization temperature increased, reaching the maximum value (9 mg/kg) at 250 ºC (Zulkurnain et al., 2012). At 270 ºC a slight decrease in 3-MCPD amounts was observed probably due to the degradation of 3-MCPD in other compounds, as it was previously demonstrated (Matthäus et al., 2011).

The effect of deodorization with different concentrations of formic acid and with strip steam obtained from water were evaluated in crude PO (240 ºC, 2 h) (Matthäus et al., 2011). GEs content remained stable (0.5 mg/kg), while 3-MCPD esters significantly decreased from 2.9 mg/kg (crude PO deodorized with water) to 2.1 (crude PO deodorized with 10% formic acid), suggesting that GEs are more stable in the oil matrix (Matthäus et al., 2011).

The formation of 3-MCPD esters in four different “grades” of crude PO (premium quality, superior quality, standard quality I and standard quality II) was studied to evaluate whether refining, deodorising and bleaching are linked with the formation of 3-MCPD (Zulkurnain et

al., 2012). In crude POs, for standard quality II, trace amounts of 3-MCPD were detected. With respect to refined (degumming at 70 ºC, 20 min with 0.05% phosphoric acid; bleaching at 100 ºC, 30 min, with 1% activated bleaching clay; and deodorization at 260 ºC, 90 min) POs, the amount of 3-MCPD in PO standard quality II was five times higher than for crude POs (Zulkurnain et al., 2012).

Frying conditions

Refined, bleached and deodorized PO samples from Malaysia were evaluated regarding their 3-MCPD esters content during frying (160 and 180 ºC) of French fries, for a total of 5 frying cycles per day, during 5 days, being the oil used continuously without replenishment (Wong et al., 2017). In addition, authors have performed frying assays with three concentrations (1%, 3% and 5%) of sodium chloride. In general, the 3-MCPD esters content decreased when the number of frying cycles increased, suggesting that 3-MCPD can be oxidized during prolonged heating (Wong et al., 2017). With respect to the temperature, the 3-MCPD content in oils subjected to 180 ºC was higher than for 160 ºC, indicating that higher temperatures led to increased amounts of 3-MCPD esters. Also, frying oils with 3% of sodium chloride have higher 3-MCPD esters content than those without sodium chloride or with low concentrations (Wong et al., 2017).

Hammouda et al. (2017) have evaluated the effect of repeated frying cycles on 3-MCPD occurrence in blends of refined olive pomace oil and refined PO in different proportions. After 16 h of deep-frying no endogenous formation of 3-MCPD esters was observed, being the values higher for the fresh PO (1.3 mg/kg) than for the heated blend (0.70 mg/kg) (Hammouda, Zribi, Mansour, Matthäus, & Bouaziz, 2017). Authors justify this result with the fact that when the frying cycles increased, 3-MCPD esters are possibly decomposed and oxidized.

Glycidyl fatty acid esters occurrence

Table 31 shows data concerning the occurrence of GEs in PO and PO products. In 2010, different vegetable oils were acquired in Germany, from national and international suppliers regarding the content of GEs (Kuhlmann, 2011). Higher GEs levels in PO (with a mean value 3.7 mg/kg) were determined comparatively to soybean, rapeseed and sunflower oils (0.2 and 0.3 mg/kg). Later, the same research group has collected samples of refined sunflower oils (n=11), refined POs, palm stearin and palm mid fraction (n=6), and refined rapeseed oils (n=5) and assessed their content in GEs. PO samples have higher concentrations (mean value 1.87 mg/kg) than sunflower oils (mean value 0.23 mg/kg) and rapeseed oils (0.31 mg/kg) (Kuhlmann, 2016).

Extra virgin olive oil, refined PO, refined palm/rapeseed/corn oil, refined rice oil and refined rice/rapeseed oil were analysed regarding their content in GEs (Shiro et al., 2011). The most abundant GEs in refined PO was glycidyl oleate (12.7 mg/kg) followed by glycidyl palmitate (8.6 mg/kg). In comparison with the other analysed oils, the amounts of GEs decreased in the following order: refined rice oil (27.8 mg/kg) > refined PO (26.3 mg/kg) > refined rice/rapeseed oil (15.6 mg/kg) > refined palm/rapeseed/corn oil (8.92 mg/kg) (Shiro et al., 2011).

Hori et al. (2012) evaluated five GEs in soybean oil, rapeseed oil, corn oil, crude PO and three samples of refined PO. Crude PO was the unique sample where GEs were not detected, while the highest amounts were observed in corn oil (2.09 mg/kg (Hori et al., 2012b). In the analysed refined POs, the values varied between 1.2 and 1.84 mg/kg, much lower than the values previously reported by Shiro et al., 2011.

Craft et al. (2012) analysed 15 samples of refined PO and palm olein blend that were commercially available and were produced by a commercial laboratory from Germany. The GEs content showed remarkable variations (0.77 to 17.3 mg/kg) according to sample origin (Craft & Nagy, 2012). Retail and/or industrial edible oils and fats (n=116) were analysed regarding the same topic, namely glycidyl laureate, glycidyl myristate, glycidyl palmitate, glycidyl oleate, glycidyl linoleate, glycidyl linolenate and glycidyl stearate (MacMahon et al., 2013a). The total GEs in PO fractions decreased in the following order: palm olein (mean = 6.03 mg/kg) > palm stearin (mean = 3.58 mg/kg) > PO (mean = 3.50 mg/kg) > PKO (mean = 0.52 mg/kg) > crude PO (<LOQ) (MacMahon et al., 2013a). In comparison with the other edible oils and fats, analysed palm olein had a much higher content than the others.

Seven GEs were evaluated in refined PO, refined soybean oil and olive oil (Steenbergen et al., 2013). Refined PO presented a much higher total of GEs content (30.2 mg/kg) than the other oils (1.56 and 4.31 mg/kg for soybean and olive, respectively). In addition, the major GEs were different according to the sample in evaluation. For instance, the major GEs quantified in refined PO were glycidyl palmitate (9.82 mg/kg) and glycidyl linoleate (4.56 mg/kg), while for refined soybean oil it was glycidyl linoleate (0.96 mg/kg) and glycidyl oleate (0.27 mg/kg), and for olive oil glycidyl oleate (3.14 mg/kg) and glycidyl linoleate (0.75 mg/kg), taking into account their fatty acids composition (Steenbergen et al., 2013).

In 2012, twenty samples of fresh vegetable oils (rapeseed oil n=12, sunflower oil n=4 and PO n=4) were purchased from Polish markets and were screened regarding their GEs composition (Aniołowska & Kita, 2016a). GEs content in PO samples varied between 5.82 and 44.3 mg/kg, being glycidyl oleate the most abundant compound. In comparison with the

other vegetable oils analysed, it was possible to confirm that the uppermost levels of these compounds were found in PO samples (Aniołowska & Kita, 2016a).

Different stages/conditions of oils processing

Different temperatures (210 to 250 ºC), as well as different periods of time (3 h or 6 h) were used to evaluate the GEs formed in PO samples during deodorization (Weißhaar & Perz, 2010). The results indicate a temperature dependence on the occurrence of GEs, with higher temperatures (250 ºC) for the same period of time (6 h) leading to higher amounts of GEs (2.9 mg/kg). On the other hand, the GEs content of PO deodorized at 250 ºC was higher for samples subjected to 3 h (4.4 mg/kg) of deodorization than for 6 h (2.9 mg/kg), probably due to the formation of other compounds (Weißhaar & Perz, 2010).

Hrncirik et al. (2011) also evaluated the effect of deodorization conditions (180 or 230 ºC) on the occurrence of GEs in bleached PO, neutralized/bleached PO and rapeseed oil samples. The GEs content significantly increased when higher temperatures were applied (230 ºC) (Hrncirik & van Duijn, 2011). Regarding the bleached PO, a temperature and time dependence was observed for GEs occurrence, with higher values (2.1 mg/kg) obtained after 5 h at 230 ºC (Hrncirik & van Duijn, 2011).

The formation mechanism of GEs was investigated in soybean oil, camellia oil and PO during laboratory-scale preparation and refining (Cheng et al., 2016). Regarding the extraction conditions applied, three different methods were selected to perform comparisons, namely hot-pressed extraction, cold pressed extraction and solvent extraction. The GEs content in PO was higher after deodorization (200 ºC, 1 h) for all the types of extraction, varying between 3.02 and 3.82 mg/kg for solvent extraction and hot-pressed extraction, respectively (Cheng et al., 2016). For solvent extraction experiments, deodorization was conducted at different temperatures (160, 180, 200, 220, 240 and 260 ºC) for 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 h (Cheng et al., 2016). A temperature dependence on the formation of GEs was reported, taking into account that after 1 h, the GEs levels in PO increased from around 2 mg/kg (160 ºC) to 3.38 mg/kg. Furthermore, for temperatures higher than 180 ºC, it was observed that the GEs formation after 1 h followed a gradual decrease, while for temperatures between 160 and 180 ºC, GEs levels in PO continuously increased with the heating time, reaching the maximum values after 2.5 h (Cheng et al., 2016).

Table 31. Chloropropanols and glycidyl fatty acid esters in palm oil products.

