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Technological developments for enhanced nutritional, bioactive and biochemical olive oil processed potatoes

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ABSTRACT

Potatoes are among the most consumed vegetables, being an important source of bioactive components in the diet. However, they require thermal processing to become edible, with partial loss of bioactive compounds. The most common processing technique is frying, and it is usual to use olive oil for this purpose in Mediterranean countries. However, doubts remain as to whether olive oil is equally suitable for frying like other widely consumed vegetable oils worldwide, particularly those equally rich in monounsaturated fatty acids (MUFA). Moreover, in classical frying, there is a high incorporation of lipids, with consequent increase in calories from fat and potential health effects, with a growing demand for healthier processing alternatives.

This work aimed to answer these questions simultaneously, evaluating the effect of different olive oil categories and diverse technological processes on the nutritional quality, safety and acceptability of potatoes.

A study on the effect of prolonged classical frying of fresh potatoes (8h/day, 175ºC) was initially carried out up to the legal limit of rejection for total polar compounds (TPC), aiming to compare several MUFA rich oils, which included extra virgin olive (EVOO), peanut (PO) and canola oils (CO). This study allowed to observe the progressive degradation of the three vegetable oils, at the same time as the effects on the nutritional quality and safety of the French fries. A similar degradation pattern was observed between PO and CO, allowing 18 h (PO) at 20 h (CO) for frying, while EVOO did not reach the rejection limit (25% TPC) even after 28 h of heating. However, the degradation of the unsaturated fatty acids was equivalent in the three vegetable oils, as well as the amount of oxidized triglycerides formed, and all antioxidants analyzed were consumed in the first 8-12 hours of frying, including in EVOO. From the point of view of the fried potato, there were significant losses of bioactive components with the three oils, particularly vitamin C and antioxidant activity. Although the potatoes fried in CO had a more balanced fatty acid profile, they also showed higher incorporation of total lipids and volatile aldehydes derived from polyunsaturated fatty acids oxidation, in addition to a lower sensory acceptability, a factor that has been shown to be dependent on cultural preferences. Acrylamide content was not affected by prolonged frying or oil type, but the sensory panel clearly distinguished the progressive sensorial degradation of the potatoes with the frying time and the oil type. It was concluded that the higher bioactive potential of EVOO, namely its richness in phenolic compounds, is lost in the first few hours of frying, but for prolonged frying EVOO is more stable to thermal oxidation than PO or CO.

It was also intended to understand the impact of low-fat frying systems on the nutritional and sensory quality of potatoes. Using traditional frying as control, two commercial

alternatives of low-fat frying (Actifry® and Airfryer®) and two domestic alternatives (microwave-grill and conventional oven) were compared, using olive oil (OO) and widely used vegetable oils. Fat reduction reached 70% in the commercial alternatives and 80% in the domestic ones, associated with a direct reduction of caloric intake, of vegetable oils components (vitamin E, fatty acids), as well as lipid degradation compounds (oxidized triglycerides, polymers, aldehydes, etc.), while preserving/enriching potatoes in ascorbic acid, phenolic compounds and carotenoids in comparison with classical frying. With all the processes, there was lower lipid oxidation with the use of olive oil. A significantly lower formation of acrylamide was observed in the microwave-grill (-57%) and oven (-87%) when compared to traditional frying. Despite the sensory preference for traditional fried potatoes and the clear influence of the oil type, taste and odor qualities were determinant for acceptability of processed potatoes than color. Overall, all tested systems proved to be healthy alternatives to classical frying, with effective production of low-fat potatoes (<3g of lipids *per* 100g) enriched in bioactive compounds.

Finally, aware of the complexity of choosing among the commercial olive oil categories for frying, a comparative study was implemented between the two most commercially important categories, EVOO and OO, under classical frying, commercial systems and microwave-grill. All potatoes processed in EVOO presented higher preservation (p<0.05) of ascorbic acid, tocopherols and phenolic compounds than in OO. However, for classical frying, and only in terms of lipid oxidation, the use of OO resulted in a lower content of oxidized lipids in the potato than EVOO. Acrylamide content was not associated to the type of olive oil, being more influenced by the type of thermal process used. The sensory panel was unable to distinguish the olive oil categories after processing. Globally, it was found that EVOO is strongly recommended for low-fat processing, enriching potatoes in bioactive nutrients, while OO may represent an economic advantage for classical deep-frying.

With the demonstration of diverse ways to enhance the nutritional quality and safety of potatoes processed in olive oil, it will be important to complement these studies from a biological perspective, namely on cardiovascular risk factors, *in vivo* antioxidant activity, together with the individual characterization of phenolic compounds, carotenoids, and bioavailability of its most important bioactive components.

Keywords: Potatoes, olive oil, vegetable oils, frying, air-frying, microwave, ascorbic acid, sensory quality, acrylamide, oxidation, volatile compounds.

RESUMO

As batatas estão entre os vegetais mais consumidos, sendo uma importante fonte de componentes bioativos na dieta. No entanto, necessitam de processamento térmico para se tornarem edíveis, o que pode originar perda parcial desses compostos. A técnica de processamento mais comum é a fritura, sendo usual nos países Mediterrânicos utilizar-se azeite para este efeito. No entanto, persistem dúvidas se este é igualmente adequado para fritura como outros óleos vegetais amplamente consumidos mundialmente, particularmente os igualmente ricos em ácidos gordos monoinsaturados (MUFA). Para além disso, na fritura clássica ocorre uma elevada incorporação de lípidos nos alimentos, com consequente aumento do aporte nutricional e potenciais efeitos deletérios na saúde, havendo uma procura crescente por alternativas de processamento mais saudáveis.

Esse trabalho pretendeu dar resposta simultânea a estas questões, avaliando o efeito de diferentes categorias de azeite e processos tecnológicos na qualidade nutricional, segurança e aceitabilidade da batata.

Realizou-se um estudo sobre o efeito da fritura clássica prolongada de batatas frescas (8h/dia, 175ºC), até ao limite legal de rejeição para compostos polares totais (TPC), comparando diversos óleos ricos em MUFA, onde se incluiu azeite virgem extra (EVOO), óleo de amendoim (PO) e de canola (CO). Este estudo permitiu acompanhar a degradação progressiva dos três óleos vegetais, ao mesmo tempo que se estudaram as implicações na qualidade nutricional e segurança das batatas fritas. Observou-se um padrão de degradação semelhante entre PO e CO, permitindo 18 h (PO) a 20 h (CO) de fritura, enquanto o EVOO não atingiu o limite de rejeição (25% TPC), mesmo após 28 h de aquecimento. Contudo, a extensão de degradação dos ácidos gordos insaturados foi equivalente nos três óleos, bem como a quantidade de triglicerídeos oxidados, e todos os antioxidantes analisados foram degradados nas primeiras 8-12 h de fritura, inclusive no EVOO. Do ponto de vista da batata, ocorreram perdas bioativas significativas com todos os óleos, nomeadamente de vitamina C e de atividade antioxidante. Embora as batatas fritas em CO apresentassem um perfil de ácidos gordos mais equilibrado, também exibiram maior incorporação de lípidos e de aldeídos voláteis derivados da oxidação de ácidos gordos polinsaturados, para além de terem tido menor aceitação sensorial, um fator que se mostrou dependente das preferências culturais. O teor de acrilamida não foi afetado pela fritura prolongada nem pelo tipo de óleo, mas o painel sensorial distinguiu claramente a degradação sensorial progressiva das batatas com o tempo de fritura e o tipo de óleo. Concluiu-se que o maior potencial bioativo do EVOO, nomeadamente a riqueza em compostos fenólicos, se perde nas primeiras horas de fritura, mas para fritura prolongada, este se comporta de forma mais estável do ponto de vista oxidativo do que o PO ou CO.

Pretendeu-se igualmente entender o impacto dos sistemas de fritura com baixo teor de gordura na qualidade nutricional e sensorial de batata, usando azeite e óleos vegetais amplamente consumidos. Utilizando a fritura clássica como controlo, compararam-se duas alternativas comerciais de fritura com baixo teor de gordura (Actifry® e Airfryer®) e duas alternativas domésticas (micro-ondas com grill e forno convencional). A redução da gordura atingiu 70% nas alternativas comerciais e 80% nas domésticas, associada a uma redução direta de ingestão calórica e de componentes dos óleos vegetais (vitamina E, ácidos gordos), bem como compostos de degradação dos mesmos (triglicerídeos oxidados, polímeros, aldeídos, etc.), ao mesmo tempo que se verificou uma maior preservação/enriquecimento em ácido ascórbico, compostos fenólicos e carotenóides do que na fritura clássica. Em todos os processos ocorreu menor oxidação lipídica com a utilização de azeite. Uma formação significativamente menor de acrilamida foi observada no micro-ondas (-57%) e no forno (-87%) comparativamente à fritura clássica. Apesar da preferência sensorial pela batata frita clássica e da clara influência do tipo de óleo, as qualidades de sabor e odor foram mais determinantes para a aceitabilidade do que a cor. Globalmente, todos os sistemas testados demonstraram ser alternativas saudáveis, com produção de batatas com baixo teor em gordura (<3g de lípidos por 100g), enriquecidas em compostos bioativos.

Por fim, cientes da complexidade da escolha das categorias comerciais de azeite para fritura, realizou-se um estudo comparativo entre as duas categorias comercialmente mais importantes, EVOO e OO, sob fritura tradicional, sistemas comerciais e micro-ondas com grill. Todas as batatas processadas em EVOO apresentaram uma preservação superior (p<0.05) do ácido ascórbico, tocoferóis e compostos fenólicos do que em OO. No entanto, para a fritura clássica, e apenas em termos de oxidação lipídica, o uso de OO resultou num menor teor de lípidos oxidados na batata. O teor em acrilamida não se mostrou associado ao tipo de azeite, sendo mais influenciado pelo tipo de processamento. O painel sensorial não conseguiu distinguir as categorias após processamento. Globalmente, verificou-se que o EVOO é fortemente recomendado para processamentos com baixo teor de gordura, enriquecendo as batatas em nutrientes bioativos, enquanto o OO pode representar uma vantagem económica para a fritura clássica.

Demonstrada a forma de potenciar a qualidade nutricional e segurança alimentar de batatas processadas em azeite, será importante continuar os estudos do ponto de vista biológico, nomeadamente sobre fatores de risco cardiovasculares, atividade antioxidante *in vivo*, bem como a caracterização individual dos compostos fenólicos, carotenóides e biodisponibilidade dos componentes biologicamente mais importantes.

Palavras-chave: Batatas, azeite, óleos vegetais, fritura, fritura de ar, micro-ondas, ácido ascórbico, qualidade sensorial, acrilamida, oxidação, compostos voláteis.

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Scientific Awards

Best poster "Deep or air frying? A comparative study with different vegetable oils." 1st International Symposium on Lipid Oxidation and Antioxidants, 2016, Porto.

*Presenting author.

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LIST OF ABBREVIATIONS

*a**	Redness
AA	Ascorbic acid
ACT	Actifry®
AF	Air-frying
AIR	Airfryer®
ANOVA	Analysis of variance
AOAC	Association of Official Agricultural Chemists
AR	Absolute retention
*b**	Yellowness
*C**	Chrome
CCD	Central composite design
CO	Canola oil
CROO	Crude refined olive oil
DAD	Diode array detector
DF	Deep-frying
DG	Diglycerides
DPPH	2,2-diphenyl-1-picrylhydrazyl radical
DPTG	Dimeric and polymeric triglycerides
DVB/CAR/PDMS	Divinylbenzene/Carboxen/Polydimethylsiloxane
DW	Dry weight
EFSA	European Food Safety Authority
ELSD	Evaporative light scattering detector
EVOO	Extra virgin olive oil
FA	Fatty acid composition
FAME	Fatty acid methyl esters
FAO	Food and Agriculture Organization
FD	Fluorescent detector
FFA	Free fatty acids
FID	Flame ionization detector
FOS	Food oil sensor®
FR	Frequent replenishment
FW	Fresh weight
GAE	Gallic acid equivalents
GC	Gas chromatography
HPLC	High performance liquid chromatography
HPSEC	High pressure size exclusion chromatography
HS	Headspace
IOC	International Olive Oil Council
IS	Internal standard
ISE	Internal standard equivalents
ISO	International Organization for Standardization
*L**	Lightness
MS	Mass spectrometry

MUFA	Monounsaturated fatty acids
MWG	Microwave-grill
n.d.	Not detected
n.s.	Not significant
NO	New oil
NR	Null replenishment
OO	Olive oil
OTG	Oxidized triglycerides
OV	Oven
PAV	*p*-anisidine value
PC	Polar compounds
PCA	Principal component analysis
PLSR1	Partial least squares regression
PO	Peanut oil
PoO	Pomace oil
PUFA	Polyunsaturated fatty acids
PV	Peroxide value
R^2adj	R-squared adjusted
R^2pred	R-squared predicted
RDA	Recommended dietary allowance
RID	Refractive index detector
RMSEP	Root mean square error of prediction
ROO	Refined olive oil
RPM	Rotation per minute
RSD	Relative standard deviation
RT	Room temperature
SFA	Saturated fatty acids
SFO	Sunflower oil
SIM	Single Ion Monitoring
SO	Soybean oil
SPE	Solid phase extraction
SPME	Solid phase microextraction
TAG	Triacylglycerols
TFA	*Trans* fatty acid
TIC	Total Ion Chromatogram
TPC	Total polar compounds
UV-Vis	Extinction coefficients
VOO	Virgin olive oil
WHO	World Health Organization
ΔE	Colour change

PART I

THEORETICAL REVIEW AND OBJECTIVES

CHAPTER 1. General Introduction

The potato plant is a perennial herb belonging to the family *Solanaceae* and *Solanum tuberosum* L. species, which produces one of the most important crops for human consumption (Singh and Kaur, 2009). Originally from South America, in particular in the Andes where it has been cultivated for more than 10,000 years (Camire *et al.*, 2009), it was unknown to the rest of the world until 500 years ago. With more than 4,000 varieties described, and being cultivated in more than 160 countries, it is now the fourth most important food crop in the world after maize, wheat, and rice (Singh and Kaur, 2009; Camire *et al.*, 2009). Its world production exceeded 381 million tons in 2014, wherein Asia and Europe are the largest producing regions (> 80%) (FAO, 2017). Its annual per capita supply in 2013 achieved almost 83 kg in Europe, 54 kg in North America, being lower in Latin America (29 kg), Asia (29 kg), and Africa (19 kg) (FAO, 2017).
The chemical composition of raw white potato is condensed in Table 1.1, expressed both on a fresh (FW) and dry weight (DW) basis.

Table 1.1 Nutritional composition of raw potato, *per* 100g.

Nutrients	FW	DW	Bioactive compounds	FW	DW
Moisture	72-85g	-	Phenolic compounds	137-965mg	596-4196mg
Carbohydrates	13-18g	55-79g	Phenolic acids	58-303mg	252-1319mg
Dietary fibre	1.0-2.0g	4.3-8.7g	Flavonoids	0.03-0.06mg	0.13-0.26mg
Protein	0.6-2.1g	2.6-9.1g	Anthocyanins	58-542mg	253-2357mg
Lipid	0.1-0.2g	0.3-0.9g	Vitamin C	5-54mg	20-235mg
Potassium	280-564mg	1217-2452mg	Vitamin E	0.02-0.1mg	0.07-0.4mg
Phosphorus	30-60mg	130-261mg	Carotenoids	0.1-5.8mg	0.2-23.3mg
Magnesium	14-18mg	61-78mg	Glycoalkaloids	1-20mg	4-87mg

Note: contents originally reported in another unit than mg/100g FW were recalculated using an average moisture content of 77%. FW - fresh weight; DW - dry weight.
Adapted from: Burgos *et al*., 2012, 2013; Elzbieta, 2012; Evers and Deuβer, 2012; Haase and Weber, 2003; Han *et al.,* 2004; Kalogeropoulos *et al.,* 2007a; Mulinacci *et al*., 2008; Navarre *et al.*, 2010; Perla *et al*.,2012; Singh and Kaur, 2009; Tajner-Czopek *et al*., 2012.

Potatoes are rich in carbohydrates, but in comparison with other carbohydrate-rich staple foods, potatoes nutrient density is low because of their high water content, of almost 80%. Potatoes are very low in fat and, although not regarded as an important source of protein (0.6-2.1% on FW basis), they have a high biological value (90-100) (Camire *et al.*, 2009). Potatoes are also rich in essential minerals, and recent findings highlight that potatoes are an important dietary source of bioactive compounds, secondary plant metabolites that may delay the onset of chronic diseases, such as arthritis, atherosclerosis, cancer, cardiovascular diseases, diabetes, gastrointestinal disorders, and neurodegenerative dysfunctions (Camire *et al.*, 2009; Visvanathan *et al.*, 2016). These potato components include vitamin C and E, carotenoids, phenolic compounds (phenolic

acids, flavonoids, anthocyanins), among others (Singh and Kaur, 2009; Visvanathan *et al.*, 2016). A balanced ingestion of potatoes might have an essential role in human health, but it needs to be cooked to become edible, particularly due to the presence of toxic glycoalkaloids (Tajner-Czopek *et al.*, 2012), and indigestibility of its ungelatinized starch. In addition, thermal processing contributes to food safety, and is responsible for the occurrence of physicochemical changes in the product, determinant for its acceptability and nutritional quality (Stadler and Lineback, 2009). The most popular cooking methods include boiling, baking, steaming, roasting, deep-frying, microwave, depending mostly on local cultural habits. Among these, the most common processing technique worldwide is frying (Pedreschi, 2012), due to its speed and operational simplicity, and formation of unique sensorial attributes (Gertz, 2014). Therefore, the heating medium (oil) used in this technique also determines the nutrient amounts truly available to consumers. In addition, the shadow on fried food nutritional impact persists, despite the absence of clear evidences of deleterious effects of vegetable oils on cardiovascular health (Sayon-Orea *et al.*, 2015).

Many types of vegetable oils are available for frying, being the most consumed worldwide, in million metric tons (2015/2016), palm (61.0), soybean (51.4), rapeseed (canola) (27.8), sunflower (14.9), palm kernel (7.0), peanut (5.5), cottonseed (4.5), coconut (3.3), and olive oil (2.9) (Statista, 2017). Oil selection for deep-frying purposes should be based on several factors, including its ability to withstand high temperatures for prolonged times, palatability and nutritional properties. Therefore, thermo-oxidative stability is an important criterion, being largely dependent on the fatty acid composition, together with the presence of minor compounds in the unsaponifiable fraction that might delay/induce oxidation. In this sense, vegetable oils with predominantly saturated fatty acids (SFA) are usually more stable, but are still suspect of being of higher risk for cardiovascular diseases (Sayon-Orea *et al.*, 2015). On the opposed side, oils rich in polyunsaturated fatty acids (PUFA) are advantageous from the nutritional point of view, but are generally more unstable at frying temperatures (Petersen *et al.*, 2013), with a premature loss of frying performance, together with the formation of degradation compounds, whose safety stills under debate. Therefore, recommendations on oils with a balanced proportion of SFA and PUFA and a high content of monounsaturated fatty acids (MUFA), particularly oleic acid, have increased (Bastida and Sánchez-Muniz, 2015). However, while industry has increased options in this field, particularly high-oleic modified oils, as high-oleic sunflower, high-oleic soybean or more recently even high-oleic safflower oils, these products are not yet available for domestic frying. On the shelf, consumers and small restaurants have usually access to oil blends based on sunflower and soybean oils, rich in PUFA, while the oils naturally richer in MUFA are usually restricted to peanut oil, olive oil and canola oil, highly dependent on geographical availability and tradition, and usually at higher prices than the blends. In

addition, doubts on olive oil adequacy for frying persist, particularly virgin olive oil, due to the absence of refining.

Olive oil use, originally limited to the Mediterranean regions, is expanding to other countries in Northern Europe, as well as United States, Argentina, Chile, Mexico and Australia, mainly as a result its sensorial attributes and potential health benefits (Gunstone, 2002; García-González *et al.*, 2008; Boskou, 2009). Olive oil is usually obtained by cold-pressing of olive fruits, followed eventually by washing with water, filtering and centrifugation. In comparison with other common vegetable oils, obtained by solvent extraction and further submitted to diverse refining processes, this virgin-olive oil retains much of the original fruit components. Therefore, while most marketed vegetable oils are mainly triacylglycerols (TAG) mixtures, olive oil still presents other natural fruit components, as pigments, phenolic compounds and volatile compounds. In addition to the health benefits of a rich monounsaturated fat increasingly documented in the literature (Gillingham *et al.*, 2011), the presence of these minor compounds enforces an added value to virgin olive oil, increasing its potential bioactivity (Stark and Madar, 2002; Waterman and Lockwood, 2007).

Several olive oil grades are commercially available. Its classification is usually based on both sensorial attributes and chemical parameters that give a general overview on its quality and authenticity. These characteristics are regulated by several organizations, including the European Commission itself, the International Olive Oil Council (IOC), or the Codex Alimentarius established by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO). Extra-virgin olive oil (EVOO) and virgin olive oil (VOO) are the most prized olive oil grades, being consensual that their quality attributes are maximized when consumed without being subjected to any thermal treatment. Therefore, these olive oil categories should be preferably added as final seasoning in fresh salads, soups, or more elaborated dishes. Indeed, several thermal effects occur when olive oil is used as the cooking base, as in roasting, sautéing (pan-frying), stir-frying, or even deep-frying (Waterman and Lockwood 2007; Boskou, 2009). Besides, in opposition to other refined vegetable oils, these effects will also disturb olive oil minor compounds. Several authors have addressed this issue, in particular the heating effect on olive oil nutritional and biological properties. Such knowledge is essential if one intends to know the adequate temperatures for each olive oil category and processing method, the interaction with the food under cooking, the adequate rejection time or the shelf-life of the processed food, aiming the maintenance of the original nutritional features of crude olive oil.

Aware that for the preparation of potatoes in olive oil with enhanced nutritional, bioactive and biochemical features, from both components, potatoes and olive oil, have determinant parts, a review on the effect of thermal processing on potato bioactive compounds as well as on olive oil quality attributes is detailed in the next chapters.

CHAPTER 2. Effect of thermal processing on potatoes compounds

Parts of the text of this chapter were published in the following book chapter:

Santos CSP, Cunha S, Casal S (2015) Bioactive Components in Potatoes as Influenced by Thermal Processing. In *Processing and Impact on Active Components in Food*. VR Preedy (Ed). UK, Elsevier, Chapter 14, pp. 111-119. (Paper 1).

Together with the agronomic practices, genotype, or postharvest storage methods, which are known to influence the concentration of potato constituents (Singh and Kaur, 2009), subsequent thermal processing determines the amounts truly available to consumers. Therefore, this chapter will focus on a detailed discussion of the influence of thermal processing on potato nutrients, particularly its bioactive compounds, taking into account the specificities of each component.

Thermal processing is among the most common methods of food preservation and is often simultaneously a condition for consumption, as in the case of potatoes, which need to be cooked to become palatable. Therefore, while contributing for food safety, it is responsible for the occurrence of physicochemical changes in the product, determinant for consumer acceptability and, above all, its nutritional quality (Stadler and Lineback, 2009). Diverse thermal processing techniques are used, applying different temperature/times combinations, heat sources, direct/indirect food contact, etc. Besides the effect of temperature increase, the heating medium is of particular importance, as in the case of water in boiling or oil during frying, where marked exchanges are expected to occur, or forced air heating in oven cooking that can also induce severe alterations, particularly oxidation of sensitive nutrients.

From the consumer point of view, the physical changes are the more visible ones, namely colour and texture, conditioning its perception of quality. Cooked potatoes colour is the result of chemical browning, due to formation of new compounds during thermal processing, and enzymatic browning achieved before thermal inactivation of the enzymes. While the former is determined by the availability of reducing sugars, free amino acids, the extension of the thermal treatment, etc., the latter in mostly conditioned by raw potatoes pre-treatments such as blanching (Singh and Kaur, 2009; Pedreschi, 2012). The textural changes are also a visible result of heat and mass transfer, together with chemical reactions, particularly starch gelatinization, being evaluated by attributes such as firmness, hardness, softness and adhesiveness (Singh and Kaur, 2009). Chemical changes will also regulate digestibility and availability of nutrients in the final potato product: starch gelatinization, for instance, increases carbohydrate availability, while the formation of resistant starch reduces the efficiency of endogenous digestive enzymes; protein denaturation increases its digestibility and simultaneously destroys anti-nutritional factors such as trypsin inhibitors; minerals might be leached during water cooking and can react with other dietary components becoming less or even non-available (Stadler and Lineback, 2009). All these effects should be taken into account when each single food is evaluated for nutritional purposes. More than the chemical composition of raw products, the influence of each thermal process on its content and availability is determinant.

Phenolic compounds are recognized as increasingly important in the diet due to their antioxidant activity. Therefore, a particular attention will be given to this class, including phenolic acids, flavonoids, and anthocyanins. In addition, vitamin C is the major vitamin in raw potato, with highly variable amounts (Table 1.1), depending on potato variety, environmental, and storage conditions (Singh and Kaur, 2009). Despite not being among the richest sources of vitamin C, the fact that is consumed in large quantities makes it an important source of vitamin C in human diet, a fact not always recognized by consumers. Therefore, the effect of thermal processing on this vitamin is particularly important, as it will determine the ingested amounts. Vitamin E will also be discussed, based on its potential enrichment after processing with vegetable oils. Carotenoids, being particularly sensitive molecules, were also included in this revision. Finally, the cooking effect on glycoalkaloids as well as on formation of compounds potentially hazardous (i.e. acrylamide) is discussed.

2.1. Phenolic compounds

In plants, phenolic compounds main functions are associated with defence against pathogens (bacteria, fungi, and viruses). However, they also participate in enzyme-catalysed browning reactions that contribute to colour, taste, and nutritional quality of potatoes (Singh and Kaur, 2009; Perla *et al.*, 2012). In humans, ingestion of phenolic compounds is increasingly associated with a wealth of health promotion effects (Singh and Kaur, 2009). Potatoes, although not being classified as a rich source of phenolic compounds, are the third daily source of these antioxidants in the American population (Chun *et al.*, 2005). It is therefore worth acquiring an in-depth knowledge of the possible effects of thermal treatments on their contents.

Under the designation of phenolic compounds, two important classes are included: phenolic acids and flavonoids. A confusing variety of data, however, is found in the literature, mostly because of the analytical techniques used. While most studies make only a global estimation by using unspecific spectrophotometric methods, few evaluate in detail individual phenolic acids and flavonoids.

Some reported results for total phenolic compounds variations with domestic cooking are present in Table 2.1. Generally, boiling is responsible for a higher loss of phenolic compounds, particularly in peeled potatoes, probably due to leaching. The phenolic compounds are apparently preserved after baking (Navarre *et al.*, 2010) but only if processed with the peel (Perla *et al.*, 2012). The same can be discussed for microwave, but with comparatively smaller losses even if processed without peel (Navarre *et al.*, 2010, Perla *et al.*, 2012). As to frying, losses are generally low, but slightly higher than those observed in microwave (Tian *et al.*, 2016), and seem to be variety dependent, with higher preservation in red varieties than purple ones (Kita *et al.*, 2013). The fat used for frying could

also have some influence on these losses, but no data on their interaction was found. In comparison, the recent air frying systems seem to cause greater loss than classical frying, probably due to the increased processing time of the former (18 min vs 3 min, respectively) (Tian *et al.*, 2016). However, the highest source of variation is the initial amount in raw potatoes, which is directly dependent on the variety used, higher in pigmented cultivars in comparison with white ones (Mulinacci *et al.*, 2008).

When the phenolic acids are analysed individually, chlorogenic acid is clearly the main compound, followed by caffeic acid (Mattila and Hellström, 2007). However, their changes during thermal processing follow the same pattern observed for total phenols: boiled peeled potato present lower values than unpeeled ones (Mattila and Hellström, 2007); baking exhibited higher preservation (Im *et al.*, 2008), as did frying and microwave cooking (Blessington *et al.*, 2010). Boiling with salt (3%) induced higher losses of phenolic acids (Im *et al.*, 2008).

The main flavonoids in potato are anthocyanins, catechin, and epicatechin. In opposition to the observed for phenolic acids, boiling preserved flavonoids better in comparison with baking or microwaving (Perla *et al.*, 2012), providing that they are processed with the peel. Blessington *et al.* (2010) observed a general increase in all cooked samples, particularly epicatechin after microwave cooking. Only quercetin dehydrate was reduced in all methods, in comparison with the raw potatoes, but these results should be interpreted with caution as they were compared on a FW basis. Anthocyanins, in particular, are responsible for many of the blue, red and violet colours in plants, including potatoes, with a marked influence in sensorial acceptance (Singh and Kaur, 2009). In plants, they contribute to self-protection and help in chemotaxonomic characterization. To humans, the potential health benefits derive from their antioxidant activity, which plays an important role in the prevention of neuronal and cardiovascular diseases, cancer and diabetes (Lachman *et al.*, 2012). When observed on a FW basis, as reported by Mulinacci *et al.* (2008), anthocyanins decreased after processing by boiling and microwave, particularly in the latter. On a DW basis, and for a specific purple variety, Burgos *et al.* (2013) reported reduced alterations by boiling. In a more detailed study, Lachman *et al.* (2012) reported a generalized increase after unpeeled cooking, by boiling, steaming, microwaving and roasting. In this case, however, microwaving losses were comparatively higher. After thermal processing, on average, anthocyanins content were higher in red and purple varieties, and lower in white and light purple varieties (Mulinacci *et al.*, 2008; Kaspar *et al.*, 2012; Lachman *et al.*, 2012; Burgos *et al.*, 2013). Frying is responsible for a heavy degradation of anthocyanins (38-70%) (Kita *et al.*, 2013).

Table 2.1 Total phenolic compounds by potato varieties *versus* thermal processing, in mg/100g dry matter.

Potatoes varieties	Raw	Thermal Processing							References
		Boiled	Baked	Microwaved	Fried	Air-fried	Stir-fried	Steamed	
Dakota Pearl	92	80	91	87	-	-	-	-	Xu *et al*., 2009 (with skin)
Goldrosh	235	224	203	190	-	-	-	-	
Nordonna	206	173	171	125	-	-	-	-	
Norkotah	304	177	212	203	-	-	-	-	
Red Norland	209	179	180	182	-	-	-	-	
Sangre	156	106	142	122	-	-	-	-	
Viking	147	125	139	133	-	-	-	-	
Dark Red Norland	205	152	174	148	-	-	-	-	
Mesa Russet	143	71	74	75	-	-	-	-	Perla *et al*., 2012
Silverton Russet	155	71	55	60	-	-	-	-	
Colorado Rose	138	74	75	54	-	-	-	-	
Russet Nugget	94	76	58	58	-	-	-	-	
Purple Majesty	285	108	756	84	-	-	-	-	
Bintje	167	286	367	219	-	-	-	323	Navarre *et al*., 2010 (with skin)
Piccolo	170	230	278	174	-	-	183	249	
Purple Majesty	416	529	620	435	-	-	-	481	
Jesús	251	127	-	-	-	-	-	-	Tierno *et al*., 2015
Kasta	332	202	-	-	-	-	-	-	
Monalisa	154	144	-	-	-	-	-	-	
Morada	347	164	-	-	-	-	-	-	
Vitelotte	359	221	-	-	-	-	-	-	
Zamora	154	100	-	-	-	-	-	-	

Table 2.1 Continued.

Potatoes varieties	Raw	Thermal Processing							References
		Boiled	Baked	Microwaved	Fried	Air-fried	Stir-fried	Steamed	
Purple Majesty	908	600	165	321	-	-	-	565	Lemos *et al*., 2015
Unknown	191	157	-	-	129	-	138	-	Ramirez-Anaya *et al*., 2015
Gui Valley	291	-	-	267	285		-	-	Yi *et al*., 2015
Rosalinde	227	-	-	-	241	-	-	-	Kita *et al*., 2013
Herbie 26	367	-	-	-	333	-	-	-	
Highland Burgundy Red	456	-	-	-	552	-	-	-	
Salad Blue	283	-	-	-	261	-	-	-	
Vitelotte	845	-	-	-	346	-	-	-	
Valfi	227	-	-	-	232	-	-	-	
Blue Congo	271	-	-	-	174	-	-	-	
Heimeiren	1300	1208	773	1250	1117	877	358	1145	Tian *et al*., 2016

Note: contents originally reported in another unit than mg/100g dry weight were recalculated using an average moisture content of 77%.

2.2 Vitamin C

Ascorbic acid (AA) is an important organic acid in plants, with a noticeable role as a detoxifier of reactive oxygen species, particularly within photosynthesis. In humans, it is crucial for several biological functions, being involved in collagen biosynthesis, it improves the bioavailability of several micronutrients, including iron, and is a cofactor for numerous enzymes, etc. (Singh and Kaur, 2009). Dehydroascorbic acid, AA's main oxidation product, has also some biological activity, with both being designated as Vitamin C. During storage and processing, their hydrolysis to diketogulonic acid should be reduced to the minimum, as this is an inactive compound in terms of vitaminic activity.

A summary of several studies on AA changes with different thermal processing methods is present in Table 2.2. Unfortunately, most studies do not quantify dehydroascorbic acid, therefore giving reductive information on total vitamin C potential activity. When the processing methods are compared, independently of the variety, the highest losses are observed with boiling (Han *et al.*, 2004). Interestingly, when boiled with salt (3%), losses are apparently reduced (Han *et al.*, 2004), in contrast to the observations made for phenolic compounds. These losses are probably a direct consequence of leaching, because they are reduced when boiled unpeeled (Burgos *et al.*, 2009, Navarre *et al.*, 2010). Frying induces lower vitamin C losses than blanching. The absence of water leaching in the former, as water is loss mostly by evaporation, and the rapid inactivation of ascorbate oxidase enzymes contribute to this observation (Haase and Weber, 2003). However, in the recent air-frying system, losses of 90% were observed, referred by the authors to be due to both leaching during soaking and degradation during cooking process (Tian *et al.*, 2016). The thermal processes that preserved AA the most were baking (Navarre *et al.*, 2010) and microwaving (Han *et al*., 2004), similar to those achieved when potatoes are boiled with peel, but the results are variable when comparing different authors. This observation sustains that leaching, rather than temperature, should be the main detrimental factor for AA loss, and that, together with cooking, previous soaking conditions can also contribute for its loss.

Table 2.2 Ascorbic acid by potato varieties *versus* thermal processing, in mg/100g dry matter.

Potatoes varieties	Raw	Thermal Processing												References
		Blanched	Boiled	Boiled (1% salt)	Boiled (3% salt)	Baked	Braised	Microwaved	Fried	Air-fried	Stir-fried	Steamed	Pressure cooking	
Undefined	88	-	-	-	-	-	-	-	66	-	-	-	-	Haase and Weber, 2003
Undefined	95	70	-	-	-	-	-	-	-	-	-	-	-	
Dejima	56	-	7	15	24	48	30	37	10	-	35	-	33	Han *et al.*, 2004
Sumi	116	-	31	30	53	81	61	93	57	-	37	-	58	
Chaju	140	-	32	30	58	94	58	104	52	-	51	-	57	
EE-2057[#]	18	-	10	-	-	8	-	7	-	-	-	-	-	Burgos *et al.*, 2009
Rucuma o Lucuma[#]	38	-	33	-	-	19	-	9	-	-	-	-	-	
Puma Maqui[#]	28	-	21	-	-	5	-	3	-	-	-	-	-	
Morar Nayra[#]	32	-	31	-	-	21	-	13	-	-	-	-	-	
Morada Taruna[#]	33	-	22	-	-	2	-	2	-	-	-	-	-	
Maria Cruz[#]	76	-	56	-	-	24	-	18	-	-	-	-	-	
Bintje[#]	55	-	71	-	-	77	72	65	-	-	-	-	-	Navarre *et al.*, 2010
Piccolo[#]	58	-	73	-	-	71	70	67	-	-	-	-	-	
Purple Majesty[#]	44	-	52	-	-	68	57	52	-	-	-	-	-	
Heimeiren	109	-	65	-	-	31	-	101	18	10	25	83	-	Tian *et al.*, 2016

Note: contents originally reported in another unit than mg/100g dry weight were recalculated using an average moisture content of 77%.
[#]With peel.

2.3. Vitamin E

Vitamin E, the most powerful lipid-soluble natural antioxidant known (Traber and Atkinson, 2007), is the global designation given to a group of eight tocochromanols, derived from the same isoprenoid precursors as carotenoids, with important antioxidant and antiradical scavenging activities. While in photosynthetic plants they seem to have a protective effect in the chloroplasts machinery and protect plant lipids from oxidation (Traber and Atkinson, 2007), a variety of positive effects have been attributed in humans, particularly to its most active compound, *α*-tocopherol (Singh and Kaur, 2009), the main tocochromanol found in potatoes (Chun *et al.*, 2006). However, vitamin E amounts in raw potatoes are reduced (Table 1.1), as might be expected from a very low-fat food. Therefore, studies on thermal processing effects on potato native vitamin E content are scarce. Chun *et al.* (2006) reported no alterations after boiling. However, several processing methods use vegetable oils, being these among the richest sources of vitamin E. Therefore, an increase in potato vitamin E content is usually reported after frying, or for other methods with addition of vegetable oils. As an example, after shallow frying in virgin olive oil, Kalogeropoulos *et al.* (2007a) reported a significant enrichment (18%) in *α*-tocopherol comparing with raw potatoes.

2.4. Carotenoids

Carotenoids are lipid-soluble pigments naturally present in reduced amounts in potatoes, but are the mainly responsible for the flesh colour (Brown *et al.*, 2008) and therefore its natural division into white (low carotenoid content), yellow (moderate content), or orange (high content) potatoes. The carotenoid family encompasses multiple compounds, chemically divided in xanthophylls and carotenes, based on the presence or absence of oxygen in their molecules. The most abundant carotenoids in potatoes are the non-provitamin A xanthophylls, lutein and zeaxanthin, which are particularly important for eye health, with a reduced amount of β-carotene (Evers and Deuβer, 2012). Still, knowing that carotenoids are sensitive molecules, in particular to oxygen, light and heat, thermal processing should have a great influence on their amounts after cooking.

A detailed study on the effect of boiling was performed by Burgos *et al.* (2012), using potatoes from diverse coloured varieties. Independently of the initial carotenoids content in the raw potatoes, the total amount of carotenoids preserved was clearly correlated with the pulp yellow intensity. This was mostly a consequence of a reduction in violoxanthin and antheraxanthin contents, with a simultaneous increase in lutein and zeaxanthin, all on a FW basis. Still, these authors used unpeeled potatoes, contributing certainly to carotenoids protection. Blessington *et al.* (2010) compared different domestic processing methods, including boiling, frying, baking and microwave cooking, using diced unpeeled potato cubes

from different varieties. Except for boiling, again with lower amounts in some samples, no other cooking methods affected the final carotenoid content significantly. However, a carotenoid reduction was observed by frying, air-frying and stir-frying of purple-fleshed potatoes (Tian *et al.*, 2016). As to industrial processing, Kaspar *et al.* (2012) compared different drying technologies for the production of potato flakes. Generally, all processes induced higher carotenoids losses than the domestic processes, and these were more evident in drum-drying than freeze-drying, clearly due to the temperatures applied.

2.5. Glycoalkaloids

The steroidal glycoalkaloids are toxic to microorganisms, insects, animals, and, in high levels, even to humans (Camire *et al.*, 2009). Therefore, their ingestion should be avoided. Recent findings, however, have shown some beneficial effects, including reduction of blood cholesterol, protection against infection with *Salmonella typhimurium* and chemoprevention (Singh and Kaur, 2009), requiring further studies to define effects and amounts for a sustained consumer advice. Potatoes, in particular, have two glycoalkaloids, α-solanine and α-chaconine (Singh and Kaur, 2009). Upon thermal process, all studies verified a slight decrease, more visible with boiling (Mulinacci *et al.*, 2008), and frying (Tajner-Czopek *et al.*, 2012). These compounds are known to decompose only above 170°C (Takagi *et al.*, 1990). Therefore, while during frying, some destruction can occur, during boiling only leaching can justify the losses observed. Unpeeled potatoes had higher glycoalkaloid amounts (Tajner-Czopek *et al.*, 2012). Indeed, during the industrial preparation of dried potatoes granules, the highest decrease in glycoalkaloids was observed after peeling and blanching (Elzbieta, 2012).

2.6. Acrylamide

Thermal processing can provide the formation of potentially hazardous compounds, such as acrylamide. Acrylamide, initially just regarded as a common industrial chemical, was classified as a probable human carcinogen by the International Agency for Research on Cancer (WHO, 1994). However, in April 2002, Swedish researchers reported that acrylamide can be found in carbohydrate-rich foods cooked at high temperatures under low moisture conditions (Swedish National Food Administration, 2002; Tareke *et al.*, 2002).

The main pathway for the formation of acrylamide seem to involve the carbonyl group of reducing sugars and the amine group of free asparagine, through the *Maillard* reaction (Matthäus and Haase, 2014). Potato contains both precursors, enhancing the formation of acrylamide during thermal processing (Lineback *et al.*, 2012) but since the amount of reducing sugars in potatoes is largely dependent on variety and ageing, acrylamide content can be highly variable.

Processing conditions influence acrylamide content, with potato crisps having higher amounts than French fries due to higher surface/volume ratio, promoting a rapid formation during frying (Palazoglu *et al.*, 2010). Thus, the indicative value for French fries is 600 µg/kg, whereas for potato crisps is around 1000 µg/kg (Matthäus and Haase, 2014). Recent studies on potatoes processed in oven and air-frying systems showed 16% to 77% reductions (Giovanelli *et al.*, 2017; Sansano *et al.*, 2015), comparing to traditional fried potatoes. However, vacuum frying method is still the one that presented the most effective reduction - up to 94% (Moreira, 2014).

Aware that acrylamide and its metabolite glycidamide are genotoxic and carcinogenic, no level of exposure can be considered safe, and therefore, no tolerable daily intake can be imposed for acrylamide in food. European Food Safety Authority's (EFSA) experts have estimated the dose range within which acrylamide is likely to cause potential adverse effects, between 0.17 to 0.43 mg/kg body weight (bw)/day (EFSA, 2015). Therefore, the estimated mean dietary acrylamide exposure from 0.4 to 1.9 µg/kg bw per day (EFSA, 2015) gives a low margin for the most exposed consumers, and shows the relevance of fried potatoes in this acrylamide dietary panorama.