Origin/local of acquisition	Samples	Compound	Occurrence (mg/kg)	Reference
Czech Republic; retail outlets	Refined palm kernel oils	Bound 3-MCPD	1.166 - 2.821	(Karšulínová et al., 2007)
-	Fat mix (palm olein, 46%)	Total 3-MCPD esters	0.897 ± 0.043 - 2.036 ± 0.108	(Seefelder et al., 2008)
		3-MCPD monoesters	0.061 ± 0.014 - 0.21 ± 0.014	
	Fat mix 10 (palm olein, 55%)	Total 3-MCPD esters	2.218 ± 0.078 - 2.435 ± 0.157	
		3-MCPD monoesters	0.182 ± 0.035 - 0.299 ± 0.148	
Germany; oil producers	Crude palm oil (before refining)	Free 3-MCPD	0.98 ± 0.09	(Franke et al., 2009)
	Crude palm oil (after hydration)	Free 3-MCPD	0.93 ± 0.09	
	Crude palm oil (after degumming)	Free 3-MCPD	0.88 ± 0.13	
	Crude palm oil (after neutralization)	Free 3-MCPD	0.97 ± 0.10	
	Crude palm oil (after washing)	Free 3-MCPD	0.84 ± 0.04	
	Crude palm oil (after drying)	Free 3-MCPD	1.04 ± 0.03	
	Crude palm oil (after bleaching)	Free 3-MCPD	1.00 ± 0.07	
	Crude palm oil (after deodorization)	Free 3-MCPD	4.39 ± 0.13	
	Pre-refined palm oil (before refining)	Free 3-MCPD	5.15 ± 0.16	
	Pre-refined palm oil (after hydration)	Free 3-MCPD	5.46 ± 0.12	
	Pre-refined palm oil (after degumming)	Free 3-MCPD	4.89 ± 0.16	
	Pre-refined palm oil (after neutralization)	Free 3-MCPD	5.80 ± 0.04	
	Pre-refined palm oil (after washing)	Free 3-MCPD	5.37 ± 0.13	
	Pre-refined palm oil (after drying)	Free 3-MCPD	6.06 ± 0.04	
	Pre-refined palm oil (after bleaching)	Free 3-MCPD	2.48 ± 0.06	
	Pre-refined palm oil (after deodorization)	Free 3-MCPD	4.56 ± 0.10	
Germany; local retailers	Refined palm oil	Total 3-MCPD esters	2.16 - 15.2	(Weißhaar & Perz, 2010)
		Total glycidyl esters	0.32 - 6.3	
	Palm kernel oil	3-MCPD esters	1.7	
		Glycidyl esters	0.5	
	Deodorized palm oil (210 ºC, 6 h)	3-MCPD esters	2.8	
		Glycidyl esters	0.3	
	Deodorized palm oil (230 ºC, 6 h)	3-MCPD esters	2.7	
		Glycidyl esters	0.8	
	Deodorized palm oil (250 ºC, 3 h)	3-MCPD esters	3.3	
		Glycidyl esters	4.4	
	Deodorized palm oil (250 ºC, 6 h)	3-MCPD esters	3.3	
		Glycidyl esters	2.9	
-	Palm shortening (A)	Total 3-MCPD monoesters	nd	(Haines et al., 2011)
		Total 3-MCPD diesters	4.0	
		Total glycidyl esters	3.4	

	Palm shortening (B)	Total 3-MCPD monoesters	nd	
		Total 3-MCPD diesters	5.8	
		Total glycidyl esters	15.5	
	Palm shortening (C)	Total 3-MCPD monoesters	nd	
		Total 3-MCPD diesters	6.2	
		Total glycidyl esters	0.5	
	Palm canola blend	Total 3-MCPD monoesters	nd	
		Total 3-MCPD diesters	3.7	
		Total glycidyl esters	0.4	
	Palm shortening blend	Total 3-MCPD monoesters	nd	
		Total 3-MCPD diesters	nd	
		Total glycidyl esters	1.5	
	Palm olein	Total 3-MCPD monoesters	nd	
		Total 3-MCPD diesters	nd	
		Total glycidyl esters	15.6	
Europe; refineries	Bleached palm oil	3-MCPD esters	1.7	(Hrncirik & van Duijn, 2011)
		Glycidyl esters	nd	
	Neutralized/bleached palm oil	3-MCPD esters	1.5	
		Glycidyl esters	nd	
Germany; local markets from national and international suppliers	Palm kernel oil	Bound glycidyl	0.3 - 2.5	(Kuhlmann, 2011)
		3-MCPD	0.2 - 0.9	
		2-MCPD (estimated)	<0.1 - 0.5	
	Degummed and bleached palm oil	Bound glycidyl	<0.1	
		3-MCPD	1.0	
		2-MCPD (estimated)	<0.1	
	Palm oil	Bound glycidyl	0.3 - 18	
		3-MCPD	1.1 - 10	
		2-MCPD (estimated)	0.2 - 5.9	
Malaysia; local mill	Refined palm oil (degumming, standard, acid activated)	3-MCPD esters	2.82 ± 0.57	(Ramli et al., 2011)
	Refined palm oil (degumming, standard, natural)	3-MCPD esters	2.21 ± 0.37	
	Refined palm oil (degumming, water, acid activated)	3-MCPD esters	0.91 ± 0.51	
	Refined palm oil (degumming, water, natural)	3-MCPD esters	0.49 ± 0.22	
-	Refined palm oil	Glycidyl linolenate	<0.082	(Shiro et al., 2011)
		Glycidyl linoleate	3.9 ± 0.081	
		Glycidyl palmitate	8.6 ± 0.14	
		Glycidyl oleate	12.7 ± 0.26	
		Glycidyl stearate	1.0 ± 0.026	
Germany; commercial stores	Refined palm oil and palm olein	Glycidyl myristate	nd - 0.81	(Craft & Nagy, 2012)
		Glycidyl palmitate	nd - 5.31	
		Glycidyl stearate	nd - 1.65	
		Glycidyl oleate	0.14 – 9.4	
		Glycidyl linoleate	0.04 - 2.61	
		Glycidyl linolenate	nd – 0.09	
		Total Glycidyl esters	0.2 – 17.3	
Japan; industry company	Crude palm oil	Glycidyl linolenate	nd	(Hori et al., 2012a; Hori et al., 2012b)
		Glycidyl linoleate	nd	
		Glycidyl palmitate	nd	
		Glycidyl oleate	nd	
		Glycidyl stearate	nd	
		Rac 1-linoleoyl-3-MCPD	nd	
		Rac 1-palmitoyl-3-MCPD	nd	
		Rac 1-oleoyl-3-MCPD	nd	
		Rac 1,2-bis-linoleoyl-3-MCPD	nd	

		Rac 1-palmitoyl-2-linoleoyl-3-MCPD	nd	
		Rac 1-oleoyl-2-linoleoyl-3-MCPD	nd	
		Rac 1,2-bis-palmitoyl-3-MCPD	nd	
		Rac 1,2-bis-palmitoyl-2-oleoyl-3-MCPD	nd	
		Rac 1,2-bis-oleoyl-3-MCPD	nd	
	Refined palm oil	Glycidyl linolenate	nd	
		Glycidyl linoleate	0.20 ± 0.03 - 0.38 ± 0.08	
		Glycidyl palmitate	0.13 ± 0.01 - 0.21 ± 0.02	
		Glycidyl oleate	0.80 ± 0.09 - 1.26 ± 0.17	
		Glycidyl stearate	-	
		Rac 1-linoleoyl-3-MCPD	nd	
		Rac 1-palmitoyl-3-MCPD	nd - 0.06 ± 0.00	
		Rac 1-oleoyl-3-MCPD	0.35 ± 0.07 - 1.19 ± 0.11	
		Rac 1,2-bis-linoleoyl-3-MCPD	nd	
		Rac 1-palmitoyl-2-linoleoyl-3-MCPD	1.85 ± 0.31 - 7.46 ± 0.51	
		Rac 1-oleoyl-2-linoleoyl-3-MCPD	nd - 2.17 ± 0.08	
		Rac 1,2-bis-palmitoyl-3-MCPD	1.01 ± 0.14 - 8.15 ± 0.23	
		Rac 1,2-bispalmitoyl-2-oleoyl-3-MCPD	6.19 ± 1.13 - 13.59 ± 1.82	
		Rac 1,2-bis-oleoyl-3-MCPD	nd	
Malaysia; oils refineries	Crude palm oil	3-MCPD	nd - 0.9	(Razak et al., 2012)
	Bleached palm oil	3-MCPD	nd - 1.8	
	Fully refined palm oil	3-MCPD	nd - 5.8	
	Fully refined oleins	3-MCPD	nd - 4.2	
	Fully refined stearins	3-MCPD	0.3 - 1.8	
Malaysia; supermarkets	Palm olein	3-MCPD	2.0 - 2.25	
Malaysia; Industry	Crude palm oil (premium quality)	3-MCPD esters	<0.006	(Zulkurnain et al., 2012)
	Crude palm oil (superior quality)	3-MCPD esters	<0.006	
	Crude palm oil (standard quality I)	3-MCPD esters	<0.006	
	Crude palm oil (standard quality II)	3-MCPD esters	0.061 ± 0.01	
	Refined bleached and deodorized palm oil (premium quality)	3-MCPD esters	1.54 ± 0.08	
	Refined bleached and deodorized palm oil (superior quality)	3-MCPD esters	1.49 ± 0.02	
	Refined bleached and deodorized palm oil (standard quality I)	3-MCPD esters	1.72 ± 0.22	
	Refined bleached and deodorized palm oil (standard quality II)	3-MCPD esters	5.93 ± 0.41	
-	Refined, bleached and deodorized palm oil	Glycidyl palmitate	2.34 - 2.39	(Blumhorst et al., 2013)
		Glycidyl stearate	0.48 - 0.50	
		Glycidyl oleate	5.11 - 5.37	
		Glycidyl linoleate	1.33 - 1.39	
		Total Glycidyl esters	9.3 – 30.9	

United States; food manufacturers, online retailers, domestic distributors	Crude palm oil	Total 3-MCPD monoesters	<LOQ	(MacMahon et al., 2013a)
		Total 3-MCPD diesters	<LOQ	
		Total glycidyl esters	<LOQ	
	Palm oil	Total 3-MCPD monoesters	1.51-7.23	
		Total 3-MCPD diesters	0.86 - 6.17	
		Total glycidyl esters	0.33 - 10.52	
	Palm kernel oil	Total 3-MCPD monoesters	0.038 - 0.20	
		Total 3-MCPD diesters	0.038 - 0.20	
		Total glycidyl esters	0.38 - 0.65	
	Palm olein	Total 3-MCPD monoesters	1.40 - 8.43	
		Total 3-MCPD diesters	0.68 - 3.72	
		Total glycidyl esters	1.88 - 9.53	
	Palm stearin	Total 3-MCPD monoesters	3.24	
		Total 3-MCPD diesters	2.49	
		Total glycidyl esters	3.58	
-	Refined palm oil	Glycidyl laureate	0.04	(Steenbergen et al., 2013)
		Glycidyl myristate	0.17	
		Glycidyl palmitate	9.82	
		Glycidyl stearate	2.53	
		Glycidyl oleate	12.7	
		Glycidyl linoleate	4.56	
		Glycidyl linolenate	0.33	
		Total glycidyl esters	30.2	
Japan; local supermarkets	Palm oil	1-Linolenoyl-3-MCPD	<0.05	(Yamazaki et al., 2013)
		1-Linoleoyl-3-MCPD	0.23	
		1-Palmitoyl-3-MCPD	0.44	
		1-Oleoyl-3-MCPD	0.69	
		1-Stearoyl-3-MCPD	0.1	
		Total 3-MCPD monoesters	1.46	
		1,2-Dilinolenoyl-3-MCPD	<0.05	
		1,2-Dilinoleoyl-3-MCPD	0.2	
		1,2-Dipalmitoyl-3-MCPD	2.4	
		1,2-Dioleoyl-3-MCPD	2.3	
		1,2-Distearoyl-3-MCPD	<0.05	
		Total homo-diesters	4.9	
		1-Linolenoyl-2-linoleoyl-3-MCPD	<0.1	
		1-Oleoyl-2-linolenoyl-3-MCPD	<0.05	
		1-Oleoyl-2-linoleoyl-3-MCPD	0.53	
		1-Palmitoyl-2-linoleyl-3-MCPD	0.74	
		1-palmitoyl-2-linolenoyl-3-MCPD	<0.05	
		1-Oleoyl-2-palmitoyl-3-MCPD	5.6	
		1-Linolenoyl-2-stearoyl-3-MCPD	<0.05	
		1-Stearoyl-2-palmitoyl-3-MCPD	0.28	
		1-Stearoyl-2-linoleoyl-3-MCPD	0.19	
		1-Stearoyl-2-oleoyl-3-MCPD	0.7	
		Total hetero-diesters	8.04	
		Total 3-MCPD esters	14.4	
		Free 3-MCPD	2.714	
Brazil; supermarkets and oil manufacturers	Palm oil (supplier A, 6 lots)	3-MCPD free	nd - 1.05 ± 0.03	(A. P. Arisseto et al., 2014)
	Palm oil (supplier B, 6 lots)	3-MCPD free	nd - 2.95 ± 0.25	
	Palm kernel oil (6 lots)	3-MCPD free	nd - 0.17 ± 0.05	

	Palm fat for general use (6 lots)	3-MCPD free	nd - 3.31 ± 0.03	
	Palm fat for frying (6 lots)	3-MCPD free	nd -2.56 ± 0.03	
	Palm fat for pastry (6 lots)	3-MCPD free	nd - 2.60 ± 0.02	
	Palm fat for ice cream (lot 1)	3-MCPD free	nd - 2.45 ± 0.08	
	Palm oil for culinary preparations (supplier A, 2 lots)	3-MCPD free	0.25 ± 0.01 - 0.32 ± 0.02	
	Palm oil for culinary preparations (supplier B, 2 lots)	3-MCPD free	0.30 ± 0.05 - 0.33 ± 0.02	
	Palm oil for culinary preparations (supplier C, 2 lots)	3-MCPD free	nd	
China; supermarket	Palm oil (A)	3-MCPD monoesters	nd	(Zhou et al., 2014)
		3-MCPD diesteres	336 ± 3.6	
	Palm oil (B)	3-MCPD monoesters	nd	
		3-MCPD diesteres	540 ± 1.9	
-	Palm oil shortening	Glycidol equivalent	10.6	(Becalski et al., 2015)
		MCPDs equivalent	12.5	
		2-MCPD	4.03	
		3-MCPD	8.42	
	Palm oil unrefined	Glycidol equivalent	nd	
		MCPDs equivalent	nd - 0.558	
		2-MCPD	nd	
		3-MCPD	<0.1 - 0.558	
Poland; local producer	Palm oil fresh	Glycidyl palmitate	15.12 ± 0.26	(Aniołowska & Kita, 2015)
		Glycidyl stearate	1.23 ± 0.05	
		Glycidyl oleate	18.57 ± 0.40	
		Glycidyl linoleate	4.06 ± 0.08	
	Palm oil (24 h, 150 ºC)	Glycidyl palmitate	10.29 ± 0.03	
		Glycidyl stearate	1.18 ± 0.01	
		Glycidyl oleate	12.59 ± 0.03	
		Glycidyl linoleate	3.04 ± 0.01	
	Palm oil (40 h, 150 ºC)	Glycidyl palmitate	9.28 ± 0.04	
		Glycidyl stearate	0.43 ± 0.00	
		Glycidyl oleate	10.54 ± 0.04	
		Glycidyl linoleate	2.56 ± 0.03	
	Palm oil (24 h, 165 ºC)	Glycidyl palmitate	5.26 ± 0.01	
		Glycidyl stearate	0.37 ± 0.00	
		Glycidyl oleate	6.38 ± 0.03	
		Glycidyl linoleate	1.62 ± 0.00	
	Palm oil (40 h, 165 ºC)	Glycidyl palmitate	2.15 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	2.17 ± 0.01	
		Glycidyl linoleate	0.72 ± 0.00	
China; markets	Refined palm oil	3-MCPD esters	1.518	(Li et al., 2015a)
China; markets	Crude palm oil	Total monoesters	nd	(Li et al., 2015b)
		Total diesters	nd	
		3-MCPD esters direct method	nd	
		3-MCPD esters indirect method	nd	
	Refined palm oil	Total 3-MCPD monoesters	0.017	
		Total 3-MCPD diesters	0.389	
		3-MCPD esters direct method	0.423	
		3-MCPD esters indirect method	0.569	
Poland; retail outlet	Palm oil fresh	Glycidyl palmitate	2.19 ± 0.02 - 17.58 ± 0.86	(Aniołowska & Kita, 2016a)
		Glycidyl stearate	nd - 1.76 ± 0.02	

		Glycidyl oleate	2.92 ± 0.08 - 19.99 ± 0.84	
		Glycidyl linoleate	0.71 ± 0.01 - 5.00 ± 0.12	
Poland; local potato snack producer	Fresh refined, bleached and deodorized palm oil	Glycidyl palmitate	14.83 ± 0.03	(Aniołowska & Kita, 2016b)
		Glycidyl stearate	1.00 ± 0.01	
		Glycidyl oleate	16.84 ± 0.07	
		Glycidyl linoleate	3.26 ± 0.10	
	Palm oil after frying potato chips (8 h, 180 ± 5 ºC)	Glycidyl palmitate	11.77 ± 0.01	
		Glycidyl stearate	0.80 ± 0.01	
		Glycidyl oleate	12.52 ± 0.01	
		Glycidyl linoleate	2.53 ± 0.05	
	Palm oil after frying potato chips (24 h, 180 ± 5 ºC)	Glycidyl palmitate	5.85 ± 0.11	
		Glycidyl stearate	0.41 ± 0.00	
		Glycidyl oleate	6.38 ± 0.01	
		Glycidyl linoleate	1.53 ± 0.06	
	Palm oil after frying potato chips (40 h, 180 ± 5 ºC)	Glycidyl palmitate	2.02 ± 0.01	
		Glycidyl stearate	nd	
		Glycidyl oleate	2.05 ± 0.01	
		Glycidyl linoleate	0.64 ± 0.01	
	Palm oil after frying French fries (8 h, 180 ± 5 ºC)	Glycidyl palmitate	10.88 ± 0.10	
		Glycidyl stearate	0.68 ± 0.00	
		Glycidyl oleate	10.99 ± 0.01	
		Glycidyl linoleate	2.11 ± 0.01	
	Palm oil after frying French fries (24 h, 180 ± 5 ºC)	Glycidyl palmitate	4.22 ± 0.01	
		Glycidyl stearate	0.29 ± 0.01	
		Glycidyl oleate	4.94 ± 0.07	
		Glycidyl linoleate	1.13 ± 0.01	
	Palm oil after frying French fries (40 h, 180 ± 5 ºC)	Glycidyl palmitate	0.95 ± 0.01	
		Glycidyl stearate	nd	
		Glycidyl oleate	1.08 ± 0.01	
		Glycidyl linoleate	0.49 ± 0.01	
	Palm oil after frying potato pellets (8 h, 180 ± 5 ºC)	Glycidyl palmitate	8.65 ± 0.01	
		Glycidyl stearate	0.58 ± 0.01	
		Glycidyl oleate	8.66 ± 0.01	
		Glycidyl linoleate	1.75 ± 0.01	
	Palm oil after frying potato pellets (24 h, 180 ± 5 ºC)	Glycidyl palmitate	3.03 ± 0.01	
		Glycidyl stearate	0.18 ± 0.01	
		Glycidyl oleate	3.04 ± 0.01	
		Glycidyl linoleate	0.96 ± 0.01	
	Palm oil after frying potato pellets (40 h, 180 ± 5 ºC)	Glycidyl palmitate	0.83 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.94 ± 0.01	
		Glycidyl linoleate	0.19 ± 0.00	
Poland; local producer	Palm oil fresh	Glycidyl palmitate	2.19 ± 0.09	(Aniołowska & Kita, 2016c)
		Glycidyl stearate	nd	
		Glycidyl oleate	2.92 ± 0.07	
		Glycidyl linoleate	0.70 ± 0.02	
	Palm oil (24 h, 180 ± 5 ºC)	Glycidyl palmitate	0.93 ± 0.03	
		Glycidyl stearate	nd	
		Glycidyl oleate	1.87 ± 0.10	
		Glycidyl linoleate	0.47 ± 0.08	
	Palm oil (40 h, 180 ± 5 ºC)	Glycidyl palmitate	0.42 ± 0.02	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.67 ± 0.02	
		Glycidyl linoleate	0.18 ± 0.02	
	Palm olein fresh	Glycidyl palmitate	9.78 ± 0.03	
		Glycidyl stearate	0.62 ± 0.02	
		Glycidyl oleate	11.61 ± 0.00	
		Glycidyl linoleate	3.33 ± 0.08	
	Palm olein (24 h, 180 ± 5 ºC)	Glycidyl palmitate	5.95 ± 0.09	
		Glycidyl stearate	0.52 ± 0.04	
		Glycidyl oleate	7.61 ± 0.25	
		Glycidyl linoleate	1.66 ± 0.15	