2.7. Concluding remarks

- Potatoes are an important source of bioactive components in the diet, enhanced by the elevated amounts consumed worldwide.
- Raw potato varieties have significantly different amounts of bioactive components, being this the main contributing factor for the amounts ingested after cooking, independently of the thermal method used.
- Potato bioactivity is affected by thermal processing, with boiling inducing generally higher losses than steam cooking, frying, baking or microwave cooking.
- Removal of the peel before or after cooking appears to influence the bioactive compounds in cooked potatoes, particularly in boiled ones.
- There is a generalized lack of studies dealing with potato-fat interactions during processing.

CHAPTER 3. Effect of thermal processing on olive quality attributes

Parts of the text of this chapter were published in the following paper:

Santos CSP, Cruz R, Cunha SC, Casal S (2013) Effect of cooking on olive oil quality attributes. *Food Research International*. 54(2): 2016-2024. (Paper 2).

The present chapter focuses on the most important studies dealing with olive oil heating, under laboratory simulation or, when available, under real cooking conditions, while attempting to understand the most adequate processing conditions to preserve its quality and to reduce hazard formation. Moreover, identifies those issues that require further research to reinforce olive oil correct use and to potentiate its health benefits.

In order to understand olive oil behaviour under thermal processing conditions, it is fundamental to address some considerations into its chemical composition. In a brief way, some key points must be mentioned. First, olive oil is among the vegetable oils with higher MUFA in its composition, being therefore less prone to oxidation than those with higher PUFA content. Second, virgin olive oil is not subjected to any refining process, keeping important olive fruits components, namely phenolic compounds (e.g. hydroxytyrosol, tyrosol and oleuropein), pigments (e.g. chlorophylls, carotenes), hydrocarbons (e.g. squalene), sterols, phospholipids, mono- and diglycerides, fatty alcohols, waxes, and diverse aromatic compounds, all with relevant functions in olive oil stability and flavour. Third, olive oil composition is dependent on several parameters including olive variety, edaphoclimatic conditions, harvesting period and technique, fruit ripening degree, leaf removal, extraction system, etc. (Gunstone, 2002; Firestone, 2005; Pellegrini and Battino, 2006; Frankel, 2011), making each olive oil batch unique, and increasing the difficulties when attempting to standardize experimental conditions.

As abovementioned, several olive oil grades are commercially available. Olive oils can be distinguished based on its production way and characteristics (sensorial attributes and chemical composition), regulated by diverse organizations (International Olive Council, Codex *Alimentarius*, European Commission, etc.).

Virgin olive oil is obtained solely by mechanical or physical extraction. In this process, free acidity, expressed in oleic acid, can be high due to lipases activity. Therefore, together with sensorial quality, free acidity enables the distinction between extra-virgin olive oil (EVOO) and VOO, with a maximum of 0.8% and 2.0%, respectively. When higher than 2.0%, or with sensorial defects (lampante virgin olive oil), olive oil should be refined, obtaining the refined olive oils category (ROO), not commercially available in all countries, as is the case of Portugal. By blending ROO and VOO, a new commercial category is created – Olive oil (OO) (Commission Regulation (EEC) N.° 2568, 1991 and amendments).

After thermal processing of olive oil, compositional changes are expected. The most common degradations include TAG hydrolysis and polymerization, fatty acid and sterol oxidation, *Maillard* reactions with food components, among others. These reactions are common to all vegetable oils, each one with particular rates and susceptibilities, and are generally used to predict fat degradation (Warner, 2002). Therefore, the most usual parameters when testing vegetable oils thermal behaviour include physical measurements

(colour, foam, density, etc.) and several chemical indicators such as free fatty acids (FFA), polymerization degree, saturated/unsaturated fatty acid ratios, tocopherol and other phenolic degradation.

Apart from the loss of beneficial substances during thermal treatment, a great concern regarding new formed compounds under thermal stress, including oxidized fatty acids and sterols or TAG polymers, and their possible impact on human health is rising. Nevertheless, due to its richness in other components than fatty acids, virgin olive oil represents a complex pool of possible thermal reactions, including degradation and interactions of those aforementioned substances.

The present chapter focuses on the behaviour of the most important olive oil components under thermal processing, including fatty acids and several vital minor compounds. When available, comparisons with other vegetable oils will be established.

Olive oil, similar to other vegetable oils, is used in deep-frying, pan-frying, roasting, microwave cooking, etc. (Waterman and Lockwood 2007; Boskou, 2009). Each thermal processing type has particular characteristics, namely regarding temperature and confection time. Tables 3.1 and 3.2 compile several studies with olive oil cooking, grouped by processing method. Within each group, real and simulated cooking are included and compared, being supported by the analytical parameters chosen by the authors to evaluate olive oil performance.

3.1. Frying

Frying is one of the oldest methods of food preparation. It improves the sensory quality of food by formation of aroma compounds, attractive colour, crust and texture, all highly appreciated by the consumers. However, the high level of incorporated fat is an undesirable outcome, increasing caloric intake (Echarte *et al.*, 2003; Pedreschi, 2012).

Due to the high temperature and prolonged time used on repeated frying, the oils are progressively degraded by a complex series of chemical reactions including oxidation, hydrolysis, and polymerization (Choe and Min, 2007). These reactions, however, are not equivalent for all the vegetable oils, and there is a particular concern regarding virgin olive oil since its bioactive attributes might be lost during this process, despite the high resistance of its fatty acids to thermal oxidation.

The most common frying methods are deep-frying, being the food totally immersed in hot oil, and pan-frying, when the food is cooked in a pan with little amounts of oil (Bognár, 1998; Sioen *et al.*, 2006; Boskou *et al.*, 2006). Following consumers awareness to reduce fat intake, several electrical cooking systems are being commercialized, aiming to achieve products similar to the fried ones, from the sensorial point of view, with reduced fat.

Nevertheless, while frying is clearly the most studied cooking method, data regarding these new methodologies is still scarce. Indeed, the results found in the literature, and compiled in Table 3.1, only for parameters evaluating oil quality, include deep-frying and pan-frying. Both frying methods were tested with several olive oil commercial grades, at temperatures ranging from 170 to 180°C in real frying, and from 160 to 190°C in simulated frying, i.e. being the olive oil heated without any food (Table 3.1).

Deep-frying is the most common frying method, particularly in restaurants and in the food industry. While in other cooking processes the oil is used for a reduced period of time, when deep-frying is selected prolonged use occur frequently. The general guidelines for deep-frying include references to temperature, usually up to 180°C, being the time in use conditioned by the degradation achieved, based on a clear visual and sensorial inspection or, more accurately, by the regulated limits on total polar compounds (TPC) (Bastida and Sánchez-Muniz, 2002).

The FFA content, is an important measure for assessing the suitability of vegetable oils for human consumption, correlated with the global acidity perception. FFA amounts are also directly correlated with the upper temperature limits due to their lower boiling points. Heating at 180°C increase TAG hydrolysis only slightly, both under simulated conditions, (Daskalaki *et al.*, 2009; Li *et al.*, 2016, Plard *et al.*, 2016; Roodaki *et al.*, 2016), or under real frying, even in the presence of food moisture, (Andrikopoulos *et al.*, 2002; Casal *et al.*, 2010; Abenoza *et al.*, 2015; Akil *et al.*, 2015), exceeding the maximum regulated limit for EVOO and VOO only in one reference (Plard *et al.*, 2016).

Fatty acids oxidation can be assessed by the primary oxidation products, the hydroperoxides, or by secondary ones, usually unsaturated aldehydes. The peroxide value (PV) is a standard determination for primary oxidation in vegetable oils, with maximum amounts of 20 meq/kg of active oxygen (O^2) for EVOO and VOO, and lower for the olive grades with refined olive oil, as OO and ROO (Commission Regulation (EEC) N.° 2568, 1991 and amendments), as these peroxides are reduced during refining. Most simulated studies show a PV oscillation with heating time, frequently starting with an increase and then decreasing (Daskalaki *et al.*, 2009; Plard *et al.*, 2016; Roodaki *et al.*, 2016), while some just report a global reduction (Cheikhousman *et al.*, 2005.). Knowing that these primary products are volatile and highly unstable, being converted to secondary ones, this may lead to the observation of lower PV with heating time, though fatty acids oxidation still proceed. However, in the presence of food, an increase of PV was verified for VOO (Andrikopoulos *et al.*, 2002), further confirmed for different olive oil grades and monovarietal olive oils (Casal *et al.*, 2010; Abenoza *et al.*, 2015; Akil *et al.*, 2015).

Table 3.1 Literature review on olive oil studies under deep-frying and pan-frying conditions.

Type	Food	Oil type	Heating conditions	FFA	PV	PAV	UV-Vis	FA	TPC	Phenolic compounds	Vitamin E	Antioxidant activity	Volatile compounds	References
Deep-frying	Potatoes	VOO, other	170°C; 10 sessions (1 day); NR	x	x		x	x	x	x	x			Andrikopoulos *et al.*, 2002
		EVOO, ROO	180°C; 12 sessions (2/day); NR						x	x		x		Gómez-Alonso *et al.*, 2003
		EVOO, other	180°C; 75 sessions (10/day); FR					x	x	x				Romero *et al.*, 2003
		VOO, others	170°C; 8 sessions (1 day); NR										x	Boskou *et al.*, 2006
		EVOO, VOO, OO, other	170°C; 27h (9h/day); NR	x	x	x	x	x	x	x	x			Casal *et al.*, 2010
		VOO	180°C; 40 sessions (4/day); NR					x	x	x	x			Olivero-David *et al.*, 2014
		ROO, others	160-190°C; 10 sessions (1 day); NR	x		x	x	x	x	x				Zribi *et al.*, 2014
		EVOO, other	180°C; 58-70 sessions; NR	x	x		x	x	x	x	x			Abenoza *et al.*, 2015
		EVOO, others	180°C; 9 sessions (1 day); FR	x	x	x		x			x		x	Akil *et al.*, 2015
	Frozen foods	EVOO	180°C; 20 sessions (2/day); FR or NR						x					Romero *et al.*, 2000
		OO, others	180°C; 40 sessions (2/day); FR						x					Bastida and Sánchez-Muniz, 2002
	Potatoes *vs* simulation	EVOO	180°C; 40 sessions (10/day); NR						x					Kalogianni *et al.*, 2010

Table 3.1 Continued.

Type	Food	Oil type	Heating conditions	FFA	PV	PAV	UV-Vis	FA	TPC	Phenolic compounds	Vitamin E	Antioxidant activity	Volatile compounds	References
Deep-frying	Simulated	VOO	180°C; 5-25 h					x	x	x	x			Brenes *et al.*, 2002
		EVOO, OO, others	160-190°C; 2 h							x		x		Valavanidis *et al.*, 2004
		EVOO, OO, other	180°C; 1-15 h 240°C; 2-7 h										x	Fullana *et al.*, 2004
		EVOO	170°C; 3 h		x					x	x			Cheikhousman *et al.*, 2005
		EVOO, ROO,CROO	180°C; 30-180 min		x					x				Carrasco-Pancorbo *et al.*, 2007
		VOO	180°C; 10-60 min	x	x		x			x				Daskalaki *et al.*, 2009
		VOO	180°C; 30-60 min										x	Procida *et al.*, 2009
		EVOO, others	190°C; 40 h						x				x	Uriarte and Guillén, 2010
		EVOO, VOO	180°C; 1-5 h							x		x		Goulas *et al.*, 2015
		EVOO, ROO	121°C; 10-20 min; 180-220°C; 10 min	x	x			x	x	x	x			Li *et al.*, 2016
		EVOO, VOO	180°C; 210 min	x	x	x				x				Plard *et al.*, 2016
		VOO, ROO	180°C; 8 h	x	x		x	x	x	x	x			Roodaki *et al.*, 2016
Pan-frying	Potatoes	VOO, other	180°C; 10 sessions (1 day); FR	x	x		x	x	x	x	x			Andrikopoulos *et al.*, 2002
		VOO, others	175°C; 8 sessions (1 day); FR										x	Boskou *et al.*, 2006
		VOO, others	180°C; 10 sessions (1 day);NR	x		x	x	x	x	x				Zribi *et al.*, 2014
	Vegetables	VOO	170°C; 1 session/type food; NO							x	x			Kalogeropoulos *et al.*, 2007a
	Mediterranean finfish	VOO	170°C; 1 session/type food; NO							x	x			Kalogeropoulos *et al.*, 2007b
	Simulated	EVOO	180°C; 15-60 min									x	x	Messina *et al.*, 2009

CROO – crude refined olive oil; EVOO – extra virgin olive oil; FA – fatty acid composition; FFA – free fatty acids; FR – frequent replenishment; NO – new oil; NR – null replenishment; OO – olive oil; PAV – *p*-anisidine value; PV – peroxide value; ROO – refined olive oil; TPC – total polar compounds; UV-Vis – extinction coefficients; VOO – virgin olive oil.

Still, such increases were always inferior to the regulated limits for EVOO and VOO, and significantly lower in comparison with sunflower oil, processed under the same frying conditions (Casal *et al.*, 2010). As regards to the secondary oxidation products, evaluated by the *p*-anisidine value (PAV), an increasing trend was observed in one recent simulation study with EVOO samples (Plard *et al.*, 2016), as well as in the presence of food with several EVOO and OO samples (Casal *et al.*, 2010). When sunflower oil is again used as comparison, its oxidation score after 3 h of real frying was equivalent to around 27 h of frying on all the olive oils tested (Casal *et al.*, 2010). Akil *et al.* (2015), observed that, even during short-term deep-frying tests, EVOO samples showed lower secondary oxidation than other seed oils.

The UV-Vis extinctions coefficients provide also information on the oil degradation degree, particularly on changes induced by technological processes, being important quality parameters in olive oil classifications (Commission Regulation (EEC) N.° 2568, 1991 and amendments). Increased absorbance at 232 nm (K_{232}) is indicative of formation of conjugated dienes from PUFA, while at 268/270 nm, depending on the solvent used (K_{268} or K_{270}), is associated with the formation of conjugated trienes and carbonyl compounds. This double-bond conjugation increases under simulated frying (Daskalaki *et al.*, 2009; Plard *et al.*, 2016), as well as in the presence of food (Andrikopoulos *et al.*, 2002, Casal *et al.*, 2010; Abenoza *et al.*, 2015), but frequently without a clear pattern, and also with no evident differences between olive oils grades. Again, K_{232} increase is less consistent than that of K_{270}, showing that the accumulation of secondary oxidation products is more consistently associated with thermal oxidation.

The fatty acid composition is known to be only slightly altered during fresh potato frying at 180°C, with a reduction in the PUFA amounts (Andrikopoulos *et al.*, 2002; Chatzilazarou *et al.*, 2006; Casal *et al.*, 2010). For prolonged frying (40 frying sessions), Olivero-David *et al.* (2014) observed a significant decrease of both linoleic and linolenic acids (72-76% and 33-51%, respectively), and only a little deviation on oleic acid (2-4%). However, since olive oil is mostly monounsaturated (73 to 75%), this PUFA reduction is relatively small when reported in absolute amounts. Fatty acid isomerization (cis-trans) is also known to increase under heating. Indeed, a linear trans fatty acid (TFA) increment with frying time was observed during fresh potato frying at 180°C, but with total amounts below 0.5g/100g fatty acids even after 27 h of frying (Casal *et al.*, 2010) or 75 sessions using EVOO (Romero *et al.*, 2003). However, these later authors used replenishment with fresh olive oil on each frying batch, contributing to the results achieved, as each session is refilled with a new pool of olive oil protective compounds while the degradation compounds are diluted. Globally, the main factor contributing for fatty acids alteration in the bath is the food itself, derived

from the use of pre-fried potatoes or foods rich in lipids, including fish, whose lipids may alter olive oil frying performance.

A more accurate measurement of the oxidation and hydrolysis degree can be achieved by measuring TAG fractions, usually by size-exclusion chromatography, simultaneously with the evaluation of the polymerization degree, globally accounting for the total polar compounds. These TPC represent all the glyceride-derived degradation products with higher polarity than the TAG, and comprise oxidized and polymerized TAG, and hydrolysis products as diglycerides and FFA. Their quantification is used as a global indicator of fat degradation. TAG oligomers (TAG dimers plus polymers of low molecular weight) in particular, are used in some countries to determine oil discarding, namely in Belgium, Czech Republic, German, Netherlands, as this fraction is known to be potentially toxic and actively absorbed after ingestion (Bastida and Sánchez-Muniz, 2002). TPC increase linearly with frying time, but the amounts and rates are dependent on the oil composition. Under simulated frying conditions at 180-190°C, TPC formation in olive oil was comparatively lower than for other vegetable oils with an increased unsaturation degree, supporting from 24 h to more than 33 h heating until the legal rejection point was achieved (25%) (Brenes *et al.*, 2002; Uriarte and Guillén, 2010). Within the olive oils, a marked increase in the formation of TPC is also observed for those with higher PUFA degree (Brenes *et al.*, 2002). Under real frying conditions using a low food:oil ratio, all tested olive oils presenting lower TPC amounts than more unsaturated vegetable oils, and with apparently no interference by the presence of food (Bastida and Sánchez-Muniz, 2002; Kalogianni *et al.*, 2010; Casal *et al.*, 2010; Zribi *et al.*, 2014). For higher food:oil ratios, the performance seems to be aggravated (Kalogianni *et al.*, 2010). When the TPC fractions are detailed, EVOO presents lower susceptibility to the formation of oligomers in comparison with high-oleic sunflower oil, despite the good frying performance presented by both oils (Romero *et al.*, 2003). The formation of TPC in EVOO seems to increase after depletion of the phenolic compounds, suggesting a higher susceptibility to degradation for this point forward (Goméz-Alonso *et al.*, 2003). The addition of fresh EVOO during frying, as suggested by Romero *et al.* (2000) can contribute for an enhanced antioxidant capacity. Indeed, these authors compared real frying with and without replenishment, concluding that frequent replenishment allowed a higher number of frying sessions and a significantly (p<0.001) lower thermo-oxidative alteration, particularly regarding oligomer amounts.

Among the fatty acid degradation products, the formation of cyclic fatty acid monomers is also reported. Romero *et al.* (2000, 2003) studied its formation during frying with EVOO and high-oleic sunflower oil. Linear increments with the number of frying sessions were observed, being 12% lower when fresh oil was added between frying sessions. Also, 43% higher cyclic fatty acid monomers amounts were determined in high-oleic sunflower oil,

probably due to its greater linoleic acid content, which is more susceptible to their formation than oleic acid.

Phenolic compounds are among those highly scored compounds in VOO, due to their antioxidant activity and potential health effects. These substances are eliminated during refining, being therefore absent or very reduced in commercial refined vegetable oils, as well as in the olive oil mixtures with refined olive oil. Hence, these are probably the most studied chemicals in olive oil, particularly under thermal processing, since it is important to maintain these potential bioactive attributes after processing. Some authors evaluated only total polyphenolic content, while others detailed the identity and amounts of each individual compound, giving further insights into their degradation mechanisms. Indeed, these substances are affected by thermal processing, but their loss is dependent on their chemical structure and probably on their antioxidant activity (Gómez-Alonso *et al.*, 2003).

The main components of olive oil phenolic fraction are hydroxytirosol, tyrosol and their derivatives (secoiridoids). Hydroxityrosol and their derivatives are extensively lost, with a 50% reduction in EVOO after frying fresh potatoes for only 10 min at 180°C, and almost complete degradation is observed after 6 frying sessions. In opposition, tyrosol and its derivatives are less prone to degradation, still retaining 80% of its original value even after 12 frying sessions (Gómez-Alonso *et al.*, 2003), being also present as a result of hydrolysis of high molecular weight compounds within the same chemical family. Also, knowing that hydroxityrosol presents a higher antioxidant activity than tyrosol, one could justify its intensive loss with an effective activity during frying by protecting lipids from oxidation. On the other hand, tyrosol presents comparatively lower antioxidant efficiency, being therefore more retained during frying, and presenting a linear degradation rate with the frying sessions (Gómez-Alonso *et al.*, 2003; Cheikhousman *et al.*, 2005; Carrasco-Pancorbo *et al.*, 2007; Daskalaki *et al.*, 2009; Brenes *et al.*, 2002). Nevertheless, these observations cannot exclude the olive oil initial phenolic content that, being highly different between cultivars, will regulate the antioxidant capacity under thermal stress and the residual phenolic amounts after processing (Brenes *et al.*, 2002; Casal *et al.*, 2010). Also, those olive cultivars with higher linoleic acid, as “Arbequina” (Brenes *et al.*, 2002; Allouche *et al.*, 2007), might be less indicated for intensive thermal processing methodologies, as they will consume higher amounts of antioxidants. Romero *et al.* (2003) tested the influence of adding fresh oil during thermal processing and, despite confirming polyphenol degradation, 30% of the polyphenol content remained even after 75 frying sessions. The refreshment of the olive oil antioxidants seems to promote better oil stability. Phenolic compounds have also been implicated in the reduced formation of acrylamide during potato frying in olive oil (Napolitano *et al.*, 2008) and heterocyclic aromatic amines in fried red meat (Persson *et al.*, 2003), in comparison with other vegetable oils, both important from the health point of view.

Vitamin E is also an important antioxidant in all vegetable oils. Despite being a minor compound in olive oil (Brenes *et al.*, 2002; Procida *et al.*, 2009), the higher oxidation stability of olive oil, mostly due to its low PUFA content, requires comparatively lower vitamin E amounts for effective protection. Some authors further confirm that polyphenols act as vitamin E stabilizers during olive oil heating, creating an effective balance of oxidative protection between these two antioxidant families (Pellegrini *et al.*, 2001; Valavanidis et *al.*, 2004). Vitamin E degradation rate during heating at 170°C (simulated frying) was lower than hydroxytirosol, but higher than tyrosol (Cheikhousman *et al.*, 2005) counting with up to 70% losses after 3 h heating. Brenes *et al.* (2002) also reported high vitamin E losses after 5 h at 180°C, but a clear dependency on the cultivar was denoted, with the olive cultivar "Arbequina", richer in PUFA, presenting a faster degradation rate. Indeed, after 10 h heating, only residual amounts of vitamin E were detected in this olive oil, while for "Picual" cultivar equivalent levels were only found after more than 20 h of heating. Under fresh potatoes frying (170°C), vitamin E loss is also fast, being depleted after 3 to 6 h, depending on the olive cultivar. Still, for other vegetable oils with increased tocopherol amounts, as sunflower oil, total depletion of vitamin E was perceived sooner, after only 3 h frying (Casal *et al.*, 2010). Andrikopoulos *et al.* (2002) also tested fresh potato frying at 170 °C and after 10 frying sessions only 15% of vitamin E remained.

Phytosterols are a quantitatively relevant fraction in virgin olive oil unsaponifiable compounds, with both health and technological effects. Indeed, some sterols are described as contributing for a higher protection of lipid degradation under thermal stress, particularly reducing the formation of TAG polymers (Singh, 2013). As these compounds are usually reduced by refining, unrefined oils contain higher amounts of these substances. Also, they are partially transferred to food during frying, contributing for an increased consumption of phytosterols, which presents nutritional significance (Salta *et al.*, 2008). Still, oxidized phytosterols are also generated during frying, whose amounts seem to be dependent on the sterol structure, composition of the matrix, and temperature (Tabee *et al.*, 2008; Tabee *et al.*, 2009). However, studies on this matter are scarce.

Finally, the volatile fraction, formed during the heating process, apart from being important from the sensorial point of view, is rich in lipid degradation compounds. The formation of low molecular weight volatile aldehydes has been studied under vegetable oils simulated deep-frying, olive oil included, with a clear dependence on the temperatures used, rather than frying time (Fullana *et al.*, 2004). Lower amounts of aldehydes, particularly the toxic acrolein, were invariably found in olive oil (EVOO and OO) in comparison to canola oil (Fullana *et al.*, 2004). The high oleic acid content in olive oil, together with the presence of chlorophylls, pheophytins and carotenoids, seems to contribute for a reduced acrolein formation (Procida *et al.*, 2009). More recently, Uriarte and Guillén (2010) also reported

lower amounts of toxic monoaromatic hydrocarbons, alkylbenzenes and alkenylbenzenes, in olive oil, in comparison with other vegetable oils with higher polyunsaturated acyl groups. Moreover, peroxidation of linoleic and arachidonic acid to *E,E*-2,4-decadienal, a volatile compound, has been identified and quantified in different vegetable oils during potatoes frying, with lower amounts formed VOO was used in comparison with other vegetable oils. This compound is considered to be a major mutagenic and cytotoxic substance in oil fumes (Boskou *et al.*, 2006).

Pan-frying is frequently used under domestic cooking, being characterized by a higher food:oil ratio in comparison with deep-frying. These changes induce higher olive oil degradation, with increased FFA, PV, UV-Vis readings, and TPC, and increased losses of phytosterol, phenolic compounds, and vitamin E (Andrikopoulos *et al.*, 2002; Kalogeropoulos *et al.*, 2007a; Kalogeropoulos *et al.*, 2007b; Salta *et al.*, 2008; Messina *et al.*, 2009), both with and without the presence of food (Table 3.1). Still, in comparison with other vegetable oils, the trends discussed for deep-frying remain similar, i.e. olive oil presents slightly higher FFA than the refined vegetable oils, while the latter presented higher thermo-oxidative degradation rates due to the increased PUFA content (Andrikopoulos *et al.*, 2002). The higher degradation under pan-frying conditions, observed for olive oil and other vegetable oils, can be explained by the higher food:oil contact surface, higher exposure to atmospheric oxygen, and lower temperature control under processing (Andrikopoulos *et al.*, 2002). Still, in comparison with other vegetable oils, the fried food is enriched with olive oil antioxidants, as long as the olive oil is not extensively heated (Kalogeropoulos *et al.*, 2007a; 2007b).

3.2. Roasting

Roasting with olive oil is common in both domestic and industrial food preparation in Mediterranean countries (Silva *et al.*, 2010a). This procedure requires equivalent fat amounts to pan-frying, but the process is highly prone to oxidation due to the higher surface area exposed to convention hot air and processing times. Conventional oven with air-convection heating was tested in EVOO, VOO, OO, and ROO, with temperatures ranging from 180 to 230°C, mostly under simulated heating (Table 3.2). When the primary oxidation degree was evaluated by the PV, variable results were achieved, either increasing with exposure time (Caponio *et al.*, 2003; Mahmoud *et al.*, 2009) or decreasing (Albi *et al.*, 1997a). Clearer oxidation trends were observed through K_{232}, which increased with exposure time (Albi *et al.*, 1997a; Caponio *et al.*, 2003, Allouche *et al.*, 2007; Mahmoud *et al.*, 2009; Silva *et al.*, 2010a). Still, when comparing with other vegetable oils with higher unsaturation degree, olive oil was clearly more resistant to oxidation under these processing conditions (Albi *et al.*, 1997a, Caponio *et al.*, 2003). In the presence of food, as tested by

Silva *et al.* (2010a) for potatoes and meat roasting, lower oxidation degrees were observed for both primary and secondary oxidation products, highlighting the importance of studying heating effects under real cooking conditions.

The fatty acid composition was also affected by roasting with an increase in the saturated/polyunsaturated fatty acid ratios (Albi *et al.*, 1997a; Caponio *et al.*, 2003; Allouche *et al.*, 2007; Mahmoud *et al.*, 2009). Formation of minor amounts of trans-oleic acid, inferior to 0.2g/100g fatty acids was observed for all the olive oil grades, but lower than the *trans* amount in other refined vegetable oils (Albi *et al.*, 1997a; Caponio *et al.*, 2003; Mahmoud *et al.*, 2009).

TPC increased with heating time as well, particularly the oligopolymers and oxidized TAG (Caponio *et al.*, 2002). However, lower amounts were reported by Albi *et al.* (1997a) for 2 h heating at 200°C, in comparison with Caponio *et al.* (2003) for higher temperature (230°C) and reduced processing times (45 min). The temperature achieved seems to be a determinant factor in the formation of TPC, particularly the oxidation/polymerization ratios. In the presence of food, processed at 180°C for 1 h, a minor increment of TPC was reported, and no apparent differences were found between EVOO, OO or ROO (Silva *et al.*, 2010a). In opposition, the TPC clearly increased in the vegetable oils with higher unsaturation degrees (sunflower and corn oil) being once again lower in the presence of food (Silva *et al.*, 2010a).

As to the antioxidant compounds, both α-tocopherol and polyphenol contents decreased with roasting at 180°C for 2 h (Albi *et al.*, 1997a; Albi *et al.*, 1997b), being apparently influenced by oil type (Silva *et al.*, 2010a) and olive oil cultivar (Allouche *et al.*, 2007). Allouche *et al.* (2007) further examined other important minor olive oil compounds, concluding that even after 36 h of heating in a hot air oven (180°C); EVOO preserved important compounds as phytosterols, triterpenic alcohols and acids, and squalene.

3.3. Microwave

Several studies on olive oil heating under microwave are found in the literature, inclusive for different oil categories, but only under simulated heating. On these studies olive oil is heated as is, usually for up to 15 min using domestic devices with distinct powers tested, from 500W (Brenes *et al.*, 2002; Mahmoud *et al.*, 2009; Goulas *et al.*, 2015) to 1100W (Caponio *et al.*, 2002; 2003), all at 2450 MHz (Table 3.2).

Most authors were unable to provide accurate data for the temperatures achieved, since this is difficult to measure in these devices, requiring instrumental adaptation. The most common is to measure it immediately after processing, reporting usually a lower temperature course for microwave samples than with conventional heating.

Table 3.2 Literature review on olive oil roasting, microwave heating and boiling studies.

Type	Food	Oil type	Heating conditions	FFA	PV	PAV	UV-Vis	FA	TPC	Phenolic compounds	Vitamin E	Phytosterols	Antioxidant activity	References
Roasting	Simulated	VOO, OO, others	180°C; 2 h	x	x		x	x	x	x	x	x		Albi *et al.*, 1997a, 1997b
		EVOO, OO	160-190°C; 30-120 min							x	x		x	Pellegrini *et al.*, 2001
		OO	230°C; 36 min and 45 min						x					Caponio *et al.*, 2002
		VOO, others	230°C; 45 min	x	x		x	x						Caponio *et al.*, 2003
		EVOO	180°C; 2-36 h		x		x	x		x	x	x		Allouche *et al.*, 2007
		EVOO, ROO	200°C; 3-30 min		x		x	x						Mahmoud *et al.*, 2009
		EVOO, VOO	180°C; 45 and 90 min							x			x	Goulas *et al.*, 2015
	Real	EVOO, VOO, others	180°C; 1 h			x	x		x	x	x		x	Silva *et al.*, 2010a
Microwave heating	Simulated	VOO, OO, others	500W; 120 min	x	x		x	x	x	x	x	x		Albi *et al.*, 1997a, 1997b
		EVOO	750W; 10 min	x	x		x	x	x					Cossignani *et al.*, 1998
		VOO	500W; 5-10 min						x	x	x			Brenes *et al.*, 2002
		OO	1100W; 12 min and 15 min						x					Caponio *et al.*, 2002
		VOO, others	1100W; 15 min	x	x		x	x						Caponio *et al.*, 2003
		EVOO, OO, PoO	750W; 1.5-15 min	x	x	x				x				Cerretani *et al.*, 2009
		EVOO, ROO	500W; 3-30 min		x		x	x						Mahmoud *et al.*, 2009
		EVOO, VOO	1000W; 1-15 min	x	x		x				x			Malheiro *et al.*, 2011
		EVOO, VOO	500W; 5 min							x			x	Goulas *et al.*, 2015
Boiling	Simulated	VOO	109°C; 30 min					x		x	x			Brenes *et al.*, 2002
		EVOO, VOO	100°C; 40, 60 and 80 min							x			x	Goulas *et al.*, 2015
	Real	EVOO, OO	100°C; 60 min			x	x			x	x		x	Silva *et al.*, 2010b

EVOO – extra virgin olive oil; FA – fatty acid composition; FFA – free fatty acids; OO – olive oil; PAV – *p*-anisidine value; PV – peroxide value; PoO – pomace oil; TPC – total polar compounds; UV-Vis – extinction coefficients; VOO – virgin olive oil

Mahmoud *et al.* (2009) described temperatures around 200°C (500W, 15 min), Albi *et al.* (1997a; 1997b) of 170±10°C (120 min; 500W) and 206±8°C for 15 min at 1100W (Caponio *et al.*, 2003). However, Cerretani *et al.* (2009) presented significantly higher and alarming temperatures (720W; 15 min; 313°C). The effect of this heating procedure will be discussed on the basis of the chemical parameters evaluated, as compiled in Table 3.2.

Olive oil oxidation was evaluated by all authors, commonly using fast methods, as PV, PAV and UV-Vis readings. As regards to PV, Albi *et al.* (1997b) found a minor increase in VOO and OO, while Mahmoud *et al.* (2009) observed a higher increment in EVOO compared to ROO. Malheiro *et al.* (2009) verified a small increase in EVOO and VOO up to 10 min (1000W), increasing above 20 meq/kg at 15 min for VOO. Both Cossignani *et al.* (1998) and Caponio *et al.* (2003), reported significant increases in the PV with microwave heating, being equivalent to the ones observed with conventional heating (230°C) but still half of those achieved with sunflower or peanut oil heated under the same processing conditions. Notwithstanding, Cerretani *et al.* (2009), observed a PV decrease in the first 6 min. followed by constant values thereafter. Indeed, PV is not a good index for the measurement of oxidation because hydroperoxides are unstable on heating at high temperatures, due to the reduction of oxygen availability and changes in the oxidation reaction balance towards a greater formation of secondary oxidation products (Cerretani *et al.*, 2009), as previously discussed. Also, in accordance to Tan *et al.* (2001), the formation of hydroperoxides is higher at low-power settings than in the medium and high-power settings, supporting Cerretani *et al.* (2009) outcomes. On the contrary, PAV, which measures secondary oxidation products in a stable way, is a better indicator of the fatty acid oxidation. Indeed, Cerettani *et al.* (2009) found a generalized increase for all the olive oil categories. Both K_{232} and K_{270} are kept constant during the first 5-10 min, increasing significantly afterwards (Malheiro *et al.*, 2009; Mahmoud *et al.*, 2009). For standard cooking times, no absorbance differences were verified after microwave and conventional heating (Caponio *et al.*, 2003). For increased processing times, outside the usual cooking ranges, higher outcomes were reported for microwave heating (Albi *et al.*, 1997a). Still, when comparing with other vegetable oil, once again the figures are lower for olive oil (Caponio *et al.*, 2003).

Regarding fatty acid composition, a reduction of the unsaturated fractions (MUFA and PUFA) is observed (Cossignani *et al.*, 1998; Caponio *et al.*, 2003; Mahmoud *et al.*, 2009) and an apparent increase in the *trans* fatty acids content. These values, however, were similar to the ones observed with conventional heating (Albi *et al.*, 1997a; Caponio *et al.*, 2003; Mahmoud *et al.*, 2009), and lower than the ones reported for sunflower oil, high-oleic sunflower, peanut oil and lard. Cossignani *et al.* (1998) further detailed the fatty composition of each glyceride fraction with no significant alterations. Still, the stereospecific analysis of

the TAG fraction revealed that the fatty acids in the *sn*-2-position were more stable towards oxidation, which is of important nutritional significance.

TAG hydrolysis under simulated cooking in microwave are usually reduced, as reported by Albi *et al.* (1997a). Malheiro *et al.* (2009) tested two EVOO and one VOO, also describing minor FFA increases up to 15 min heating (1000W). Cerretani *et al.* (2009) compared EVOO, OO and pomace oil (PoO) and observed that the FFA content was quite constant up to 10 min (720W), increasing significantly thereafter, particularly for EVOO. Moreover, the authors stated that the water content in EVOO, due to the absence of refining and probably also some enzymatic activity, could promote TAG hydrolysis at temperatures higher than 300°C, being this out of the usual cooking range. Still, as mentioned, all these studies were performed under simulating conditions, in the absence of food. Therefore, depending on food moisture and composition, an increase in the hydrolysis degree can probably be expected, as well as lower effective temperatures due to water evaporation during the cooking process. Real cooking studies are then mandatory for effective conclusions.

Regarding oxidation, Caponio *et al.* (2002) observed a higher oxidation degree by microwave heating than by conventional heating, simulating heating times for a pizza cooking at 1100W (15 min). TPC as high as 26.8% were achieved (203°C), in opposition to 18.6% under 45 min of conventional heating (230°C). This increase occurred at expenses of TAG oligopolymers and oxidized TAG, both significantly higher in microwave heating. This fact confirms the previous results obtained by Albi *et al.* (1997b), with higher TAG modification in microwave heating in comparison with conventional, particularly polymerization. Brenes *et al.* (2002), using EVOO and only 10 min heating at 500W, found a lower TPC increase (up to 6%), indicating that power rather than heating time influences TAG degradation. In comparison with other vegetable oils, TPC verified in olive oil (VOO and OO) were lower than those obtained for sunflower oil and high-oleic sunflower oil (Albi *et al.*, 1997b).

Olive oil phenolic compounds were also evaluated by some authors. Generally, minor losses were reported after microwave heating for a short processing period. Brenes *et al.* (2002), using EVOO and gentle processing conditions (10 min, 500W), reported lower losses than those achieved during frying. In opposition, a huge decrease on total polyphenols in VOO and OO (>85%) was reported by Albi *et al.* (1997b), for prolonged heating (120 min, 500W). Cerretani *et al.* (2009), reported similar decreases for EVOO and VOO, particularly for more than 6 min heating at 720W (>255°C). These observations suggest that, for prolonged/intense microwave cooking, lower olive oil grades could present economic advantages, since most phenolic compounds are degraded, but effective studies with food are mandatory for supported conclusions.

Microwave heating also causes a decrease in olive oil *α*-tocopherol (Brenes *et al.*, 2002), or its complete disappearance (Albi *et al.*, 1997b; Malheiro *et al.*, 2009). The same pattern is observed for chlorophylls and carotenoids, particularly for more than 10 min heating (1000W) (Malheiro *et al.*, 2009). The sterolic fraction was not altered but a slight reduction in squalene (<27%) was observed by Albi *et al.* (1997a).

In general, the results point into an apparent higher oxidation when compared with conventional heating, despite being probably lower than those achieved with other vegetable oils (Malheiro *et al.*, 2011). Still, as previously mentioned, all studies were performed without the presence of food, requiring real processing conditions for correct inferences.

3.4. Boiling

Studies dealing with the behaviour of olive oil under water boiling conditions are scarce as well (Table 3.2). In several countries there is a common practice of cooking vegetable in water in the presence of small amounts of olive oil. These include soup preparation and stewing, using temperatures around 100°C, or a little higher while using pressure cookers, but lower than all the aforementioned methods. Therefore, rather than the oxidative stress imposed by temperature, hydrolytic reactions should be expected by the presence of water, as well as leaching of water soluble components.

Brenes *et al.* (2002) used VOO to simulate several cooking conditions, including boiling. Three different pHs were tested (4, 5 and 6) in order to simulate the usual pH of vegetables, and olive oil was added at 2.4% (m/v). After cooking for 30 min, the water and oil phases were analysed separately for phenolic compounds by HPLC. The authors confirmed hydrolysis of hydroxytirosol and tyrosol aglycons, as well as the migration of hydrophilic polyphenols from the oil into the water phase. Still, no losses of total phenolic compounds (water plus oil) were observed at pH 6, but a clear degradation was visible for lower pH values. Therefore, boiling acidic vegetables in the presence of olive oil might reduce its phenolic content. Still, one cannot forget that the vegetables themselves also have phenolic compounds, and these will inevitable diffuse into the boiling water and possibly interact. The tocopherol content and fatty acid composition were also evaluated with no significant changes, indicating that olive oil is not oxidized during water boiling.

Silva *et al.* (2010b) complemented the abovementioned study using real cooking conditions, with vegetables (potatoes, carrots and onions), 2% and 5% of each EVOO and OO. Olive oil was added in the beginning of the cooking process or just at 15 min from completion. Maybe unexpectedly, the presence of the vegetables in the cooking water increased polyphenols loss dramatically. The authors suggested that this loss could be a consequence of the presence of metals (Fe and Cu) in some vegetables. These losses

were also dependent of processing time, being advantageous to add the olive oil only at 15 min from conclusion. An interesting observation from these authors was that EVOO, despite presenting more phenolic compounds, showed similar amounts to OO after processing with vegetables. This can question the benefits of using EVOO in vegetables boiling processes. As to fat oxidation, the authors evaluated primary oxidation products by the K_{232}, secondary ones by PAV, and tocopherol content, concluding, similarly to Brenes *et al.* (2002) that fat was not oxidized and kept its antioxidant properties.

3.5. Concluding remarks

- By selecting a particular olive grade, such as extra-virgin, virgin or refined olive oil, distinct starting compositions may be achieved, particularly regarding bioactive and antioxidant compounds, with a direct effect on the thermal performance and final nutritional value of cooked food.
- The different cooking practices, from common frying to boiling and including microwave cooking, along with operating conditions, as time, temperature and food amounts, undoubtedly modify the olive oil chemical profile and hence, its bioactivity, but most studied deal only with simulated heating, disregarding the potential effects of food-oil interactions.
- Olive oil performance under prolonged thermal processing is usually equal or superior to other refined vegetable oils, due to its balanced composition regarding both major and minor components.
- In order to preserve virgin olive oil bioactive components, heating time should be reduced to the minimum while frequent replenishment under prolonged thermal processing can be advised.
- Future studies dealing with thermal degradation paths of minor olive oil components and their implication in human health will be of particular value to further clarify this issue.

CHAPTER 4. Background and aim of study

Based on the points discussed in the previous chapters, it has been found that potatoes are among the most widely consumed vegetables, being rich in carbohydrates and important bioactive compounds, including vitamins and antioxidants. The most common processing technique is frying and, in the Mediterranean countries, it is common to use olive oil. Despite its recognized thermal resistance, doubts about virgin olive oil adequacy for frying persist, both in comparison to other MUFA-rich oils and vegetable oils widely consumed, or even among olive oil categories commercially available. In addition, studies on the technological, nutritional and bioactive potentialities of olive oil processed potatoes are scarce, in particular using microwave and low-fat frying systems.

For these reasons, the **main aim** of this thesis was **to improve the nutritional quality and food safety of olive oil processed potatoes**, aiming to preserve or enhance potato's bioactive compounds, reduce olive oil degradation and formation of potentially hazard compounds (i.e. acrylamide), while granting consumer acceptability and clarifying virgin olive oil suitability for thermal processing.

To achieve this overall objective, the following **objectives** were proposed:

1. **To investigate the influence of prolonged potatoes frying on monounsaturated-rich vegetable oils, including olive oil, from a nutritional, quality and safety points of view**

1.1. To compare the behaviour of olive oil vs similar vegetable oils under prolonged, in order to elucidate consumers on their thermal resistance and safety;

1.2. To study the nutritional and safety impact on potatoes composition after frying, in order to perceive nutritional gains and losses based on oil choice.