	Palm olein (40 h, 180 ± 5 ºC)	Glycidyl palmitate	2.26 ± 0.05	
		Glycidyl stearate	nd	
		Glycidyl oleate	2.97 ± 0.05	
		Glycidyl linoleate	0.79 ± 0.09	
	Palm olein with high oleic sunflower oil and rapeseed oil fresh	Glycidyl palmitate	7.01 ± 0.02	
		Glycidyl stearate	0.61 ± 0.02	
		Glycidyl oleate	10.05 ± 0.10	
		Glycidyl linoleate	2.78 ± 0.22	
	Palm olein with high oleic sunflower oil and rapeseed oil (24 h, 180 ± 5 ºC)	Glycidyl palmitate	4.83 ± 0.19	
		Glycidyl stearate	nd	
		Glycidyl oleate	6.29 ± 0.12	
		Glycidyl linoleate	1.48 ± 0.12	
	Palm olein with high oleic sunflower oil and rapeseed oil (40 h, 180 ± 5 ºC)	Glycidyl palmitate	2.42 ± 0.04	
		Glycidyl stearate	nd	
		Glycidyl oleate	3.53 ± 0.13	
		Glycidyl linoleate	0.87 ± 0.14	
	Palm oil extracted from French fries (24 h, 180 ± 5 ºC)	Glycidyl palmitate	0.76 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.74 ± 0.00	
		Glycidyl linoleate	0.15 ± 0.00	
	Palm oil extracted from French fries (40 h, 180 ± 5 ºC)	Glycidyl palmitate	0.34 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.30 ± 0.01	
		Glycidyl linoleate	nd	
	Palm olein extracted from French fries (24h, 180 ± 5 ºC)	Glycidyl palmitate	1.63 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	1.43 ± 0.01	
		Glycidyl linoleate	0.28 ± 0.00	
	Palm olein extracted from French fries (40h, 180 ± 5 ºC)	Glycidyl palmitate	0.44 ± 0.01	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.42 ± 0.01	
		Glycidyl linoleate	0.14 ± 0.00	
	Palm olein with high oleic sunflower oil and rapeseed oil extracted from French fries (24 h, 180 ± 5 ºC)	Glycidyl palmitate	1.43 ± 0.01	
		Glycidyl stearate	nd	
		Glycidyl oleate	1.31 ± 0.00	
		Glycidyl linoleate	0.25 ± 0.00	
	Palm olein with high oleic sunflower oil and rapeseed oil extracted from French fries (40 h, 180 ± 5 ºC)	Glycidyl palmitate	0.34 ± 0.00	
		Glycidyl stearate	nd	
		Glycidyl oleate	0.66 ± 0.01	
		Glycidyl linoleate	0.19 ± 0.00	
China; local market	Crude palm oil (hot pressed extraction)	Glycidyl esters	1.58 ± 0.04	(Cheng et al., 2016)
	Crude palm oil (cold pressed extraction)	Glycidyl esters	0.212 ± 0.02	
	Crude palm oil (solvent extraction)	Glycidyl esters	nd	
	Degummed palm oil (hot pressed extraction)	Glycidyl esters	1.59 ± 0.04	
	Degummed palm oil (cold pressed extraction)	Glycidyl esters	0.221 ± 0.02	
	Degummed palm oil (solvent extraction)	Glycidyl esters	nd	
	Neutralized palm oil (hot pressed extraction)	Glycidyl esters	1.57 ± 0.06	
	Neutralized palm oil (cold pressed extraction)	Glycidyl esters	0.230 ± 0.01	

	Neutralized palm oil (solvent extraction)	Glycidyl esters	nd	
	Bleached palm oil (hot pressed extraction)	Glycidyl esters	1.61 ± 0.04	
	Bleached palm oil (cold pressed extraction)	Glycidyl esters	0.226 ± 0.03	
	Bleached palm oil (solvent extraction)	Glycidyl esters	nd	
	Deodorized palm oil (hot pressed extraction)	Glycidyl esters	3.82 ± 0.08	
	Deodorized palm oil (cold pressed extraction)	Glycidyl esters	3.22 ± 0.06	
	Deodorized palm oil (solvent extraction)	Glycidyl esters	3.02 ± 0.08	
Germany; local markets from national and international suppliers	Refined palm oils, palm stearin, palm mid fraction	1,3-DCP 2,3-DCP 2-MCPD 3-MCPD Glycidyl esters	nd nd 0.8 - 1.65 0.18 - 2.48 0.10 - 3.55	(Kuhlmann, 2016)
Japan; local stores	Palm, semi-solid	3-MCPD 2-MCPD Glycidyl esters	2.09 - 2.62 1.21 - 1.33 0.26 - 3.53	(Koyama et al., 2016)
	Palm, solid	3-MCPD 2-MCPD Glycidyl esters	nd - 5.15 nd - 4.07 nd - 1.67	
China; markets	Refined palm oil (fresh)	3-MCPD esters	1.94 ± 0.04	(Li et al., 2016)
	Refined palm oil (heated at 160 ºC for 1 h)	3-MCPD esters	2.36 ± 0.21	
Tunisia; National Oil Office	Fresh palm oil	3-MCPD esters Glycidyl esters	1.30 ± 0.01 4.00 ± 0.01	(Hammouda et al., 2017)
Malaysia; industry	Refined, bleached and deodorized palm oil	3-MCPD esters	3.02 ± 0.08	(Wong et al., 2017)
Europe; industry	Palm oil	3-MCPD	2.88 ± 0.04	(Zelinkova et al., 2017)
		2-MCPD Glycidyl esters	1.66 ± 0.01 0.73 ± 0.05	
	Heated palm oil	3-MCPD 2-MCPD Glycidyl esters	2.47 ± 0.06 1.54 ± 0.05 2.29 ± 0.09	

nd, not detected

Frying conditions

Aniołowska et al. (2015) evaluated the effect of frying (with and without French fries) on GEs content using PO, palm olein and a blend constituted by palm olein, high oleic sunflower oil and rapeseed oil (Aniołowska & Kita, 2015). The highest amounts of GEs were found in fresh palm olein (25.34 mg/kg), followed by the blend (20.45 mg/kg). It is notable that there was a huge difference between the aforementioned oils and PO, for which the sum of GEs for the fresh oil was 5.81 mg/kg, indicating that the fatty acids composition has influence on the GEs occurrence (Aniołowska & Kita, 2015). Concerning the influence of food on the quality of frying media, in this study, it was possible to observe that the GEs content in the oil extracted from French fries fried in PO, palm olein and a blend was around one, five and three-fold lower compared with fresh frying medium. With respect to the fat

extracted from fried French fries, the same trend was observed, since the highest values of GEs were observed when palm olein and the blend were used to fry. On the other hand, a reduction on the GEs values was observed along frying time (Aniołowska & Kita, 2015). The effects of frying foods with different water contents in PO up to 5 days (180 ºC, 8 h/day) on the oil degradation and the content of GEs was evaluated (Aniołowska & Kita, 2016b). It was found that the composition of the raw material had a considerable influence on GEs content of PO. Glycidyl oleate and glycidyl palmitate were the most abundant GEs and the levels in the fresh PO were 16.84 and 14.83 mg/kg, respectively (Aniołowska & Kita, 2016b).

For instance, a significant reduction on GEs content was observed for all the POs that were used for the different foods fried, nonetheless it was greater for potato pellets (snacks) and French fries, than for potato chips. Furthermore, authors have correlated the GEs levels with other quality parameters of frying oils and there were strong correlations with all parameters, but it was higher for diacylglycerols (r = 0.98) and total polar compounds (r = -0.98) than for the other tested parameters (Aniołowska & Kita, 2016b).

The impact of frying temperature (150, 165 and 180 ºC) on GEs occurrence in PO (used to fry potato chips) intermittently heated for 8 h/day over five consecutive days, was studied (Aniołowska & Kita, 2016c). The highest amount of GEs was reported for fresh oil (39 mg/kg), while the lowest was for frying PO that was exposed for 40 h at 180 ºC (Aniołowska & Kita, 2016c). According to their findings, GEs content decreased with increasing temperature and time, and it was strongly correlated with the degree of oil degradation (r = -0.99 for total polar compounds; r = 0.94 for diacylglycerols).

Malaysian refined, bleached and deodorized PO used to fry French fries at different temperatures, during 5 days (5 cycles/day), in the absence or presence of sodium chloride (1%, 2%, 3% and 5%) was assessed regarding the concentration of GEs (Wong et al., 2017). With the increased duration of frying cycles GEs content in frying oil increased. Contrarily to what happens with the effect of the temperature on the occurrence of 3-MCPD, a higher content of GEs was observed when low temperature (160 ºC) was applied (Wong et al., 2017). The frying system with 5% of sodium chloride had also a high content of GEs.

Mitigation strategies

Up to now, few studies have reported mitigation strategies to reduce the contents of chloropropanols and GEs. In general, three main approaches are considered to mitigate these compounds, namely (a) removal of potential precursors; (b) modifications of processing parameters; and (c) degradation or removal of the compounds in the final product (Arisseto, Marcolino, Vicente, & Sampaio, 2013). In 2010, Bornscheuer and Hesseler (2010) described a study on an enzymatic approach to remove 3-MCPD and its

esters from aqueous and biphasic systems by converting it into glycerol. It was demonstrated that the combination of halohydrin dehalogenase and epoxide hydrolase allowed the easy removal of 3-MCPD, resembling a very useful method for application in edible oils (Bornscheuer & Hesseler, 2010). For instance, Mathäus et al. (2011) proposed some strategies to mitigate or reduce the presence of 3-MCPD esters and related compounds in crude PO, such as: (a) washing the crude oil before refining to remove water-soluble chloride; and (b) to treat the crude oils with acid during deodorization. Although, authors mention that these preliminary tests were conducted at a laboratory scale and it is necessary to adapt them to an industrial scale, constituting another challenge (Matthäus et al., 2011). The results reported by Hrncirik et al. (2011) indicate that it is more feasible to decrease the amount of GEs formed during refining than 3-MCPD (Hrncirik & van Duijn, 2011). Also, authors consider that it is crucial to optimize deodorization temperature and duration to decrease the formation of these compounds. Nonetheless, it is important to consider that the applied temperatures and periods of time must be enough to remove other contaminants such as free fatty acids (Hrncirik & van Duijn, 2011).

Also, Craft et al. (2012) have evaluated different approaches to mitigate 3-MCPD diesters during PO production, such as: (a) application of glycerol and ethanol as refining aids during the deodorization step; (b) washing crude PO with ethanol/water (1:1, v/v) prior to deodorization; and (c) washing palm fruit before oil extraction (Craft et al., 2012). The most efficient strategy was washing palm fruit before oil extraction, resulting in a 95% reduction. The other methodologies were less effective, 25 – 35% reduction for application of glycerol and ethanol as refining aids during the deodorization step, and 30% for washing crude PO with ethanol/water (1:1, v/v) prior to deodorization (Craft et al., 2012).

Recently, Zhang et al. (2016) evaluated the possible mechanism of free radical scavengers on mitigation of 3-MCPD in vegetable oils. Therefore, authors have selected four antioxidants (6%, w/w), namely L-ascorbyl palmitate, α-tocopherol, lipophilic tea polyphenols and rosemary extract, to evaluate the inhibition efficiency during high deodorization temperatures (240 ºC, 60 min) (Zhang et al., 2016). The control sample of PO presented the highest content of 3-MCPD esters (2.44 mg/kg). The rosemary extract was the most efficient, achieving a reduction of 82.4% of 3-MCPD esters in PO, followed by lipophilic tea polyphenols. On the other hand, the less efficient were α-tocopherol and L-ascorbyl palmitate (Zhang et al., 2016).

Final remarks

Although chloropropanols and GEs occurrence is multifactorial, it is already possible to perform some considerations on the conditions that might prevent the formation of these undesired compounds, namely:

Removal of critical reactants from the raw material, for example washing palm fruits before oil extraction. This procedure appears to significantly reduce the presence of some precursors of chloropropanols and GEs.

Change of processing conditions for oil production, namely by controlling the deodorization temperature and keep it bellow 200 ºC. Nonetheless, processing of oils under controlled conditions is crucial to decrease the presence of other undesired compounds and assure the quality and safety of palm oil products.

Frying temperature seems to have a minor impact in GEs formation, while the main factor is the duration of frying cycles. In what concerns 3-MCPD, the levels decrease during frying, probably because it is decomposed and oxidized during prolonged heating.

Future research needs

PO and their related products are widely used by food industry in frying or as an ingredient in the formulation of other foodstuffs. Recently, concerns linked to the presence of chloropropanols and GEs in PO have emerged, since these hazardous compounds have potential risk toxicity on human health. Therefore, monitoring the presence of these compounds in PO has increased.

In the last decade, scientific and regulatory entities have enhanced the importance of these contaminants by publishing several reports on their occurrence in several foods, as well as on the risks for human health related to the presence of 3-MCPD, 2-MCPD and GEs. Nonetheless, maximum limits for the occurrence of chloropropanols and GEs in vegetable oils have not yet been published, although European Commission is finalising the new EU legislation on this matter.

The results reported herein indicate that a high variability on the chloropropanols and GEs was observed for PO products, depending on the origin, but also due to the applied processing conditions, namely deodorisation. Furthermore, it is possible to predict that these contaminants are now widely distributed in the food chain because some POs have considerable high contents, and up to now, to the best of our knowledge, it is not mandatory to evaluate the presence of chloropropanols and GEs in vegetable oils. Another gap, is the lack of studies on the impact of cooking methods on the amounts of these contaminants.

Also, realistic conditions must be applied. For instance, for frying, several of the studies reported are absent of food and apply temperatures higher than the recommended frying temperature, therefore it is not possible to accurately link these conditions with the occurrence of chloropropanols and GEs.

Concerning the potential precursors, during recent years significant achievements were reported, namely the association with the presence of chlorides and acylglycerols, but it is necessary to increase the research because there is controversy in the described information and the formation steps are not clearly.

Furthermore, regarding the appointed mitigation strategies, although some of these are effective, only few studies have dedicated their research to this subject and it is essential to increase the number of studies, as well as to test other parameters. Furthermore, it is important to evaluate these measures as a whole, ensuring that the measures to decrease these contaminants do not have a negative impact, for example by producing other undesirable compounds.

In summary, apart from what the future holds, plentiful research should be done soon, especially concerning the formation and mitigation mechanisms of these toxic compounds that need to be fully explained. Additionally, it will only be possible to realise the desired goals, if joint efforts, that must encompass food industry, governing bodies, research and academia, are taken.

References

Aini, I. N., & Miskandar, M. S. (2007). Utilization of palm oil and palm products in shortenings and margarines. *European Journal of Lipid Science and Technology*, *109*(4), 422–432. https://doi.org/10.1002/ejlt.200600232

Aniołowska, M., & Kita, A. (2015). The Effect of Type of Oil and Degree of Degradation on Glycidyl Esters Content during the Frying of French Fries. *Journal of the American Oil Chemists' Society*, *92*(11–12), 1621–1631. https://doi.org/10.1007/s11746-015-2715-3

Aniołowska, M., & Kita, A. (2016a). Monitoring of glycidyl fatty acid esters in refined vegetable oils from retail outlets by LC–MS. *Journal of the Science of Food and Agriculture*, *96*(12), 4056–4061. https://doi.org/10.1002/jsfa.7603

Aniołowska, M., & Kita, A. (2016b). The effect of frying on glicydyl esters content in palm oil. *Food Chemistry*, *203*, 95–103. https://doi.org/10.1016/j.foodchem.2016.02.028

Aniołowska, M., & Kita, A. (2016c). The effect of raw materials on thermo-oxidative stability and glycidyl ester content of palm oil during frying. *Journal of the Science of Food and Agriculture*, *96*(6), 2257–2264. https://doi.org/10.1002/jsfa.7345

Arisseto, A., Marcolino, P., Vicente, E., & Sampaio, K. A. (2013). Ésteres de cloropropanóis e de glicidol em alimentos. *Quimica Nova*, *36*(9), 1406–1415. https://doi.org/10.1590/S0100-40422013000900022

Arisseto, A. P., Marcolino, P. F. C., & Vicente, E. (2014). Determination of 3-monochloropropane-1,2-diol fatty acid esters in Brazilian vegetable oils and fats by an in-house validated method. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *31*(8), 1385–1392. https://doi.org/10.1080/19440049.2014.926400

Barcelos, E., Rios, S. de A., Cunha, R. N. V., Lopes, R., Motoike, S. Y., Babiychuk, E., … Kushnir, S. (2015). Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science*, *6*(March), 1–16. https://doi.org/10.3389/fpls.2015.00190

Becalski, A., Feng, S., Lau, B. P. Y., & Zhao, T. (2015). A pilot survey of 2- and 3-monochloropropanediol and glycidol fatty acid esters in foods on the Canadian market 2011-2013. *Journal of Food Composition and Analysis*, *37*, 58–66. https://doi.org/10.1016/j.jfca.2014.09.002

Blumhorst, M. R., Collison, M. W., Cantrill, R., Shiro, H., Masukawa, Y., Kawai, S., & Yasunaga, K. (2013). Collaborative Study for the Analysis of Glycidyl Fatty Acid Esters in Edible Oils using LC–MS. *Journal of the American Oil Chemists' Society*, *90*(4), 493–500. https://doi.org/10.1007/s11746-012-2187-7

Bornscheuer, U. T., & Hesseler, M. (2010). Enzymatic removal of 3-monochloro-1,2-propanediol(3-MCPD) and its esters from oils. *European Journal of Lipid Science and Technology*, *112*(5), 552–556. https://doi.org/10.1002/ejlt.200900245

Cheng, W., Liu, G., & Liu, X. (2016). Formation of Glycidyl Fatty Acid Esters Both in Real Edible Oils during Laboratory-Scale Refining and in Chemical Model during High Temperature Exposure. *Journal of Agricultural and Food Chemistry*, *64*(29), 5919–5927. https://doi.org/10.1021/acs.jafc.6b01520

Cheng, W. W., Liu, G. Q., Wang, L. Q., & Liu, Z. S. (2017). Glycidyl Fatty Acid Esters in Refined Edible Oils: A Review on Formation, Occurrence, Analysis, and Elimination Methods. *Comprehensive Reviews in Food Science and Food Safety*, *16*(2), 263–281. https://doi.org/10.1111/1541-4337.12251

Commission of the European Communities. (2001). Commission Regulation (EC) No. 466/2001 of 8 March 2001 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, *L 269*, 1–15.