In order to achieve the abovementioned tasks, it was needed:

- To optimize an HS-SPME/GC-MS method to evaluate several volatile families patterns and amounts in vegetable oils, before and after potatoes deep-frying;
- To develop and validate a fast and environmentally friendly method for quantification of acrylamide contents in processed potatoes by GC-MS.

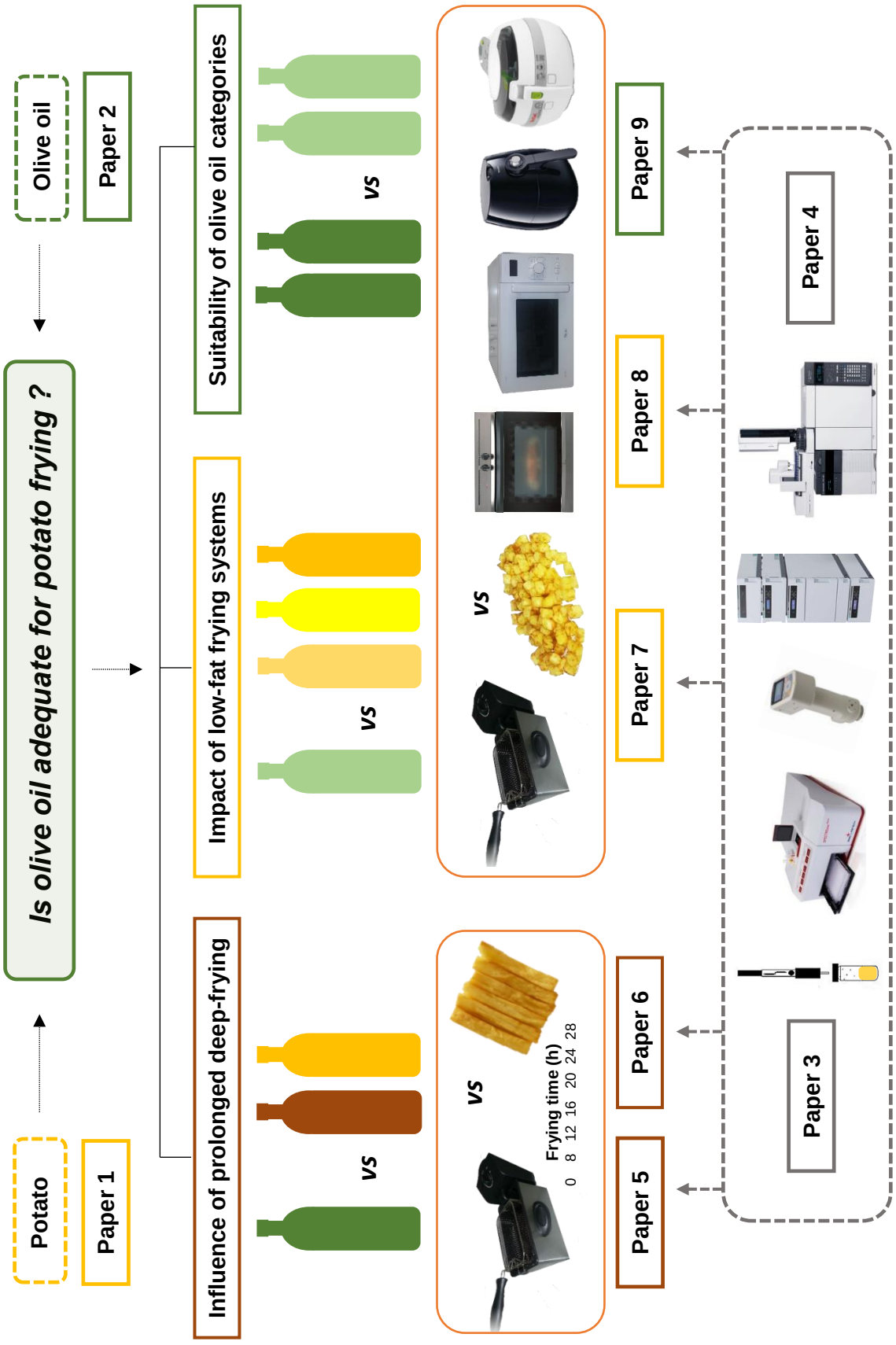
2. **To understand the impact of low-fat frying systems on potato quality**

2.1. To compare the nutritional and sensorial quality of fried potatoes by classical deep-frying, with low-fat alternatives (air-frying, microwave-grill, and oven-frying), using four of the most common vegetable oils worldwide (soybean, sunflower, canola, and olive oil), in order to understand the potential nutritional and health impact of each "frying" choice.

3. To understand the suitability of using different olive oil categories for diverse potato cooking techniques

3.1. To compare the nutritional and sensorial quality of fried potatoes from classical deep-frying and low-fat alternatives, using different commercial olive oil categories.

EXPERIMENTAL DESIGN


Potato
Olive oil
Is olive oil adequate for potato frying ?
Paper 1
Paper 2
Influence of prolonged deep-frying
Impact of low-fat frying systems
Suitability of olive oil categories
vs
vs
vs
vs
vs
Frying time (h)
0 8 12 16 20 24 28
Paper 5
Paper 6
Paper 7
Paper 8
Paper 9
Paper 3
Paper 4

PART II

MATERIALS AND METHODS

CHAPTER 5. Reagents and standards

Reagents and standards used in this thesis were purchased in diversified suppliers, and are presented in the Table 5.1 and Table 5.2. Ultrapure water (0.054 μS/cm) was supplied by a “Seral” system (SeralPur Pro 90 CN, Germany).

Table 5.1 Reagents.

Reagents		Company
1,2-dichloroetane	Chromatographic Grade	Sigma-Aldrich, USA
1,4-dioxane	Chromatographic Grade	Sigma-Aldrich, Germany
2,2-diphenyl-1-picrylhydrazyl radical (DPPH)	Analytical Grade	Sigma-Aldrich, Germany
Acetic acid (glacial)	Analytical Grade	Diverse suppliers
Acetone	Analytical Grade	Diverse suppliers
Anisidine	Analytical Grade	Merck, Germany
Butylated hydroxytoluene (BHT)	Analytical Grade	Sigma-Aldrich, USA
Chloroform	Analytical Grade	Pronalab, Portugal
Cyclohexan	Analytical Grade	Carl Roter, Germany
Diethyl ether	Analytical Grade	Sigma-Aldrich, Spain
Diethylene glycol	Analytical Grade	Sigma-Aldrich, Germany
Ethanol	Analytical Grade	Diverse suppliers
Ethyl acetate	Analytical Grade	Merck, Germany
Ethyl acetate	Chromatographic Grade	Fluka, Germany
Folin-ciocalteu	Analytical Grade	Merck, Germany
Heptane	Chromatographic Grade	Sigma-Aldrich, Germany
Hexane	Analytical Grade	Pronalab, Portugal
Hexane	Chromatographic Grade	Merck, Germany
Hydrochloric acid (HCl – 32%)	Analytical Grade	Sigma-Aldrich, Germany
Isooctane	Analytical Grade	Diverse suppliers
Metaphosphoric acid	Analytical Grade	Merck, Germany
Methanol (MeOH)	Analytical Grade	Atom Scientific, UK
Methanol	Chromatographic Grade	Sigma-Aldrich, USA
Petroleum ether	Analytical Grade	Panreac, Spain
Potassium carbonate (K_2CO_3)	Analytical Grade	Panreac, Spain
Potassium chloride (KCl)	Analytical Grade	Panreac, Spain
Potassium hydroxide (KOH)	Analytical Grade	VWR, Belgium
2-Propanol	Analytical Grade	Sigma-Aldrich, Germany
Sodium acetate (CH_3COONa)	Analytical Grade	Sigma-Aldrich, Germany
Sodium bicarbonate ($NaHCO_3$)	Analytical Grade	Sigma-Aldrich, USA
Sodium carbonate (Na_2CO_3)	Analytical Grade	Merck, Germany
Sodium chloride (NaCl)	Analytical Grade	VWR, Belgium
Sodium sulphate anhydrous (Na_2SO_4)	Analytical Grade	Sigma-Aldrich, Germany
Tetrahydrofuran (THF)	Chromatographic Grade	Merck, Germany
Tris-(2-carboxyethyl)-phosphine-hydrochloride (TCEP)	Analytical Grade	Carl Roter, Germany
Tween 80	Analytical Grade	Panreac, Spain
Xanthydrol	Analytical Grade	Sigma-Aldrich, Switzerland
α-linoleic acid	Analytical Grade	TLC, Japan
β-carotene	Analytical Grade	Sigma-Aldrich, USA

Table 5.2 Standards.

Standards	Company
1,2,3-trichloropropane	Sigma-Aldrich, USA
“0.0” and “4.0” (FOS)	Sigma-Aldrich, USA
2-methyl-2-(4,8,12 trimethyltridecyl) chroman-6-ol (tocol)	Matreya, USA
4-methyl-2-pentanol	Sigma-Aldrich, USA
Acrylamide	Sigma-Aldrich, Germany
Acrylamide $^{13}C_3$-labeled (AA-$^{13}C_3$)	Cambridge Isotope Laboratories, USA
Fatty acids methyl esters (FAME), diverse	Supelco, USA
TraceCERT, Supelco 37 Component FAME Mix CRM47885	Supelco, USA
Gallic acid	Sigma-Aldrich, USA
L-ascorbic acid	Sigma-Aldrich, USA
Monostearin	Sigma-Aldrich, USA
Tocopherols, diverse	Sigma-Aldrich, Germany
Triundecanoate	Sigma-Aldrich, USA

CHAPTER 6. Analytical methods

Parts of the text of this chapter were published in the following papers:

Molina-Garcia L, Santos CSP, Cunha SC, Casal S, Fernandes JO (2017) Comparative Fingerprint Changes of Toxic Volatiles in Low PUFA Vegetable Oils Under Deep-Frying. *Journal of the American Oil Chemists' Society*. 94(2):271-284. (Paper 3).

Molina-Garcia L, Santos CSP, Melo A, Fernandes JO, Cunha SC, Casal S (2015) Acrylamide in Chips and French Fries: a Novel and Simple Method Using Xanthydrol for Its GC-MS Determination. *Food Analytical Methods*. 8(6):1436-1445. (Paper 4).

The methods used in this thesis and its specifications are described below.

6.1. Sensory analysis

A quantitative descriptive analysis was conducted to characterize and differentiate the sensorial attributes of fried potatoes in the different cooking techniques and oils.

Voluntary participants of the Faculty of Pharmacy of University of Porto composed the sensory panel. Selection criteria included experience in diverse sensory analysis; availability; health status; food habits; and interest to participate in the study. Thus, all participants were familiar with the correct identification and assessment of the intensity of basic aqueous solutions, such as sweet, sour, salty, bitter and astringent. However, the sensory panel were trained in three pre-sessions before each assay.

The first training session was focused on identification of sensory vocabulary for potatoes capable of describing the perceptions as objectively as possible each attribute, using a 10-cm unstructured linear scale (Troncoso *et al.*, 2009). In the two subsequent training sessions the assessors verified the need to remove or include attributes, and to become familiarized with the scale and samples. In all sessions, samples were identified with random order and water was provided for assessors to clean their palates between samples. The attributes were evaluated individually by the following order: appearance, smell, take the first bite and then further chew the product, and finally assess sensations after swallowing. Overall assessment, named acceptability, was also included. Analysis of variance of data collected from the last part of training indicated that the sensory panel had ability to differentiate the products based on the defined attributes.

Finally, the evaluation of samples was performed after 3 to 5 min of being removed from each cooking device, while still hot. The samples were presented to the assessors on white plates, marked with three random digits, and the analyses took place as described above. Plates were arranged in randomized order. Responses were collected on evaluation sheets, and the specific characteristics of each assay are detailed in Table 6.1.

Table 6.1 Sensory analysis specifications by assay.

	To achieve objective 1.2	To achieve objectives 2 and 3
Sensory panel	12 assessors 10 females and 2 males	10 assessors 8 females and 2 males
	Age range of 23 to 55	
Training session	Deep-fried potatoes in fresh and used vegetable oils	Air-fried, deep-fried, microwave and oven-fried potatoes
Appearance	Colour intensity Colour homogeneity	
Odour	Odour quality Odour intensity	
Taste	Taste quality Aftertaste	
Texture	Crispiness Firmness	Adhesiveness Graininess
Evaluation session	Three samples by session Mid-afternoon (around 16:30 h)	Four samples by session Mid-morning (around 10:30 h) Mid-afternoon (around 16:30 h)

6.2. Colour

Colour was measured with a Minolta CR-400 colorimeter (Konica Minolta Optics Inc., Japan), after calibration with a white reference. Standard illuminant D6 and colour space system CIE L*a*b* were chosen to represent colour coordinate values. *L** value represents lightness-darkness dimension (0 to 100), *a** value represents red-green dimension (-120 to 120), and *b** value represents yellow-blue dimension (-120 to 120). The samples were measured under light protection due to possible interferences. Moreover, from values of colour coordinates some parameters were estimated, such as colour change (∆E) = $\sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}}$, chrome (C*) = $\sqrt{a^{*2} + b^{*2}}$, and browning index = [100(x − 0.31)]/0.172, where x = (a^* + 1.75L^*)/(5.645L^* + a^*−3.012*b**), in accordance with Yost *et al.* (2006).

6.3. Gravimetric methods

6.3.1 Moisture

Moisture was determined in potatoes by infrared drying at 105°C (Scaltec, SMO 01 model, Germany). Around 4.5 g of sample was spread into an aluminium dish and submitted to drying cycle until constant weight, being expressed in g/100g of potatoes.

6.3.2 Lipids

Two methods were used to extract lipids from potatoes, for quantification and further analysis of several compositional parameters.

Classical hot-solvent method was used using an automatic Soxhlet device (Büchi Extraction System, B-811 model, Switzerland), and petroleum ether (40-60°C) with BHT (0.01%) (AOAC, 2005). The extraction occurred from 10 g of samples during 6 h, except in raw potatoes (8 h). Total fat was estimated gravimetrically after solvent evaporation and drying under nitrogen stream (60ºC), being expressed in g/100g of potatoes, both as FW or DW. Lipids were further preserved in amber glass vials at 4ºC for fatty acid analysis and vitamin E profile and content, as will be described below.

Aiming to improve the extraction method for further analysis of sensitive oxidation parameters, a cold method was used to achieve objectives 2 and 3, according to Cruz *et al.* (2013), with minor modifications. Briefly, 10 g of homogenized fried potatoes were extracted with propan-2-ol (8 mL) and cyclohexane (10 mL), in the presence of two antioxidants: BHT (50 µL; 10 mg/mL in methanol) and ascorbic acid (50 mg). After a reduced period of manual vortexing, samples were preserved overnight under refrigeration.

The non-lipid compounds were removed by washing with aqueous potassium chloride (0.9%). After centrifugation, the upper phase (cyclohexane) was transferred to amber glass vials and the sample was further extracted with a second portion of cyclohexane. The combined supernatants were evaporated under a nitrogen stream (60°C). Again, extracted lipids were stored in amber glass vails closed under a nitrogen stream, at 4°C, until further analyses.

6.4. Dielectric constant method

Total polar compounds during the frying experiments were estimated based on the dielectric constant changes, as frequent in several restaurants and industrial frying facilities, controlling up to oil recommended disposal point (25%). A Food Oil Sensor (FOS, C-CIT Sensors AG, Switzerland), after calibration with a “zero” and “4.0” control, according to the manufacturer operation guide, was used. The true amount of total polar compounds was latter quantified by liquid-chromatography, as detailed below.

6.5. Spectrophotometric methods

6.5.1 Antioxidant Activity

For *in vitro* antioxidant activity estimation, samples extracts were prepared according to Pérez-Jiménez *et al.* (2008), using acidic methanol:water (50:50, v:v, 20 mL) followed by acetone/water (70:30, v/v, 20 mL) for potatoes (1.5 g), and direct dilution in ethyl acetate (1:3, m/v).

Gallic acid was used as reference on all assays, in general within the 0.125 to 250 µg/mL range ($R^2 > 0.998$). Results were expressed in mg Gallic acid equivalents (GAE) *per* 100g or kg for potatoes or vegetable oils.

Total reducing capacity, as a broad estimation of total phenolics, was determined by the colorimetric Folin-Ciocalteu method, adapted to 96-well microplates (Wu *et al.*, 2012), and applied to achieve objectives 1, 2 and 3. Briefly, 25 µL of extract or standard solutions were mixed with 125 µL of 10% Folin-Ciocalteu reagent in Eppendorf's, incubated for 8 min at room temperature in the dark, followed by addition of 125 µL of 7.5% Na_2CO_3 aqueous solution. After homogenization and incubation in the dark for further 30 min at room temperature, Eppendorf's were centrifuged (18000g, 5 min; Eppendorf, 5810R model, Germany) and transferred to 96-well microplates for readings at 765 nm using a UV-Visible microplate reader (BMG Labtech, Sprectrostar nano model, Germany). Blanks and standard solutions were prepared with the same solvents used for the preparation of extracts.

For the radical scavenging activity, extracts were analysed for their capacity to scavenge the stable 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (Wu *et al.*, 2012), applied to achieve objectives 1 and 2. Concisely, 40 µL of extract or standard solution were mixed with a methanolic solution of DPPH (0.065 mM; 200 µL), homogenized and incubated in the dark for 30 min at room temperature, centrifuged (18000g, 5 min; Eppendorf, 5810R model, Germany) and transferred to 96-well microplate for absorbance recordings at 517 nm. Again, blanks and standards solution were adjusted to the solvents used.

The *β*-carotene/linoleic acid bleaching assay was also applied to achieve objective 1 (Fukumoto and Mazza, 2000). Briefly, 20 mg of linoleic acid, 200 mg of Tween 80 and 1 mL of *β*-carotene solution (0.4 mg/mL in chloroform stabilized with ethanol), were vortexed for 2 min, and chloroform removed using a stream of nitrogen for 30 min. A total of 50 mL of air-sparged demineralized water (during 30 min) was then added to the mixture, which was vortexed to form a clear solution. In a 96-well microplate, 20 µL of each solvent (control), sample or standard solution plus 200 µL of the prepared β-carotene solution were added. The assay was monitoring at 470 nm using the abovementioned microplate kinetic reader, at 45°C, for every 10 min up to 60 min, with 5 min agitation before each reading.

6.5.2 Carotenoids

Carotenoids in potatoes (2.5 g) and vegetable oils (1.0 g) were extracted with acetone:hexane (40:60, v:v), and the optical density of the supernatant was measured at 663 nm, 645 nm, 505 nm and 453 nm (Shimadzu, UV-1800 model, Japan). The carotenoids content was estimated by following formula: β-carotene (mg/100 ml) = 0,216 x A663 - 1,220

x A645 - 0,304 x A505 + 0,453 x A453, according to Nagata and Yamashita (1992), being the results expressed in µg/100g of potatoes or vegetables oils or mg/kg of vegetable oils.

6.5.3 Anisidine value

Total contents of secondary oxidation products in vegetable oils, namely *α*- and *β*-unsaturated aldehydes, were estimated by the PAV, according to ISO 6885:2006 (2006). Anisidine crystals were prepared according to the same standard, as well as the anisidine solution in acetic acid at 0.25g/100mL. The amount of sample used in the assay was first tested to get absorbance from 0.1 to 0.7 at 350 nm (Shimadzu, UV-1800 model, Japan). Under this standard, the anisidine value has no dimensions, being equivalent to one hundred times the increase in absorbance, measured at a wavelength of 350 nm in a 10 mm cell, of a test solution when reacted with *p*-anisidine under the test conditions specified.

6.5.4 Extinction coefficients

Extinction coefficients at 232 nm and 270 nm (K_{232} and K_{270}, respectively) were determined according to analytical method described in the Commission Regulation (EEC) N.° 2568 (1991) and amendments. Briefly, an accurate amount of sample is dissolved in isooctane, and its absorbance read at 232 nm and in the 270 nm region (Shimadzu, UV-1800 model, Japan). ΔK was also calculated on the basis of the same method.

6.6. High Performance Liquid Chromatographic methods

6.6.1 Total ascorbic acid

Total ascorbic acid was analysed in potatoes by reversed-phase HPLC, after reduction of dehydroascorbic acid to ascorbic acid, with minor adjustments from literature methods (de Velde et al., 2012; Chebrolu et al., 2012). Briefly, 2.0 g of sample were extracted with TCEP (2.5 mM), containing 3% of metaphosphoric acid and 8% of glacial acetic acid, under light protection. Methanol was added to the extracts, followed by centrifugation (18000g, 0°C, 5 min; Eppendorf, 5810R model, Germany), and analysed up to 48 h after extraction, according to the chromatographic conditions presented in Table 6.2.

Quantification was based on external calibration standard with *L*-ascorbic acid, subjected to the entire extraction procedure, with linear dynamic range from 5 to 75 µg/mL ($R^2 > 0.999$) to achieved objective 1.2, and from 3 to 16 µg/mL ($R^2 > 0.999$) to achieved objectives 2 and 3. The results were expressed in mg/100g of potatoes.

Table 6.2 Chromatographic conditions for total ascorbic acid analysis.

	Total ascorbic acid
Equipment	Gilson (France) or Jasco (Japan)
Detector	DAD λ 266 nm Varian Prostar (USA) or Jasco MD-2015 PLUS (Japan)
Column	C18 Spherisorb ODS-2 (Waters, 3 µm, 150 x 4.6 mm, Ireland)
Mobile phase	Gradient - A: Acetate buffer (30 mM) and B: Aqueous methanol (30:70, v/v)
Flow rate	0.6mL/min, room temperature (RT)

6.6.2 Tocopherols

Tocopherols were determined in an accurate sample of vegetable oils and lipid extracts (6.3.2.) of potatoes by normal-phase HPLC, based on the ISO 9936:2006 (2006), with the additional use of an internal standard (IS) - tocol. The chromatographic conditions are detailed in Table 6.3.

Table 6.3 Chromatographic conditions for tocopherols analysis.

	Tocopherols
Equipment	Jasco (Japan)
Detector	FD λexc. 290 nm/ λem. 330 nm Jasco FP-2020 Plus (Japan)
Column	Supelcosil LC-SI (Supelco, 3 µm, 75 x 3.0 mm, USA)
Mobile phase	Isocratic: Hexane/1,4-Dioxane (97.5:2.5, v/v)
Flow rate	0.7 mL/min, RT

Individual calibration curves were prepared for each individual tocopherol standard, with mean linear dynamic range from 1 to 39 µg ($R^2 > 0.998$) to *α*-tocopherol, from 1 to 35 µg ($R^2 > 0.999$) to *β*-tocopherol, from 1 to 43 µg ($R^2 > 0.999$) to *γ*-tocopherol, and from 1 to 46 µg ($R^2 > 0.999$) to *δ*-tocopherol. The results were expressed on a mass basis for vegetable oils and potatoes, the later taking into account the amount of extracted lipids.

6.6.3 Polar compounds

Determination of polar compounds (PC) and its fractions in vegetables oils, such as dimeric and polymeric triglycerides (DPTG), oxidized triglycerides (OTG), diglycerides (DG), and FFA were performed according to Márquez-Ruiz *et al.* (1996), using monostearin as IS. The quantification of PC was based on Márquez-Ruiz *et al.* (1996), while the fractions were calculated based on Dobarganes *et al.* (2000).

Briefly, an accurate amount of IS solution prepared in THF was weighted into a glass vial, and the solvent was evaporated, followed by the addition of an exact amount of the sample, and the volume completed up to 4 mL with petroleum ether. A portion of the solution (2 mL) was applied to a SPE cartridge of silica (1g, Finisterre, Teknokroma, Spain), previously conditioned with hexane:diethyl ether (87:13, v/v, 10 mL). The neutral lipids were eluted with hexane:diethyl ether (87:13, v/v, 10 mL), while the polar compounds were extracted with diethyl ether (10 mL) and, after evaporation under a nitrogen stream, take up in 1 mL of THF. To achieve objectives 2 and 3, lipid extracts of potatoes were also analysed. The results were expressed in g/100g of lipids. The chromatographic conditions are showed in Table 6.4.

Table 6.4 Chromatographic conditions for polar compounds analysis.

	Polar compounds
Equipment	Jasco (Japan)
Detector	ELSD Sedere, Sedex 75 (France) or RID Gilson, 132 model (France)
Column	Phenomenex (Phenogel, 100 Å, 5 µm, 600 x 7.8 mm, USA)
Mobile phase	Isocratic: THF
Flow rate	1 mL/min, RT

6.7. Gas Chromatographic methods

6.7.1 Fatty acids composition

Fatty acids composition of vegetable oils and lipid extracts of potatoes were evaluated by GC-FID, according to chromatographic conditions presented in Table 6.5. A cold transmethylation was used to convert fatty acids glycerides into FAME (ISO 12966-2:2011, 2011), with or without addition of an IS (triundecanoate).

Fatty acids identification was based on individual standards, and FID response was calibrated on the basis of a certified quantitative reference mixture of FAME (Supelco-CRM47885). The fatty acids were calculated on a relative percentage basis or absolute

amounts (on the basis of the IS) and the expressed in g/100g of potatoes, vegetable oils or extracted lipids.

Table 6.5 Chromatographic conditions for fatty acids analysis.

		Fatty acids
Equipment		Chrompack CP 9001, Chrompack CP-9050 autosampler (The Netherlands)
Detector		FID
Columns		CP-Sil 88 column (Chrompack-Varian, 0.19 μm 50 m × 0.25 mm, the Netherlands) FAME CP-Select CB column (Agilent, 0.25 μm, 50 m x 0.25 mm, USA)
Gas		Helium, 120 kPa, constant flow
Temperatures	**Injector**	250 °C, 1 μL
	Oven	140 °C (5 min hold) 5 °C/min to 220 °C (15 min hold) Total run time: 35 min
	Detector	270 °C

6.7.2 Volatile compounds

An HS-SPME technique coupled with GC-MS (Table 6.6) was developed and optimized for the separation and identification of volatile compounds (Molina-Garcia *et al.*, 2017, paper 3). The compounds 4-methyl-2-pentanol and 1,2,3-trichloropropane were chosen as IS, since they are not usually present in the volatile fraction of the oils analysed (Liu *et al.*, 2011, Vichi *et al.*, 2003) and give rise to well-separated peaks under the selected chromatographic conditions.

Briefly, an accurate amount of vegetable oil (1.5 g) was placed into a 20 mL glass vial (La-Pha-Pack GmBH, Germany) with a magnetic glass stir bar (VWR, Germany), spiked with 4 μg of 4-methyl-2-pentanol and 6 μg of 1,2,3-trichloropropane from IS working solutions and immediately closed with an aluminium cover with Teflon septum (La-PhaPack GmBH, Germany). The vial was then inserted into a metal block stabilized at 50°C and heated during 5 min in order to allow equilibration of the volatiles released into the headspace, while stirring (200 rpm) (Cimarec, ThermoScientific, USA). Extraction of volatiles is known to be enhanced by sample agitation, with magnetic stirring widely used by other authors (Sghaier *et al.*, 2016), usually around 200 rpm (Liu *et al.*, 2011, Cecchi and Alfei, 2013; Cajka *et al.*, 2013). A special reference should be made to the stir bars used, because the usual Teflon coated ones might retain components from the oil matrix, introducing an external source of error and contamination (data not shown). The problem was solved by using glass covered stir bars.

After equilibration, a manual SPME holder (Supelco, USA) containing the fiber (DVB/CAR/PDMS 50/30 µm film thickness; Supelco, USA) was inserted into the vial and exposed to the headspace for 30 min, maintaining constant temperature and stirring conditions. The mixed DVB/CAR/PDMS fibers were selected due to the ability for isolation of compounds with different physical-chemical properties and proven suitability for vegetable oils (Cecchi and Alfei, 2013).

For potatoes analysis, the previous method was adapted and validated in the potato matrix. Again, two IS (0.2 µg each from solution in ethanol, as above) were added to an accurate amount of potatoes mass (1.5 g), followed by 7 mL of NaCl aq. solution (15%). The mixture was stabilized at 65°C during 5 min (glass stir-bar, 400 rpm). A manual SPME holder exposed the fiber (as above) to the headspace for 30 min, maintaining constant temperature and stirring conditions.

On both vegetable oils and potatoes, volatiles were thermally desorbed for 5 min in the injector port of the GC-MS system. The MS system was routinely set in full scan mode, with scans from m/z 20 to 450, at 2 scans/s. Agilent ChemStation (version D.0200SP1) was used for data collection/processing and GC-MS control.

Table 6.6 Chromatographic conditions for volatile compounds analysis.

Volatile compounds		
Equipment		Agilent 6890 (USA)
Detector		MS, Agilent 5973B (USA)
Column		SPB-5 (Supelco, 1 µm, 60 m × 0.32 mm, USA)
Gas		Helium
Internal pressure		70 kPa
Temperature	**Injector**	270 °C
	Oven	40 °C (5 min hold) 4 °C/min to 150 °C 10 °C/min to 240 °C (3.5 min hold) Total run time: 45 min
	Transfer line	250 °C
Electron ionization		70 eV

The volatile compounds were identified by comparing the respective mass spectra with a mass spectral database (WILEY7n.L). In addition, identification was complemented by matching relative retention times with data found in the literature. With a retention time of 17.2 min, 4-methyl-2-pentanol was used for semi-quantification of compounds with a

retention time lower than 24 min, while 1,2,3-trichloropropane, being retained for 25.5 min, was used for the less volatile compounds. The relative levels of the investigated compounds were calculated from the peak area ratios of the compounds of interest to the peak area of the IS, therefore reported on internal standard equivalents (ISE).

On the basis of the optimized conditions, reproducibility was tested. Figure 6.1 shows chromatograms of volatiles extracted from two different olive oils sub-samples treated in the same way. Identical volatiles profile can be observed, with variability inferior to 10% of relative standard deviation (RSD) (data not shown), attesting the method's accuracy. Therefore, this methodology allows a direct comparison of different oil matrices, supporting further developments into more general methods for volatiles quantification, enabling more efficient comparison of results between research teams.

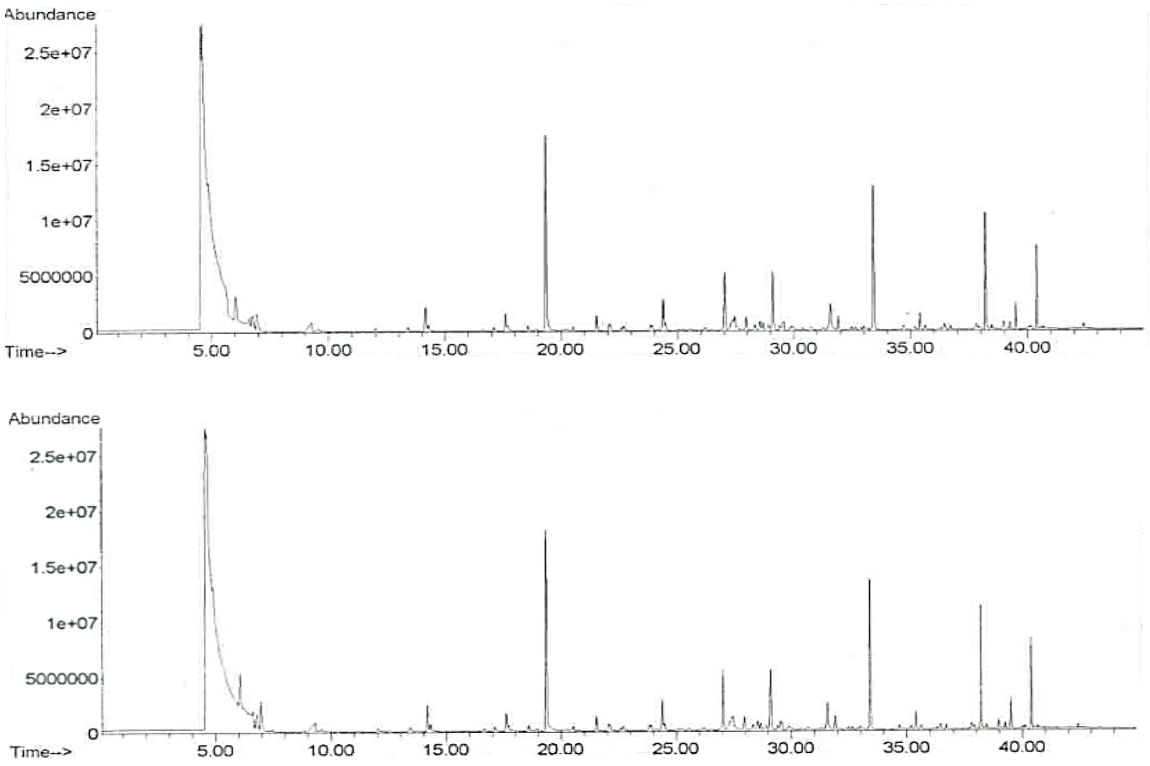


Figure 6.1 Example of chromatograms of replicate heated EVOO under the optimized HS-SPME conditions.

The non-aldehyde compounds analysed comprised alkanes, alkenes, alkylbenzenes, ketones, carboxylic acids, furan derivatives, pyrazines, pyridine, pyrimidine, and pyrrole, while the aldehyde family included alkanals, alkenals, and alkadienals.

6.7.3 Acrylamide

A fast, simple, environmentally friendly, and reliable method was developed and validated for determining acrylamide contents in fried potatoes by GC-MS (Molina-García et al., 2015, paper 4), using xanthydrol as derivative (Lim and Shin 2013; Tsukakoshi *et al.*, 2012; Yamazaki *et al.*, 2012). The derivatization procedure applied was characterized by a substitution reaction of amide groups of both acrylamide and AA-$^{13}C_3$ with xanthydrol to produce xanthyl-acrylamide and xanthyl-AA-$^{13}C_3$ as shown in Figure 6.2 (Yamazaki *et al.*, 2012).

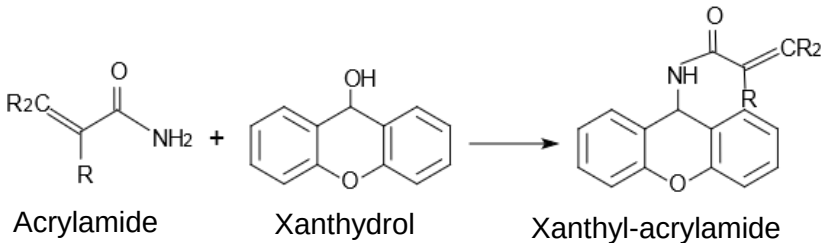


Figure 6.2 Derivative reaction of acrylamide with xanthydrol (based on Yamazaki *et al.*, 2012).

Acrylamide was extracted from potato samples by a liquid extraction procedure with water and 1,2-dichloroetane, at room temperature, allowing the simultaneous extraction of both acrylamide and fats and a clear liquid phase separation. Thus, 2 g of sample was placed in a centrifuge tube, spiked with 2 µg of AA-$^{13}C_3$ from the IS working solution, and left in contact for a few minutes. Next, 20 mL of water and 5 mL of 1,2-dichloroetane were added and shaken in a homogenizer (Edmund Bühler, model KL2, Germany) for 15 min before centrifugation (2991g, RT, 5 min; Heraeus sepatech, Labofuge Ae). After separation of the upper aqueous phase, 5 mL of both water and 1,2-dichloroetane were added again, shaken for further 5 min and centrifuged as above, and the aqueous phase was again separated. The supernatants were combined, and mixed with 6 mL of diethylene glycol solution (10 % in MeOH v/v) to prevent loss of acrylamide due to vaporization (Yamazaki *et al.*, 2012), and concentrated in a rotary evaporator (Büchi Rotavapor, model RE 111 and 461 water bath, Switzerland), until a final volume of 5 mL.

In order to eliminate possible triglyceride remains, the concentrated extract was frozen at -80°C during 3 min and immediately centrifuged (2991 g, 4°C, 10 min; Heraeus sepatech, Labofuge Ae), discarding possible precipitates and reserving the supernatant for derivatization. Consequently, clear extracts were obtained in a fast and effective way, without the necessity of previous clean-up steps by SPE.

Derivatization conditions were optimized made through a statistical design using a real sample of French fries. First, the main determinants for acrylamide derivatization were identified using a full factorial design (24) with the variables under study: temperature, time, derivatization agent amount, and amount of acid (HCl, 1.5 mol/L). The factorial design was composed by 5 center points, and range levels for variables were between 20 and 60°C for temperature, 15 to 60 min for time of derivatization, and 0.3 to 2 mL and 1 to 3 mL for amount of derivatization agent solution (xanthydrol) and amount of HCl solution, respectively. Afterward, the significant variables were optimized using a central composite design (CCD) whose range parameters are detailed in Table 6.7.

Table 6.7 Independent variables with their values used in CCD optimization of derivatization conditions.

Symbol	Variable	Unit	Coded levels				
			-1.682	-1	0	1	1.682
x1	Temperature	ºC	6.36	20	40	60	73.64
x2	Time	min	6.36	20	40	60	73.64
x3	Derivatization agent amount	mL	0.12	0.60	1.30	2.00	2.48

The experimental CCD consisted of a 23 full factorial design, with 5 center points and two axial points on the axis of each design variable at a distance of α=1.682 from the design center. The complete design consisted in a total of 19 combinations including 5 replicates of the center point. The experiments were performed in a random manner at different combinations of these parameters using statistically designed experiments. The response was the xanthyl-acrylamide peak area.

Figure 6.3 shows a Pareto chart where the magnitude of effects of each variable under study is detailed. Results indicated that the statistical significant effects on the peak area of the xanthyl-acrylamide were temperature, time, and derivatizing agent amount. The amount of HCl had no significant effects on the peak area of acrylamide; so, it was fixed for further experiments at 1 mL, the lower tested amount of HCl to reduce the dilution effect of this variable. After the search for main effects, a curvature was detected in the two-level factorial designs, enabling a CCD design test to generate a response surface map in optimization of derivatization step.

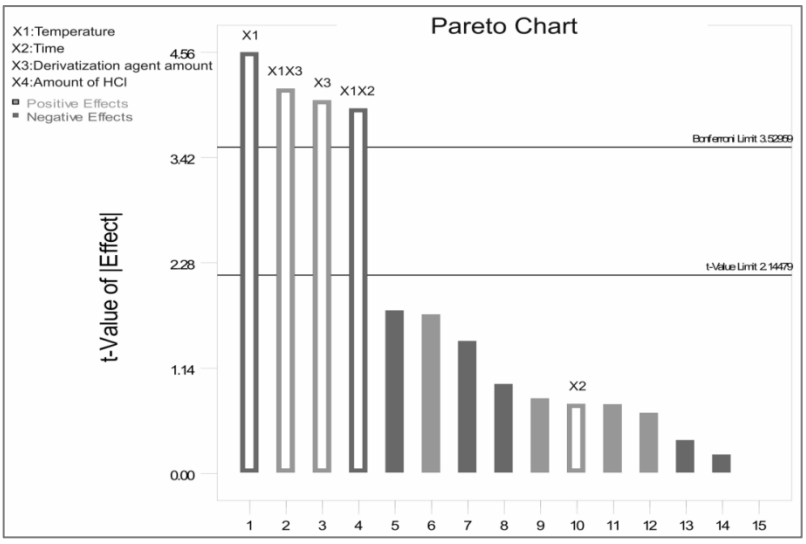


Figure 6.3 Pareto chart with the magnitude of the chosen main effects in derivatization procedure; unfilled bars represent the statistical significant effects obtained. Gray color bars show positive effects whereas negative effects are in black color.

The range parameters for temperature (X_1), time (X_2), and derivatizing agent amount (X_3) are shown in Table 6.8. Regression analysis was executed to fit the response function, and the final model was obtained. Good fitness was obtained for a quadratic model with adequate and significant response surface. Since the values of R^2pred and R^2adj attained were 0.7773 and 0.9291, respectively, a ratio of 13.796 was achieved, which indicates an adequate signal. From ANOVA, the adequacy and significance of the quadratic model were evaluated. The Model F value was 27.21, indicating the high significance of the model, being only a 0.01% possibility that this value is so large that could occur due to noise. The terms X_2, X_3, X_1X_2, X_2X_3, X_1^2, X_2^2, and X_3^2 were the significant, with *Prob>F* values of less than 0.05. The “Lack of fit value” *of Prob>F* was 0.3135, indicating nonsignificant lack of fit (p>0.05), meaning that the model is valid for the present study.

Response surfaces obtained for experimental design allowed prediction of response function (xanthyl-acrylamide peak area) based on the effects of temperature (X_1), time (X_2), and derivatizing agent amount (X_3) (Fig. 6.4). Figure 6.4A shows surface and contour plot demonstrating the effects of time and temperature while derivatization agent amount is kept constant at 1.65 mL. Figure 6.4B shows surface and contour plot of the effects of derivatizing agent amount and temperature at constant time of 50 min. According to this optimization study, higher responses were obtained with temperatures in the middle of the range, with high time of derivatization, and with higher derivatizing agent amount.

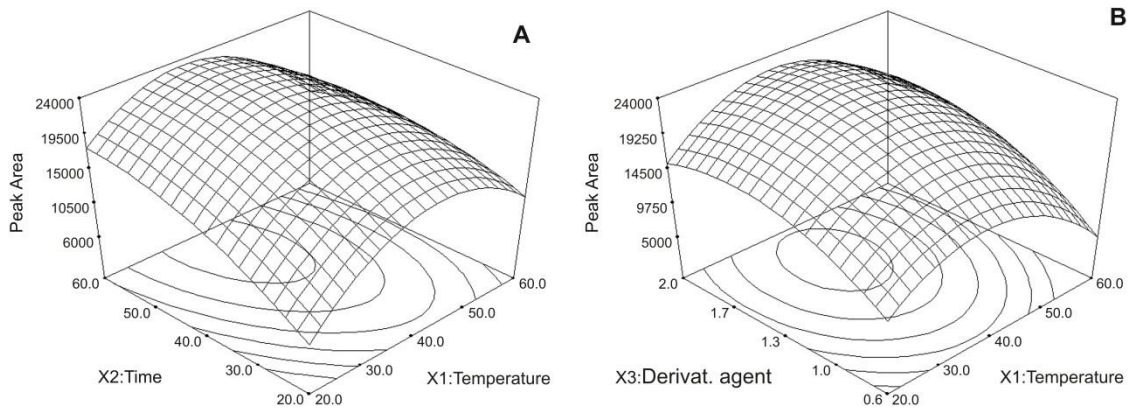


Figure 6.4 Response surface for the central composite design of derivatization procedure. A) Combined effect of temperature and time on the peak area at constant derivatizing agent amount (1.65 mL). B) Combined effect of temperature and derivatizing agent amount on the peak area at constant time (50 min).

Therefore, the derivatization optimum conditions obtained were: water bath at 40°C, in dark glass, during 50 min, with 1.65 mL of xanthydrol (MeOH solution at 5%), and 1.0 mL of HCl (aqueous solution at 1.5 mol/L).

After derivatization, the solution was slightly alkalinized to pH 9.0, by addition of 0.7 mL of KOH aqueous solution at 2.5 mol/L, and buffered with 200 mg of $NaHCO_3/K_2CO_3$ (2:11 w/w). Then, 2 mL of NaCl solution at 1 g/mL was added, and the acrylamide derivative was extracted twice with 1 mL of ethyl acetate, being vigorously shaken and centrifuged (2991 g, RT, 5 min; Heraeus sepatech, Labofuge Ae). Finally, 1 mL of the combined organic layer was transferred to a 2 ml injection vial, concentrated to 0.5 mL under a gentle stream of nitrogen at RT, and injected twice (1 µL). The chromatographic conditions are presented in Table 6.8.

The mode of acquisition selected was single ion monitoring (SIM). Acrylamide derivative (xanthyl-acrylamide) was detected using the quantification ion at m/z 251 and confirmation ions at m/z 234 and 207 while AA-$^{13}C_3$ derivative (xanthyl-AA-$^{13}C_3$) was detected by quantification ion at m/z 254 and confirmation ions at m/z 237 and 209 (Lim and Shin 2013; Yamazaki *et al.*, 2012).

Table 6.8 Chromatographic conditions for acrylamide analysis.

Acrylamide		
Equipment		Agilent 6890 (USA)
Detector		MS Agilent 5973B (USA)
Column		DB-XLB (Agilent, 0.10 µm, 30 m x 0.25 mm, USA)
Gas		Helium
Internal pressure		70 kPa
Temperature	**Injector**	250 °C
	Oven	85 °C (1 min hold) 18 °C/min to 280 °C (4.17 min hold) Total run time 16 min
	Transfer line	280 °C
Electron ionization		70 eV

A total ion chromatogram (TIC) and SIM chromatograms of xanthyl-acrylamide, corresponding to a fried potato sample containing 921±73 µg/kg of acrylamide, are shown in Figure 6.5. Peak identification was confirmed by retention time and comparison with authentic standards.

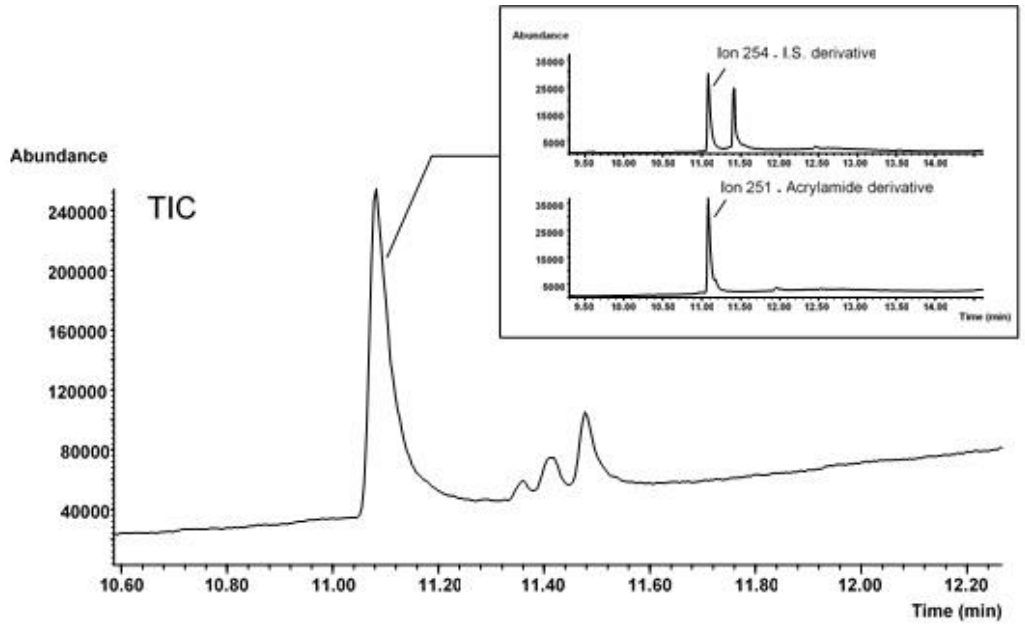


Figure 6.5 Example of TIC and SIM chromatograms of derivatized acrylamide and $AA^{13}C_3$ (IS) of fried potatoes.

The analytical parameters of the optimized method are shown in Table 6.9. Moreover, in order to validate the efficiency of the global method developed, a toasted bread reference sample (ERM-BD 273, IRMM) was analysed in triplicate. A total of 426 µg/kg was quantified, corresponding to 100.2% of the declared value (425±29 µg/kg).

Table 6.9 Analytical parameters.

Parameter	Value
Linear dynamic range (µg/kg)	10 - 1000
Calibration graph	
Intercept	0.0679
Slope	0.0012
Correlation coefficient	0.9997
Detection limit (µg/kg)	4
Quantification limit (µg kg)	10
RSD (%)[a]	
Intraday	6.9
Interday	7.2

[a] n=6: RSD - relative standard deviation

6.8. Statistical analysis

Results were expressed as mean and standard deviation, or as median (minimum – maximum), if normal distribution was verified or not, respectively.

Significant differences, between vegetable oils or frying time, were determined verifying normal distribution of the residuals and the homogeneity of variance by Shapiro-Wilk's ($n < 50$), and Levene's tests, respectively. The dependent variables were analysed using a one-way ANOVA with or without Welch's correction, depending if the requirement of the variance homogeneity was verified or not. Furthermore, if a statistical significant effect was found, the means comparison were observed by Tukey's or Duncan and Dunnett T3 or Tamhane's T2 as post hoc tests, depending if equal variances were assumed or not, respectively. The dependent variables with non-normal distribution were analysed by Kruskal-Wallis. Between two samples, a t-Test or z-Test were applied for means comparison. Moreover, parametric bivariate correlation (Pearson) and non-parametric bivariate correlation (Spearman) were established between parameters.

The statistical tests were performed using Statistical Package for the Social Sciences software, version 20 (IBM Corporation, Chicago, IL) or XLSTAT 2016 with statistical significance set at $p < 0.05$.

CHAPTER 7. Samples

Two potato varieties were used in this thesis, chosen on the basis of their frying aptitude and seasonal availability in the local market (Porto, Portugal):

- White potatoes (*Solanum tuberosum* L., Fontane variety) – characterized by yellow skin, light yellow flesh, oval shape, and medium-late maturity. Fresh potatoes were manually peeled, cut into toothpicks (1 x 1 x 4 cm), washed in plain water for 5 min, and drained before cooking, used to achieve objective 1;
- Red potatoes (*Solanum tuberosum* L., Mozart variety) - characterized by red skin, yellow flesh, oval shape, and late maturity. Fresh potatoes were manually peeled, cut into cubes (1 x 1 x 1 cm), washed in plain water for 5 min, and drained before cooking techniques, used to achieve objectives 2 and 3.

The chemical characterization of each fresh potato varieties is detailed in Table 7.1.

Several vegetable oil were used in this thesis, all commercially available:

- EVOO and peanut oil (PO), both commercially available in the local market, and canola oil (CO) acquired from international market (Paris, France), were used to achieve objective 1;
- Soybean oil (SO), sunflower oil (SFO), and OO, all widely available in the local market, and CO2 acquired from international market (Pforzheim, Germany), were used to achieve objective 2,
- Two different EVOO and OO, all widely available in the local market, were used to achieve objective 3.

The chemical characteristics of the fresh oils are compiled in Table 7.2.

Table 7.1 Characteristics of fresh potato varieties: moisture, lipids, tocopherols, total ascorbic acid, total carotenoids, total phenolics, and antioxidant activity in fresh weight.

Potatoes varieties		**Fontane**	**Mozart**
Moisture	g/100g	82.1±0.2	80.8±0.5
Lipids	g/100g	0.1±0.0	0.4±0.0
Tocopherols	µg/100g	20±0.2	20±0.2
Total ascorbic acid	mg/100g	31.1±1.0	6.8±0.1
Total Carotenoids	µg/100 g	178±5	79±13
Total Phenolics	mg GAE/100 g	5.9±0.3	22.1±0.6
DPPH	mg GAE/100 g	7.9±0.1	6.1±0.9
β-carotene/linoleic acid	mg GAE/100 g	7.2±0.0	-

DPPH – 2,2-diphenyl-1-picrylhydrazyl radical; GAE – Gallic acid equivalents.

Table 7.2 Characteristics of fresh oils: fatty acids composition, vitamins, antioxidant activity, and degradation indicators.

		To achieve objective 1			To achieve objective 2				To achieve objective 3			
		EVOO	**PO**	**CO**	**SO**	**SFO**	**CO**	**OO**	**EVOO1**	**EVOO2**	**OO1**	**OO2**
SFA	g/100g	15.9±0.2	17.8±0.1	7.5±0.0	15.6±0.0	10.6±0.1	7.3±0.5	15.7±0.2	15.4±0.3^{a}	16.8±0.0^{c}	15.8±0.1^{b}	15.6±0.1^{ab}
MUFA	g/100g	74.5±0.2	55.6±0.1	63.4±0.0	27.3±0.2	46.8±0.8	62.5±0.4	77.0±0.3	74.6±0.2^{a}	74.7±0.0^{a}	76.7±0.1^{b}	77.5±0.0^{c}
PUFA	g/100g	9.3±0.1	25.7±0.1	28.4±0.0	55.6±0.2	42.0±0.9	27.9±0.2	7.0±0.2	9.7±0.0^{d}	8.1±0.0^{c}	7.0±0.0^{b}	6.5±0.1^{a}
TFA	g/100g	0.03±0.00	0.18±0.00	0.26±0.01	0.37±0.03	0.17±0.01	0.43±0.17	0.09±0.02	0.02±0.00^{a}	0.03±0.00^{a}	0.10±0.01^{c}	0.09±0.00^{b}
PUFA/SFA		0.59±0.01	1.44±0.02	3.77±0.01	3.56±0.02	3.94±0.12	3.84±0.26	0.44±0.01	0.63±0.01^{d}	0.48±0.00^{c}	0.44±0.00^{b}	0.41±0.00^{a}
Tocopherols	mg/100g	37±1	54±1	81±2	71±4	67±1	116±9	29±2	35±1	38±4	33±1	32±3
Total carotenoids	µg/100g	1484±37	67±7	59±3	25±3	30±4	19±3	480±6	1313±22^{c}	1142±62^{b}	478±5^{a}	471±9^{a}
Total phenolics	mg GAE/100g	56±7	3±0	3±0	6±0	6±0	6±0	14±0	51±3^{d}	36±4^{c}	13±0^{b}	10±0^{a}
DPPH	mg GAE/100g	5±0	6±0	7±0	8±0	7±0	8±0	7±0	6±0^{a}	6±0^{a}	7±0^{b}	7±0^{b}
K_{232}		2.2±0.0	4.7±0.1	3.6 0.3	-	-	-	-	2.18±0.09^{b}	1.91±0.00^{a}	1.96±0.07^{a}	1.93±0.04^{a}
K_{270}		0.2±0.0	3.0±0.0	0.7±0.1	-	-	-	-	0.19±0.01^{a}	0.18±0.00^{a}	0.29±0.03^{b}	0.28±0.02^{b}
ΔK		-0.01±0.00	0.27±0.01	-0.01±0.00	-	-	-	-	-0.01±0.00^{a}	-0.01±0.00^{a}	0.01±0.00^{b}	0.01±0.00^{b}
PAV*		17±2	28±1	24±0	1±0	3±0	1±0	3±0	9±0^{c}	9±1^{c}	4±0^{b}	3±0^{a}
TPC	g/100g	1.4±0.3	3.0±0.5	2.1±0.3	5.1±0.6	3.6±0.2	3.2±0.1	4.2±0.2	2.8±0.2^{a}	3.2±0.0^{b}	3.6±0.2^{c}	4.1±0.4^{c}
DPTG	g/100g	n.d.	n.d.	n.d.	0.1±0.0	0.1±0.0	0.1±0.0	0.1±0.0	n.d.	n.d.	n.d.	n.d.
OTG	g/100g	0.2±0.0	0.5±0.0	0.7±0.0	2.4±0.5	1.1±0.1	1.0±0.0	1.2±0.3	0.8±0.0	0.6±0.0	0.8±0.1	1.2±0.4
DG	g/100g	0.8±0.0	2.1±0.1	0.9±0.3	1.2±0.0	1.5±0.0	0.7±0.0	1.8±0.1	1.0±0.1^{a}	1.7±0.0^{c}	1.7±0.1^{c}	1.4±0.0^{b}
FFA	g/100g	0.3±0.0	0.2±0.0	0.3±0.0	0.5±0.0	0.6±0.0	0.7±0.0	0.5±0.0	0.4±0.0^{a}	0.5±0.0^{b}	0.5±0.0^{ab}	0.6±0.0^{c}

$^{a-d}$ statistically significant differences between olive oil categories (p<0.05);

* expressed as 100 times the optical density measured at 350 nm in a 1 cm cuvette of a solution containing 1.00 g of the oil in 100 mL, according to method;

CO - canola oil; DG - diglycerides; DPPH - 2,2-diphenyl-1-picrylhydrazyl radical; DPTG - dimeric and polymeric triglycerides; EVOO - extra virgin olive oil; FFA - free fatty acids; GAE - Gallic acid equivalents; MUFA - monounsaturated fatty acids; n.d. – not detected; OO - olive oil; OTG - oxidized triglycerides; PAV - *p*-anisidine value; PUFA - polyunsaturated fatty acids; SFA - saturated fatty acids; SFO - sunflower oil; SO - soybean oil; TFA - *trans* fatty acids; TPC - total polar compounds.

CHAPTER 8. Cooking techniques

The cooking techniques used in this thesis and its specifications are described below. On all procedures, except deep-frying, the oil:potato ratio was kept similar to enable direct comparisons. Also, all processing methods were previously optimized (data not shown) to find condition that provided similar final appearances on all processes. For potato analysis, samples were ground in an electric grinder (Flama, Cesar, Portugal), and stored at -20°C.

8.1. Deep-frying

Three fryers (TRISTAR, FR-6929 model, The Netherlands, nominal power of 800 W) with adjustable temperature up to 190°C, 1.75 L capacity, and maximum load of 200 g *per* L, were used for deep-frying (DF) assays. Temperature was periodically controlled with a digital thermometer.

To achieve objective 1, the frying assays were designed to simulate prolonged restaurant frying, heating the oil for 8 h a day (intermittent thermal stress - 8 h heating, and 16 h cooling, covered). A portion of potatoes toothpicks (50 g) was fried in 1.5 L of each vegetable oil, at 175°C, during 6 min, for every 30 min, during 8 h per day, up to 28 h of frying, the rejection time defined on the basis of dielectric constant changes, as will be detailed in the method's section.

To achieve objectives 2 and 3, a portion of potatoes cubes (200 g) was fried in 1.5 L of vegetable oils, at 175°C, during 6 min.

8.2. Air-frying

Air-frying (AF) was tested in two different equipment's: Actifry® (ACT) (Tefal, SERIE001 model, France, nominal power of 1400W), and Airfryer® (AIR) (Philips, Viva Collection HD9220 model, The Netherlands, nominal power of 1425W).

A portion of potatoes cubes (300 g) was thoroughly homogenised with 3.6 g of each vegetable oil (1:83 ratio), and fried during 20-25 min (ACT) or 15-20 min (AIR; manual agitation at 10 and 15 min), following the equipment's specifications.

8.3. Boiling

Boiling was performed as control process of physicochemical parameters in the objective 2.

A portion of potatoes cubes (300 g) was added to boiling water, and cooked during 5 min. After water drain, vegetable oils were added in similar amounts to those used in air-frying.

8.4. Microwave-grill

Microwave-grill (MWG) (Whirlpool, GT285/WH model, USA, nominal power of 1900W) was used for low-fat potatoes “frying”. The formation of crust and typical colour of fried potatoes was reached using the grill function, together with crisp plate, as recommended by the manufacturer.

A portion of potatoes cubes (200 g) was thoroughly involved in 2.4 g of each vegetable oil (1:83 ratio), cooked during 5 min (microwave function, output power at 700W), and crisped during 10 min (grill function and crisp plate, output power at 900W). Potatoes were manually shaken at 10 min and 12.5 min.

8.5. Oven

A domestic convection oven (OV) (Teka, HI-435 model, Germany, nominal power of 1900W) was used for low-fat potatoes “frying”.

A portion of potatoes cubes (200 g) was involved in 2.4 g of vegetable oils (1:83 ratio), and cooked under greaseproof paper, commercially available for the purpose, during 30 min, at 190 °C. Potatoes were manually shaken at 15 and 22.5 min.

PART III

RESULTS AND DISCUSSION

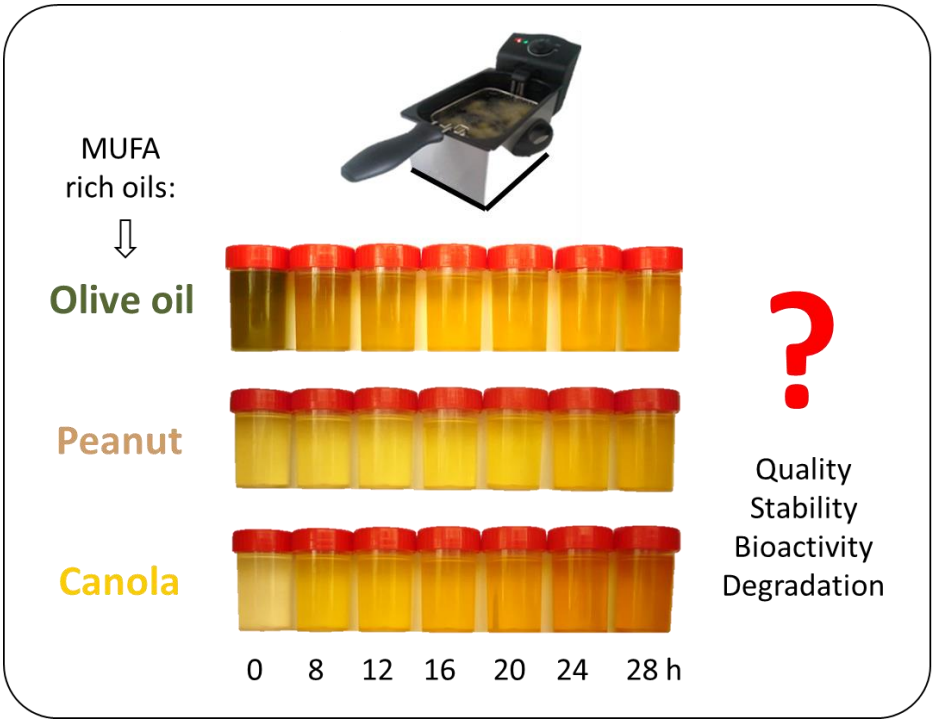
CHAPTER 9. The influence of prolonged potatoes frying on monounsaturated-rich vegetable oils

Parts of the text of this chapter were submitted to publication:

Santos CSP, Molina-García L, Cunha SC, Fernandes JO, Casal S (2017) Impact of potatoes deep-frying on common monounsaturated-rich vegetable oils: a comparative study. (Paper 5).

Santos CSP, Molina-García L, Cunha SC, Casal S (2018) Fried potatoes: impact of prolonged frying in monounsaturated oils, *Food Chemistry*. 243: 192-201 (Paper 6).

9.1. Impact of potatoes deep-frying on common monounsaturated-rich vegetable oils: a comparative study



9.1.1 Background and aim of study

Frying is the most common potato processing technique worldwide. However, oil selection for deep-frying purposes should be based on several factors, including its ability to withstand high temperatures for prolonged times, palatability and nutritional properties. In this sense, recommendations on oils high content of MUFA, particularly oleic acid, have increased.

Most works on the oxidative stability of oleic-rich oils under potatoes deep-frying take common PUFA rich oils as reference, as soybean or sunflower oil (Abenoza *et al.*, 2015; Aladedunye and Przybylski 2009; Andrikopoulos *et al.*, 2002; Casal *et al.*, 2010; Olivero-David *et al.*, 2014; Petersen *et al.*, 2013; Serjouie *et al.*, 2010; Taha *et al.*,. 2014; Xu *et al.*, 2015). However, few performed direct comparisons of naturally high-MUFA and none on these three naturally MUFA-rich oils available for domestic/restaurant frying: peanut, canola and olive oil. Although being expectable that all three oils will be stable under frying, the minor differences that could impact on their nutritional and health properties can only be perceived with a direct comparison under processing conditions similar to those performed under real frying.

Therefore, the aim of the present work was to compare EVOO, PO, and CO during real intermittent fresh potatoes deep-frying at 175°C, with a focus on the oils quality through time, in order to elucidate consumers on their choices, both from the quality and safety points of view.

9.1.2 Sampling

Three commercial oils were chosen: EVOO, PO and CO. The deep-frying assays were performed according to Chapter 8. Oils samples were collected every 4 h, except on the first day, closed under nitrogen and stored at 4°C for further analyses, namely colour, polar compounds by HPSEC, *p*-anisidine value, extinction coefficients, fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, and volatile compounds, all described in Chapter 6. All analytical determinations were performed in triplicate (n = 3).

9.1.3 Results and Discussion

9.1.3.1 Colour

Colour is the “first” attribute inspected in frying oils, directly available to food processors who, based on the oil initial characteristics and typical use, are able to determine that colour alteration, particularly darkening, is a sign of oil degradation (Lalas, 2009). However, it might not always correlate directly with oil degradation, particularly with the total polar compounds, a mandatory parameter to frying control in several countries.

Instrumental colour coordinate values were measured on all samples oils (Table 9.1). Before frying, significant differences between samples were shown, according to each oil inherent characteristics, including the typical greenish shades in EVOO due to chlorophyll presence, and light yellow in refined PO and CO, lighter in the latter. During deep-frying, significant differences within and between samples were observed. Generally, *a** increased in all vegetable oils, due to redden hues increase. *L** and *b** presented opposite patterns in the oils, with a reduction in PO and CO and an increased in EVOO. In addition, *ΔE* increased in all vegetable oils, but PO colour was more stable, followed by EVOO (higher *b** changes), while greater variations were perceived in CO (on all colour coordinates). These colour alterations are the combined result of polymerization and oxidation, including the degradation of natural pigments in unrefined EVOO, and formation of browning compounds from *Maillard* reaction (Aladedunye and Przybylski 2009; Lalas 2009).

Table 9.1 Degradation indicators changes in vegetable oils during intermittent fresh potatoes deep-frying.

Oil types	Frying time	*L**	*a**	*b**	*ΔE*	PAV	K_{232}	K_{270}	TFA (relative %)
	0 h	$17.8\pm0.0^{a,A}$	$0.5\pm0.0^{a,A}$	$0.9\pm0.0^{a,A}$	-	$17\pm2^{a,A}$	$2.2\pm0.0^{a,A}$	$0.2\pm0.0^{a,A}$	$0.03\pm0.00^{a,A}$
	8 h	$17.7\pm0.1^{a,A}$	$1.5\pm0.0^{a,B}$	$2.3\pm0.0^{a,B}$	$1.7\pm0.0^{b,A}$	$88\pm9^{a,BC}$	$10.0\pm0.7^{a,B}$	$2.2\pm0.2^{a,B}$	$0.16\pm0.01^{a,B}$
	12 h	$18.0\pm0.2^{a,ABC}$	$2.0\pm0.1^{a,C}$	$3.1\pm0.0^{a,C}$	$2.7\pm0.0^{b,B}$	$87\pm5^{a,B}$	$11.5\pm1.8^{a,B}$	$2.4\pm0.4^{a,BC}$	$0.24\pm0.05^{a,BC}$
EVOO	16 h	$18.0\pm0.2^{a,ABC}$	$2.3\pm0.0^{a,D}$	$4.3\pm0.0^{a,D}$	$3.8\pm0.0^{b,C}$	$114\pm2^{a,D}$	$12.1\pm0.2^{a,B}$	$2.5\pm0.2^{a,BC}$	$0.26\pm0.00^{a,C}$
	20 h	$18.9\pm0.1^{a,B}$	$2.2\pm0.1^{a,CD}$	$3.8\pm0.0^{a,E}$	$3.9\pm0.0^{b,D}$	$100\pm4^{a,BCD}$	$17.0\pm1.2^{a,C}$	$2.9\pm0.1^{a,C}$	$0.30\pm0.04^{a,C}$
	24 h	$19.6\pm0.0^{a,C}$	$2.8\pm0.0^{a,E}$	$4.5\pm0.0^{a,F}$	$4.6\pm0.0^{b,E}$	$109\pm13^{a,CD}$	$17.0\pm0.2^{a,C}$	$3.0\pm0.1^{a,C}$	$0.42\pm0.03^{a,D}$
	28 h	$19.2\pm0.1^{a,BC}$	$3.0\pm0.1^{a,E}$	$4.8\pm0.0^{a,G}$	$4.9\pm0.0^{a,G}$	$135\pm10^{a,E}$	$16.4\pm2.0^{a,C}$	$2.9\pm0.5^{a,C}$	$0.51\pm0.02^{a,E}$
	0 h	$23.6\pm0.1^{b,D}$	$2.5\pm0.0^{c,AB}$	$9.1\pm0.0^{c,B}$	-	$28\pm1^{c,A}$	$4.7\pm0.1^{c,A}$	$3.0\pm0.0^{c,A}$	$0.18\pm0.00^{b,A}$
	8 h	$22.8\pm0.0^{c,C}$	$2.2\pm0.0^{b,A}$	$9.4\pm0.0^{c,C}$	$0.9\pm0.1^{a,A}$	$149\pm2^{b,B}$	$21.7\pm1.5^{c,B}$	$4.5\pm0.1^{b,C}$	$0.33\pm0.00^{b,B}$
	12 h	$22.6\pm0.0^{c,C}$	$2.3\pm0.0^{b,A}$	$9.3\pm0.0^{c,C}$	$1.1\pm0.1^{a,A}$	$153\pm3^{b,B}$	$21.5\pm0.7^{c,B}$	$3.8\pm0.1^{b,B}$	$0.40\pm0.02^{b,C}$
PO	16 h	$21.7\pm0.0^{c,AB}$	$2.6\pm0.0^{b,B}$	$9.3\pm0.0^{c,C}$	$1.9\pm0.1^{a,B}$	$206\pm1^{b,C}$	$26.5\pm3.2^{c,C}$	$4.2\pm0.4^{b,BC}$	$0.43\pm0.01^{b,D}$
	20 h	$22.0\pm0.0^{c,B}$	$3.2\pm0.0^{b,C}$	$9.3\pm0.0^{c,C}$	$2.0\pm0.1^{a,B}$	$209\pm7^{b,C}$	$27.4\pm0.5^{c,C}$	$4.4\pm0.2^{b,BC}$	$0.45\pm0.01^{b,D}$
	24 h	$21.5\pm0.0^{c,A}$	$3.7\pm0.0^{b,D}$	$8.2\pm0.0^{c,A}$	$2.5\pm0.1^{a,C}$	$206\pm10^{b,C}$	$29.8\pm0.9^{c,CD}$	$5.3\pm0.1^{c,D}$	$0.53\pm0.00^{b,E}$
	28 h	$21.6\pm0.2^{b,ABC}$	$4.3\pm0.1^{b,D}$	$8.1\pm0.1^{c,A}$	$2.8\pm0.3^{a,C}$	$207\pm11^{b,C}$	$32.4\pm1.4^{c,D}$	$5.4\pm0.4^{b,D}$	$0.55\pm0.02^{ab,E}$
	0 h	$24.5\pm0.1^{c,D}$	$1.7\pm0.0^{b,A}$	$8.7\pm0.0^{b,E}$	-	$24\pm0^{b,A}$	$3.6\pm0.3^{b,A}$	$0.7\pm0.1^{b,A}$	$0.26\pm0.01^{c,A}$
	8 h	$22.2\pm0.1^{b,C}$	$3.6\pm0.1^{c,B}$	$9.2\pm0.1^{b,E}$	$3.0\pm0.2^{c,A}$	$178\pm20^{b,B}$	$12.6\pm1.4^{b,B}$	$4.2\pm1.0^{b,B}$	$0.38\pm0.01^{c,B}$
	12 h	$21.9\pm0.0^{b,C}$	$4.0\pm0.0^{c,C}$	$8.7\pm0.0^{b,E}$	$3.4\pm0.1^{c,B}$	$237\pm19^{c,C}$	$14.9\pm0.6^{b,B}$	$4.3\pm0.2^{c,B}$	$0.39\pm0.05^{b,BC}$
CO	16 h	$20.5\pm0.0^{b,B}$	$4.2\pm0.0^{c,D}$	$7.7\pm0.0^{b,D}$	$4.8\pm0.1^{c,C}$	$256\pm11^{c,C}$	$18.7\pm1.0^{b,C}$	$4.6\pm0.4^{b,B}$	$0.45\pm0.01^{c,BCD}$
	20 h	$20.6\pm0.0^{b,B}$	$4.8\pm0.0^{c,E}$	$7.2\pm0.0^{b,C}$	$5.5\pm0.1^{c,C}$	$252\pm7^{c,C}$	$18.9\pm0.0^{b,C}$	$4.8\pm0.1^{c,B}$	$0.47\pm0.04^{b,CD}$
	24 h	$20.2\pm0.0^{b,A}$	$5.2\pm0.0^{c,F}$	$6.0\pm0.0^{b,B}$	$6.2\pm0.1^{c,D}$	$275\pm20^{c,C}$	$20.5\pm1.9^{b,C}$	$5.0\pm0.2^{b,B}$	$0.51\pm0.03^{b,DE}$
	28 h	$19.6\pm0.1^{a,A}$	$5.3\pm0.0^{c,F}$	$5.7\pm0.0^{b,A}$	$6.8\pm0.1^{c,E}$	$257\pm11^{c,C}$	$20.3\pm1.8^{b,C}$	$5.0\pm0.10^{b,B}$	$0.57\pm0.01^{b,E}$

Superscript different letters indicate statistically significant differences ($p < 0.05$): small letters between different vegetable oils for the same frying time and large letters within each vegetable oil during frying time.
CO – canola oil; EVOO – extra virgin olive oil; PAV – *p*-anisidine value; PO – peanut oil; TFA – trans fatty acid; ΔE – colour change; a* - redness; b* yellowness; L* - lightness.

9.1.3.2 Polar compounds

The formation of “polar” compounds, derived mainly from triglycerides degradation, is recognized as one of the most reliable global assessment of oils performance, since it includes products derived from thermal, oxidative and hydrolytic changes (Choe and Min 2007), generally quantified as TPC. Several European countries have established regulatory limits for TPC in frying oils, wherein the rejection point is between 24-27%, being 25% in Portugal (Fox, 2001). Together with the amounts of TPC and its fractions (Fig. 9.1), linear adjustments with frying time were assessed (Table 9.2). The comparisons between linear adjustments in the three vegetable oils were checked by analysis of covariance.

Before deep-frying, all oils presented a similar TPC content, below 3g/100g (Fig. 9.1A). A gradual increase was clearly observed during deep-frying, with PO and CO presenting similar TPC progressions and rejection points (25g/100g - at 18 h and 20 h, respectively), while EVOO presented a slower formation of TPC with time, achieving 22g/100g at 28 h. These observations are supported by the linear adjustments presented in Table 9.2 and their statistical differences. These rejection points were in agreement with the literature (Abenoza *et al.*, 2015; Casal *et al.*, 2010, Serjouie *et al*., 2010), except when replenishment is used, delaying the TPC limits by repetitive dilution (Aladedunye and Przybylski, 2009).

Within the TPC, dimeric and polymeric triglycerides (DPTG) are large non-volatile molecules that provide stable information on the oil degradation under deep-frying conditions, taking many countries to impose an additional limit of 12 g/100g for this fraction. In our tested oils, DPTG formation trends were similar to those obtained for the TPC, with PO and CO exceeding this 12 g/100g limit at 16-18 h, while EVOO remained below this limit up to the final assayed oil sample at 28 h (Fig. 9.1B). Again, in accordance with the linear adjustments for DPTG during intermittent deep-frying, EVOO was differentiated from the other vegetable oils (Table 9.2). Oxidized triglycerides (OTG) are probably the molecules that raise more concern from the health point of view, being readily absorbed and having potential effects on lipid metabolism (Li *et al*., 2016). According to Fig. 9.1C, equivalent amounts of OTG were observed on the three oils through the entire assay. This observation was reconfirmed by linear adjustments for OTG during intermittent deep-frying, without differentiation between the tested oils (Table 9.2).

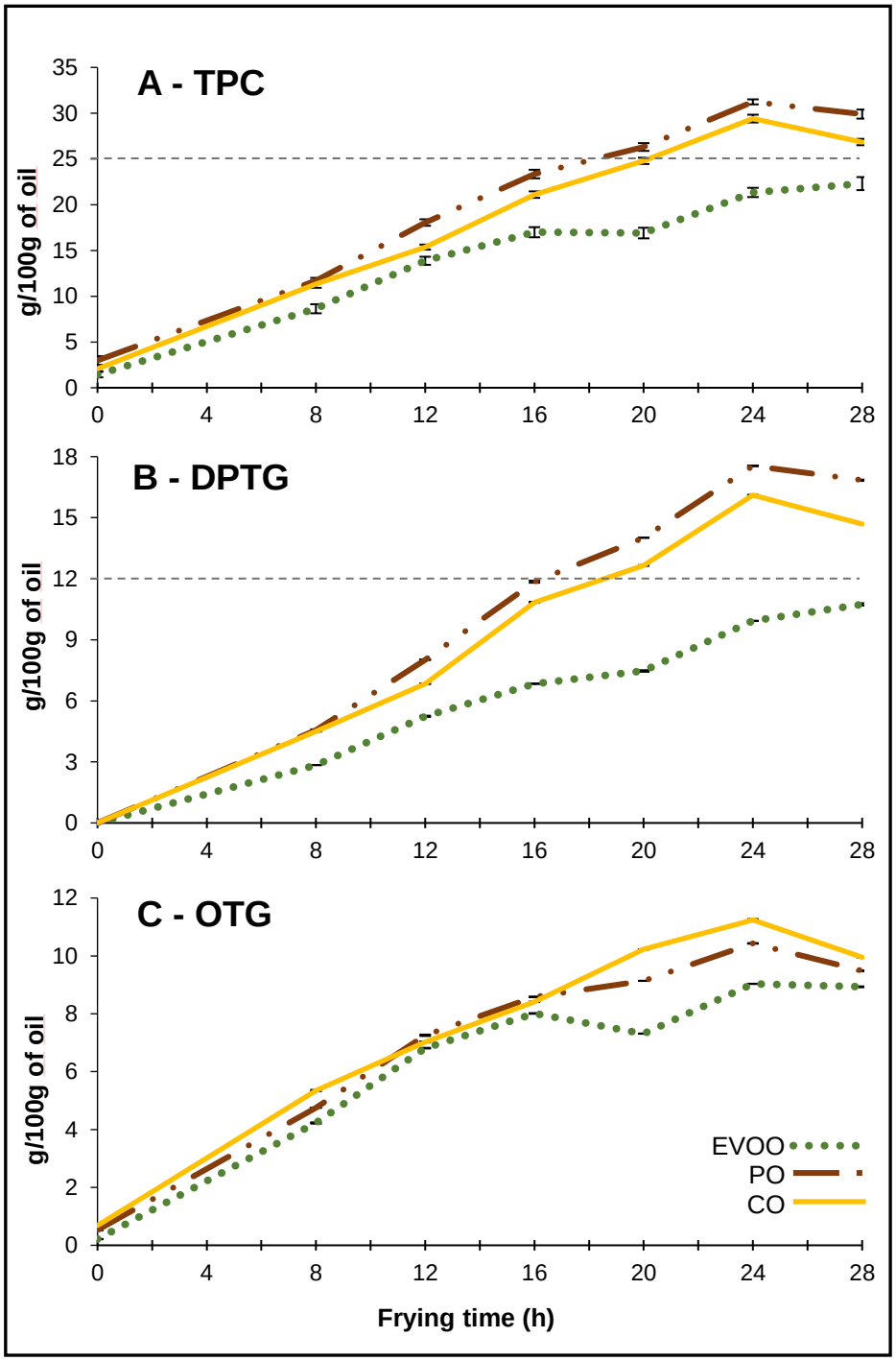


Figure 9.1 Changes in the vegetable oils during intermittent fresh potatoes deep-frying in EVOO, PO and CO: A) TPC; B) DPTG; C) OTG.

Table 9.2 Linear adjustments between frying time and TPC, DPTG, and OTG.

	Oil types	r^2	B	Intercept [a]	Slope [a]	*p* [b]	EVOO *vs* PO [c]	EVOO *vs* CO [c]	PO *vs* CO [c]
TPC	**EVOO**	0.908	0.953	1.5 (-1.4; 4.4)	3.2 (2.6; 3.9)	0.000	0.000	0.001	n.s.
	PO	0.921	0.960	2.2 (-1.5; 6.0)	4.6 (3.7; 5.4)	0.000			
	CO	0.907	0.952	1.6 (-2.3; 5.4)	4.3 (3.4; 5.1)	0.000			
DPTG	**EVOO**	0.969	0.984	-0.8 (-1.7; 0.1)	1.7 (1.5; 1.9)	0.000	0.000	0.000	n.s.
	PO	0.951	0.975	-1.4 (-3.3; 0.5)	2.9 (2.5; 3.4)	0.000			
	CO	0.933	0.966	-1.1 (-3.0; 0.9)	2.6 (2.2; 3.0)	0.000			
OTG	**EVOO**	0.784	0.885	1.2 (-0.7; 3.1)	1.3 (0.9; 1.7)	0.000	n.s.	n.s.	n.s.
	PO	0.800	0.894	1.5 (-0.6; 3.4)	1.4 (1.0; 1.9)	0.000			
	CO	0.822	0.907	1.4 (-0.6; 3.4)	1.5 (1.1; 2.0)	0.000			

[a] values of mean and values of 95 % Confidence Interval; [b] *p* value of linear regression adjustment; [c] *p* value of significant differences between linear adjustments. n.s. - not significant.

9.1.3.3 Other oxidation indicators

Table 9.1 also details the PAV evolution with frying time for the three oils. Regardless of the oil, a significant increase of PAV ($p<0.05$) was verified. However, EVOO presented a significantly lower content than the other vegetable oils for all time intervals tested ($p<0.05$). A higher formation rate was verified in the first 8-12 h of deep-frying, more significant in CO, followed by a general stabilization. This could be derived from the equilibrium between formation of carbonyls from hydroperoxides degradation and loss of carbonyls by volatilization of smaller molecules or other chemical reactions (Farhoosh *et al.*, 2012). Effectively, various studies demonstrated the PAV increase with frying time, even after short-time (Casal *et al*., 2010; Petersen *et al*., 2013; Taha *et al*., 2014; Xu *et al*., 2015), again providing that no replenishment is performed.

Oxidation can also be deduced from structural alterations in unsaturated fatty acids double bonds position and conformation, namely formation of conjugated dienes (K_{232}) or even conjugated trienes (K_{270}), the later indicative of more profound oxidation (Farhoosh *et al*., 2012), or by isomerization to trans-fatty acids (Tsuzuki *et al*., 2010). During deep-frying, K_{232} increased significantly ($p<0.05$) on all oils, particularly in the first 8 h of frying (Table 9.1). For the same frying time, EVOO presented always lower K_{232} values ($p<0.05$), followed by CO, with significantly higher amounts in PO ($p<0.05$). The K_{270} trend did not allowed the previous distinction, with similar amounts between CO and PO, both with higher amounts than EVOO.

Regarding the TFA contents (Table 9.1), they were always below 0.6%, therefore of no concern from an health point of view and in agreement with other frying studies developed with these vegetable oils (Aladedunye and Przybylski, 2009; Casal *et al*., 2010). The initial EVOO amounts (0.03%) are lower than PO and CO due to the absence of refining but

during frying the amounts of TFA increased consistently (p<0.05) on the three oils, with very similar amounts at 28 h. Aware that these structural fatty acid changes reflect mainly fatty acid oxidation, as observed in other studies (Abenoza *et al*., 2015; Andrikopoulos *et al*., 2002; Casal *et al*., 2010; Serjouie *et al.*, 2010), a detailed study of the fatty composition was performed (Table 9.3).

Oleic acid (C18:1n-9) was the major fatty acid in all samples, the basis of their initial choice for this study, classifying them from the nutritional point as fats with a monounsaturated basis. However, different (p<0.05) initial MUFA amounts were shown: EVOO (71%), CO (58%) and PO (53%) (Table 9.3). Focusing on the PUFA, the ones that could be more implicated in the oxidation parameters discussed earlier, linoleic acid (C18:2n-6) had distinguished patterns, being lower in EVOO and similar on both PO and CO. In opposition, linolenic acid (C18:3n-3) was present in low amounts on both EVOO and PO, being significantly higher in CO. Therefore, despite the similar total PUFA contents in PO and CO, the C18:3n-3/C18:2n-6 ratio was higher in CO while the MUFA/PUFA ratio was higher in EVOO and similar in PO and CO (Table 9.3).

Table 9.3 Fatty acids composition (relative %) in the tested vegetable oils during intermittent fresh potatoes deep-frying.