Commission of the European Communities. (2006). Commission Regulation (EC) No. 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuffs. *Official Journal of the European Communities*, *L 364*, 5–24.

Corley, R. H. V. (2009). How much palm oil do we need? *Environmental Science and Policy*, *12*(2), 134–139. https://doi.org/10.1016/j.envsci.2008.10.011

Craft, B. D., & Nagy, K. (2012). Mitigation of MCPD-ester and glycidyl-ester levels during the production of refined palm oil. *Lipid Technology*, *24*(7), 155–157. https://doi.org/10.1002/lite.201200207

Craft, B. D., Nagy, K., Sandoz, L., & Destaillats, F. (2012). Factors impacting the formation of Monochloropropanediol (MCPD) fatty acid diesters during palm (*Elaeis guineensis*) oil production. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *29*(3), 354–361. https://doi.org/10.1080/19440049.2011.639034

Crews, C., Chiodini, A., Granvogl, M., Hamlet, C., Hrnčiřík, K., Kuhlmann, J., … Seefelder, W. (2013). Analytical approaches for MCPD esters and glycidyl esters in food and biological samples: A review and future perspectives. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *30*(1), 11–45. https://doi.org/10.1080/19440049.2012.720385

Destaillats, F., Craft, B. D., Sandoz, L., & Nagy, K. (2012). Formation mechanisms of Monochloropropanediol (MCPD) fatty acid diesters in refined palm (*Elaeis guineensis*) oil and related fractions. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *29*(1), 29–37. https://doi.org/10.1080/19440049.2011.633493

Divinová V., Svejkovská B., Doležal M., V. J. (2004). Determination of free and bound 3-chloropropane-1,2-diol by gas chromatography with mass spectrometric detection using deuterated 3-chloropropane-1,2-diol as internal standard. *Czech Journal of Food Science*, (22), 182–189. https://doi.org/10.17221/3422-CJFS

Dubois, V., Breton, S., Linder, M., Fanni, J., & Parmentier, M. (2007). Fatty acids profile of 80 vegetable oils with regard to their nutritional potential. *European Journal of Lipid Science and Technology, 109*, 710-732. https://doi.org/10.1002/ejlt.200700040

Edem, D. O. (2002). Palm oil: Biochemical, physiological, nutritional, hematological, and toxicological aspects: A review. *Plant Foods for Human Nutrition*, *57*(3–4), 319–341. https://doi.org/10.1023/A:1021828132707

European Commission. (2014). Commission Recommendation of 10 September 2014 on monitoring of the presence of 2 and 3-monochloropropane-1,2-diol (2 and 3-MCPD), 2- and 3-MCPD fatty acid esters and glycidyl fatty acid esters in food. *Official Journal of the European Union*, *L 271*, 93–95.

European Food safety Authority. (2013). Analysis of occurrence of 3-monochloropropane-1, 2-diol (3-MCPD) in food in Europe in the years 2009-2011 and preliminary exposure assessment. *EFSA Journal*, *11*(9), 3381. Retrieved from http://doi.wiley.com/10.2903/j.efsa.2013.3381

European Food Safety Authority. (2016). Risks for human health related to the presence of 3- and 2-monochloropropanediol (MCPD), and their fatty acid esters, and glycidyl fatty acid esters in food. *EFSA Journal*, *14*(5). Retrieved from http://doi.wiley.com/10.2903/j.efsa.2016.4426

European Food Safety Authority. (2018). Update of the risk assessment on 3-monochloropropane diol and its fatty acid esters. *EFSA Journal*, *16*(1), 1–48. https://doi.org/10.2903/j.efsa.2018.5083

FAOSTAT. (2017). Food and Agriculture Organization of the United Nations, Statistics Division, URL http://faostat3.fao.org/home/E. Accessed 07.01.18.

Food and Agriculture Organization of the United Nations and World Health Organization. (2002). *Evaluation of Certain Food Additives and Contaminants*. *Joint FAO/WHO Expert Committee on Food Additives. WHO technical report series*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/10079756

Food and Agriculture Organization of the United Nations and World Health Organization. (2017). *Evaluation of certain contaminants in food. oint FAO/WHO Expert Committee on Food Additives. WHO technical report series*. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/21699062

Franke, K., Strijowski, U., Fleck, G., & Pudel, F. (2009). Influence of chemical refining process and oil type on bound 3-chloro-1,2-propanediol contents in palm oil and rapeseed oil. *LWT - Food Science and Technology*, *42*(10), 1751–1754. https://doi.org/10.1016/j.lwt.2009.05.021

Gee, P. T. (2007). Analytical characteristics of crude and refined palm oil and fractions. European *Journal of Lipid Science and Technology, 109*, 373-379. https://doi.org/10.1002/ejlt.200600264

Haines, T. D., Adlaf, K. J., Pierceall, R. M., Lee, I., Venkitasubramanian, P., & Collison, M. W. (2011). Direct determination of MCPD fatty acid esters and glycidyl fatty acid esters in vegetable oils by LC-TOFMS. *Journal of the American Oil Chemists' Society*, *88*(1), 1–14. https://doi.org/10.1007/s11746-010-1732-5

Hamlet, C., Sadd, P., & Crews, C. (2002). Occurrence of 3-chloro-propane-1, 2-diol (3-MCPD) and related compounds in foods: a review. *Food Additives & Contaminants*, *19*(August), 619–631. https://doi.org/10.1080/0265203021013239

Hammouda, I. Ben, Zribi, A., Mansour, A. Ben, Matthäus, B., & Bouaziz, M. (2017). Effect of deep-frying on 3-MCPD esters and glycidyl esters contents and quality control of refined olive pomace oil blended with refined palm oil. *European Food Research and Technology*, *243*(7), 1219–1227. https://doi.org/10.1007/s00217-016-2836-4

Hori, K., Koriyama, N., Omori, H., Kuriyama, M., Arishima, T., & Tsumura, K. (2012a). Simultaneous determination of 3-MCPD fatty acid esters and glycidol fatty acid esters in edible oils using liquid chromatography time-of-flight mass spectrometry. *LWT - Food Science and Technology*, *48*(2), 204–208. https://doi.org/10.1016/j.lwt.2012.03.014

Hori, K., Matsubara, A., Uchikata, T., Tsumura, K., Fukusaki, E., & Bamba, T. (2012b). High-throughput and sensitive analysis of 3-monochloropropane-1,2-diol fatty acid esters in edible oils by supercritical fluid chromatography/tandem mass spectrometry. *Journal of Chromatography A*, *1250*, 99–104. https://doi.org/10.1016/j.chroma.2012.06.017

Hrncirik, K., & van Duijn, G. (2011). An initial study on the formation of 3-MCPD esters during oil refining. *European Journal of Lipid Science and Technology*, *113*(3), 374–379. https://doi.org/10.1002/ejlt.201000317

Jedrkiewicz, R., Głowacz, A., Gromadzka, J., & Namieśnik, J. (2016a). Determination of 3-MCPD and 2-MCPD esters in edible oils, fish oils and lipid fractions of margarines available on Polish market. *Food Control*, *59*, 487–492. https://doi.org/10.1016/j.foodcont.2015.05.039

Jędrkiewicz, R., Kupska, M., Głowacz, A., Gromadzka, J., & Namieśnik, J. (2016b). 3-MCPD: A Worldwide Problem of Food Chemistry. *Critical Reviews in Food Science and Nutrition*, *56*(14), 2268–2277. https://doi.org/10.1080/10408398.2013.829414

Karšulínová, L., Folprechtová, B., Doležal, M., Dostálová, J., & Velíšek, J. (2007). Analysis of the lipid fractions of coffee creamers, cream aerosols, and bouillon cubes for their health risk associated constituents. *Czech Journal of Food Sciences*, *25*(5), 257–264.

Koyama, K., Miyazaki, K., Abe, K., Egawa, Y., Kido, H., Kitta, T., … Watanabe, Y. (2016). Collaborative Study of an Indirect Enzymatic Method for the Simultaneous Analysis of 3-MCPD, 2-MCPD, and Glycidyl Esters in Edible Oils. *Journal of Oleo Science*, *65*(7), 557–568. https://doi.org/10.5650/jos.ess16021

Kuhlmann, J. (2011). Determination of bound 2,3-epoxy-1-propanol (glycidol) and bound monochloropropanediol (MCPD) in refined oils. *European Journal of Lipid Science and Technology*, *113*(3), 335–344. https://doi.org/10.1002/ejlt.201000313

Kuhlmann, J. (2016). Analysis and occurrence of dichloropropanol fatty acid esters and related process-induced contaminants in edible oils and fats. *European Journal of Lipid Science and Technology*, *118*(3), 382–395. https://doi.org/10.1002/ejlt.201400518

Li, C., Li, L., Jia, H., Wang, Y., Shen, M., Nie, S., & Xie, M. (2016). Formation and reduction of 3-monochloropropane-1,2-diol esters in peanut oil during physical refining. *Food Chemistry*, *199*, 605–611. https://doi.org/10.1016/j.foodchem.2015.12.015

Li, C., Nie, S. P., Zhou, Y. Qiang, & Xie, M. Y. (2015a). Exposure assessment of 3-monochloropropane-1, 2-diol esters from edible oils and fats in China. *Food and Chemical Toxicology*, *75*, 8–13. https://doi.org/10.1016/j.fct.2014.10.003

Li, H., Chen, D., Miao, H., Zhao, Y., Shen, J., & Wu, Y. (2015b). Direct determination of fatty acid esters of 3-chloro-1, 2-propanediol in edible vegetable oils by isotope dilution - ultra high performance liquid chromatography - triple quadrupole mass spectrometry. *Journal of Chromatography A*, *1410*, 99–109. https://doi.org/10.1016/j.chroma.2015.07.080

Lin, S. W. (2011). Palm oil. In F. D. Gunstone (Ed.), *Vegetable oils in food technology: composition, properties and uses* (2nd ed., pp. 25–58). Wiley-Blackwell. https://doi.org/10.1002/9781444339925.ch3

Macmahon, S., Begley, T. H., & Diachenko, G. W. (2013). Analysis of processing contaminants in edible oils. Part 2. Liquid chromatography-tandem mass spectrometry method for the direct detection of 3-monochloropropanediol and 2-monochloropropanediol diesters. *Journal of Agricultural and Food Chemistry*, *61*(20), 4748–4757. https://doi.org/10.1021/jf400581g

MacMahon, S., Begley, T. H., & Diachenko, G. W. (2013a). Occurrence of 3-MCPD and glycidyl esters in edible oils in the United States. *Food Additives and Contaminants - Part A Chemistry, Analysis, Control, Exposure and Risk Assessment*, *30*(12), 2081–2092. https://doi.org/10.1080/19440049.2013.840805

MacMahon, S., Mazzola, E., Begley, T. H., & Diachenko, G. W. (2013b). Analysis of processing contaminants in edible oils. Part 1. Liquid chromatography-tandem mass spectrometry method for the direct detection of 3-monochloropropanediol monoesters and glycidyl esters. *Journal of Agricultural and Food Chemistry*, *61*(20), 4737–4747. https://doi.org/10.1021/jf4005803

Manicini, A., Imperlini, E., Nigro, E., Montagenese, C., Daniele, A., Orrù, S., & Buono, P. (2015). Biological and nutritional properties of palm oil and palmitic acid: effects on health. *Molecules, 20*, 17339-17361. https:// doi.org/10.3390/molecules200917339

Matthäus, B., Pudel, F., Fehling, P., Vosmann, K., & Freudenstein, A. (2011). Strategies for the reduction of 3-MCPD esters and related compounds in vegetable oils. *European Journal of Lipid Science and Technology*, *113*(3), 380–386. https://doi.org/10.1002/ejlt.201000300

Ramli, M. R., Siew, W. L., Ibrahim, N. A., Hussein, R., Kuntom, A., Razak, R. A. A., & Nesaretnam, K. (2011). Effects of degumming and bleaching on 3-MCPD esters formation during physical refining. *Journal of the American Oil Chemists' Society*, *88*(11), 1839–1844. https://doi.org/10.1007/s11746-011-1858-0

Razak, R. A., Kuntom, A., Siew, W. L., Ibrahim, N. A., Ramli, M. R., Hussein, R., & Nesaretnam, K. (2012). Detection and monitoring of 3-monochloropropane-1,2-diol (3-MCPD) esters in cooking oils. *Food Control*, *25*(1), 355–360. https://doi.org/10.1016/j.foodcont.2011.10.058

Scientific Committee on Food (SCF). (2001). *Opinion of the scientific committee on food on 3-monochloropropane-1,2-diol (3-MCPD). Updating the SCF opinion of 1994. Scientific Committee on Food*. https://doi.org/10.1111/1467-8608.00251

Seefelder, W., Scholz, G., & Schilter, B. (2011). Structural diversity of dietary fatty esters of chloropropanols and related substances. *European Journal of Lipid Science and Technology*, *113*(3), 319–322. https://doi.org/10.1002/ejlt.201000319

Seefelder, W., Varga, N., Studer, A., Williamson, G., Scanlan, F. P., & Stadler, R. H. (2008). Esters of 3-chloro-1,2-propanediol (3-MCPD) in vegetable oils: Significance in the formation of 3-MCPD. *Food Additives and Contaminants - Part A Chemistry, Analysis,*

Control, Exposure and Risk Assessment, *25*(4), 391–400. https://doi.org/10.1080/02652030701385241

Shiro, H., Kondo, N., Kibune, N., & Masukawa, Y. (2011). Direct method for quantification of glycidol fatty acid esters in edible oils. *European Journal of Lipid Science and Technology*, *113*(3), 356–360. https://doi.org/10.1002/ejlt.201000395

Šmidrkal, J., Tesařová, M., Hrádková, I., Beřcíková, M., Adamčíková, A., Filip V. (2016). Mechanism of formation of 3-chloropropan-1,2-diol (3-MCPD) esters under conditions of the vegetable oil refining. *Food Chemistry, 211*, 124-129. https://doi.org/10.1016/j.foodchem.2016.05.039.

Steenbergen, H., Hrnčiřík, K., Ermacora, A., de Koning, S., & Janssen, H. G. (2013). Direct analysis of intact glycidyl fatty acid esters in edible oils using gas chromatography-mass spectrometry. *Journal of Chromatography A*, *1313*, 202–211. https://doi.org/10.1016/j.chroma.2013.06.056

US Department of Agriculture, Agricultural Research Service, Nutrient Data Laboratory. (2017). USDA National Nutrient Database for Standard Reference, Release 28. Nutrient Data Laboratory. URL: http://www.ars.usda.gov/ba/bhnrc/ndl. Accessed 10.01.18.

Weißhaar, R. (2008a). Determination of total 3-chloropropane-1,2-diol (3-MCPD) in edible oils by cleavage of MCPD esters with sodium methoxide. *European Journal of Lipid Science and Technology*, *110*(2), 183–186. https://doi.org/10.1002/ejlt.200700197

Weißhaar, R. (2008b). 3-MCPD-esters in edible fats and oils - a new worldwide problem. *European Journal of Lipid Science and Technology*, *110*, 671–672. https://doi.org/10.1002/ejlt.200800154

Weißhaar, R., & Perz, R. (2010). Fatty acid esters of glycidol in refined fats and oils. *European Journal of Lipid Science and Technology*, *112*(2), 158–165. https://doi.org/10.1002/ejlt.200900137

Wong, Y. H., Muhamad, H., Abas, F., Lai, O. M., Nyam, K. L., & Tan, C. P. (2017). Effects of temperature and NaCl on the formation of 3-MCPD esters and glycidyl esters in refined, bleached and deodorized palm olein during deep-fat frying of potato chips. *Food Chemistry*, *219*, 126–130. https://doi.org/10.1016/j.foodchem.2016.09.130

Yamazaki, K., Ogiso, M., Isagawa, S., Urushiyama, T., Ukena, T., & Kibune, N. (2013). A new, direct analytical method using LC-MS/MS for fatty acid esters of 3-chloro-1,2-propanediol (3-MCPD esters) in edible oils. *Food Additives and Contaminants - Part A*

Chemistry, Analysis, Control, Exposure and Risk Assessment, *30*(1), 52–68. https://doi.org/10.1080/19440049.2012.713031

Zelinkova, Z., Giri, A., & Wenzl, T. (2017). Assessment of critical steps of a GC/MS based indirect analytical method for the determination of fatty acid esters of monochloropropanediols (MCPDEs) and of glycidol (GEs). *Food Control*, *77*, 65–75. https://doi.org/10.1016/j.foodcont.2017.01.024

Zhang, H., Jin, P., Zhang, M., Cheong, L., Hu, P., Zhao, Y., … Xu, X. (2016). Mitigation of 3 - Monochloro-1,2-propanediol Ester Formation by Radical Scavengers. *Journal of Agricultural and Food Chemistry,* *64*(29), 5887–5892. https://doi.org/10.1021/acs.jafc.6b02016

Zhou, H., Jin, Q., Wang, X., & Xu, X. (2014). Direct measurement of 3-chloropropane-1,2-diol fatty acid esters in oils and fats by HPLC method. *Food Control*, *36*(1), 111–118. https://doi.org/10.1016/j.foodcont.2013.07.041

Zulkurnain, M., Ming, O., Abdul, R., Arbi, I., Chuan, T., & Ping, C. (2012). The effects of physical refining on the formation of 3-monochloropropane-1 , 2-diol esters in relation to palm oil minor components. *Food Chemistry*, *135*(2), 799–805. https://doi.org/10.1016/j.foodchem.2012.04.144

CAPÍTULO 5. Considerações finais e perspetivas futuras

Neste capítulo são apresentadas algumas conclusões parciais sobre os resultados obtidos em cada capítulo, culminando com considerações finais sobre o trabalho desenvolvido, onde se faz uma análise sobre as potencialidades dos resultados e a sua aplicação. Numa perspetiva de continuidade deste trabalho, apresentam-se também perspetivas futuras.