Oil types	Frying time	C16:0	C18:1n-9	C18:2n-6	C18:3n-3	MUFA/PUFA
	0 h	12.0±0.1^{c,A}	70.7±0.5^{c,A}	8.6±0.0^{a,G}	0.8±0.0^{b,E}	7.87±0.02^{c,A}
	8 h	12.9±0.1^{c,B}	70.9±0.0^{c,AB}	7.2±0.0^{a,F}	0.6±0.0^{b,D}	9.60±0.04^{c,B}
	12 h	12.8±0.1^{c,B}	71.3±0.4^{c,AB}	6.6±0.0^{a,E}	0.5±0.0^{b,C}	10.45±0.03^{c,C}
EVOO	16 h	13.0±0.0^{c,B}	71.5±0.0^{c,B}	6.2±0.0^{a,D}	0.4±0.0^{b,B}	11.39±0.01^{c,D}
	20 h	13.2±0.0^{c,C}	71.4±0.0^{c,AB}	6.0±0.0^{a,C}	0.4±0.0^{b,B}	11.75±0.02^{b,E}
	24 h	13.4±0.1^{c,CD}	71.2±0.2^{c,AB}	5.5±0.0^{a,B}	0.3±0.0^{b,A}	12.68±0.03^{b,F}
	28 h	13.5±0.1^{c,D}	70.9±0.2^{c,AB}	5.4±0.1^{a,A}	0.3±0.0^{b,A}	12.94±0.12^{b,G}
	0 h	10.6±0.1^{b,A}	52.9±0.0^{a,A}	25.5±0.0^{c,G}	0.2±0.0^{a,D}	2.16±0.00^{a,A}
	8 h	11.3±0.0^{b,B}	53.9±0.0^{a,B}	22.7±0.0^{c,F}	0.1±0.0^{a,BC}	2.47±0.00^{a,B}
	12 h	11.4±0.0^{b,BC}	54.4±0.0^{a,C}	22.0±0.1^{c,E}	0.1±0.0^{a,C}	2.57±0.01^{a,C}
PO	16 h	11.7±0.0^{b,CD}	54.7±0.04^{a,D}	20.8±0.0^{c,D}	0.1±0.0^{a,AB}	2.73±0.00^{a,D}
	20 h	11.9±0.1^{b,DE}	54.7±0.08^{a,D}	20.1±0.1^{c,C}	0.1±0.0^{a,A}	2.82±0.00^{a,E}
	24 h	12.3±0.2^{b,F}	55.1±0.02^{a,E}	19.1±0.0^{c,A}	0.1±0.0^{a,A}	3.00±0.00^{a,G}
	28 h	11.9±0.0^{b,E}	54.9±0.03^{a,F}	19.7±0.0^{c,B}	0.1±0.0^{a,A}	2.90±0.01^{a,F}
	0 h	4.7±0.0^{a,A}	57.8±0.1^{b,A}	19.0±0.0^{b,E}	9.3±0.0^{c,E}	2.24±0.00^{b,A}
	8 h	5.0±0.0^{a,B}	59.4±0.1^{b,B}	17.8±0.1^{b,D}	7.9±0.0^{c,D}	2.54±0.02^{b,B}
	12 h	5.3±0.2^{a,C}	59.6±0.3^{b,B}	17.3±0.1^{b,C}	7.4±0.0^{c,C}	2.64±0.00^{b,C}
CO	16 h	5.2±0.0^{a,C}	60.7±0.1^{b,C}	16.9±0.0^{b,B}	6.8±0.0^{c,B}	2.78±0.00^{b,D}
	20 h	5.3±0.0^{a,CD}	60.7±0.0^{b,C}	16.8±0.1^{b,B}	6.7±0.0^{c,B}	2.82±0.01^{a,E}
	24 h	5.5±0.1^{a,D}	61.6±0.0^{b,D}	16.4±0.1^{b,A}	6.3±0.2^{c,A}	2.98±0.00^{a,G}
	28 h	5.4±0.0^{a,CD}	61.3±0.0^{b,D}	16.3±0.0^{b,A}	6.3±0.1^{c,A}	2.91±0.01^{a,F}

Superscript different letters indicate statistically significant differences (p < 0.05): small letters between different vegetable oils for the same frying time and large letters within each vegetable oil during frying time.
C16:0 - palmitic acid; C18:1n-9 - oleic acid; C18:2n-6 - linoleic acid; C18:3n-3 - linolenic acid; CO - canola oil; EVOO - extra virgin olive oil; MUFA/PUFA – monounsaturated fatty acids/polyunsaturated fatty acids ratio; PO – peanut oil.

During deep-frying, the relative proportion of MUFA to PUFA increased appreciably (p<0.05) on all oils (Table 9.3) due to PUFA loss. Using palmitic acid as reference due to its increased stability to oxidation (Aladedunye and Przybylski, 2009), standardized losses were calculated based on Table 9.3 data. Losses were proportional to the amounts of each fatty acid, therefore with higher reduction of oleic acid in EVOO (8.5%) in comparison with PO (4.1%) and CO (4.9%), higher losses of linolenic acid (C18:2n-6) in PO (7.9%) and linolenic acid (18:3n-3) in CO (3.2%). Interestingly, and despite the differences in the major fatty acids amounts, total fatty acids degradation extent (sum of MUFA and PUFA losses) was similar on the three oils, with 12.2% in PO, 12.9% in EVOO and 13.3% in CO. Therefore, the effect of frying on the fatty acids should also be affected by other components in the lipid matrix, namely antioxidant compounds, justifying their study, as detailed below.

9.1.3.4 Minor antioxidants compounds and antioxidant activity

The antioxidant compounds studied included tocopherols, phenolic compounds and *β*-carotene, together with some antioxidant activity assays (Table 9.4).

Tocopherols are among the most important natural lipophilic antioxidants. Before frying, CO contained the highest total tocopherol amounts (810 mg/kg) followed by PO (535 mg/kg), and EVOO (366 mg/kg) (p<0.05), within the ranges presented in the literature (Boskou, 2011; Codex *Alimentarius*, 2015). A fast degradation was observed on all oils in the first frying hours, with total losses between 8 h to 12 h (Table 9.4). These results are in agreement with the literature, with intense tocopherol loss, even for reduced frying times (Casal *et al*., 2010; Olivero-David *et al*., 2014; Taha *et al*., 2014; Xu *et al*., 2015), probably derived from its scavenging activity of peroxyl radicals (Aladedunye and Przybylski, 2013).

Total phenolic compounds were estimated by the Folin-Ciocalteau method, as described in the Chapter 6. As expected, EVOO presented the highest total content before heating (p<0.05), due to absence of refining process and sample origin (Boskou, 2011). However, their potential effect as antioxidants was transient, with 83% loss after 8 h of frying for EVOO (p<0.05) and complete degradation for PO and CO. This is also in accordance with the literature (Abenoza *et al*., 2015; Andrikopoulos *et al*., 2002; Casal *et al*., 2010; Olivero-David *et al*., 2014), supporting that deep-frying process promotes total phenolics loss, probably by a combination of steam distillation, thermal degradation, or its use as effective antioxidant (Abenoza *et al*., 2015).

Concerning *β*-carotene, it can also react with radicals from decomposition of lipid hydroperoxides to form stable *β*-carotene radicals (Aladedunye and Przybylski, 2013). Before deep-frying, EVOO presented the highest contents of *β*-carotene (p<0.05), probably due to absence refining process (Boskou, 2011). After deep-frying, similar amounts were determined on the three oils, indicative that most of the *β*-carotene originally present in

EVOO was also loss in the first 8 h of frying. The slight *β*-carotene increase observed in all vegetable oils ($p<0.05$) after 8 h was probably derived from cumulative interactions with potatoes, naturally containing *β*-carotene, already observed in a previous study (Casal *et al*., 2010). This increase can also support *a** colour readings, previously discussed.

The antioxidant activity assays tested were based in hydrogen atom transfer (*β*-carotene/linoleic acid bleaching) and both electron and hydrogen atom transfer (DPPH), as presented in Table 9.4.

Table 9.4 Antioxidants and antioxidant activity changes in vegetable oils during intermittent fresh potatoes deep-frying.

Oil types	Frying time	Tocopherols (mg/kg)	Total Phenolics (mg GAE /kg)	*β*-carotene (mg/kg)	DPPH (mg GAE /kg)	*β*-carotene/ linoleic acid (mg GAE /kg)
	0 h	366.4±12.4^{a,B}	563.9±67.0^{b,B}	14.8±0.4^{b,D}	56.2±0.6^{a,C}	88.5±0.3^{c,C}
	8 h	36.8±0.0^{a,A}	96.2±1.6^{A}	1.7±0.0^{b,AB}	5.6±0.2^{a,B}	23.6±0.02^{c,B}
	12 h	n.d.	n.d.	1.4±0.1^{c,A}	1.5±0.2^{a,A}	2.6±0.05^{c,A}
EVOO	16 h	n.d.	n.d.	1.4±0.0^{b,A}	n.d.	n.d.
	20 h	n.d.	n.d.	1.5±0.1^{A}	n.d.	n.d.
	24 h	n.d.	n.d.	2.0±0.0^{b,B}	n.d.	n.d.
	28 h	n.d.	n.d.	2.6±0.1^{c,C}	n.d.	n.d.
	0 h	535.2±6.0^{b,B}	28.7±0.4^{a}	0.7±0.1^{a,A}	62.0±0.6^{b,C}	62.8±0.8^{a,C}
	8 h	45.6±1.7^{b,A}	n.d.	0.7±0.1^{a,A}	16.2±0.3^{b,B}	11.3±0.2^{a,B}
	12 h	n.d.	n.d.	0.7±0.1^{a,A}	2.4±0.2^{b,A}	1.4±0.05^{a,A}
PO	16 h	n.d.	n.d.	0.6±0.0^{a,A}	n.d.	n.d.
	20 h	n.d.	n.d.	1.2±0.3^{B}	n.d.	n.d.
	24 h	n.d.	n.d.	1.6±0.1^{a,C}	n.d.	n.d.
	28 h	n.d.	n.d.	1.4±0.0^{a,BC}	n.d.	n.d.
	0 h	810.2±21.9^{c,C}	33.1±1.2^{a}	0.6±0.0^{a,A}	71.8±0.8^{c,C}	69.3±0.7^{b,C}
	8 h	110.6±3.1^{c,B}	n.d.	0.7±0.0^{a,A}	22.5±0.1^{c,B}	16.0±0.2^{b,B}
	12 h	63.2±0.0^{A}	n.d.	1.1±0.1^{b,B}	3.7±0.2^{c,A}	2.3±0.01^{b,A}
CO	16 h	n.d.	n.d.	1.4±0.0^{b,BC}	n.d.	n.d.
	20 h	n.d.	n.d.	1.7±0.2^{D}	n.d.	n.d.
	24 h	n.d.	n.d.	1.6±0.1^{a,CD}	n.d.	n.d.
	28 h	n.d.	n.d.	1.7±0.1^{b,D}	n.d.	n.d.

Superscript different letters indicate statistically significant differences ($p<0.05$): small letters between different vegetable oils for the same frying time and large letters within each vegetable oil during frying time.
CO - canola oil; DPPH - 2,2-diphenyl-1-picrylhydrazyl radical; EVOO - extra virgin olive oil; GAE – Gallic acid equivalents; n.d. – not detected; PO - peanut oil.

Regarding the DPPH assay, CO presented the highest activity (p<0.05). After 8 h of deep-frying, a significant decrease was observed on all oils (p<0.05), of 90%, 74%, and 69% for EVOO, PO and CO, respectively. In opposition, in the *β*-carotene/linoleic acid bleaching assay, EVOO presented the highest activity (p<0.05), but again with a significant reduction at 12 h (p<0.05) on all oils, of 73%, 82%, and 77% for EVOO, PO and CO, and total absence of activity on all oils from this point forward, in agreement with previous absence of all minor antioxidants assayed.

Therefore, independently of oil type, the antioxidant activity decreased very fast under real deep-frying, as did the most important compounds with recognized antioxidant activity. Interestingly, the degradation of minor antioxidant compounds occurred in the first hours of frying, but no increased oxidation rate was observed thereafter, as supported by the PAV or OTG results previously discussed.

9.1.3.5 Volatile compounds

We have further explored the volatile fraction, as its constituents might be formed from fatty acid degradation, as well as from degradation of other minor constituents, both from oils and potatoes. HS-SPME is shown to be the among most popular techniques for the volatile compounds of edible oils (Sghaier *et al.,* 2016), but few studies were developed under deep-frying real conditions (Omar *et al*., 2007; Zhang *et al*., 2015; Petersen *et al*., 2013).

The volatile compounds analysed, grouped by chemical families, are presented in Table 9.5, while selected compounds are detailed in the Table 9.6. Before deep-frying, EVOO presented a more complex volatile profile, with the presence of “fresh” volatiles, such as alkanes, sesquiterpenes, alkanals, alkenals, alkadienals and alcohols, produced through biogenic pathways (Kalua *et al*., 2007), whereas PO and CO shown a poorer profile, as expected due to losses along the refining process. Alkanes and alkylbenzenes were presented in all oils, more pronounced in PO *vs* EVOO/CO (p<0.05). During deep-frying, some volatile families decreased or disappeared in the first hours, and an increment of others was verified, with clear within and between sample differences. Alkanals, alkenals and alkadienals were the families presenting greater amounts in all vegetable oils during frying, in accordance with other studies (Omar *et al*., 2007; Petersen *et al*., 2013). After 28 h of deep-frying, EVOO exhibit more alkanals (p<0.05), PO more alkenals (p<0.05) and CO more alkadienals (p<0.05), which is in accordance with their PUFA patterns and losses.

Table 9.5 Changes of volatile families (mg/kg of ISE) in vegetable oils during intermittent fresh potato deep-frying.

Oil types	Frying time	Alkanes	Alkenes	Sesquiterpenes	Alkylbenzenes	Alkanals	Alkenals	Alkadienals	Ketones	Alcohols	Carboxylic acids	Furan derivatives	Total
EVOO	0 h	6.2±0.7^{a,B}	2.7±0.3^{D}	1.0±0.2	17.9±3.1^{C}	1.5±0.1^{b,A}	8.4±0.2^{A}	0.5±0.1^{A}	n.d.	5.1±0.3^{A}	n.d.	n.d.	43±4^{b,A}
	8 h	6.4±0.1^{c,B}	1.1±0.0^{BC}	n.d.	41.3±7.4^{b,C}	85.7±0.9^{c,D}	107.7±1.1^{b,D}	25.8±0.02^{a,D}	1.8±0.1^{C}	11.2±0.7^{b,C}	3.7±0.3^{a,A}	2.4±0.02^{b,AB}	287±9^{b,D}
	12 h	0.5±0.2^{a,A}	1.3±0.3^{C}	n.d.	0.3±0.0^{a,A}	83.8±9.8^{b,CD}	103.8±4.7^{b,D}	24.3±1.1^{a,D}	1.5±0.1^{b,B}	7.7±0.4^{b,B}	4.0±0.6^{b,AB}	3.7±0.5^{b,C}	231±17^{b,C}
	16 h	0.6±0.1^{A}	0.7±0.1^{AB}	n.d.	0.4±0.0^{AB}	66.4±8.0^{b,BC}	78.4±9.5^{a,BC}	15.3±1.9^{a,C}	1.3±0.1^{b,AB}	7.9±1.2^{b,B}	5.6±0.8^{a,ABC}	2.3±0.3^{a,AB}	179±22^{a,B}
	20 h	0.6±0.0^{b,A}	0.7±0.1^{AB}	n.d.	0.5±0.0^{B}	77.9±2.2^{c,BCD}	83.3±3.0^{b,C}	15.7±0.7^{a,C}	1.4±0.04^{c,B}	7.8±0.3^{b,B}	6.3±0.2^{a,C}	2.5±0.2^{b,B}	197±6^{b,BC}
	24 h	0.8±0.1^{b,A}	0.5±0.1^{A}	n.d.	0.9±0.5^{AB}	61.5±10.7^{b,B}	62.0±12.6^{a,B}	11.4±2.3^{a,B}	1.0±0.2^{ab,A}	6.5±1.3^{b,AB}	6.0±1.7^{a,BC}	1.9±0.5^{a,AB}	152±30^{a,B}
	28 h	0.7±0.1^{b,A}	0.6±0.0^{A}	n.d.	n.d.	66.8±2.5^{c,BC}	61.5±1.8^{b,B}	11.8±0.3^{a,B}	1.0±0.04^{b,A}	6.5±0.2^{c,AB}	4.1±0.2^{a,ABC}	1.6±0.1^{a,A}	155±5^{ab,B}
PO	0 h	10.7±1.1^{a,C}	n.d.	n.d.	23.0±4.6^{B}	0.8±0.1^{a,A}	n.d.	n.d.	3.1±0.7^{ABC}	n.d.	0.8±0.2^{A}	n.d.	38±5^{b,A}
	8 h	5.6±0.1^{b,B}	n.d.	n.d.	16.5±0.8^{a,B}	56.1±4.5^{b,C}	118.0±9.2^{b,C}	46.4±3.7^{b,B}	1.6±0.2^{C}	14.5±0.9^{c,D}	12.0±3.1^{b,B}	3.5±0.2^{c,A}	274±21^{b,CD}
	12 h	0.6±0.1^{a,A}	n.d.	n.d.	0.4±0.0^{a,A}	43.6±3.0^{a,B}	125.2±4.9^{c,C}	48.0±2.9^{b,B}	0.4±0.02^{a,A}	12.9±0.1^{c,D}	9.6±0.2^{c,B}	4.6±0.2^{c,B}	245±11^{b,C}
	16 h	n.d.	n.d.	n.d.	n.d.	69.9±2.0^{b,D}	131.5±0.6^{b,C}	44.1±2.1^{b,B}	0.4±0.1^{a,AB}	14.5±0.8^{c,D}	25.8±1.6^{b,C}	3.7±0.3^{b,A}	290±3^{b,D}
	20 h	n.d.	n.d.	n.d.	n.d.	51.7±0.2^{b,C}	76.2±3.8^{b,A}	26.7±0.4^{b,A}	0.5±0.0^{a,A}	7.8±0.8^{b,B}	14.3±3.1^{b,B}	3.0±0.3^{b,A}	180±8^{b,B}
	24 h	n.d.	n.d.	n.d.	n.d.	72.0±4.1^{b,D}	95.5±11.0^{b,B}	31.6±2.4^{b,A}	0.7±0.0^{a,BC}	10.3±1.1^{c,C}	27.8±4.2^{b,C}	3.7±0.6^{b,A}	242±24^{b,C}
	28 h	0.5±0.1^{a,A}	n.d.	n.d.	n.d.	50.9±1.5^{b,BC}	71.6±2.1^{c,A}	27.8±1.5^{b,A}	0.4±0.0^{a,A}	5.2±0.2^{b,A}	9.7±2.8^{b,B}	3.8±0.1^{c,BC}	170±9^{b,B}
CO	0 h	6.0±1.2^{a,ABCD}	n.d.	n.d.	13.8±3.2^{A}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	20±4^{a,A}
	8 h	3.6±0.1^{a,D}	n.d.	n.d.	11.5±0.0^{a,A}	40.3±2.2^{a,BC}	63.1±4.4^{a,BC}	66.6±4.8^{c,B}	n.d.	1.0±0.1^{a,AB}	0.7±0.2^{a,A}	1.8±0.2^{a,AB}	189±12^{a,C}
	12 h	8.3±0.9^{b,D}	n.d.	n.d.	24.7±4.5^{b,B}	28.3±2.7^{a,A}	59.6±7.3^{a,BC}	41.1±5.4^{b,A}	n.d.	3.4±0.5^{a,C}	2.7±0.2^{a,B}	1.6±0.2^{a,A}	170±22^{a,CD}
	16 h	0.5±0.04^{C}	n.d.	n.d.	n.d.	38.0±0.3^{a,BC}	64.7±1.2^{a,C}	42.0±1.7^{b,A}	1.3±0.3^{b}	0.9±0.1^{a,AB}	7.6±0.3^{a,D}	2.1±0.1^{a,BC}	157±2^{a,BCD}
	20 h	0.5±0.1^{a,ABC}	n.d.	n.d.	n.d.	33.9±4.8^{a,AB}	43.9±6.2^{a,A}	35.5±5.7^{c,A}	0.8±0.0^{b}	0.6±0.0^{a,A}	4.6±0.4^{a,C}	1.9±0.2^{a,AB}	122±17^{b,B}
	24 h	0.3±0.1^{a,AB}	n.d.	n.d.	n.d.	43.8±3.3^{a,C}	66.9±4.1^{a,C}	44.9±4.0^{c,A}	1.3±0.3^{b}	1.5±0.0^{a,B}	9.5±1.4^{a,E}	2.8±0.2^{ab,D}	171±13^{a,CD}
	28 h	0.3±0.04^{a,A}	n.d.	n.d.	n.d.	39.3±3.0^{a,BC}	51.3±3.7^{a,AB}	40.2±2.6^{c,A}	n.d.	1.1±0.1^{a,AB}	5.0±0.2^{a,C}	2.4±0.1^{b,CD}	140±10^{a,BC}

Superscript different letters indicate statistically significant differences (p<0.05): small letters between different vegetable oils for the same frying time and large letters within each vegetable oil during frying time.
CO - canola oil; EVOO - extra virgin olive oil; ISE – internal standard equivalents; n.d. - not detected; PO - peanut oil.

However, from a health of view, these compounds are generally not regarded as similar, with potential deleterious effects increasing in the order alkanals < alkenals < alkadienals (Katragadda *et al*., 2010). Interestingly, while alkylbenzenes, benzaldehyde, 2-propenal (acrolein), 2-butenal (crotonal), and *E,E*-2,4-decadienal, are well known toxic compounds (Dung *et al*., 2006; Hecht *et al*., 2015; LoPachin and Gavin, 2014), low amounts of 2,4-decadienal (*Z,E*- and *E,E*-2,4-decadienal) are determinant for the typical deep-fried flavour, while 2-heptenal and *E*-2-octenal are implicated in the typical deep-fried odour (Warner, 2009).

Globally, EVOO presented lower levels of all these volatile degradation compounds (Table 9.6), except total alkylbenzenes at 8 h (Table 9.5). Benzaldehyde and crotonal were characteristics of CO, whereas *E,E*-2,4-decadienal was detected in higher amounts in PO. Acrolein presented high oscillations, probably due its volatility (boiling point 52.5 °C), identified in PO after 8 h, and after 16 h in EVOO and CO, with lower amounts in EVOO (p<0.05).

The off-flavours emitted (Table 9.6) were mainly C18:1n-9 degradation products (octanal, nonanal, *E*-2-decenal, and 2-undecenal – a fruity, plastic or waxy odours), expected from its higher prevalence, but also from C18:2n-6 (hexanal, and 2-pentylfuran – a grassy, plastic or fruity odours) and C18:3n-3 (acrolein, and *E,E*-2,4-heptadienal – a fishy, acrid or oily odours) (Neff *et al*., 2000).

Significant Pearson's correlations were verified between several volatile families, such as alkenals and PAV for PO (r^2= 0.651, p<0.05), and CO (r^2= 0.864, p≤0.001) as well as alkadienals for PO (r^2= 0.594, p<0.05), and CO (r^2 =0.664, p<0.05). Also, significant Person's correlation between volatile compounds, namely *E*-2-decenal and TPC (r^2= 0.634, p<0.05) and DPTG (r^2= 0.601, p<0.05) was only verified for CO. These correlations between volatile compounds and common lipid degradation parameters are in agreement with literature (Petersen *et al*., 2013).

Table 9.6 Changes in selected volatile compounds (mg/kg of ISE) in vegetable oils during intermittent fresh potato deep-frying.

Oil type	Frying time	Hexanal	Octanal	Nonanal	2-Propenal	2-Butenal	2-Heptenal	2-Octenal	2-Decenal	2-Undecenal	*E,E*-2,4-Decadienal	*Z,E*-2,4-Decadienal	*E,E*-2,4-Heptadienal	2-Pentylfuran	Benzaldehyde
EVOO	0 h	0.6±0.0^{b,A}	n.d.	0.9±0.1^{b,A}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	8 h	4.7±0.8^{a,B}	15.0±0.2^{c,AB}	58.4±0.1^{c,D}	n.d.	n.d.	15.1±0.8^{a,CD}	7.3±0.2^{a,BC}	45.6±1.1^{c,C}	33.1±1.0^{c,C}	14.1±0.1^{a,C}	6.3±0.1^{a,D}	3.9±0.2^{a,B}	1.4±0.1^{a,A}	n.d.
	12 h	7.1±1.6^{b,BC}	15.8±1.4^{c,B}	56.9±6.4^{c,D}	n.d.	n.d.	17.7±2.4^{a,D}	8.4±1.2^{b,C}	41.0±0.5^{c,C}	29.5±0.3^{c,C}	12.8±0.3^{a,C}	5.3±0.2^{a,C}	4.4±0.8^{a,B}	2.0±0.3^{a,B}	n.d.
	16 h	7.0±1.4^{a,BC}	13.5±1.2^{c,AB}	41.8±4.6^{c,BC}	2.5±0.6^{a}	0.4±0.1^{a}	12.8±1.5^{a,BC}	6.1±0.8^{a,AB}	29.8±3.3^{c,AB}	21.0±2.3^{b,AB}	8.2±0.9^{a,B}	3.7±0.5^{a,AB}	2.4±0.3^{a,A}	1.7±0.2^{a,AB}	n.d.
	20 h	8.4±0.2^{C}	14.9±0.9^{c,AB}	49.3±1.4^{b,C}	4.0±0.7	n.d.	12.3±0.7^{a,ABC}	6.6±0.3^{a,ABC}	31.6±0.7^{c,B}	22.3±0.6^{c,B}	8.6±0.2^{a,B}	4.1±0.2^{a,B}	2.5±0.2^{a,A}	1.8±0.1^{a,AB}	n.d.
	24 h	8.8±0.7^{a,C}	11.8±2.5^{b,A}	35.6±7.0^{b,B}	3.0±0.8	n.d.	8.6±1.8^{a,A}	4.9±1.0^{a,A}	23.8±4.8^{b,A}	16.6±3.3^{A}	5.8±1.2^{a,A}	2.9±0.5^{a,A}	1.6±0.3^{a,A}	1.4±0.3^{a,A}	n.d.
	28 h	8.4±0.4^{a,C}	13.2±0.7^{c,AB}	39.9±1.2^{c,BC}	n.d.	n.d.	9.2±0.6^{a,AB}	5.2±0.1^{a,A}	24.6±0.5^{c,A}	17.6±0.7^{c,AB}	6.1±0.1^{a,A}	3.0±0.1^{a,A}	1.6±0.0^{a,A}	1.6±0.1^{a,AB}	n.d.
PO	0 h	0.4±0.1^{a,A}	n.d.	0.5±0.0^{a,A}	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	8 h	12.4±0.3^{b,B}	9.2±0.7^{b,BC}	29.1±3.4^{b,D}	3.6±0.5^{B}	n.d.	45.8±2.5^{c,C}	14.7±1.4^{b,BC}	30.8±3.2^{b,C}	17.9±1.4^{b,CD}	33.9±2.7^{c,B}	10.6±0.8^{b,B}	n.d.	3.5±0.2^{b,A}	n.d.
	12 h	n.d.	8.9±0.6^{b,BC}	29.5±2.5^{b,D}	2.6±0.2^{A}	n.d.	55.8±1.5^{b,D}	18.3±0.3^{c,C}	24.9±1.5^{b,B}	18.2±1.3^{b,D}	33.2±2.1^{b,B}	10.7±0.6^{b,B}	n.d.	4.6±0.2^{b,B}	n.d.
	16 h	27.7±1.2^{c,D}	10.2±0.1^{b,C}	24.5±0.3^{b,CD}	4.5±0.4^{b,B}	n.d.	55.3±0.6^{c,D}	22.1±1.0^{b,D}	24.8±0.6^{b,B}	18.6±0.8^{b,D}	30.5±1.4^{c,B}	10.1±0.7^{b,B}	n.d.	3.7±0.3^{b,A}	n.d.
	20 h	20.6±1.7^{b,C}	7.3±0.3^{b,A}	18.4±1.8^{a,B}	n.d.	n.d.	33.4±2.3^{b,B}	12.5±0.7^{b,AB}	15.1±0.6^{b,A}	11.4±0.1^{b,A}	18.6±0.4^{b,A}	6.0±0.02^{b,A}	n.d.	3.0±0.3^{b,A}	n.d.
	24 h	33.6±0.1^{b,E}	9.7±0.7^{ab,C}	20.4±2.9^{a,BC}	n.d.	n.d.	39.0±3.5^{c,B}	17.6±2.9^{b,C}	19.1±2.4^{ab,A}	14.9±1.7^{BC}	22.1±2.5^{c,A}	7.1±0.7^{c,A}	n.d.	3.7±0.6^{c,A}	n.d.
	28 h	12.9±1.5^{b,B}	8.2±0.1^{b,B}	25.7±0.5^{b,CD}	2.4±0.4^{A}	n.d.	27.3±0.5^{c,A}	9.6±0.1^{c,A}	16.5±0.4^{b,A}	12.1±0.8^{b,AB}	20.0±1.2^{c,A}	6.8±0.3^{c,A}	n.d.	3.8±0.1^{c,AB}	n.d.
CO	0 h	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	8 h	4.5±0.4^{a,A}	6.0±0.4^{a,AB}	22.1±2.3^{a,D}	n.d.	2.3±0.2^{B}	22.2±1.1^{b,B}	6.8±0.5^{a,AB}	15.3±1.7^{a,C}	11.7±1.3^{a,D}	19.0±1.9^{b,B}	6.0±0.7^{a,C}	32.1±1.7^{b,B}	1.8±0.2^{a,AB}	5.9±0.1^{C}
	12 h	4.1±0.4^{a,A}	5.2±0.4^{a,A}	15.5±2.3^{a,ABC}	n.d.	0.7±0.01^{A}	17.0±0.7^{a,A}	5.8±0.7^{a,A}	11.0±2.2^{a,AB}	8.5±1.4^{a,B}	12.3±2.4^{a,A}	4.0±0.8^{a,AB}	18.7±1.6^{b,A}	1.6±0.2^{a,A}	3.1±0.4^{A}
	16 h	10.1±0.8^{b,D}	5.4±0.1^{a,A}	13.3±0.8^{c,A}	4.5±0.2^{b,B}	3.2±0.1^{b,C}	22.0±0.2^{b,B}	7.9±0.1^{a,BC}	12.6±0.5^{a,ABC}	9.2±0.4^{a,BC}	12.3±0.7^{b,A}	4.3±0.2^{a,AB}	18.0±0.6^{b,A}	1.7±0.0^{a,A}	3.9±0.1^{AB}
	20 h	7.2±0.9^{a,BC}	4.9±0.6^{a,A}	14.6±2.0^{a,AB}	3.8±0.6^{AB}	1.9±0.4^{B}	15.6±1.8^{a,A}	5.6±0.8^{a,A}	10.0±1.5^{a,A}	3.3±0.5^{a,A}	10.2±1.5^{a,A}	3.3±0.5^{a,A}	16.4±2.8^{b,A}	1.6±0.1^{a,A}	3.4±0.7^{AB}
	24 h	8.6±0.6^{a,CD}	7.2±0.9^{a,B}	19.3±2.7^{a,BCD}	2.7±0.3^{A}	2.2±0.3^{B}	22.6±1.5^{b,B}	8.6±1.1^{a,C}	14.8±1.3^{a,BC}	11.1±0.7^{CD}	14.2±1.4^{b,A}	5.0±0.5^{b,BC}	18.3±1.4^{b,A}	2.4±0.3^{b,C}	4.3±0.6^{B}
	28 h	6.0±0.8^{a,B}	6.3±0.2^{a,AB}	20.7±1.3^{a,CD}	2.5±0.8^{A}	1.2±0.2^{A}	16.8±0.8^{b,A}	6.3±0.4^{b,AB}	12.1±0.7^{a,ABC}	8.7±0.4^{c,BC}	12.4±0.8^{b,A}	4.1±0.2^{b,AB}	17.6±1.3^{b,A}	2.1±0.0^{b,BC}	3.6±0.1^{AB}

Superscript different letters indicate statistically significant differences (p<0.05): small letters between different vegetable oils for the same frying time and large letters within each vegetable oil during frying time.
CO - canola oil; EVOO - extra virgin olive oil; ISE – internal standard equivalents; n.d. - not detected; PO - peanut oil.

9.1.3.6 Global analysis of parameters

To find which parameters better summarize the impact of intermittent fresh potatoes deep-frying in the three oils, and their potential differences, a PCA was performed.

It allowed explaining 64% of total data variance by using two principal dimensions based on physical-chemical parameters analysed during frying time, as represented in Figure 9.2.

Dimension 1, which comprises 42% of the total variance, was characterized in the positive region by the major degradation indicators, namely TPC and its fractions, PAV, K_{232}, K_{270}, ΔE and a^*, alkadienals, alkenals, furan derivatives and carboxylic acids, while the negative region is influenced by more protective effects, as total tocopherols, antioxidant activity and phenolics, alkanes, alkylbenzenes, sesquiterpenes, and alkenes. Dimension 2, which justifies 22% of the total variance observed, was characterized in the positive region by L^*, b^*, C18:2n-6, and C18:3n-3, while alkenes, alkanals, C18:1n-9, C16:0, and β-carotene were increased in the negative region. Thus, six distinct groups are represented, with a clear distinction of EVOO from the other two oils, the later with higher values in dimension 2 through the entire frying time, and a similar evolution trend through dimension 1 with frying, towards increased oxidation markers. Globally, at 28 h, EVOO has lower values of dimension 1 than the other two oils. This supports a lower oxidation of EVOO against the other two oils.

These PCA results corroborate the abovementioned discussion between samples during deep-frying, such as increased formation of primary and secondary oxidation compounds, total polar compounds and its fractions for PO and CO in comparison to EVOO, while a distinction of the two (CO and PO) is not clearly perceptible.

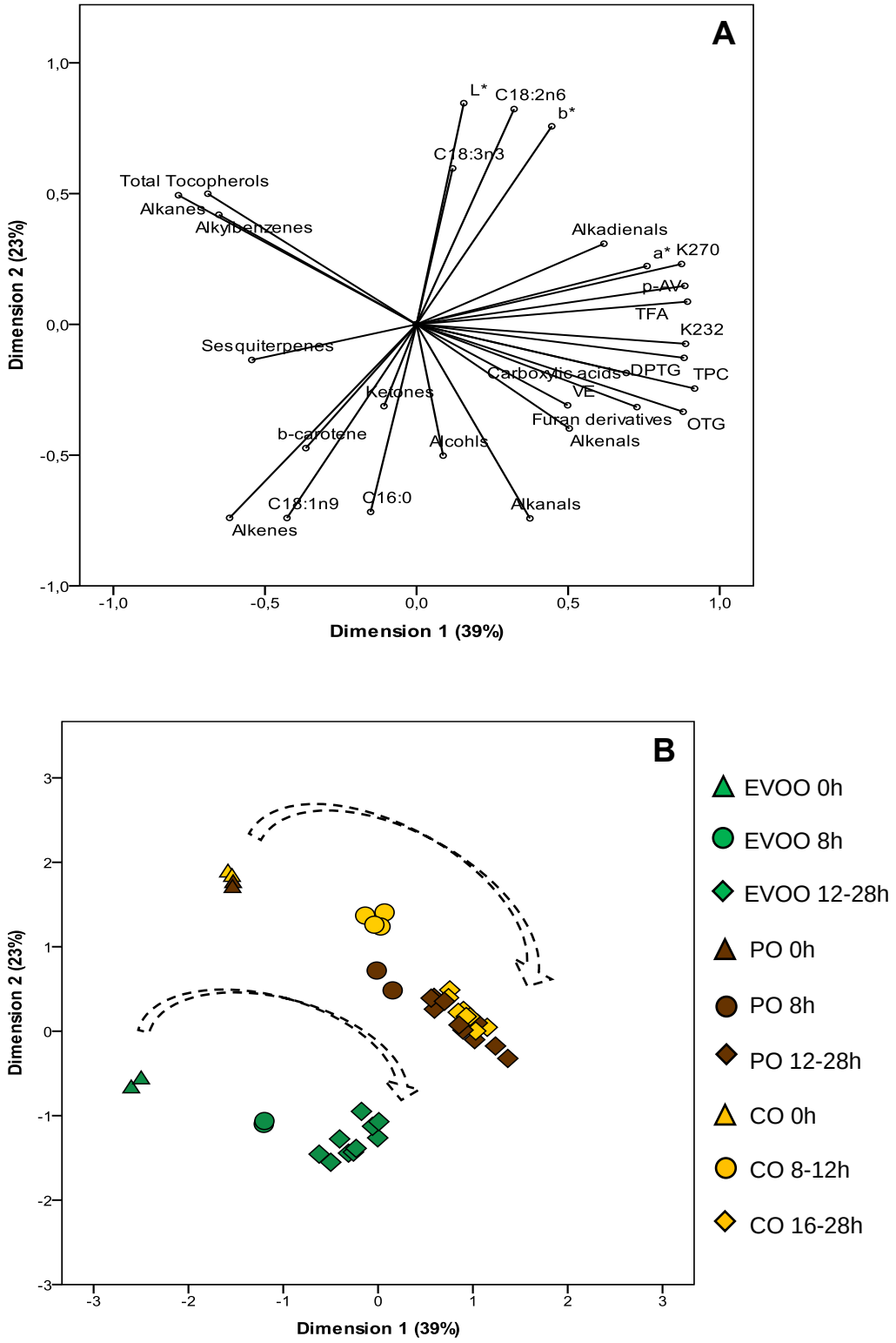


Figure 9.2 Principal component analysis of vegetable oils during intermittent fresh potatoes deep-frying: A) loadings of variables; B) Samples of EVOO, PO and CO at different frying times.

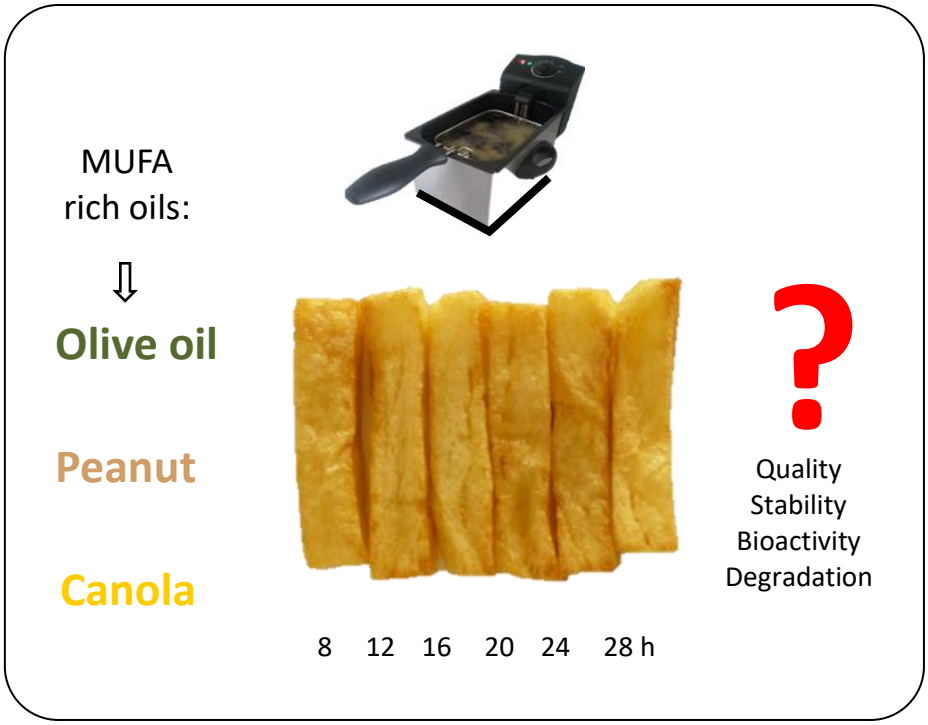
9.1.4 Concluding remarks

The results of present work demonstrated that fatty acids oxidation was similar on the three oils, but other degradation parameters pointed into a lower degradation of EVOO against PO and CO, while the distinction of these two was less clear. As to EVOO, it presents lower formation of primary and secondary oxidation compounds, total polar compounds and potentially harmful volatile compounds than PO and CO, for the same frying time and conditions, but is losing the potential nutritional and bioactive advantages derived from the antioxidant pool soon upon frying (8-12 h). However, higher total content of off-flavours were also quantified in EVOO, probably related with this same degradation of minor compounds, which might be associated with the strong odours described by some consumers when using EVOO for frying. Interestingly, between PO and CO differences were less clear, probably derived from their equivalent total PUFA content. However, PO presented higher total content of typical deep-fried flavour, associated with the recognized general good sensorial acceptance of peanut oil for frying, but it also presented higher amounts of secondary oxidation products and potentially unhealthy volatile compounds, requiring attention not to surpass legal limits regarding oil degradation to avoid increasing its amounts.

Independently of oil type, the antioxidants pool and activity decreased almost completely during the first 8 to 12 hours of frying. Therefore, based on the oxidation indicators studied through time, the minor antioxidants evaluated were not key determinants in oil protection from oxidation. Also, the presence of higher amounts C18:3n-3 in CO was not a determinant for its increased degradation, even regarding oxidation parameters, with a close behaviour to PO, richer in C18:2n-6.

From the consumer point of view and regarding EVOO, if the natural minor antioxidants, including phenolic compounds, are perceived as nutritionally important, heating time should be restricted, without exceeding 8 h, preferably less. In addition, further studies on nutritional and sensorial changes in the potatoes should be investigated, including formation of acrylamide, for a better comparison of these three oils from the nutritional and safety points of view.

9.2. Fried potatoes: impact of prolonged frying in monounsaturated oils



9.2.1 Background and aim of study

Potatoes are an important dietary source of bioactive compounds and essential minerals. However, loses are expected to occur during cooking, as well as interactions with the heating medium used in the oil-based techniques, therefore contributing for the final composition of the processed potato.

When searching for the nutritional impact from the consumer's perspective, on the fried food, data on the nutritional and sensory changes are scarce and those who do, evaluate potatoes quality after frying on fresh oils and not with prolonged frying, when the oil baths began to degrade (Carlson and Tabacchi, 1986; Han *et al*., 2004; Salta *et al*., 2008; Tian *et al*., 2016).

Therefore, the aim of the present work was to compare the nutritional and sensory quality of fresh potatoes during intermittent deep-frying in three MUFA-rich oils commercially available to consumers: peanut oil (PO), canola (CO) and extra virgin olive oil (EVOO), up to oil recommended disposal point (25% of TPC). This study focuses on the nutritional, sensory, and potential health impact of the increasingly degraded fat being incorporated in the fresh potatoes and interacting with the most important potato bioactive compounds, while attempting to compare the gains and losses from using different monounsaturated-rich oils recommended for frying.

9.2.2 Sampling

Three commercial oils were chosen: EVOO, PO and CO. The deep-frying assays were performed according to Chapter 8. Potatoes samples were collected every 4 h, except on the first day, and immediately analysed by the sensory panel and instrumental colour, followed by moisture and total ascorbic acid. Further analyses, namely lipid content, fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, acrylamide, and volatile compounds, all detailed in Chapter 6, were evaluated in frozen samples. Fresh vegetable oils were also analysed for some composition parameters (fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, and volatile compounds). All analytical determinations were performed in triplicate (n = 3).

9.2.3 Results and Discussion

The nutritional and sensory quality of fried potatoes is associated with diverse chemical (moisture and fat contents), physical (colour), and structural (crispiness attribute) parameters (Pedreschi, 2012). Thus, each of these parameters is discussed in detail below.

9.2.3.1 Impact on nutritional quality of fresh white potatoes

Potatoes moisture reduced from 82.1% (Table 9.7), within the characteristic range of potatoes with frying aptitude (Pedreschi, 2012), to 48.5-58.2%, with a great variability over the tested conditions, corresponding almost to a duplication of the dry mass amount of potato on a 100g basis, and therefore an expected enrichment on all potatoes components. Regarding the three oils tested, despite the significant differences observed for some frying time (p<0.05), no clear pattern was perceived. However, an apparent moisture reduction was observed with time, with the lowest moisture contents observed at 28 h for all samples (p<0.05), with strong negative Pearson correlations for PO (r= -0.862, p<0.001) and CO (r = -0.766, p<0.01). This could be derived from achieving a higher degree of doneness over time. With all processing condition constant over time, it could be a consequence of oil degradation, known to influence several physical properties that influence its thermal efficiency, as surface tension or convective heat transfer coefficients (Tseng *et al*., 1996). The decreased oil:potato ratio over time, imposed by the absence of replenishment, could also have contributed for this effect.

Moisture reduction was inevitably accompanied by fat absorption, with final contents ranging from 7.6 to 11.2% (Table 9.7), but without any correlation with frying time, and with similar amounts at the first (8 h) and last (28 h) samplings. Indeed, previous authors have demonstrated that one of the most significant parameters conditioning oil absorption in potatoes frying is temperature (Pedreschi, 2012), kept constant during all the assays. However, significant differences (p<0.05) were observed with CO, exhibiting slightly higher lipid amounts at all samplings (+0.8 to +2.2%), therefore not associated with oil degradation. Some authors have studied the influence of oil type on fat uptake, but the results are not consistent, nor the mechanism proposed for the differences observed (Kita and Lisinska, 2005). Again, inherent characteristic of the oils, as fatty acid composition, viscosity, and specific heat characteristics (Ziaiif *et al*., 2008) (not evaluated), have been associated with oil absorption. Therefore, this could be a specific characteristic of the oil brand used and, to clarify it, different brands of the three oils could have been used simultaneously. Both moisture and fat content were similar to those of French fries from different varieties and fried in diverse vegetable oils (Salta *et al*., 2008).

Table 9.7 Changes in fresh white potato composition during intermittent deep-frying.

		Oils	Potatoes	Fried at						
		Fresh	Raw	8 h	12 h	16 h	20 h	24 h	28 h	r
Moisture g/100 g	**EVOO**	-	82.1 ±0.2	54.5±0.3^{a,B}	58.2±0.3^{c,D}	51.8±0.1^{a,A}	56.0±0.3^{c,C}	54.4±0.2^{b,B}	52.2±0.2^{b,A}	-0.426^{NS}
	PO			56.0±0.1^{b,C}	56.9±0.1^{b,D}	56.7±0.0^{c,D}	54.0±0.1^{a,B}	54.3±0.1^{b,B}	52.5±0.3^{b,A}	-0.862***
	CO			55.0±0.1^{a,E}	56.6±0.1^{a,F}	52.9±0.2^{b,B}	54.7±0.1^{b,D}	53.6±0.1^{a,C}	48.5±0.1^{a,A}	-0.766***
Lipids g/ 100g	**EVOO**	-	0.1 ±0.0	9.0±0.0^{a,BC}	7.6±0.0^{a,A}	9.8±0.0^{b,D}	8.7±0.2^{a,B}	8.8±0.1^{b,B}	9.3±0.2^{a,C}	0.289^{NS}
	PO			9.4±0.0^{b,C}	8.1±0.0^{b,A}	8.9±0.2^{a,B}	9.1±0.1^{b,B}	8.3±0.1^{a,A}	9.4±0.0^{a,C}	0.071^{NS}
	CO			11.2±0.2^{c,E}	9.4±0.0^{c,A}	10.8±0.1^{c,CD}	10.6±0.2^{c,C}	9.9±0.1^{c,B}	11.1±0.1^{b,DE}	0.050^{NS}
of which **C18:1n-9**	**EVOO**	70.7±0.7	-	6.4±0.0^{b,E}	6.2±0.0^{c,DE}	6.1±0.0^{b,CD}	6.0±0.0^{b,BC}	5.8±0.0^{c,AB}	5.7±0.1^{b,A}	-0.965***
	PO	52.9±0.0		5.0±0.0^{a,D}	4.3±0.0^{a,B}	4.6±0.1^{a,C}	4.6±0.0^{a,C}	4.1±0.0^{a,A}	4.7±0.0^{a,C}	-0.382^{NS}
	CO	57.9±0.1		6.6±0.0^{c,D}	5.3±0.1^{b,A}	6.1±0.1^{b,CD}	6.0±0.2^{b,BC}	5.6±0.1^{b,AB}	5.9±0.2^{b,BC}	-0.304^{NS}
C18:2n-6	**EVOO**	8.6±0.0	-	0.7±0.0^{a,F}	0.6±0.0^{a,E}	0.6±0.0^{a,D}	0.5±0.0^{a,C}	0.5±0.0^{a,B}	0.5±0.0^{a,A}	-0.983***
	PO	25.5±0.1		2.3±0.0^{c,E}	1.8±0.0^{c,D}	1.8±0.0^{b,CD}	1.8±0.0^{b,C}	1.5±0.0^{b,A}	1.7±0.0^{b,B}	-0.797***
	CO	19.1±0.0		2.1±0.0^{b,D}	1.6±0.0^{b,AB}	1.7±0.0^{b,C}	1.7±0.1^{b,BC}	1.5±0.0^{b,A}	1.6±0.1^{b,ABC}	-0.664**
C18:3n-3	**EVOO**	0.8±0.0	-	0.06±0.00^{b,E}	0.05±0.00^{b,D}	0.04±0.00^{b,CD}	0.04±0.00^{a,BC}	0.04±0.00^{a,AB}	0.03±0.00^{a,A}	-0.964***
	PO	0.2±0.0		0.02±0.00^{a,C}	0.02±0.00^{a,B}	0.02±0.00^{a,B}	0.02±0.00^{a,BC}	0.01±0.00^{a,A}	0.02±0.00^{a,B}	-0.582**
	CO	9.3±0.0		0.93±0.01^{c,D}	0.68±0.01^{c,BC}	0.70±0.01^{c,C}	0.68±0.03^{b,BC}	0.58±0.02^{b,A}	0.64±0.03^{b,AB}	-0.780***
Ascorbic acid mg/100g	**EVOO**	-	31.1 ±1.0	46.3±0.9^{ab,C}	32.8±3.6^{B}	31.9±2.6^{a,B}	12.8±0.2^{b,A}	16.2±0.7^{a,A}	17.5±1.7^{ab,A}	-0.872***
	PO			44.7±1.4^{a,C}	37.8±1.7^{B}	45.4±4.1^{b,C}	12.5±0.0^{b,A}	17.5±0.2^{b,A}	16.4±0.3^{a,A}	-0.823***
	CO			47.9±0.5^{b,F}	35.0±1.5^{D}	40.8±0.7^{b,E}	11.0±0.1^{a,A}	16.6±0.3^{ab,B}	19.1±0.2^{b,C}	-0.822***
Tocopherols µg/100g	**EVOO**	36.6±1.2*	20 ±0.2	362±3^{a,C}	283±4^{a,A}	388±6^{a,D}	347±4^{a,B}	397±3^{a,D}	449±2^{a,E}	0.708***
	PO	53.5±0.6*		868±3^{b,C}	545±6^{b,B}	470±14^{b,A}	540±1^{b,B}	561±1^{b,B}	790±7^{b,C}	-0.091^{NS}
	CO	81.0±2.1*		3337±10^{c,F}	1173±3^{c,D}	847±9^{c,B}	1012±8^{c,C}	751±1^{c,A}	1530±11^{c,E}	-0.560*
Total carotenoids µg/100g	**EVOO**	1484±37	178 ±5	114±4^{a,A}	157±9^{C}	116±3^{a,A}	168±4^{C}	130±3^{a,B}	228±1^{a,C}	0.669**
	PO	67±7		134±11^{ab,A}	160±12^{B}	153±2^{b,AB}	189±5^{C}	168±2^{b,B}	237±0^{a,D}	0.839***
	CO	59±3		154±13^{b,A}	151±14^{A}	189±0^{c,B}	190±14^{B}	194±2^{c,B}	282±6^{b,C}	0.851***
Total phenolics mg GAE/100g	**EVOO**	56.4±6.7	5.9 ±0.3	14.3±0.0^{b,ABC}	14.4±2.0^{BC}	11.4±0.2^{a,A}	13.9±0.6^{AB}	14.0±1.0^{a,AB}	17.1±1.1^{C}	0.404^{NS}
	PO	2.9±0.0		8.7±0.5^{a,A}	12.1±0.4^{B}	13.9±0.6^{b,BC}	13.0±0.4^{BC}	16.3±0.8^{ab,E}	15.2±0.7^{DE}	0.864***
	CO	3.2±0.1		10.1±1.4^{a,A}	12.4±0.1^{AB}	13.6±1.6^{ab,BC}	12.6±0.7^{ABC}	18.3±0.8^{b,D}	15.3±0.5^{C}	0.771***
DPPH mg GAE/100g	**EVOO**	5.6±0.1	7.9 ±0.1	15.1±0.2^{a,B}	2.9±0.0^{b,A}	n.d.	n.d.	n.d.	n.d.	-0.745***
	PO	6.2±0.1		16.5±0.5^{b,B}	2.3±0.1^{a,A}	n.d.	n.d.	n.d.	n.d.	-0.723***
	CO	7.2±0.1		18.0±0.5^{c,B}	3.5±0.0^{c,A}	n.d.	n.d.	n.d.	n.d.	-0.747***
β-carotene/ linoleic acid mg GAE/100g	**EVOO**	8.8±0.0	7.2 ±0.0	8.2±0.0^{c,B}	2.8±0.0^{b,A}	n.d.	n.d.	n.d.	n.d.	-0.798***
	PO	6.3±0.1		6.8±0.2^{a,B}	1.4±0.0^{a,A}	n.d.	n.d.	n.d.	n.d.	-0.749***
	CO	6.9±0.1		7.2±0.0^{b,B}	2.8±0.0^{b,A}	n.d.	n.d.	n.d.	n.d.	-0.808***
Acrylamide µg/100 g	**EVOO**	-	-	77±8^{A}	85±1^{a,ABC}	82±3^{a,AB}	96±3^{b,CD}	107±2^{b,D}	92±1^{BC}	0.724***
	PO			75±3^{A}	88±1^{b,A}	125±10^{b,C}	93±3^{ab,B}	93±3^{a,B}	96±2^{B}	0.281^{NS}
	CO			62±2^{A}	103±5^{c,C}	105±7^{b,C}	78±6^{a,AB}	99±6^{ab,BC}	106±9^{C}	0.492*

*in mg/100g for vegetable oils;

$^{a-c}$ Statistically significant differences between vegetable oils or $^{A-F}$ between frying time (p<0.05);

r - Pearson correlation with frying time: significant at the *0.05, **0.01, and ***0.001 level (2-tailed), NS – not significant.

C18:1n-9 - oleic acid; C18:2n-6 - linoleic acid; C18:3n-3 - linolenic acid; CO - canola oil; DPPH - 2,2-diphenyl-1-picrylhydrazyl radical; EVOO - extra virgin olive oil; GAE - Gallic acid equivalents; PO - peanut oil.

Due to the reduced amounts of raw potatoes lipids (0.1% - Table 9.7), the main fatty acids after processing reflect directly the frying oil composition (Table 9.7), as expected (Hosseini *et al.*, 2016). Based on the high content of MUFA on the three oils, oleic acid was consistently the major one on all potato samples, slightly lower when fried in PO (4.1-5.2 g/100g) than EVOO (5.7-6.4 g/100g) and CO (5.3-6.6 g/100g), with strong negative Pearson correlations with frying time for EVOO (r= -0.965, p<0.001). The polyunsaturated fatty acids reduced with increased frying time, as observed for instance by the strong negative Pearson correlations with frying time for C18:2n-6/C16:0 ratio (EVOO: r= -0.980, PO: r= -0.961, CO: r= -0.949, all at p<0.001), indicative of fatty acid oxidation. Linolenic acid, present in comparative higher amounts in CO, reduced slightly with frying time (Table 9.7).

Regarding TFA, also common indicators of oil thermal degradation, despite being generally low on all potato extracts (below 0.6% in the extracted lipids) (Fig. 9.3), the amounts increased consistently with frying time (p<0.05), retaining the significant differences already observed on the fresh oils, with potatoes fried in EVOO having lower TFA content at all sampling times, while potatoes in CO had significantly higher amounts (p<0.05), closely followed by PO. Also, the amounts found are equivalent to those previously observed in the oils (Subchapter 9.1, Table 9.1). These results were corroborated by strong positive Pearson correlations between TFA and frying time on the three oils (EVOO: r= 0.965, PO: r =0.927, CO: r= 0.898, all at p<0.001), in accordance with published data for palm superolein and olive oil during 48 h (Romano *et al.*, 2012).

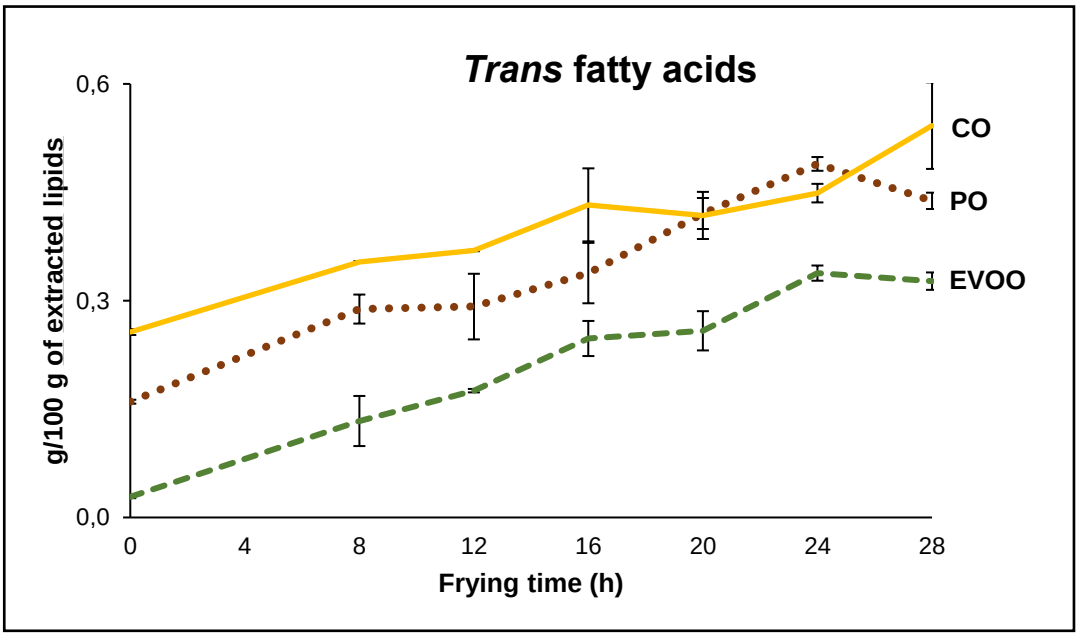


Figure 9.3 Changes of TFA during intermittent fresh white potatoes deep-frying in EVOO, PO and CO.

Despite the high frying temperature, the food temperature is not known to exceed 100°C, and cooking takes place in a short time, with expected small nutritional losses compared to other confection methods (Decker and Ferruzzi, 2013). Indeed, most studies on prolonged frying compare the oils degradation over time, inferring that the absorption of degraded oil, instead of fresh one, will compromise only the potatoes lipid content and composition. Without being false, this is just part of the problem, as most of these degraded compounds are not chemically stable, and will inevitable interact with potatoes' most sensitive components, as vitamins, and antioxidants, potentiating degradation beyond the expected. This is particularly true for ascorbic acid. Potatoes are rich sources of ascorbic acid in our diet (Camire *et al*., 2009), but it is partially loss during the thermal and oxidative stress imposed by food processing. As showed from Table 9.7, the 31 mg/100g of ascorbic acid present in fresh potato reduced drastically with frying time. The apparent increase at 8 h of frying, observed on the three oils, was not sufficient to compensate moisture decrease, indicating already a great percentage of loss in the first frying hours. From this point forward, frying induced higher ascorbic acid losses, indicating that the frying oil is under a higher oxidative stress, and its degradation compounds, namely free radicals, interact with potatoes ascorbic acid (Carlson and Tabacchi, 1986; Han *et al.*, 2004). Strong negative Pearson confirm these increased ascorbic acid losses with time (EVOO: r= -0.872; PO: r = -0.823; CO: r = -0.822, all at $p<0.001$). In this case, no differences were observed between the tested oils.

For total free carotenoids, present on the fresh potatoes and oils (particularly in EVOO) (Table 9.7), a slight decrease was observed in the first samplings (8-12 h), followed by a full recovery on the last sampling hours, with positive Pearson correlation in the potatoes fried in the three oils (EVOO: r= 0.669, $p<0.01$; PO: r= 0.839, $p<0.001$; CO: r= 0.851, $p<0.001$). It is interesting to denote that, despite the huge differences observed in the fresh oils, with 20 times higher carotenoids in EVOO than PO or CO, no differences were observed between fried potatoes, even if the different amounts of incorporated lipid are taken into account. This apparent carotenoids stability is consistent with the data from Blessington *et al*. (2010), and the increased pattern with time can be supported by moisture loss, also higher at 28h, together with possible partial hydrolysis of carotenoids bonded to potatoes compounds (Burmeister *et al*., 2011), without excluding the possibility of contribution from coloured Maillard reaction products. However, no distinction was made between xanthophylls and carotenes, both present in the potatoes and oils, whose balance might alter during processing and influence total carotenoids content and profile (Burmeister *et al*., 2011).

Regarding tocochromanols, collectively known as vitamin E, they are present in very low amounts in raw potatoes (20 µg/100g of *α*-tocopherol). The higher amounts after frying

cannot be explained by moisture loss alone, and therefore seem to be incorporated in a direct proportion of their content in the vegetable oils (Table 9.7), with higher amounts in CO, followed by PO and EVOO (p<0.05). This pattern was kept up to the last sampling hour, with potatoes fried in CO having higher tocopherol amounts and EVOO lower (p<0.05). However, with increased frying time, different patterns were observed, with a slight but significant increase when fried on EVOO (r= 0.708, p<0.001), a significant decrease in CO (r= -0.560, p<0.05), and non-significant variations with PO. This is indicative of a higher preservation of vitamin E during EVOO frying. The apparent increase over the last sampling could be just a direct consequence of the higher lipids amounts observed, as previously discussed.

Regarding phenolic compounds, again present on both potatoes and vegetable oils, particularly in EVOO, their content on the fried potatoes increased in comparison with the raw ones on all samplings, more than doubling the initial amounts, as expected from moisture loss. Indeed, on a dry basis, from an initial 33 mg/100g in raw potato, potatoes fried in PO heated for 28 h kept 32 mg/100g and CO kept 30 mg/100g, indicative of high stability. Despite the recognized loss of oil phenolic compounds with frying time in subchapter 9.1 (Casal *et al*., 2010), with EVOO heated for 28 h the potatoes were still being enriched in phenolic compounds, with 36 mg/100g, on a dry basis. When compared with the literature, a huge variation of total phenolic amounts is found, particularly derived from their estimation being based on the reaction with the Folin-Ciocalteu reagent, as in the present article, and also derived from the standard phenolic used as references, usually chlorogenic, caffeic or gallic acids. However, frying is known to be conservative regarding these compounds (Blessington *et al*., 2010), where with the confirmation that this protective effect seems to be prolonged even with heavily degraded oil.

Regarding the antioxidant activity of the fried potatoes, two different methods were tested: the capacity to inhibit oxidation of *β*-carotene by reactive oxygen species released from oxidized linoleic acid, and the capacity to release electrons for inhibition of radicals. Both potato compounds (ascorbic acid, phenolic compounds, carotenoids, etc.) and vegetable oil compounds (phenolics, carotenoids, tocochromanols, phytosterols, etc.) are expected to contribute for the collective antioxidant activity observed with these assays. Although without major differences between the three fresh oils with both assays, potatoes sampled at 8 h showed differences in their antioxidant activity, with higher activity with the DPPH assay with CO fried potatoes, and higher *β*-carotene/linoleic acid bleaching activity in EVOO. However, on both assays the antioxidant activity was below the quantification limit from the 16 h forward, contradicting the expected antioxidant capacity derived from the presence of antioxidants on the potatoes, namely ascorbic acid, carotenoids and phenolic compounds. This could occur because the tests were made with potatoes extracts where

the degraded oil is in contact with potato bioactive compounds, and might compete for the antioxidant capacity of the potato compounds during the assay, therefore being not available to react with the DPPH radical or to counteract with the Reactive Oxygen Species released from linoleic acid. This is particularly interesting from the nutritional point of view, showing that despite the presence of interesting bioactive compounds in the fried food, as carotenoids and phenolic compounds, the total balance of effects will be potentially ineffective in our body, or even potential pro-oxidative.

9.2.3.2 Impact on acrylamide formation in fresh white potatoes

As a possible carcinogenic in humans (Group 2A), this compound has received much attention from the scientific community and consumers in general, with fried potatoes positioned among the highest sources (Matthäus and Haase, 2014).

Raw potatoes acrylamide contents were below the quantification limit (1 µg/100g), as expected because its formation occurs when they are cooked at high temperature (>120°C) (Matthäus and Haase, 2014).The results for fried potatoes are presented in Table 9.7. Slight variations between samples were verified with the three oils and during frying time, ranging from 62 to 125 µg/100g. These results are near to indicative acrylamide values for ready-to-eat French fries (60 µg/100g), and potato crisps (100 µg/100g) (Matthäus and Haase, 2014). Fried potatoes at 8 h had similar acrylamide content between oils (p>0.05), and correspond to the lowest values. The highest acrylamide levels were observed with fried potatoes at 16 h in PO (p<0.05), closely followed by CO at 16 h and 28 h or EVOO at 24 h. Globally, a high variability was observed in acrylamide amounts, without an association with oil type, but with an apparent increase with frying hours, particularly for EVOO and CO. Literature data are not consistent on these issues, with most authors reporting no association with oil type (Matthäus and Haase, 2014), although Zhang *et al.* (2015), on a 600 frying cycles study with six different oils addressed the possible influence of oil heat transfer coefficients in acrylamide content. Regarding the influence of oil quality, again no consistent associations are found in the literature for simulated or potatoes frying, but with an highlight that the food type may have some influence (Matthäus and Haase, 2014), On all reports, variability between replicates is usually high, which might hamper statistical significances.

9.2.3.3 Impact on volatile compounds of fresh white potatoes

The development of desirable and undesirable flavours in fried potatoes is a combined effect of oil alteration, breakdown products of potatoes constituents, and compounds produced by potato/oil interactions (van Loon *et al*., 2005).

Volatiles emitted from raw potatoes have origin in lipid degradation, due to its lypoxygenase content (van Loon *et al*., 2005), while those from fried potatoes have various origins, including lipid degradation, sugar/Maillard reaction, sulfur and terpenes degradations (van Loon *et al*., 2005; Comandini *et al*., 2011).

Regarding the volatile chemical families analysed (Table 9.8), grouped as non-aldehyde and aldehydes, raw potatoes had the lowest total volatile content, wherein aldehydes (alkanals and alkenals) were predominant. During deep-frying, the total volatile contents increased in all fried potatoes, reflected in positive Pearson correlations with frying time (EVOO: r= 0.605, $p<0.01$; PO: r= 0.596, $p<0.01$; CO: r= 0.854, $p<0.001$). However, some oscillations were verified in the individual families and groups, derived probably from partial losses by evaporation during sampling (Matthäus, 2006), as well as from the inherent characteristics of the SPME methodology. This method evaluates the volatiles released and equilibrated in the headspace with the fiber and not its true total content, only achieved by purge-and trap methodologies, being therefore more useful for comparison, as pursued in the present work. The maximum content of total volatiles was observed at 24 h for potatoes fried in EVOO, resulting mostly from oleic acid oxidation (alkanes and alkanals) and sugar/*Maillard* reaction degradation products (pyrazines), corresponding to the sample also with highest acrylamide content. At 28 h of frying, no differences were found between samples for total volatiles ($p>0.05$).

Pyrazines were the main non-aldehyde group, with strong Pearson correlations with the highest correlations with frying time on the three oils, but without distinct differences between them. These compounds result mostly from sugar/*Maillard* reactions degradation from the potatoes (Romano *et al*., 2012), potentially accumulating in the oils with frying time.

For aldehydes, different patterns were observed with the three oils along the frying time, with higher alkanals formation in EVOO, similar alkenals on the three oils, and higher alkadienals in PO and CO. This is consistent with the fatty acid profile of the oils (Table 9.7), with higher saturated fatty acids in EVOO, similar monounsaturated on the three oils, and higher linolenic acid in PO and CO.

In terms of volatiles typical of fried potatoes flavour, *E,E*-2,4-decadienal, derived from peroxidation of linoleic acid (Boskou *et al*., 2006), was predominant in CO and PO (Fig. 9.4), while 2,5-dimethylpyrazine (data not shown), formed by *Maillard* reaction, was the main typical flavour in EVOO fried potatoes.

Table 9.8 Changes of volatile chemical families (µg/100 g of ISE) during intermittent fresh white potatoes deep-frying.

		Oils	**Potatoes**	**Fried at**						
		Fresh	Raw	8 h	12 h	16 h	20 h	24 h	28 h	r
Alkylbenzenes	**EVOO**	1789 ±311	36 ±4	81 ±6^{a,A}	66 ±3^{a,A}	319 ±9^{b,D}	114 ±16^{a,B}	185 ±6^{a,C}	165 ±18^{a,C}	0.327^{NS}
	PO	2297 ±464		174 ±36^{b,A}	185 ±4^{b,AB}	202 ±11^{a,ABC}	177 ±4^{b,AB}	220 ±13^{b,BC}	243 ±6^{b,C}	0.735***
	CO	1376 ±322		194 ±18^{b,AB}	178 ±3^{b,B}	212 ±30^{a,AB}	198 ±21^{b,AB}	250 ±17^{b,B}	324 ±36^{c,C}	0.788***
Carboxylic Acids	**EVOO**	n.d.	n.d.	12 ±1^{a,A}	18 ±5^{a,A}	23 ±2^{a,A}	26 ±5^{a,A}	434 ±52^{C}	204 ±41^{b,B}	0.684**
	PO	80 ±24		124 ±23^{b,C}	89 ±1^{b,B}	90 ±1^{b,B}	53 ±0^{b,A}	n.d.	n.d.	-0.952***
	CO	n.d.		n.d.	n.d.	n.d.	n.d.	n.d.	62 ±2^{a}	0.654**
Furan derivatives	**EVOO**	n.d.	17 ±0	9 ±0^{a,A}	19 ±0^{a,C}	56 ±1^{b,D}	18 ±0.^{a,C}	21 ±0^{a,C}	15 ±2^{a,B}	-0.005^{NS}
	PO	n.d.		26 ±0^{c,A}	28 ±0^{c,A}	27 ±4^{a,A}	63 ±2^{c,C}	35 ±4^{b,B}	35 ±2^{b,B}	0.364^{NS}
	CO	n.d.		20 ±3^{b,A}	25 ±1^{b,AB}	23 ±2^{a,AB}	26 ±0^{b,B}	25 ±3^{a,AB}	33 ±3^{b,C}	0.788***
Pyrazine	**EVOO**	n.d.	n.d.	488 ±16^{a,A}	506 ±21^{a,A}	1160 ±34^{b,B}	1069 ±223^{B}	1707 ±78^{c,C}	1529 ±13^{ab,C}	0.907***
	PO	n.d.		838 ±28^{b,A}	977 ±5^{b,B}	953 ±86^{a,AB}	1035 ±7^{B}	1279 ±51^{a,C}	1329 ±15^{a,C}	0.930***
	CO	n.d.		776 ±51^{b,A}	954 ±25^{b,A}	904 ±46^{a}	984 ±42^{A}	1511 ±30^{b,B}	1746 ±181^{b,C}	0.894***
Pyrrole	**EVOO**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	32 ±2	27 ±6^{a}	0.796***
	PO	n.d.		n.d.	n.d.	32 ±4^{B}	24 ±0^{b,A}	30 ±0^{B}	45 ±3^{ab,C}	0.898***
	CO	n.d.		n.d.	22 ±1^{A}	28 ±0^{A}	20 ±1^{a,A}	37 ±4^{A}	56 ±16^{b,B}	0.860***
TOTAL Non-Aldehydes	**EVOO**	3290 ±349	83 ±7	635 ±17^{a,A}	642 ±21^{a,A}	1597 ±31^{b,BC}	1292 ±198^{B}	2079 ±95^{c,D}	1756 ±13^{a,C}	0.851***
	PO	3753 ±483		1039 ±7^{b,A}	1190 ±7^{b,B}	1214 ±85^{a,B}	1299 ±8^{B}	1563 ±55^{a,C}	1783 ±22^{a,D}	0.946***
	CO	1974 ±442		990 ±58^{b,A}	1179 ±24^{b,A}	1168 ±63^{a,A}	1228 ±53^{A}	1823 ±12^{b,B}	2208 ±197^{b,C}	0.891***
Alkanals	**EVOO**	105 ±79	337 ±160	580 ±5^{b,A}	645 ±14^{b,A}	1725 ±30^{b,D}	819 ±67^{b,B}	1193 ±37^{c,C}	910 ±27^{c,B}	0.299^{NS}
	PO	85 ±6		592 ±30^{b,CD}	625 ±16^{b,D}	511 ±20^{a,B}	431 ±1^{a,A}	558 ±6^{b,B}	536 ±9^{a,B}	-0.434^{NS}
	CO	n.d.		437 ±23^{a,AB}	380 ±1^{a,A}	474 ±21^{a,B}	466 ±41^{a,B}	428 ±2^{a,AB}	629 ±23^{b,C}	0.671**
Alkenals	**EVOO**	843 ±19	117 ±60	301 ±10^{a,A}	31 ±2^{a,A}	1608 ±48^{c,D}	503 ±39^{a,B}	693 ±94^{b,C}	751 ±98^{C}	0.250^{NS}
	PO	n.d.		556 ±81^{b,A}	764 ±17^{c,C}	756 ±37^{b,C}	612 ±14^{b,AB}	689 ±37^{b,BC}	679 ±28^{BC}	0.147^{NS}
	CO	n.d.		435 ±33^{b,A}	470 ±23^{b,A}	575 ±76^{a,AB}	562 ±52^{ab,AB}	519 ±24^{a,AB}	655 ±86^{B}	0.710***
Alkadienals	**EVOO**	480 ±50	18 ±10	128 ±6^{a,AB}	110 ±0^{a,A}	524 ±24^{a,E}	188 ±21^{a,BC}	245 ±32^{a,CD}	268 ±34^{a,D}	0.270^{NS}
	PO	n.d.		240 ±31^{b,A}	1313 ±44^{c,D}	1122 ±72^{c,C}	926 ±29^{c,B}	1018 ±63^{c,C}	972 ±39^{b,B}	0.374^{NS}
	CO	n.d.		854 ±68^{c,AB}	661 ±25^{b,A}	740 ±76^{b,AB}	727 ±88^{b,AB}	692 ±34^{b,AB}	865 ±100^{b,B}	0.069^{NS}
TOTAL Aldehydes	**EVOO**	1427 ±97	472 ±231	1009 ±17^{a,A}	1070 ±13^{a,A}	3857 ±83^{c,D}	1510 ±104^{a,B}	2131 ±73^{b,C}	1929 ±130^{C}	0.276^{NS}
	PO	85 ±6		1388 ±66^{b,A}	2702 ±37^{c,D}	2389 ±105^{b,C}	1969 ±35^{b,B}	2265 ±87^{b,C}	2187 ±62^{BC}	0.268^{NS}
	CO	n.d.		1725 ±102^{c,A}	1511 ±41^{b,A}	1788 ±141^{a,AB}	1756 ±148^{ab,AB}	1639 ±48^{a,A}	2149 ±170^{B}	0.526*
TOTAL Volatiles	**EVOO**	4717 ±413	554 ±234	1669 ±43^{a,A}	1734 ±46^{a,A}	5477 ±62^{c,D}	2858 ±369^{B}	6297 ±806^{b,D}	3889 ±216^{C}	0.605**
	PO	3838 ±489		2551 ±66^{b,A}	3981 ±52^{c,C}	3694 ±234^{b,C}	3321 ±32^{B}	3828 ±174^{a,C}	3970 ±102^{C}	0.596**
	CO	1974 ±442		2716 ±196^{b,A}	2690 ±80^{b,A}	2956 ±251^{a,A}	2983 ±246^{A}	3714 ±66^{a,B}	4464 ±426^{C}	0.864***

$^{a-c}$ Statistically significant differences between vegetable oils or $^{A-D}$ between frying time (p<0.05).

r - Pearson correlation with frying time: significant at the *0.05, **0.01, and ***0.001 level (2-tailed), NS – not significant.

CO - canola oil; EVOO - extra virgin olive oil; ISE – internal standard equivalents; n.d. - not detected; PO - peanut oil.

A huge variation with time was found in *E,E*-2,4-decadienal amounts, ranging from 80 to 1100 µg/100g of potatoes, within the literature ranges (Boskou *et al*., 2006). The minimum value was observed for fried potatoes in EVOO, while the maximum values were observed for fried potatoes in CO and PO, particularly this last, in agreement with its higher linoleic acid content. *E,E*-2,4-decadienal amounts should be regarded with caution due to its potential toxicity (Boskou *et al*., 2006).

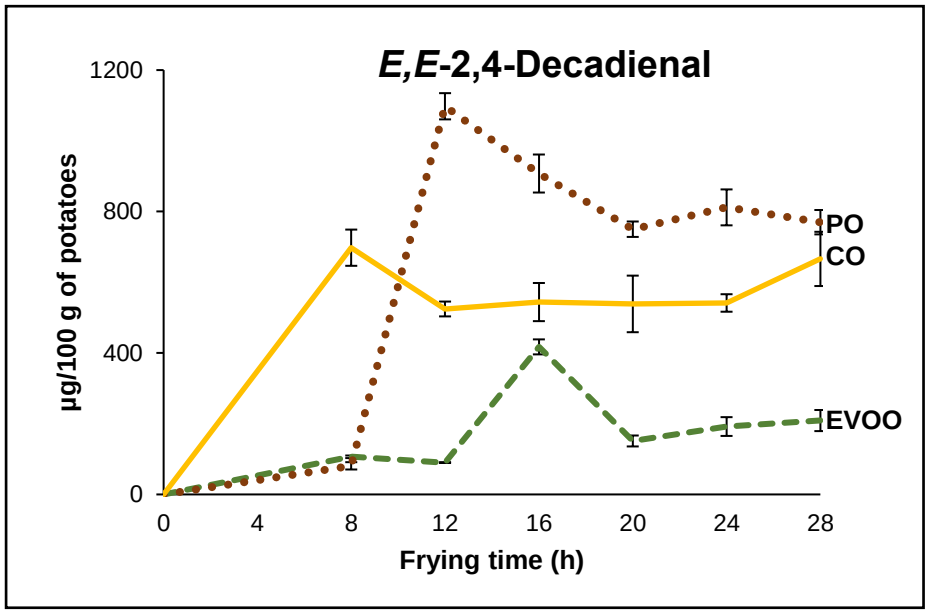


Figure 9.4 Changes of *E,E*-2,4-decadienal during intermittent fresh white potatoes deep-frying in EVOO, PO and CO.

In terms of undesirable flavour, nonanal was the most representative in fried potatoes in EVOO, while 2-undecenal was more noticeable in potatoes fried in PO and CO, on average (data not shown). These volatile compounds have been described as resulting from oxidation of different oleic acid hydroperoxides (Perkins, 2007), the main fatty acid of fried potatoes. Interestingly, hexanal (data not shown), associated to linoleic acid oxidation and to the loss of "positive" odour attributes in fried potatoes (Comandini *et al*., 2011), was detected in higher amounts in the raw potatoes, probably favoured by the increased equilibrium in the headspace derived from the lower total amount of volatiles. It decreased up to the 20 h, increasing again thereafter in EVOO and PO. These variations were already described by Alizadeh *et al.* (2015) under different frying conditions.

For EVOO fried potatoes, strong positive Pearson correlation was found between total of volatile compounds formed by sugar/*Maillard* reaction degradation and acrylamide ($r = 0.834$, $p<0.01$) at 24 h, particularly with pyrazines ($r = 0.797$, $p<0.01$), supporting the probable association of the maximum emission of volatiles with the highest acrylamide content.

9.2.3.4 Impact on colour and sensory analysis of fresh white potatoes

Several studies report the importance of oil absorption during frying and its implication on the sensory quality of fried foods and acceptability (Decker and Ferruzzi, 2013; Matthäus, 2006). In this sense, we have carried out sensory analysis of fried potatoes in the different vegetable oils, over frying time. Instrumental measure of colour was also included to corroborate the sensory findings regarding this attribute. Indeed, colour development in fried potatoes is strictly related to consumer perception of quality (Pedreschi, 2012) and several variables may affect it, including oil type, temperature, frying time, and sample dimensions (Krokida *et al*., 2001).

According to the literature, the ideal tonality for fried potatoes is between -5 and 0 for coordinate *a**, and higher than 10 for coordinate *b** (Krokida *et al.*, 2001), while higher *L** value indicates lighter colour, desirable in fried potatoes (Garayo and Moreira, 2002). Although frying time was identical for per batch (6 min), instrumental colour determinations made on the surface reflected some variability. Regardless of the oil type or frying time, coordinate *a** was generally higher ($4 < a^* < 10$), while coordinate *b** was in accordance, and *L** was always superior to 45. On average, and in comparison with raw peeled potatoes [*L** (72), *a** (-1), *b** (30)], deep-frying promoted a decrease of coordinate *L** (-16) and an increase of coordinates *a** (+8) and *b** (+4), intensifying the red tones, as expected (Pedreschi, 2012). With increased frying time, coordinate b* (yellow) was kept similar between samples ($p>0.05$) while coordinate *a** presented an irregular pattern. Still, at 28 h, potatoes fried in PO presented the higher colour changes [ΔE (28), *L** (52), *a** (10), *b** (37)], while fried potatoes in CO presented the lowest [ΔE (18), *L** (62), *a** (6), *b** (37)]. Therefore, weak correlations with frying time were only observed with PO for *L** (r= -0.539, $p<0.05$) and ΔE (r= -0.485, $p<0.05$). Regarding the browning index, all samples were within the 44-49 range, without association with frying time or oil type.

For the sensory analysis, Figure 9.5 resumes the information on the three oils for the same day (A-C), and Figure 9.6 resumes the variations for the same oil over the three days (A-C). As previously explained, potatoes fried in the oils heated for 28 h were not given to the panel as these had more than 25% of TPC.

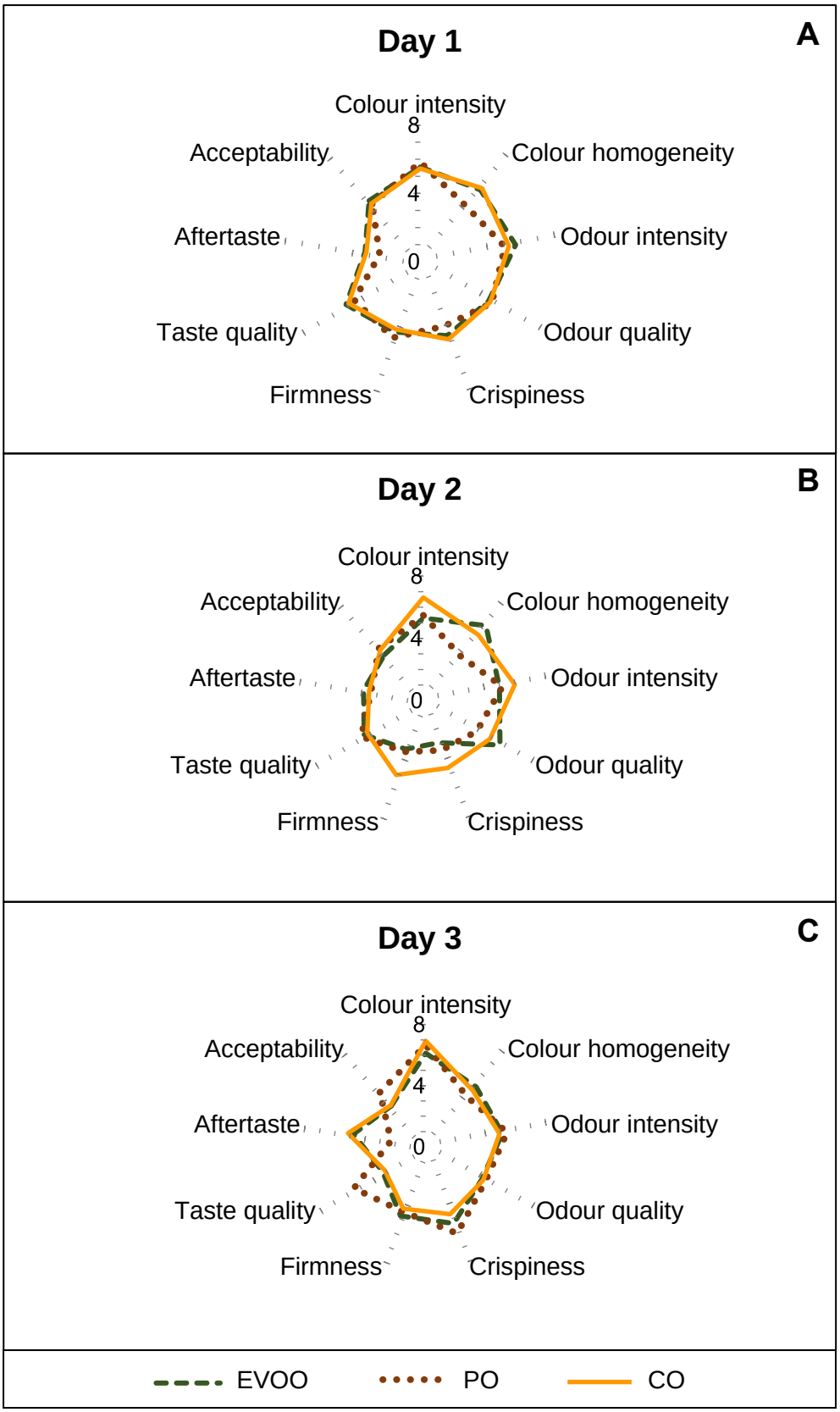


Figure 9.5 Mean sensory attribute scores during intermittent fresh white potatoes deep-frying in EVOO, PO and CO, comparison of: A) Day 1, B) Day 2, and C) Day 3.