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5.1 Conclusões parciais

Influência do processamento

Os métodos de confeção influenciam significativamente a qualidade nutricional dos alimentos processados. Com este trabalho, verificou-se que, apesar das últimas recomendações preconizarem a utilização do forno em detrimento da fritura, se desconhecem muitas das alterações que podem ocorrer. Sobretudo porque a temperatura e o tempo recomendados para a confeção de alimentos processados pré-preparados no forno (200 ºC, 15 minutos) é muito superior às condições recomendadas para a fritura (180 ºC, 5 minutos).

Relativamente à fritura industrial, avaliou-se a qualidade de salgados prontos-a-comer, bem como de batatas fritas de restauração. Observou-se que, de uma maneira geral, os óleos vegetais usados são maioritariamente constituídos por ácidos gordos insaturados. Assim, durante a fritura, estes alimentos absorveram sobretudo gordura que, do ponto de vista da saúde do consumidor, representa um menor impacto negativo. Uma possível explicação para esta situação é a utilização preponderante de gorduras e óleos de origem vegetal, em detrimento das gorduras de origem animal, por parte da indústria alimentar.

Teores de sal, gordura e composição em ácidos gordos

Outro objetivo deste trabalho era avaliar os teores de sal, de gordura e a composição em ácidos gordos de alimentos processados. Os produtos selecionados para este trabalho, como já anteriormente referido foram agrupados em 9 categorias: produtos de pastelaria, produtos de panificação, *snacks*, *fast-food*, refeições prontas-a-comer, molhos, frutos secos e oleaginosas, batatas fritas e cereais.

Em relação aos teores de sal, de uma forma geral, existe uma grande variedade de alimentos processados que continuam a apresentar teores elevados, o que pode representar uma preocupação acrescida para a saúde. No que diz respeito à composição da gordura destes alimentos, observou-se que existe uma tendência para melhorar, mas alguns alimentos apresentam teores elevados de gordura saturada, nomeadamente os produtos de pastelaria e de panificação. Por outro lado, os teores de ácidos gordos *trans* diminuíram significativamente o que é extremamente relevante e representa um objetivo alcançado por parte da indústria e das autoridades competentes.

Produtos similares

A qualidade nutricional de produtos similares de marcas diferentes e com características distintas (exemplo com e sem glúten) também foi avaliada. Observou-se que para alguns produtos similares existem diferenças significativas (por vezes mais do dobro) em relação aos teores de sal, gordura e/ou composição em ácidos gordos. Estas diferenças também podem ser observadas quando se compara a qualidade nutricional de produtos sem glúten e com glúten. As discrepâncias foram relevantes para todos os parâmetros em estudo e não é possível associar a marca ou uma característica específica de um produto à sua qualidade nutricional.

Rotulagem

Foi também analisada a conformidade da rotulagem dos alimentos processados selecionados, tendo em consideração a legislação em vigor. Verificaram-se diferenças relativamente baixas entre os valores declarados no rótulo e os determinados analiticamente. No entanto, no caso do sal foram encontradas diferenças superiores, e que devem merecer especial atenção.

Após esta análise, verificou-se que a maioria dos produtos das categorias dos *snacks*, *fast-food* e refeições prontas a comer, não cumpre as tolerâncias fixadas para os ácidos gordos saturados. Por outro lado, para as categorias dos cereais e dos molhos, todos os valores cumpriam as tolerâncias fixadas para os nutrientes em estudo.

4-Hidroxi-2-alcenais

A formação e ocorrência dos 4-hidroxi-2-alcenais está diretamente relacionada com a presença de ácidos gordos polinsaturados, ómega-3 e ómega-6. Verificou-se que os óleos brutos contêm maiores quantidades destes compostos do que os refinados, o que indica que é importante o processamento dos óleos para melhorar a sua qualidade e segurança.

Para as outras categorias de alimentos, verificou-se que, de uma forma geral, os alimentos com maiores teores dos referidos ácidos gordos, têm maiores quantidades destes compostos. Este aspeto pode ser relevante, na medida em que existe uma tendência para enriquecer alguns produtos com este tipo de ácidos gordos.

Em relação à fritura e ocorrência destes compostos, verificou-se que temperaturas acima dos valores recomendados estão relacionadas com quantidades superiores destes compostos, sobretudo em óleos polinsaturados.

Cloropropanóis e ésteres glicidílicos de ácidos gordos

Em geral, observou-se uma grande variação nos teores destes contaminantes nos diferentes óleos vegetais, mas também para o mesmo tipo de matriz. Este aspeto pode estar relacionado com a origem das matérias-primas, mas também com as condições de processamento dos óleos, em especial a desodorização.

Neste trabalho foi dada especial atenção à presença destes contaminantes no óleo de palma, uma vez que é o mais usado pela indústria. Encontraram-se teores preocupantes em alguns óleos de palma, mas é de destacar, sobretudo, a elevada variabilidade e controvérsia dos resultados. Este aspeto é relevante, uma vez que este óleo é um ingrediente de inúmeros alimentos processados, podendo haver dispersas pela cadeia alimentar quantidades preocupantes destes compostos.

5.2 Considerações finais

O presente trabalho centrou-se no aprofundamento e discussão do equilíbrio nutricional e da segurança dos alimentos processados, bem como na avaliação do seu potencial impacto na saúde, com base em orientações nacionais e internacionais.

Assim, foram desenvolvidas diferentes tarefas que tiveram por finalidade concretizar os vários objetivos específicos, previamente descritos. Durante o desenvolvimento deste trabalho foi necessário ajustar os objetivos em função dos resultados que iam sendo obtidos. É de realçar o papel ativo que tiveram as orientadoras desta tese e os revisores das diversas revistas às quais foram submetidos os artigos. Estes contributos foram, sem dúvida, fundamentais para ampliar o sentido crítico, da experiência e da interpretação dos resultados.

Verificou-se que, apesar da imensa literatura no que diz respeito ao estudo dos alimentos processados, existe ainda um desconhecimento considerável. Desta forma, considera-se que é fundamental continuar a desenvolver linhas de investigação nesta área, sobretudo porque todos os dias são lançados inúmeros produtos alimentares “novos”, sujeitos a diferentes técnicas de processamento, mas também porque os produtos já existentes estão continuamente a sofrer alterações na sua formulação e importa perceber o impacto das mesmas na sua qualidade nutricional.

Deve ser dada uma atenção especial ao impacto dos métodos de confeção na qualidade e segurança destes alimentos. Para além disso, é fundamental que sejam usadas condições realísticas na aplicação destes métodos, nomeadamente temperatura, tempo e equipamento adequado. Só assim é possível ter uma visão completa e realística do seu impacto e, a partir daí, poder elaborar estratégias e orientações que diminuam a formação de compostos indesejáveis e que contribuam para uma alimentação equilibrada e saudável.

Os parâmetros avaliados neste estudo permitem afirmar que é prioritário reduzir os teores de sal em algumas categorias de alimentos processados. No entanto, também é possível concluir que os teores de ácidos gordos *trans* diminuíram significativamente nos últimos anos. É notório um esforço para os alimentos terem uma composição lipídica com menor impacto na saúde dos consumidores, nomeadamente substituindo gorduras parcialmente hidrogenadas e ricas em ácidos gordos saturados, por óleos ricos em ácidos gordos insaturados.

Outra área de grande relevo é a reformulação da composição dos alimentos processados. Este trabalho demonstrou que é possível ter produtos similares, com características organoléticas aceites pelo consumidor, com melhor equilíbrio nutricional e mais seguros. Desta forma, considera-se que é fundamental usar a partilha do conhecimento científico e desenvolver esforços conjuntos que contribuam para a reformulação destes produtos.

Espera-se que os resultados apresentados nesta tese possam ser úteis para a continuação da investigação, que contribuam para o avanço do conhecimento científico, mas também que possam constituir um desafio para a indústria alimentar e para as entidades com competências em saúde.

Em suma, é necessário envolver as instituições académicas, os investigadores, e a indústria para termos mais dados sobre ocorrência de nutrientes e compostos emergentes em alimentos processados, mas também incluir outros profissionais para termos dados válidos de consumo. Só assim será possível fazer uma avaliação de risco de exposição e do potencial impacto na saúde associado ao consumo dos alimentos processados. A informação sobre a qualidade nutricional e segurança dos alimentos processados é fundamental para a formulação de políticas alimentares e de saúde que visem melhorar o estado nutricional da população.

5.3 Perspetivas futuras

Os alimentos processados são consumidos por todas as faixas etárias da população, mas em especial pelas crianças e jovens. Por serem reconhecidos como uma fonte de sal, gordura e açúcar é crucial aprofundar o conhecimento científico nesta área, através da prática de uma investigação rigorosa, responsável e sem conflito de interesses.

Com este trabalho enfatizou-se que cada vez mais é preciso que as entidades com competências em saúde e outras colaborem entre si, através da partilha do conhecimento. Dada a quantidade de alimentos que foram incluídos neste trabalho, não foi possível dar cumprimento a uma área de grande interesse e pouco explorada, nomeadamente os 4-hidroxi-2-alcenais, cloropropanóis e ésteres glicidílicos de ácidos gordos. Portanto, para dar continuidade a este trabalho, seria crucial explorar este tema, avaliando a sua ocorrência em alimentos processados, mas sobretudo determinando a influência dos métodos de processamento industrial e de confeção na sua presença.

Esta informação é fundamental para posteriormente poderem ser delineados trabalhos de investigação na área das estratégias de mitigação destes compostos indesejáveis, que contribuam efetivamente para a sua redução e que permitam alcançar o objetivo primordial de se proporcionar uma alimentação equilibrada e segura.