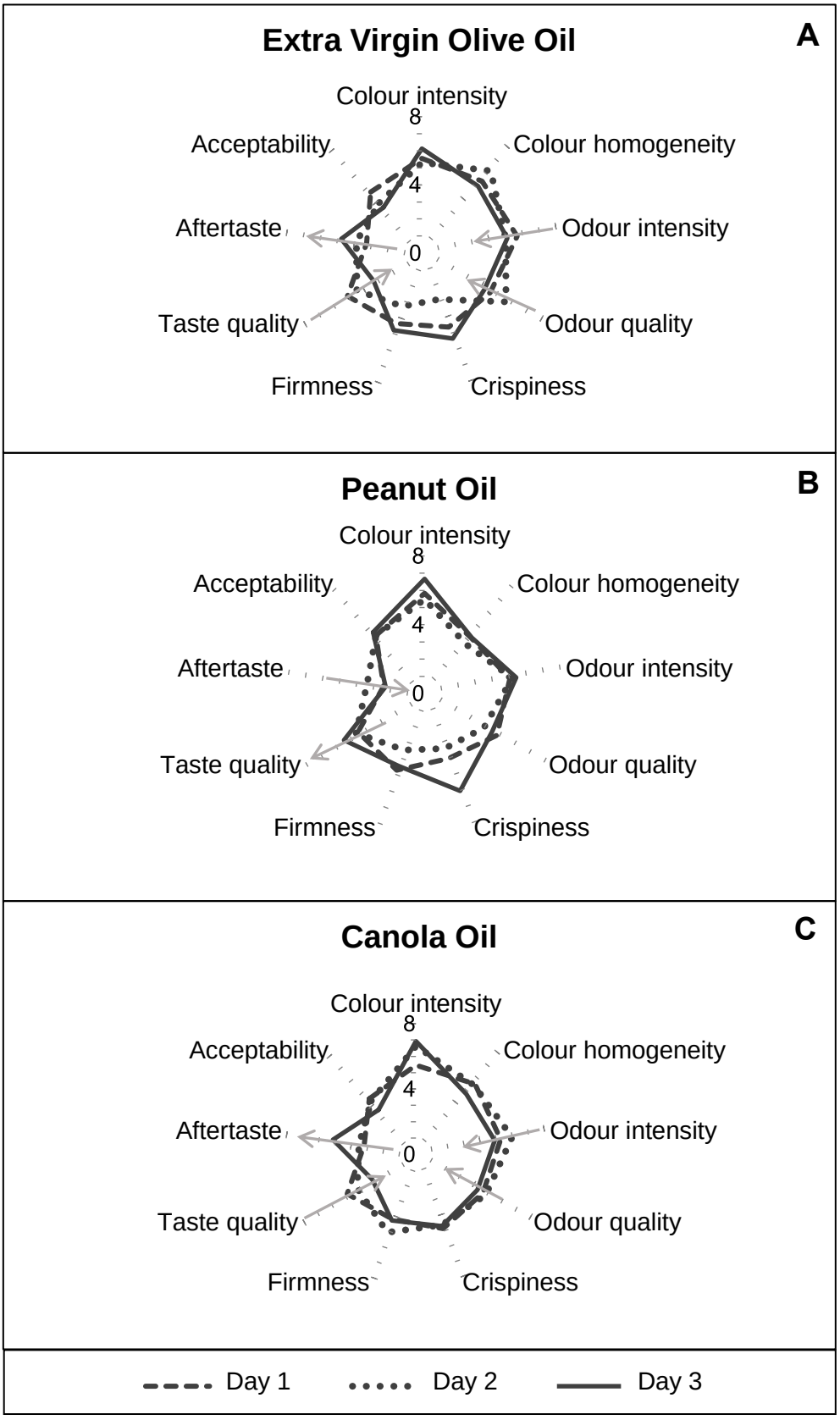


Figure 9.6 Mean sensory attribute scores during intermittent fresh white potatoes deep-frying at day 1, 2 and 3, evolution of: A) Extra Virgin Olive Oil, B) Peanut Oil, and C) Canola Oil.

Despite the recognized differences in the oils natural taste, with EVOO presenting a bitter, astringent and pungent flavour due to its rich phenols composition (Servili *et al*., 2004) while refined oils have more neutral taste (Matthäus, 2006), the sensory panel did not detect marked differences between samples in the first day (p>0.05) (Fig. 9.5A). However, increased frying time, and consequently oil degradation, promoted perceptible changes on sensory attributes of fried potatoes. In the second day (Fig. 9.5B), the sensory panel detected statistically significant differences (p<0.05) associated to colour intensity (CO > PO, EVOO), colour homogeneity (EVOO, CO > PO), odour quality (EVOO, CO > PO), crispiness (CO > PO, EVOO), and firmness (CO > EVOO, PO). However, in the last day (Fig. 9.5C), differences between samples were only associated to taste quality (PO > EVOO, CO).

When each oil was analysed separately, panellists were able to detect degradation in EVOO (Fig. 9.6A) with significant decrease (p<0.05) for taste quality over time (day 1, day 2 > day 3), as well as in CO (Fig. 9.6C), while no significant negative evolution was perceived in PO (Fig. 9.6B). Aftertaste increased contributed clearly for the taste quality reduction in EVOO and CO, together with a reduction on odour intensity and quality, again not perceived in PO. Only colour intensity was consistent on the three oils, with a perceived increased over time, despite being significant only for CO (Fig. 9.6C) (day 3, day 2 > day 1), in agreement with the instrumental colour readings. This evolution was corroborated with weak Pearson correlations, negative for taste quality in EVOO (r= -0.399, p<0.05), positive for crispiness in PO (r= 0.428, p<0.01), and again positive for colour intensity in CO (r =0.492, p<0.01). Regarding acceptability, statistically significant differences were not found in each day or oil type, but a weak negative Pearson correlation between frying time and acceptability was verified for EVOO (r= -0.349, p<0.05).

In addition, Pearson and Spearman correlations were performed to understand what sensory attributes affected fried potatoes acceptability on each day to ascertain the vegetable oil type influences. Only moderate to strong positive Pearson correlations were found. In the first and second days' acceptability was associated to taste quality, for EVOO (r= 0.817, p<0.001 and r= 0.674, p<0.05), PO (r= 0.791, p<0.01 and r= 0.939, p<0.001), and CO (r= 0.675, p<0.05 and r= 0.927, p<0.001). In the third day, EVOO was associated to odour and taste qualities (r= 0.769, p<0.01, and r= 0.905, p<0.001, respectively); PO was associated to odour quality and firmness (r= 0.763, p<0.01, and r= 0.837, p<0.001, respectively); and in CO associated to taste quality (r= 0.843, p<0.001). Although acceptability is also linked to perception of colour, namely golden-yellow colour acquired during deep-frying, in this work no correlations were found. However, the relationship between sensorial (subjective) and instrumental analysis (objective) was studied by Pearson correlations. In general, weak correlations between colour intensity and L^*

coordinate (r= -0.479, p<0.05), and between colour homogeneity and a^* coordinate (r =0.416, p<0.05) were found on the first day, increasing its significance on the third day (r= -0.764 and 0.749, respectively, p<0.05) revealing more consistent patterns among assessors.

From the assessor's perspective, other sensorial attributes than colour were determinant for acceptability of fried potatoes, such as taste and odour qualities, firmness, and probably taste habits, as CO is still not common in Portugal.

9.2.4 Concluding remarks

The impact of prolonged frying on potatoes nutritional and sensory qualities was studied, including vitamins and antioxidant activity. The results for fried potato composition are compiled in Table 9.9, using as reference the samples at 8 h and 28 h of frying to demonstrate the highest gains and losses with prolonged frying. Globally, these results show that fried potatoes bioactive compounds are heavily affected by the degradation extent of the frying oil.

From a health point of view, it was particularly noticeable that frying in used oil, even if close to the recommended limits for frying, has highly negative effects on fried potatoes composition. Vitamin C was significantly reduced with frying time, from around 60% of dietary recommendation at 8 h to only 20%, on a 100g dose basis. Additionally, the potential antioxidant activity of the product was heavily affected, being almost absent after 12 h of frying, half of the average frying time allowed in this assay. Indeed, despite containing several antioxidants compounds, as ascorbic acid itself, vitamin E, and phenolic compounds, these were inefficient in the in vitro tests for antioxidant activity, giving an interesting perception on what can occur in the body once ingested, with the degraded lipids consuming all the potential antioxidant activity of the product. Therefore, they cannot be regarded as being truly available once ingested being interesting to perform bioavailability studies in the future to corroborate it. It also highlights for the permissiveness of the 25 % total polar compounds limit for frying oils. Additionally, the significant impact of fried potatoes on the daily ingestion of acrylamide was reinforced (0.4 to 1.9 µg/kg body weight per day) (EFSA, 2015).

Regarding the three monounsaturated-rich oils, the differences observed were reduced. From a nutritional point of view, CO was more equilibrated, with interesting amounts of essential fatty acids and vitamin E, superior during all the frying sessions. However, by being richer in polyunsaturated fatty acids, more prone to oxidation, higher amounts of lipid oxidation were inevitably observed in CO and PO. Regarding EVOO, its richness in phenolic compounds, one of its main positive health highlights in comparison

with other monounsaturated fats, originated fries with higher phenolic compounds, particularly in the first frying hours.

Table 9.9 Resume of the nutritional impact on potatoes lipids and antioxidants as a result of prolonged frying in different monounsaturated-rich oils (per 100g of fried potatoes and % of dietary recommendations for adults).

Fried potatoes	EVOO		PO		CO		Reference dietary recommendations *per* day
	8 h	28 h	8 h	28 h	8 h	28 h	
Lipids (g), of which	9.0	9.3	9.4	9.4	11.2	11.1	70 g*
SFA (g)	1.5	1.6	1.7	1.7	0.9	0.9	<20 g*
MUFA (g)	6.7	6.2	5.3	4.9	7.2	6.5	>20 g*
PUFA (g)	0.8	0.5	2.3	1.7	3.0	2.3	>20 g*
Trans (g)	0.01	0.03	0.03	0.04	0.04	0.06	< 2 g*
Vitamin C (mg)	46 (58%)	18 (23%)	45 (56%)	16 (20%)	48 (60%)	19 (24%)	80 mg*
Vitamin E (mg)	0.4 (3%)	0.5 (4%)	0.9 (8%)	0.8 (7%)	3.3 (28%)	1.5 (13%)	12 mg*
Phenolic compounds (mg)	14	17	9	15	10	15	NR
Antioxidant activity	+	-	+	-	+	-	NR
Acrylamide# (µg)	77	92	75	96	62	105	NR
Volatile aldehydes (mg)	1.0	1.9	1.4	2.2	1.7	2.2	NR

*Regulation (EU) N.° 1169, 2011;
#EFSA estimated the mean dietary acrylamide exposures from 0.4 to 1.9 µg/kg body weight per day (EFSA, 2015).
CO - canola oil; EVOO - extra virgin olive oil; MUFA - monounsaturated fatty acid; NR - no recommendations; PO - peanut oil; PUFA - polyunsaturated fatty acid; SFA - saturated fatty acid.

CHAPTER 10. Impact of low-fat frying systems on potato quality

Parts of the text of this chapter were published or submitted to publication:

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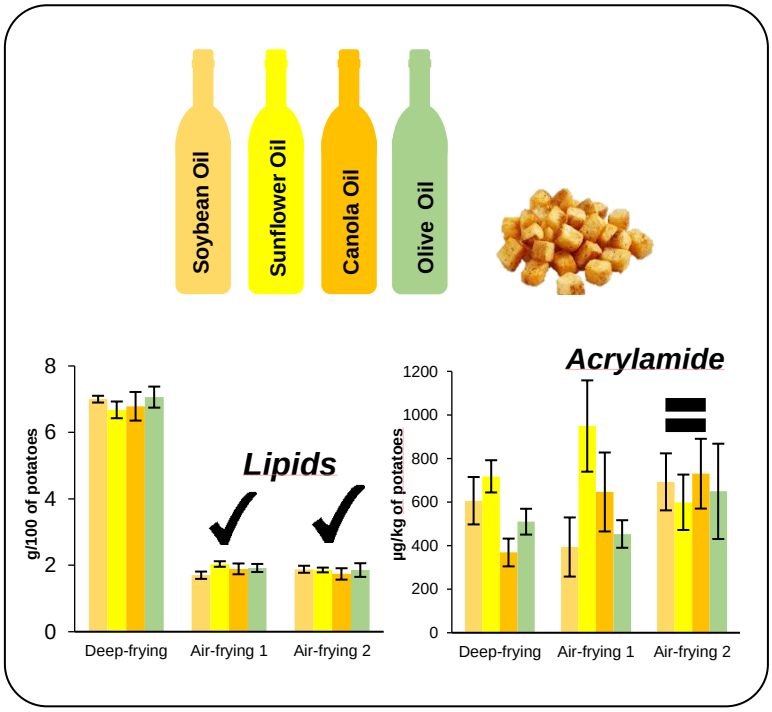
Santos CSP, Cunha SC, Casal S (2017) Can microwave and conventional oven be low fat "frying" alternatives? (Paper 8).

10.1. Deep or Air frying? A comparative study with different vegetable oils



10.1.1 Background and aim of study

In recent years, air-frying has emerged as an alternative process to deep-frying, producing fried food with small amounts of fat by external deposition of oil droplets dispersed in a hot air stream, inside a closed chamber. Several domestic air-frying equipment's are now available on the market, some completing with continuous agitation and direct contact with anti-adherent surfaces, but all designs provide uniform heat transfer rates between air and the product being fried. Apart from the well-established fat reduction, reported to achieve as much as 90% (Shaker, 2015), its acceptability depends, among other features, on the sensorial properties of the final product, particularly its similarity with deep-frying.

Some recent studies were developed on air-frying, on a comparative basis with deep-frying, using only one equipment and one vegetable oil. These studies have focused mostly on mass transfer and volume changes (Andres *et al*., 2013), mechanical and optical properties (Heredia *et al*., 2014), moisture content, oil uptake and its physicochemical properties (Shaker, 2015), acrylamide (Sansano *et al*., 2015), starch gelatinization profile, microstructure and sensory characteristics (Teruel *et al*., 2015), and phytochemical composition and antioxidant activity (Tian *et al*., 2016). To our knowledge, no scientific studies comparing different air-frying equipment's and/or different vegetable oils were published.

The aim of this work was to compare classical deep fried potatoes with two air-frying equipment's, testing four common frying oils – soybean (SO), sunflower (SFO), canola (CO), and olive oil (OO) – on a global approach of physical, chemical, and sensory data.

10.1.2 Sampling

The cooking process, including air-frying (ACT and AIR), boiling, and deep-frying, were performed according to Chapter 8. Each cooking process was performed in duplicate, on two different days, using two different bottles of each vegetable oil. Boiling was used as a process control, with equivalent amounts of fat added only after processing, after water drainage. Potatoes samples were immediately analysed by the sensory panel and for instrumental colour, followed by moisture and total ascorbic acid analysis. Further analyses performed, namely lipid content, fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, acrylamide, and degradation indicators (as p-anisidine value and polar compounds), are all detailed in Chapter 6. Fresh vegetable oils were also analysed for some composition parameters (fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, *p*-anisidine value, and polar compounds). All analytical determinations were performed in duplicate for each processing day (n = 4).

10.1.3 Results and discussion

10.1.3.1 Impact of different frying processes on potatoes physical characteristics

Colour of processed potatoes is strictly implicated in their acceptance, being influenced by oil type, temperature, frying time, and sample size (Krokida *et al*., 2001). Instrumental colour coordinates (CIE-*L*a*b**) for control and frying processes are summarized in Table 10.1.

Table 10.1 Effect of different frying processes on potatoes instrumental colour.

		*L**	*a**	*b**	*C**	*ΔE*
Control	**SO**	53.64 (28.46; 67.98)	-4.76^{A} (-8.74; -3.62)	15.74^{A} (4.28; 25.49)	16.87^{A} (6.67; 26.94)	-
	SFO	46.19 (36.78; 53.54)	-6.19^{A} (-7.73; -4.17)	15.06^{A} (8.42; 19.45)	16.41^{A} (9.42; 20.93)	-
	CO	50.26^{AB} (38.31; 63.47)	-6.01^{A} (-7.71; -4.72)	14.55^{A} (10.12; 16.87)	15.79^{A} (11.17; 18.55)	-
	OO	52.42 (29.66; 62.30)	-5.73^{A} (-8.67; -4.65)	14.09^{A} (6.87; 20.60)	15.66^{A} (8.53; 22.35)	-
Deep-frying	**SO**	48.74^{ab} (40.54; 60.29)	3.65^{b,C} (1.39; 7.73)	29.22^{B} (23.53; 36.43)	29.76^{B} (23.57; 36.77)	28.35^{ab,B} (19.38; 33.20)
	SFO	52.78^{abc} (43.61; 64.21)	3.74^{b,B} (0.80; 8.97)	30.39^{B} (25.85; 34.79)	30.70^{B} (25.86; 34.95)	21.98^{a} (11.90; 32.98)
	CO	58.19^{c,B} (46.47; 64.80)	0.42^{a,B} (-2.05; 3.90)	27.98^{B} (25.44; 32.53)	28.20^{B} (25.48; 32.53)	52.17^{c} (36.27; 63.22)
	OO	51.22^{a} (28.43; 61.35)	4.92^{b,C} (0.52; 6.76)	30.54^{C} (27.32; 33.81)	30.71^{C} (27.83; 34.16)	28.71^{b,B} (24.02; 34.11)
Actifry	**SO**	52.95 (35.83; 61.80)	-1.29^{B} (-6.60; 5.45)	27.27^{B} (22.66; 32.99)	27.49^{B} (22.70; 32.99)	22.15^{a,A} (8.23; 32.16)
	SFO	48.90 (39.21; 56.50)	1.77^{B} (-2.68; 6.30)	27.54^{B} (21.34; 34.70)	27.91^{B} (21.51; 35.09)	18.77^{a} (10.32; 20.27)
	CO	48.03^{A} (43.46; 56.58)	2.62^{B} (-2.90; 7.33)	27.81^{B} (20.57; 35.08)	27.98^{B} (20.77; 35.84)	48.35^{b} (35.01; 66.44)
	OO	47.51 (42.05; 59.41)	-0.65^{B} (-6.76; 7.38)	28.31^{B} (20.51; 30.90)	28.38^{B} (20.96; 31.77)	17.91^{a,A} (13.94; 27.92)
Airfryer	**SO**	54.10 (37.32; 64.99)	3.23^{BC} (-4.55; 7.03)	27.55^{B} (22.57; 34.70)	27.95^{B} (23.02; 35.23)	20.55^{a,A} (13.58; 32.89)
	SFO	49.66 (29.16; 61.20)	1.22^{B} (-1.68; 8.27)	28.47^{B} (19.68; 32.57)	29.25^{B} (19.72; 32.66)	21.15^{a} (12.82; 30.30)
	CO	47.85^{A} (37.52; 55.99)	1.07^{B} (-3.48; 10.22)	26.72^{B} (22.42; 29.81)	27.08^{B} (22.46; 30.57)	47.12^{b} (33.95; 64.51)
	OO	52.57 (34.64; 60.39)	0.34^{B} (-1.56; 2.31)	27.80^{B} (21.76; 30.07)	27.81^{B} (21.76; 30.09)	19.42^{a,A} (10.53; 25.96)

Superscript different letters indicate statistically significant differences ($p < 0.05$): small letters between vegetable oils and large letters between frying processes.

*a** - redness; *b** - yellowness; *C** - chrome; CO - canola oil; *L** - lightness; OO - olive oil; SFO - sunflower oil; SO - soybean oil; *ΔE* - colour change.

Control potatoes were light yellow, independent of oil type (p>0.05). Values of coordinate b^*, range of yellows, were twice higher in fried potatoes compared to control. L^* decreased slightly with the different frying processes, but was not sufficient to show statistical significant differences by oil type (p>0.05), except for CO (p<0.05), while the other colour coordinates were all lower (p<0.05).

Regarding the different frying processes, a high variability was verified for coordinate a, namely in ACT, maybe explained by the direct contact with the surface of chamber. However, deep-frying in SO and OO presented higher a^* levels than air-frying. These observations are in agreement with the literature (Heredia *et al*., 2014), since air-frying is associated with a lower rate of chemical reactions and non-enzymatic browning, despite requiring more processing time to achieve the characteristic colour of deep-fried potatoes (Teruel *et al*., 2015).

Krokida *et al*. (2001) considered the ideal tonality for fried potatoes between -5 and 0 for coordinate a^*, with values of coordinate b^* higher than 10. In this study, values of coordinate a^* were generally higher, while values of coordinate b^* were in accordance, regardless of oil type or frying processes.

Values of coordinate C^* were very similar between frying processes. Regarding ΔE, potatoes fried in CO presented statistically higher values on all frying processes (p<0.05). When the frying processes are compared for each oil, higher ΔE were observed with deep-frying in SO and OO (p<0.05), without variations on the other oils.

10.1.3.2 Impact of different frying processes on potatoes chemical composition

In fried potatoes, moisture and oil contents are among the most important components from the nutritional and sensory points of view (Heredia *et al*., 2014). As previously, explained, boiled potatoes were used as control, while deep-frying was the reference for the air-frying systems. Regarding moisture, boiled potatoes presented, as expected, higher moisture (Fig. 10.1A – 80% on average), independently oil type (p<0.05).

A general decrease in the moisture content was achieved, with reduced differences between all frying processes (average of 60%). Globally, these results are in accordance with moisture amounts reported for these frying technologies (Andres *et al*., 2013). However, for each oil individually, except CO, the results were not always equivalents between processes, with potatoes fried in SO having similar moisture amounts after deep-frying and Airfryer (p>0.05), and both SFO and OO after deep-frying and Actifry (p>0.05).

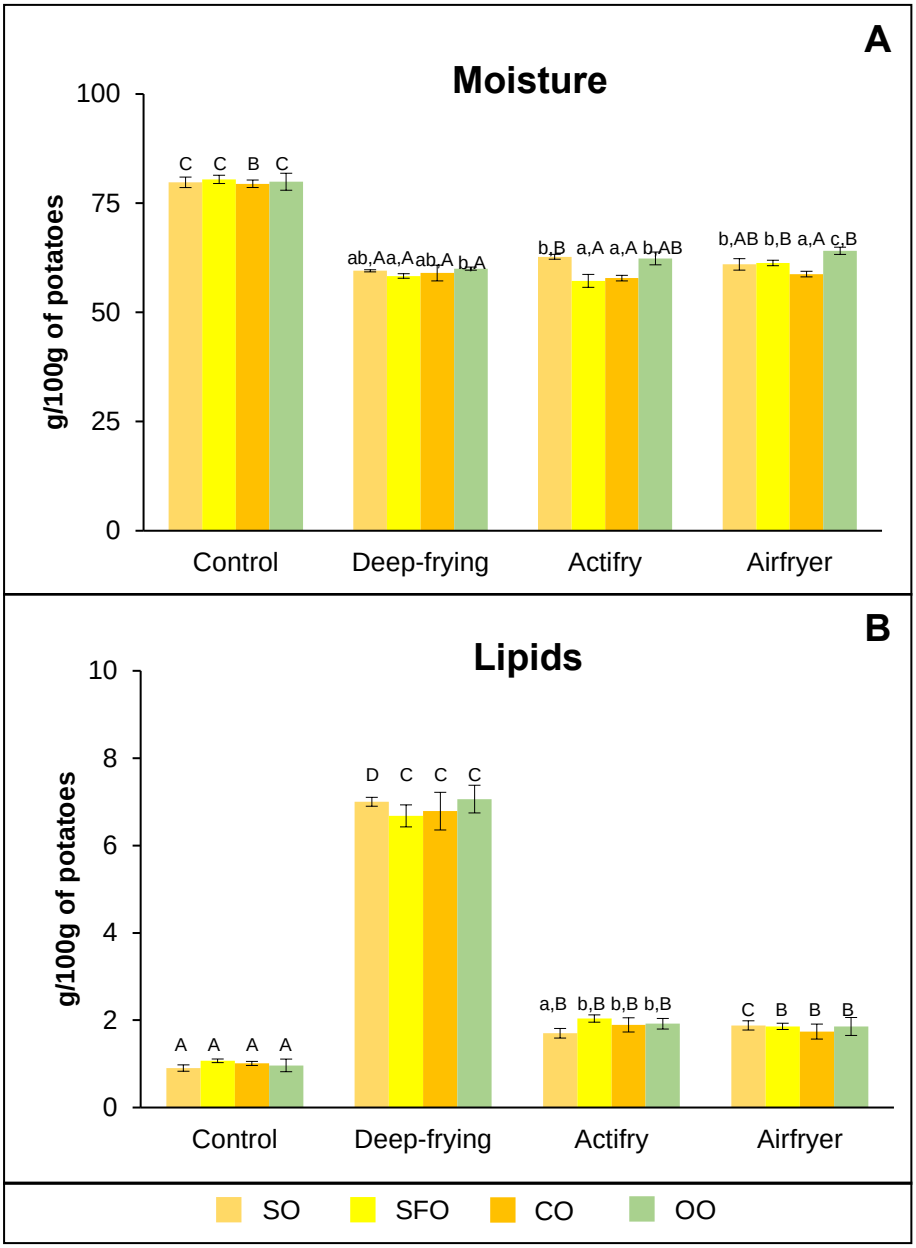


Figure 10.1 Effect of different frying processes on potatoes moisture (A) and lipids (B). [a-c] for significant differences (p<0.05) between vegetables oils; [A-C] for significant differences (p<0.05) between frying processes.

In terms of lipid amounts (Fig. 10.1B), a reduction of 70% was observed in both air-frying equipment's in comparison with deep-frying, from average amounts of 7 g/100g to 2 g/100g, corresponding to less 45 kcal/100g, with a strong nutritional impact. These differences between deep-frying and air-frying were already explained (Teruel *et al.*, 2015), and the results presented in this study are in agreement with the literature (Shaker, 2015; Teruel *et al*., 2015). Only small variations were perceived between processes and oil type, wherein SO exhibited the lowest lipid contents (p<0.05).

The fatty acids composition of fried potatoes (Table 10.2) reflects directly the oil composition (Table 7.2), due to the reduced significance of raw potatoes lipids (0.36% - Table 7.1). The amount of TFA *per* 100 g of potatoes (Table 10.2) was consistently higher in deep-fried potatoes (p<0.05), but similar between the other processes (p>0.05). However, potatoes fried in OO presented smaller TFA content in comparison with other vegetable oils (p<0.05), while the higher TFA values were observed for fried potatoes in CO, already with high TFA amounts before heating (0.4%). This should be interpreted with caution, being a direct consequence of the incorporated lipid amounts, because when the TFA results are compared on an extracted oil basis (data not shown), these TFA represent only 0.1–0.5%, similar to the amounts presented in the unheated oils (Table 7.2). This is also a direct indication that one frying session is not enough to induce significant fatty acid isomerization, on both deep-frying (6 min) and air-frying sessions (15–25 min).

Table 10.2 Effect of different frying processes on the main fatty acids (g/100g of potatoes).

		C16:0	C18:1n-9	C18:2n-6	C18:3n-3	TFA
Control	SO	0.12 ± 0.06^{A}	0.24 ± 0.11^{A}	$0.48\pm0.22^{c,A}$	$0.06\pm0.02^{ab,A}$	0.004 ± 0.002^{A}
	SFO	0.07 ± 0.02^{A}	0.43 ± 0.16^{A}	$0.41\pm0.15^{bc,A}$	$0.01\pm0.00^{a,A}$	0.002 ± 0.001^{A}
	CO	0.05 ± 0.01^{A}	0.51 ± 0.15^{A}	$0.18\pm0.05^{ab,A}$	$0.08\pm0.02^{b,A}$	0.005 ± 0.003^{A}
	OO	0.08 ± 0.05^{A}	0.48 ± 0.28^{A}	$0.05\pm0.03^{a,A}$	$0.01\pm0.00^{a,A}$	0.001 ± 0.000^{A}
Deep-frying	SO	$0.68\pm0.04^{b,B}$	$1.53\pm0.15^{a,C}$	$2.92\pm0.15^{c,B}$	$0.32\pm0.01^{b,B}$	$0.026\pm0.003^{b,B}$
	SFO	$0.42\pm0.08^{a,B}$	$2.74\pm0.31^{b,C}$	$2.50\pm0.30^{c,C}$	$0.03\pm0.01^{a,B}$	$0.013\pm0.002^{a,B}$
	CO	$0.27\pm0.01^{a,C}$	$3.38\pm0.03^{bc,C}$	$1.10\pm0.03^{b,C}$	$0.48\pm0.02^{c,C}$	$0.035\pm0.003^{c,B}$
	OO	$0.69\pm0.06^{c,B}$	$4.23\pm0.45^{c,B}$	$0.36\pm0.03^{a,C}$	$0.04\pm0.00^{a,C}$	$0.009\pm0.002^{a,B}$
Actifry	SO	$0.15\pm0.03^{c,A}$	$0.46\pm0.16^{a,B}$	$0.46\pm0.01^{c,A}$	$0.06\pm0.00^{c,A}$	$0.004\pm0.000^{b,A}$
	SFO	$0.11\pm0.02^{b,A}$	$0.78\pm0.16^{b,B}$	$0.74\pm0.16^{c,B}$	$0.01\pm0.00^{a,A}$	$0.003\pm0.001^{ab,A}$
	CO	$0.07\pm0.00^{a,B}$	$0.80\pm0.06^{c,B}$	$0.29\pm0.02^{b,B}$	$0.13\pm0.01^{d,B}$	$0.008\pm0.001^{c,A}$
	OO	$0.17\pm0.02^{c,A}$	$0.95\pm0.11^{c,A}$	$0.11\pm0.01^{a,B}$	$0.02\pm0.00^{b,B}$	$0.002\pm0.000^{a,A}$
Airfryer	SO	$0.13\pm0.02^{c,A}$	$0.27\pm0.04^{a,AB}$	$0.54\pm0.09^{c,A}$	$0.06\pm0.01^{b,A}$	$0.004\pm0.001^{b,A}$
	SFO	$0.08\pm0.01^{b,A}$	$0.54\pm0.09^{b,AB}$	$0.51\pm0.08^{c,AB}$	$0.01\pm0.00^{a,A}$	$0.002\pm0.000^{a,A}$
	CO	$0.06\pm0.01^{a,A}$	$0.62\pm0.04^{b,AB}$	$0.22\pm0.02^{b,AB}$	$0.09\pm0.01^{c,A}$	$0.007\pm0.001^{c,A}$
	OO	$0.14\pm0.01^{c,A}$	$0.79\pm0.06^{c,A}$	$0.09\pm0.01^{a,B}$	$0.01\pm0.00^{a,B}$	$0.001\pm0.000^{a,A}$

Superscript different letters indicate statistically significant differences (p < 0.05): small letters between vegetable oils and large letters between frying processes.
C16:0 - palmitic acid; C18:1n-9 - oleic acid; C18:2n-6 - linoleic acid; C18:3n-3 - linolenic acid; CO - canola oil; OO - olive oil; SFO - sunflower oil; SO - soybean oil; TFA - *trans* fatty acid.

Tocopherols are naturally present in vegetable oils, and therefore expectedly present in the potatoes, on direct proportion of the amounts present in the fresh oils (Table 7.2) and of incorporated lipids (Fig. 10.1B). Thus, OO presented consistently lower amounts of tocopherols than the other three oils (Table 10.3), for all the processes. Also, higher amounts were achieved in deep-frying (p<0.05).

As expected, total ascorbic acid content decreased from 6.75 mg/100g in fresh potatoes (Table 7.1) to values around 1 mg/100g after potatoes cooking. However, its content was higher in boiled potatoes (p<0.05), but similar between oil types (p>0.05). In addition, deep-frying process promoted higher losses (69%) than air-frying process (60%), wherein the lowest average losses were achieved in the Airfryer, with OO (51%). Although raw potatoes had carotenoids, namely *β*-carotene (Table 7.1), as did the boiled samples, increased amounts were achieved after all frying processes by fat incorporation (p<0.05), being similar between oil types (p>0.05), except Actifry potatoes (p<0.05), a probable indicator of potentially less oxidation during the frying process.

Regarding antioxidant activity, statistically significant differences between cooked potatoes and oil type were verified. For example, total phenolics were reduced in deep-fried potatoes in comparison with the control ones (between 3% in OO and 18% in SFO), while an apparent increase was observed in air-fried potatoes indicative of lower phenolic degradation with this processes. As expected, potatoes fried in OO presented higher phenolic amounts, consistently with the fresh oil (Table 7.2). However, an apparent decreased in DPPH radical scavenging activity, as Gallic acid equivalents, was observed on all frying processes when comparing to control (p<0.05), with consistently higher values observed with CO (p<0.05).

Spearman correlations between antioxidant activity and vitamins were separately performed for the different frying processes, to determine the key contributors to antioxidant activity. Regardless of the oil type, total tocopherols had positive correlations with the DPPH results (r_s=0.682, p<0.01 for Actifry, and r_s=0.603, p<0.05 for Airfryer), while total phenolics and DPPH correlated negatively for all frying processes (deep-frying: r_s= -0.624, p<0.01, Actifry: r_s= -0.715, p<0.01, and Airfryer: r_s= -0.676, p<0.01), indicative that the phenolic compounds are probably not the main contributors for the antioxidant activity observed.

Acrylamide, a recognized processing contaminant in fried potatoes, was also studied, being represented in Figure 10.2. Despite being absent in the control samples, because its formation generally occurs above 120°C and under low-moisture conditions (Molina-García *et al*., 2015), values from 369 to 949 µg/kg were detected in the fried samples. Regarding Fig. 10.2, a clear pattern was not observed between processes or oils. However, Airfryer was the most consistent equipment in terms of acrylamide amounts, while OO was more consistent between processes. The higher variation in the Actifry system is probably explained by the direct contact with the chamber surface, consistent with a greater variability of brownish/reddish tones from *Maillard* reaction, probably associated with the colour variation previously discussed.

Table 10.3 Effect of different frying processes on tocopherols, total ascorbic acid, total carotenoids, and antioxidant activity in potatoes.

		Tocopherols	Total ascorbic acid	Total carotenoids	Total phenolics	DPPH
		mg/100g	mg/100g	µg/100g	mg GAE/100g	mg GAE/100g
Control	SO	$0.54^{b,A}$ (0.43-0.62)	3.32^{B} (3.31-3.41)	36^{A} (295-55)	$22.94^{b,B}$ (22.03-23.84)	$10.57^{b,C}$ (10.01-11.14)
	SFO	$0.62^{b,A}$ (0.52-0.75)	3.13^{B} (3.02-3.28)	42^{A} (40-48)	$25.08^{c,B}$ (23.39-26.77)	$10.19^{b,C}$ (9.51-10.86)
	CO	$0.54^{b,A}$ (0.49-0.60)	3.06^{B} (2.20-3.70)	34^{A} (26-37)	$14.14^{a,B}$ (13.79-14.49)	$15.15^{c,BC}$ (15.03-15.26)
	OO	$0.23^{a,A}$ (0.22-0.24)	3.49^{B} (3.36-3.84)	58^{A} (45-76)	$47.54^{d,B}$ (46.76-48.33)	$7.52^{a,B}$ (7.42-7.63)
Deep-frying	SO	$4.60^{b,C}$ (4.14-4.81)	0.88^{A} (0.50-1.34)	167^{BC} (151-198)	$20.46^{b,A}$ (19.76-21.38)	$9.06^{a,B}$ (8.50-9.63)
	SFO	$3.79^{b,C}$ (3.39-3.99)	0.90^{A} (0.65-1.13)	141^{B} (133-164)	$20.56^{b,A}$ (18.99-22.18)	$8.38^{a,B}$ (7.67-9.10)
	CO	$2.87^{b,D}$ (2.46-3.27)	1.22^{A} (1.00-1.56)	169^{B} (149-179)	$13.11^{a,A}$ (12.84-13.81)	$15.49^{b,C}$ (15.07-15.78)
	OO	$0.51^{a,B}$ (0.37-0.68)	0.97^{A} (0.64-1.03)	$180.^{B}$ (148-211)	$46.08^{c,A}$ (43.06-46.57)	$7.67^{a,B}$ (7.33-8.53)
Actifry	SO	$1.02^{a,B}$ (0.99-1.05)	1.38^{A} (0.69-1.97)	$189^{bc,C}$ (161-214)	$27.96^{b,D}$ (27.26-28.88)	$7.56^{b,A}$ (7.04-8.07)
	SFO	$1.41^{b,B}$ (1.10-1.73)	1.14^{A} (1.01-1.34)	$145^{a,B}$ (118-172)	$25.56^{b,B}$ (23.99-27.18)	$7.14^{b,A}$ (6.49-7.86)
	CO	$1.21^{b,C}$ (1.19-1.37)	1.09^{A} (0.71-1.15)	$155^{ab,B}$ (149-164)	$19.11^{a,C}$ (18.84-19.81)	$14.57^{c,B}$ (14.23-14.98)
	OO	$0.49^{a,B}$ (0.43-0.53)	1.00^{A} (0.49-1.51)	$196^{c,B}$ (184-210)	$52.36^{c,C}$ (51.08-53.88)	$6.10^{a,A}$ (5.61-6.78)
Airfryer	SO	$1.24^{b,B}$ (1.01-1.50)	1.51^{A} (0.87-2.05)	146^{B} (135-151)	$26.46^{b,C}$ (25.76-27.38)	$6.98^{a,A}$ (6.57-7.34)
	SFO	$1.18^{b,B}$ (0.99-1.51)	1.31^{A} (0.85-1.35)	132^{B} (114-152)	$27.06^{b,B}$ (25.49-28.68)	$6.41^{a,A}$ (5.67-7.26)
	CO	$1.03^{b,B}$ (0.85-1.04)	1.28^{A} (1.12-1.49)	136^{B} (122-153)	$20.61^{a,D}$ (20.34-21.31)	$14.01^{b,A}$ (13.51-14.20)
	OO	$0.47^{a,B}$ (0.44-0.50)	1.72^{AB} (0.98-2.66)	167^{B} (153-177)	$54.10^{c,C}$ (53.11-55.09)	$5.51^{a,A}$ (5.17-6.56)

Superscript different letters indicate statistically significant differences ($p < 0.05$): small letters between vegetable oils and large letters between frying processes.
DPPH - 2,2-diphenyl-1-picrylhydrazyl radical; CO - canola oil; GAE - Gallic acid equivalents; OO - olive oil; SFO - sunflower oil; SO - soybean oil.

In fact, acrylamide formation is a complex mechanism that includes processing parameters (frying time, and oil temperature), oil characteristics (type, oil oxidation, and hydrolysis products), and potato composition (variety, agronomic conditions, postharvest storage, asparagine and reducing sugars, *Maillard* reaction, moisture, and oil uptake) (Vinci *et al*., 2012; Yang *et al*., 2016; Zhang *et al*., 2015). Moreover, vitamins and antioxidant activity may inhibit their formation (Zeng *et al*., 2009; Serpen and Gokmen, 2009).

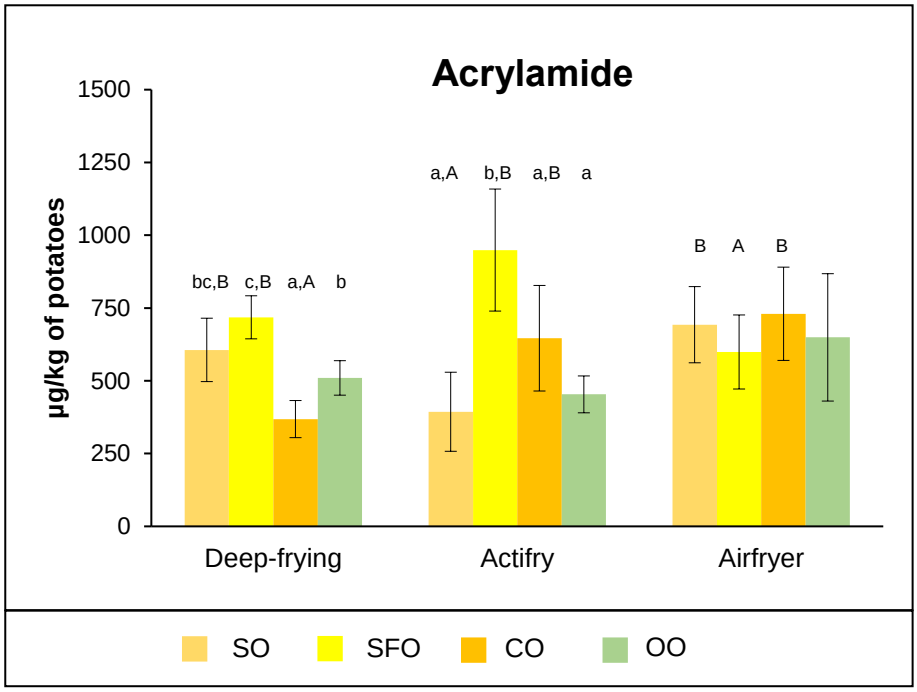


Figure 10.2 Effect of different frying processes on acrylamide (µg/kg) in potatoes. [a-c] for significant differences (p<0.05) between vegetables oils; [A-B] for significant differences (p<0.05) between frying processes.

10.1.3.3 Impact of different frying processes on lipid degradation

The lipids extracted from fried potatoes were analysed for several indicators of fat degradation, including PAV and PC, as detailed in Table 10.4.

Focusing on PAV, lipids from deep-fried potatoes presented a higher fat degradation state in comparison with all other processes (p<0.05), while no significant differences were observed between the two air-frying equipment's (p>0.05) and also between these two and the control, indicative of the low lipid degradation induced by air-frying. When the different oils are compared, lower lipid degradation was consistently observed in OO (p<0.05).

Polar compounds, indicative of triglycerides degradation, are used in several countries for frying oils quality control, with a legal maximum usually around 25% for TPC (Portaria N.° 1135, 1995), and/or 12% from DPTG (Brühl, 2014). Higher TPC contents were observed with deep-frying, while both air-frying equipment's presented similar amounts to the boiled samples (p>0.05). As this study corresponds to only one frying cycle on each oil batch, from 6 min in deep-frying to 15-25 min in air-frying, the TPC increase is expectedly low in comparison with the legal limits.

Table 10.4 Effect of different frying processes on indicators of incorporated fat degradation (g/100 g of potatoes for PC, DPTG and OTG).

		PAV	TPC	DPTG	OTG
Control	SO	$4.8^{b,A}$ (4.4-5.8)	$3.76^{b,A}$ (3.56-3.95)	$0.08^{b,A}$ (0.08-0.09)	$1.31^{b,C}$ (1.23-1.35)
	SFO	$2.3^{a,A}$ (1.8-2.7)	$3.73^{b,A}$ (3.51-4.07)	$0.08^{b,A}$ (0.07-0.09)	$1.14^{ab,A}$ (0.96-1.36)
	CO	$3.7^{ab,A}$ (3.0-4.0)	$3.06^{a,A}$ (2.83-3.26)	$0.05^{a,A}$ (0.04-0.06)	$0.88^{a,A}$ (0.81-0.96)
	OO	$4.2^{b,A}$ (3.2-5.8)	3.56^{ab} (2.70-4.41)	n.d.	1.22^{ab} (0.47-1.95)
Deep-frying	SO	$44.6^{b,B}$ (27.4-49.6)	$7.49^{b,B}$ (7.08-8.19)	$1.93^{b,B}$ (1.64-2.42)	$3.11^{b,C}$ (2.92-3.31)
	SFO	$44.5^{b,C}$ (38.4-50.9)	$7.86^{b,B}$ (7.06-8.53)	$2.43^{b,B}$ (2.01-2.82)	$2.86^{b,B}$ (2.51-3.18)
	CO	$53.5^{b,C}$ (51.5-53.7)	$6.43^{ab,B}$ (5.76-7.10)	$1.69^{ab,C}$ (1.34-2.01)	$2.62^{ab,C}$ (2.20-3.05)
	OO	$17.1^{a,B}$ (14.3-18.4)	5.88^{a} (5.54-6.21)	$1.07^{a,B}$ (0.82-1.19)	2.00^{a} (1.78-2.22)
Actifry	SO	$2.6^{a,A}$ (1.2-3.4)	4.26^{A} (4.21-4.31)	0.12^{A} (0.04-0.15)	1.39^{AB} (1.08-1.68)
	SFO	$5.6^{b,B}$ (4.3-6.1)	3.94^{A} (3.66-4.16)	0.09^{A} (0.08-0.11)	1.19^{A} (0.94-1.41)
	CO	$3.2^{a,A}$ (2.1-3.7)	3.58^{A} (3.05-4.17)	0.07^{AB} (0.05-0.08)	1.27^{AB} (0.80-1.66)
	OO	$2.6^{a,A}$ (2.2-3.2)	4.48 (3.47-5.56)	0.06^{A} (0.05-0.07)	1.61 (0.77-2.56)
Airfryer	SO	$3.5^{a,A}$ (2.5-4.0)	$4.43^{ab,A}$ (4.12-4.72)	$0.14^{b,A}$ (0.13-0.15)	$1.77^{ab,B}$ (1.60-1.86)
	SFO	$6.6^{c,B}$ (5.7-9.2)	$4.31^{ab,A}$ (3.68-4.73)	$0.17^{b,A}$ (0.12-0.19)	$1.63^{ab,A}$ (1.18-1.93)
	CO	$5.3^{bc,B}$ (4.9-7.1)	$3.59^{a,AB}$ (3.51-3.64)	$0.11^{a,B}$ (0.09-0.11)	$1.35^{a,B}$ (1.31-1.39)
	OO	$4.0^{ab,A}$ (3.4-5.0)	4.75^{b} (3.90-5.77)	$0.08^{a,A}$ (0.07-0.10)	1.98^{b} (1.06-2.98)

Superscript different letters indicate statistically significant differences ($p < 0.05$): small letters between vegetable oils and large letters between frying processes.
CO - canola oil; DPTG - dimeric and polymeric triglycerides; OO - olive oil; OTG - oxidized triacylglycerols; PAV - *p*-anisidine value; SFO - sunflower oil; SO - soybean oil; TPC - total polar compounds.

As to the TPC main fractions, OTG were the main degradation products, followed by DPTG (Table 10.4), following the same trend observed in TPC: higher amounts in deep-frying and reduced differences between the remaining processes and oils. However, when these fractions are compared on a relative basis (to TPC) more clear differences are perceived. Indeed, the OTG fraction, globally varying from 29 to 42%, was statistically equivalent between deep-frying and the Airfryer, while lower amounts (p<0.05) are observed for both Actifry and the control. Also, the DPTG fraction represents from 18 to 31% in deep-frying, while less than 4% was observed on all the other processes (p<0.05). These differences in the DPTG are consistent with the reduced degradation induced by the air-frying process, while the comparatively higher OTG in the Airfryer is consistent with a

frying technology based solely on hot air flowing, while the Actifry device combines air stream with direct contact with anti-adherent surfaces. The only study on degradation indicators with the air-frying process used solely one device (Actifry) and oil (SFO) (Heredia *et al*., 2014). Lipid oxidation was evaluated by the peroxide value, not adequate for heated samples, but DPTG values were similar.

10.1.3.4 Impact of different frying processes on global sensory characteristics

One of the main concerns in the development of equipment focused on low-fat products is the sensory aspect. In fact, oil uptake by potatoes is important to crust and colour formations, and improvement of palatability, including flavour (Heredia *et al*., 2014).

The sensory panel evaluated appearance, odour, taste, and flavour in the different frying processes, based on ten attributes (Fig. 10.3). Graininess was not significantly different by frying processes or oil type ($p>0.05$). Adhesiveness was only significantly different for potatoes in SO, higher in air-frying than deep-frying ($p<0.05$), while crispy was only significantly different for potatoes in CO, higher in deep-frying than air-frying ($p<0.05$). Regardless of the oil type, colour attributes and odour intensity presented higher scores in deep-frying than air-frying ($p<0.05$). Considering that SFO is the vegetable oil most frequently used in Portugal for potato frying (Casal *et al*., 2010), it was scored with superior odour quality as well as taste quality in deep-frying, but smaller in air-frying ($p<0.05$). Potatoes fried in CO (deep-frying and Airfryer) showed inferior odour attributes than other vegetable oils ($p<0.05$), probably due to increased fatty acid oxidation, consistent with its increased PUFA content. Potatoes deep-frying in CO had also the smallest taste quality, but higher in air-frying process ($p<0.05$). Aftertaste attributes was similar between oils within each frying process ($p>0.05$), but higher score in deep-frying than air-frying processes were observed with SO and OO ($p<0.05$), maybe due to off-flavour developed from its fatty acids.

In terms of acceptability, statistically significant differences were observed by oil type ($p<0.05$) for both frying technologies, probably influenced by cultural habits. As expected, fried potatoes in SFO presented the best score for deep-frying, while fried potatoes in CO presented the worst score. In opposition, air-frying in SFO presented the worst score, while the best score was achieved with SO. Consequently, statistically significant differences by frying process ($p<0.05$) were only observed for SFO. Curiously, fried potatoes in OO were similar to the best potatoes for both frying processes, but a high variability was observed within the panel.

The external appearance of fried potatoes by frying process is shown in Figure 10.4. In fact, lower colour homogeneity was verified in air-frying process, particularly in Actifry, as a result of direct contact with the surface chamber. This observation is consistent with instrumental colour evaluation, as previously discussed.

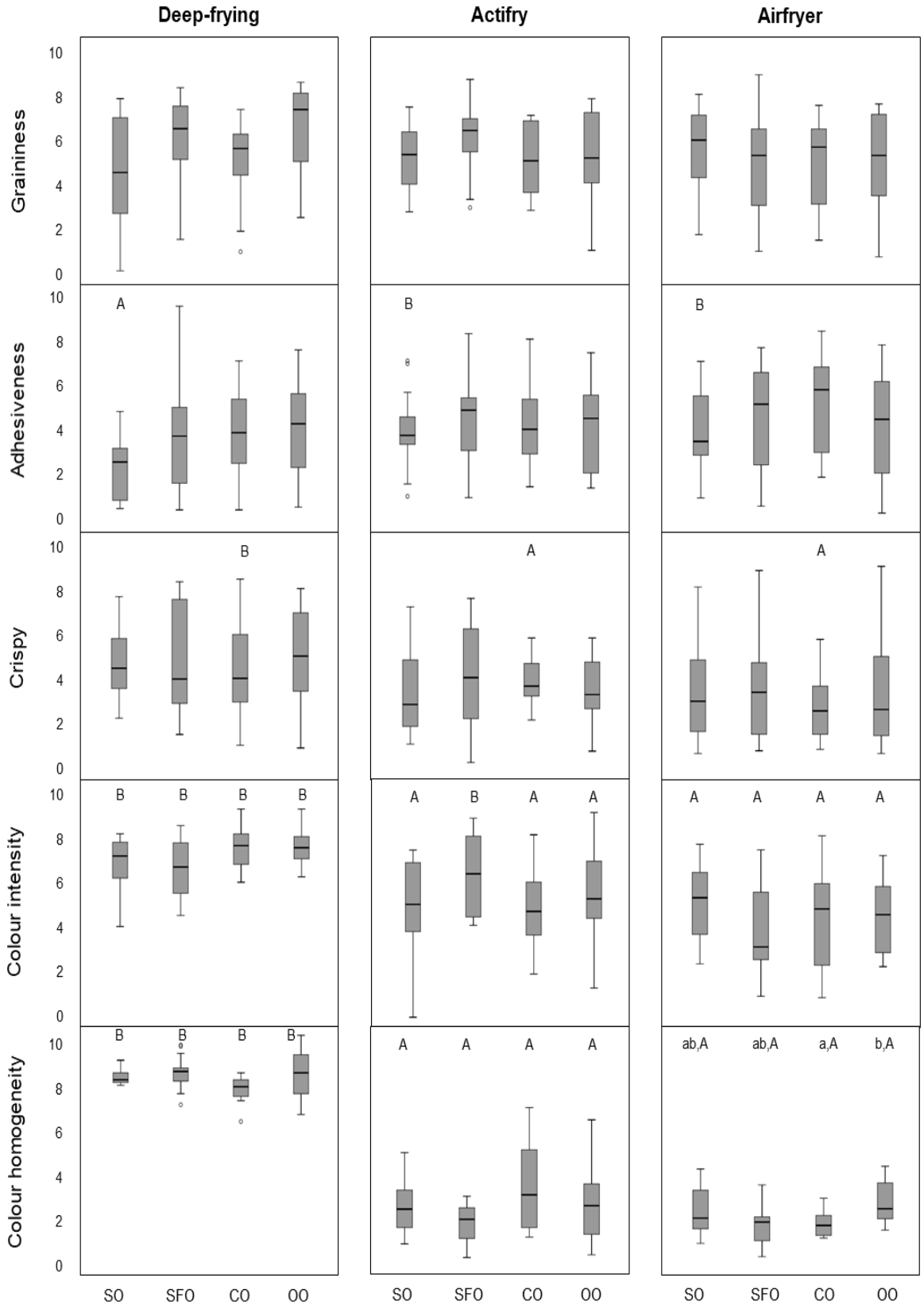


Figure 10.3 Boxplot of quantitative descriptive sensory analysis of potatoes in different frying process: deep-frying, Actifry and Airfryer.

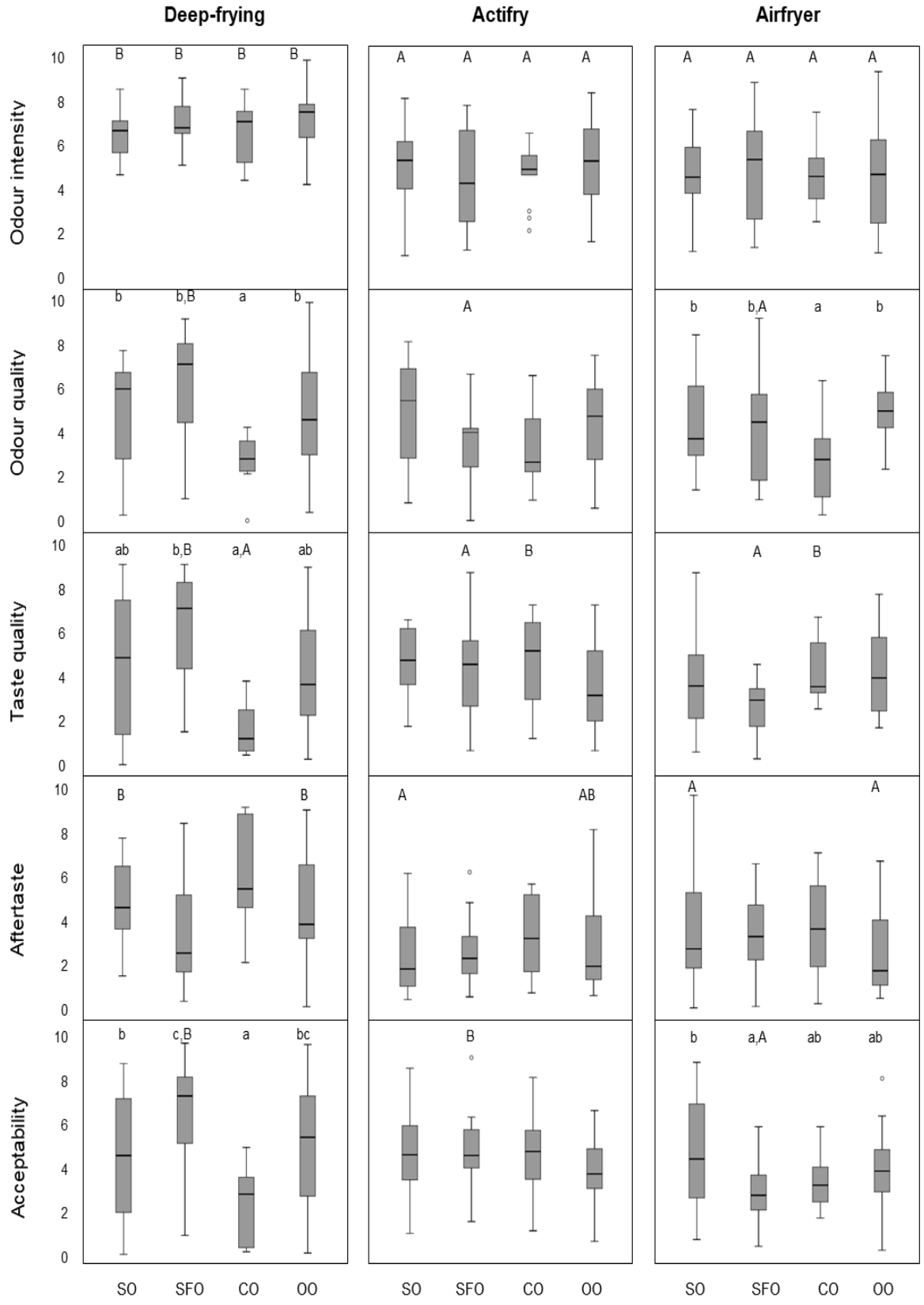


Figure 10.3 Continued.

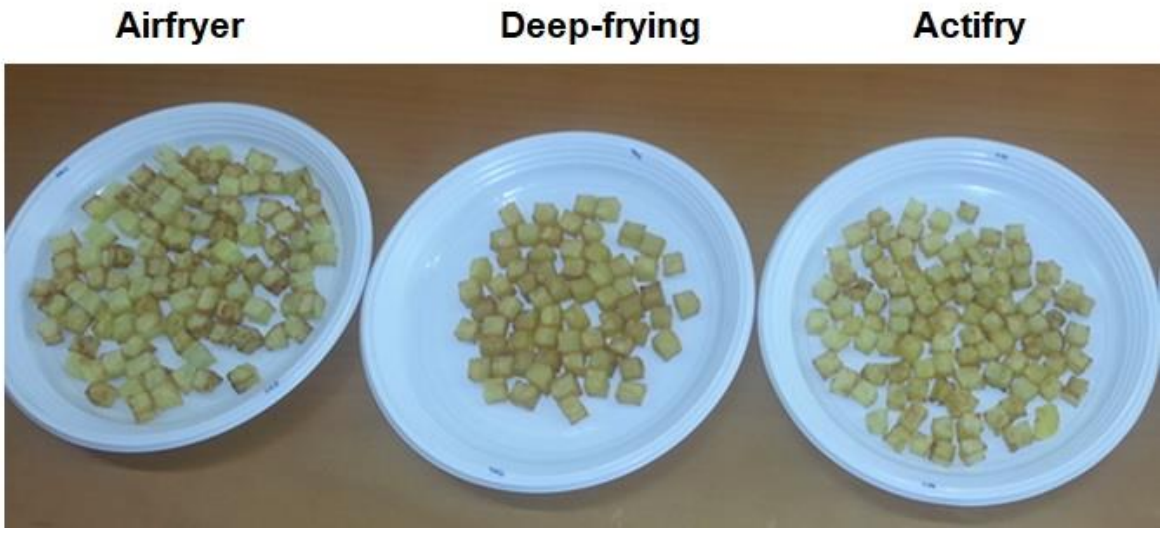


Figure 10.4 Example of fried potatoes in OO: Airfryer, deep-frying and Actifry.

Additionally, Spearman correlations were separately performed (Table 10.5) to understand what sensory attributes affected fried potatoes acceptability, by oil type. Moderate to strong and positive correlations as well as moderate and negative correlations were found. Except for SFO and CO in Airfryer, taste quality attribute was the most determinant for all vegetable oils and processes. Inversely, aftertaste had influence in the deep-frying with CO and OO, Actifry with SFO, and Airfryer with SO. Despite the low colour homogeneity verified for the air-frying process, this attribute correlated positively with acceptability Actifry in OO and Airfryer in SFO and CO.

Moreover, a multivariate regression technique, partial least squares regression type 1 (PLSR1), using the Unscrambler software 10.4 (CAMO Technologies, Woodbridge, NJ, USA), was applied to investigate relationships between acceptability (Y-matrix) and other sensory attributes (X-matrix) in terms of prediction of Y-variable from X-variables. Internal full cross-validation was used for developing the models and validating it, resulting in calibration and validation coefficients. Significant variables in the PLSR1 predictions were identified by the modified Jack-knife uncertainty test, and the models were recalculated using only the significant variables. The root-mean-square error of prediction (RMSEP), that represents the average prediction error expected for new samples based on the same units as the original response variables, were determined for each model.

Table 10.5 Spearman correlation between acceptability and sensory attributes.

		Graininess	Adhesiveness	Crispy	Color intensity	Color homogeneity	Odor intensity	Odor quality	Taste quality	Aftertaste
Deep-frying	SO	0.361	-0.018	0.790**	0.083	0.082	0.441	0.417	0.608**	-0.359
	SFO	0.388	0.003	0.309	-0.199	0.248	0.633**	0.550*	0.904**	-0.324
	CO	0.528*	0.248	0.392	0.029	0.090	-0.073	0.728**	0.537*	-0.513*
	OO	0.633**	-0.129	0.318	0.248	0.507*	0.128	0.669**	0.784**	-0.583*
Actifry	SO	0.095	0.304	0.394	0.418	0.376	0.113	0.372	0.863**	-0.084
	SFO	0.411	-0.052	0.538*	0.110	0.159	0.190	0.426	0.669**	-0.512*
	CO	0.048	0.120	0.322	0.084	0.414	0.214	0.426	0.889**	-0.205
	OO	0.022	0.385	0.035	0.200	0.514*	-0.012	0.336	0.718**	0.019
Airfryer	SO	-0.006	-0.466*	0.188	0.123	-0.046	0.520	0.102	0.498*	-0.627**
	SFO	0.252	0.582**	-0.017	0.129	0.606**	-0.203	-0.343	0.387	0.292
	CO	0.363	-0.081	0.096	0.496*	0.502*	-0.112	0.392	0.407	0.273
	OO	0.411	0.325	-0.193	0.168	0.455	0.191	0.323	0.539*	-0.094

Statistically significant differences at *$p \leq 0.05$; ** $p \leq 0.01$.
CO – canola oil; OO – olive oil; SFO – sunflower oil; SO – soybean oil.

The PLSR1 (Table 10.6) reinforced the results of bivariate correlation discussed above, particularly in the quality of taste and odour. Calibration coefficients (≥0.7) that expressed the strength of the current models were better in deep-frying potatoes, except with CO, as well as validation coefficients (≥0.7), which depicted the current models ability to predict new samples. However, the RMSEP values were below two for all models (on a scale of 0–10 cm), maybe due to variability of sensory analysis influenced by food habits of assessors. In fact, from the assessor's perspective, taste, and odour qualities were more determinant for acceptability of fried potatoes than colour.

Table 10.6 Results of PLSR1 between sensory attributes (X-variables) and acceptability (Y-variables) for different frying processes potatoes.

		Positive Correlations	Negative Correlations	Calibration	Validation	RMSEP
Deep-frying	SO	Crispy Graininess Taste quality	-	0.84	0.77	1.19
	SFO	Taste quality	-	0.95	0.77	1.27
	CO	Odor quality Taste quality	-	0.62	0.35	1.83
	OO	Odor quality Graininess Taste quality	-	0.77	0.71	1.50
Actifry	SO	Taste quality	-	0.89	0.59	1.22
	SFO	Graininess Taste quality	Aftertaste	0.34	0.17	1.98
	CO	Taste quality	-	0.78	0.70	1.12
	OO	-	-	0.37	0.05	1.60
Airfryer	SO	Odor intensity	-	0.85	0.37	1.94
	SFO	Adhesiveness	-	0.32	0.12	1.23
	CO	Color intensity Color homogeneity	-	0.45	0.33	1.03
	OO	Odor intensity Odor quality Taste quality	-	0.53	0.38	1.50

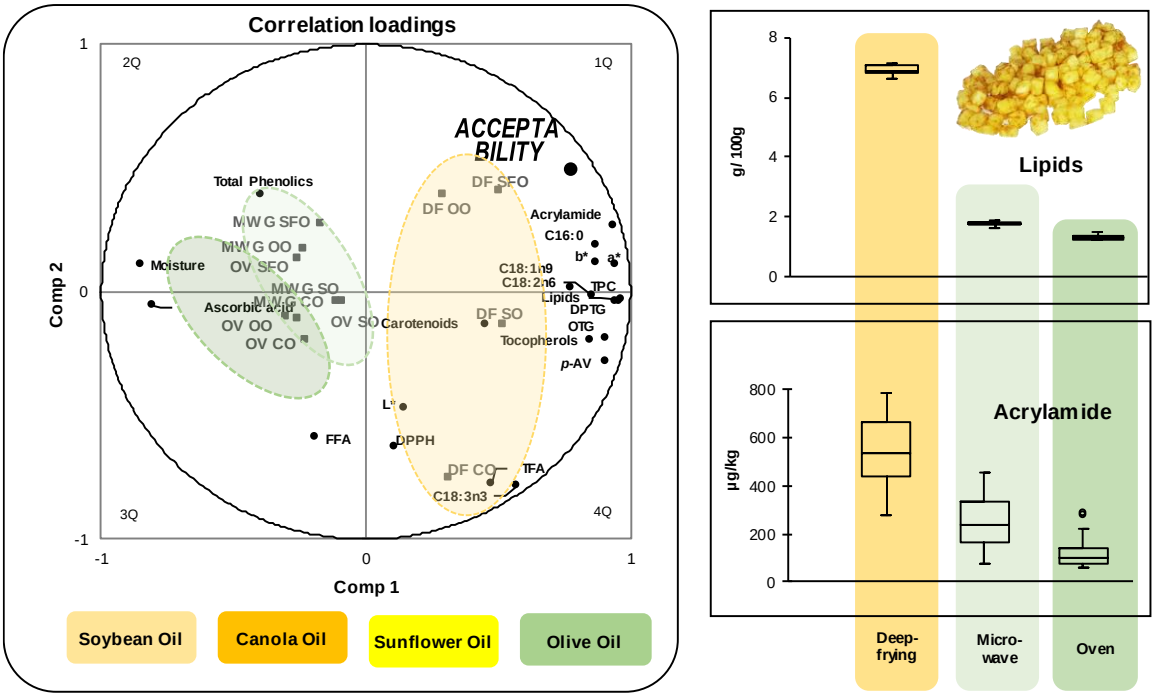
CO – canola oil; OO – olive oil; RMSEP - Root mean square error of prediction; SFO – sunflower oil; SO – soybean oil.

10.1.4 Concluding remarks

The results of this study showed that “air-frying” systems showed better nutritional quality than deep-frying, including fat content (a reduction of 70%), ascorbic acid, and lipid degradation. No influence on acrylamide formation or phenolic compounds degradation was perceived. When the two air-frying equipment’s are compared, the exclusive air-flow technology induced slightly higher lipid degradation than the combined one. From the oils perspective, olive oil (OO), a commercial mixture of refined with virgin olive oil, presented generally lower lipid degradation rate in comparison with other vegetable oils, but higher variability in the sensory analysis, indicative of a strong influence of food habits. Globally, a good acceptance was achieved, with taste and odour qualities being more important than colour, and consequently being the oil choice more determinant than the frying processes.

Thus, air-frying processes show health benefits for consumers, with a reduced oxidation, particularly with OO, also with ecological advantages due to reduced amount of oil used and no effluents after frying.

10.2. Can microwave and conventional oven be low-fat “frying” alternatives?



10.2.1 Background and aim of study

In subchapter 10.1, recent air-frying systems used to produce “fried” potatoes with reduced fat amounts were studied. Still, not all people have access or want to invest on such equipment’s, with common domestic devices as microwave-grill and conventional ovens being used for low-fat potatoes “frying” alternatives. However, these were recently tested with ready-to-use frozen French fries (Giovanelli *et al*., 2017), showing an effective reduction in the fat content in comparison with deep-frying, but not being regarded as true low-fat alternatives, with final fat amounts ranging from 7 to 11g/100g in fat due to the fat already incorporated in the pre-fried potatoes. Additionally, when using pre-fried potatoes, consumers cannot influence the final potatoes fat composition.

Microwave is widely used at a domestic level, being characterized by short processing times, but it lacks the formation of the typical browning colours due to the inherent characteristics of the processing principles, based on interaction of electromagnetic waves with the dielectric properties of the food (Malheiro *et al*., 2011). However, the microwave industry developed grill/crisp alternatives, being their combination now accessible on most domestic microwave devices. Conventional oven cooking, on the other hand, uses hot air convection, with some secondary radiation emerging from oven walls and some conduction from the baking pan, with heat conduction occurring from the external to the internal parts of the food. In traditional deep-frying processes heat is transferred also mainly by convection, but with a higher efficiency than the former due to the direct contact of the food with the heated oil (Pedreschi, 2012). Thus, the heat and mass transfer characteristics of foods cooked by microwave are quite different from those associated with conventional oven cooking, and both from traditional deep-frying, influencing not only the chemical composition of the processed food but also its sensory properties and hence acceptability.

Considering the abovementioned observations, the aim of this work was to compare in terms of composition, fat degradation, bioactivity and acceptance, potatoes from classical deep-frying, with low-fat domestic alternatives (microwave-grill and oven procedures). Aware that the oil used from frying has also a strong influence on both acceptability and composition, we have compared four of the most commonly available vegetable oils worldwide (soybean, sunflower, canola, and olive oil).

10.2.2 Sampling

The cooking processes, including microwave-grill, oven, and deep-frying, were performed according to Chapter 8. Each cooking process was performed in duplicate, on two different days, using two different bottles of each vegetable oil. Potatoes samples were immediately analysed by the sensory panel and for instrumental colour, followed by moisture and total ascorbic acid. Further analyses performed, namely lipid content, fatty

acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, acrylamide, and degradation indicators (as *p*-anisidine value and polar compounds), all detailed in Chapter 6. Fresh vegetable oils were also analysed for some composition parameters (fatty acids composition, tocopherols, carotenoids, total phenolics, antioxidant activity, *p*-anisidine value, and polar compounds). All analytical determinations were performed in duplicate for each processing day (n = 4).

10.2.3 Results and Discussion

10.2.3.1 Physicochemical parameters that impact on sensory attributes and global preference

Moisture, initially at 81%, decreased with all frying processes, with reduced but statistically significant differences ($p<0.05$) between DF (59%), and both MWG and OV (62%) (Fig. 10.5A). In terms of incorporated lipids, an average of 6.9% was quantified in DF, against only 1.8% in MWG and 1.3% in OV (Fig. 10.5B), corresponding to 70 to 85% reduction of lipids, being regarded as true low-fat approaches (<3g/100g). These moisture/oil contents are equivalent to those achieved with commercial low-fat air-frying systems when using fresh potatoes (Santos *et al*., 2017) but lower than when using pre-fried potatoes, with significant amounts of fat already incorporated before processing (Giovanelli *et al*., 2017). No statistical differences were observed between the different oils tested.

The golden colour developed in the potatoes surface, achieved by non-enzymatic Maillard reactions, is a quality standard in DF (Pedreschi, 2012). Imposed by the different heating sources and mechanisms, differences in colour intensity and homogeneity are inevitable. An objective analysis of the instrumental coordinates, including lightness (L^*), and colour components for green-red (a^*) and blue-yellow (b^*) showed similar average values for L^* on all frying processes ($p>0.05$) (Fig. 10.6), despite their different fat contents.

The average scores for each colour coordinate were in agreement with the recommended tones for fried potatoes: between -5 and 0 for a^* and superior to 10 for b^* (Krokida *et al*., 2001), but differences between processes were perceived ($p<0.05$), both for a^* [DF (3) > MWG (-2) > OV (-4)], and b^* [DF (29) > MWG (26) > OV (24)]. When a^* dispersion (redness) is compared for the two low-fat approaches, lower homogeneity is perceived in MWG in comparison with OV.

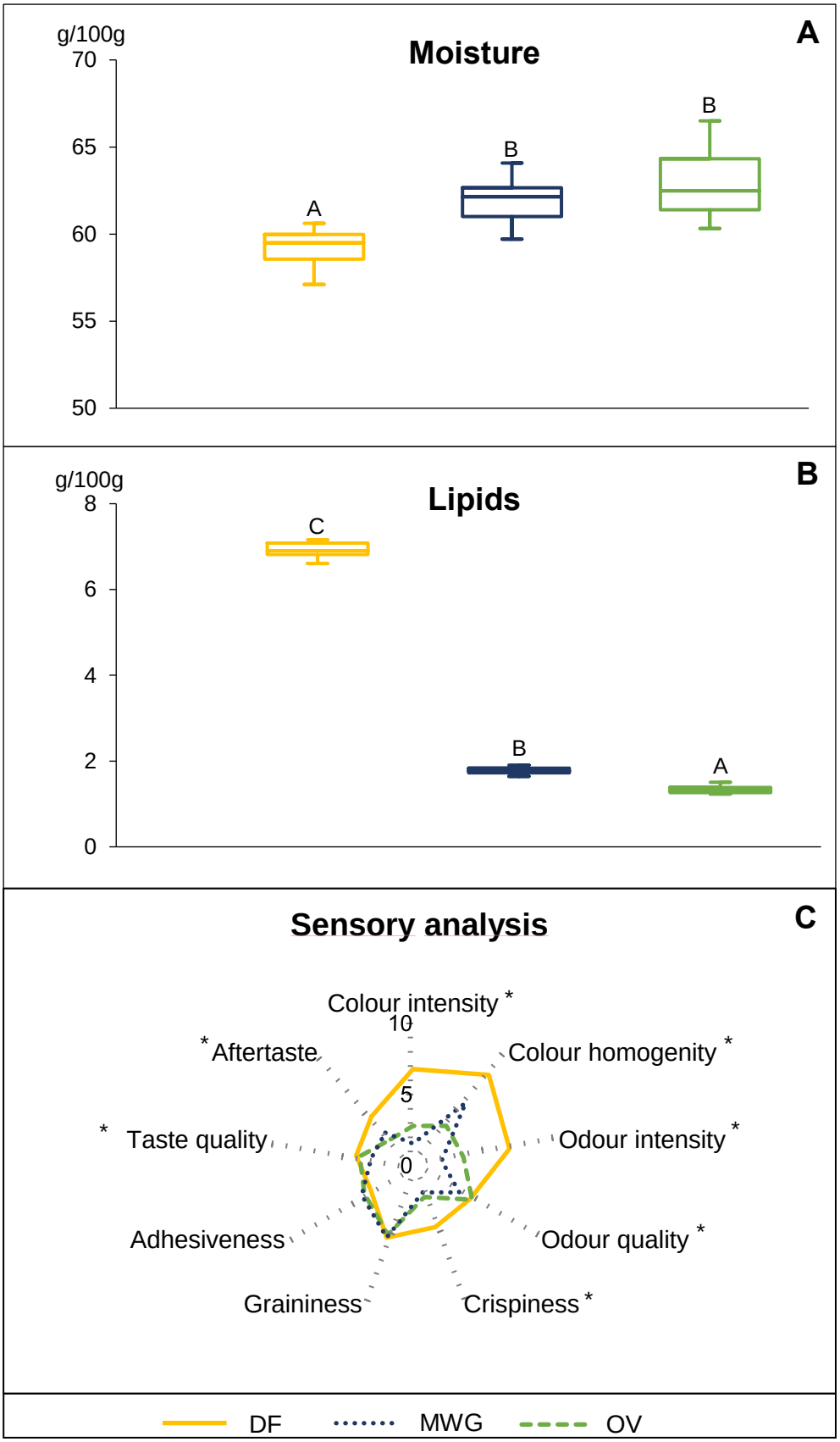


Figure 10.5 Potatoes moisture (A) and lipids (B) contents, and sensory analysis (C) ($^{A\text{-}C}$ for significant differences (p<0.05) between frying processes; * for significant differences (p<0.05) between frying processes).

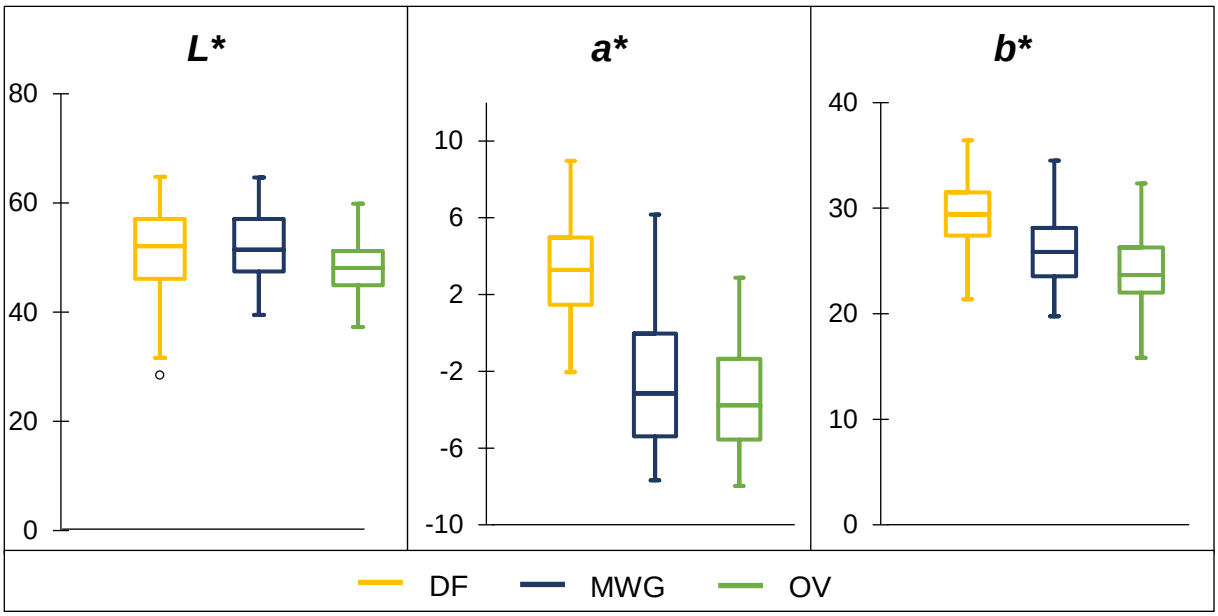


Figure 10.6 Instrumental colour coordinates for each processing method.

Independently of the oil type (Fig. 10.5C), most sensorial attributes presented higher scores for classical deep-frying (p<0.05), including colour and odour intensities (DF>MWG>OV), colour homogeneity (DF>OV>MWG), odour quality (DF, MWG>OV), crispness and aftertaste (DF>MWG, OV), as well as taste quality (DF, MWG>DF, OV), while adhesively and graininess were similar between processes (p>0.05). The lower crispness scores on both low-fat approaches are directly associated with water loss and fat incorporation data, previously discussed, essential for crust formation (Melema, 2003). Data also shows that, even with frequent turnover, oven and microwave-grill are unable to achieve DF colour homogeneity due to the direct contact with the metal recipient surfaces.

Still, when MWG and OV are compared, MWG presented better odour and colour intensities, as well as taste and colour qualities, but lower colour homogeneity, in agreement with the instrumental colour measures. Crispiness and aftertaste were also similar on both low-fat approaches. Global acceptability corroborated the individual scores, with higher acceptability for DF (4.5), followed by MWG (3.4) and OV (2.5). The MWG scores were within the range of commercial air-frying devices (3.5-4.3) tested under similar processing condition (Santos et al., 2017). When the different oils are compared under DF, a general preference for SFO was perceived (6.1/10 global mean score) in opposition to CO (3.2/10), but no significant differences were observed in the low-fat processes, probably due to the low amounts of incorporated fat (Fig. 10.7). The differences between oils in DF reveal the influence of rooted food habits on the sensory panel, in this case more used to sunflower and olive oil and clearly less amenable to canola oil.

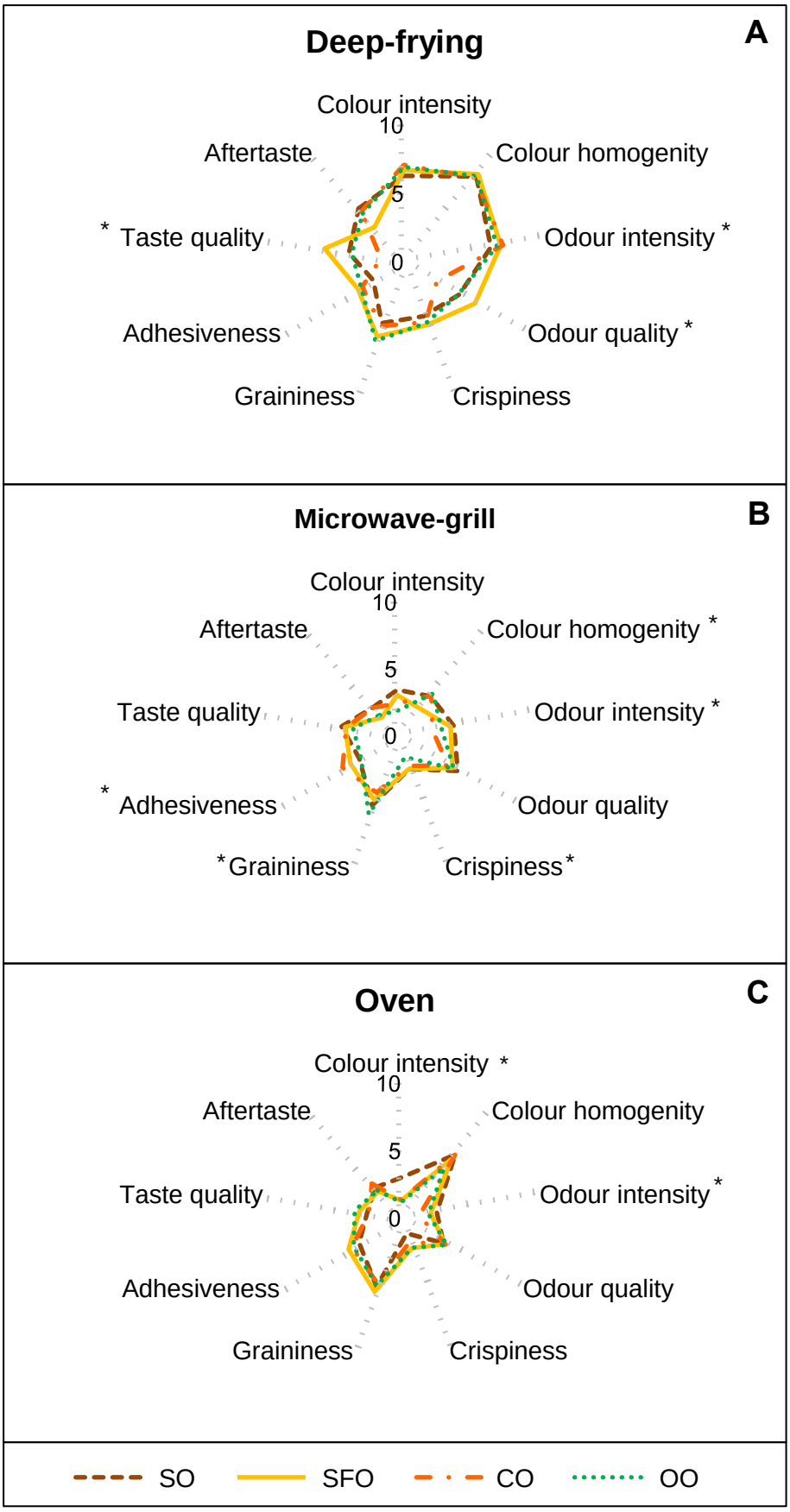


Figure 10.7 Sensory profiles of fried potatoes: A) deep-frying; B) microwave-grill; and C) oven (* for significant differences (p<0.05) between vegetable oils).

10.2.3.2 Process and oil type influence on nutritional quality

Side-by-side with the amounts of incorporated lipids, the intrinsic characteristics of each vegetable oil influences directly the nutritional quality of the final product. On the other hand, the thermal process applied will also influence on the loss of potato bioactive compounds, as ascorbic acid, together with the formation of new compounds, including lipids oxidation products and acrylamide. A separate discussion of these parameters is detailed below.

10.2.3.2.1 Impact on health attributes

When the oils extracted from processed potatoes are compared in terms of fatty acid composition, a dominance of oleic acid in OO fried potatoes, of linolenic acid in SO and SFO and linolenic acid in CO and SO was observed on all the processes (Table 10.7), consistent with the composition of the fresh oils (Table 7.2), with absolute contents in the potatoes determined by the total lipid amounts. This confirms that, by using fresh potatoes, the oils choice is totally reflected in the final product, even in low-fat approaches, which cannot be granted when using commercial pre-fried potatoes.

Potatoes are an excellent source of ascorbic acid (Love and Pavek, 2008). However, the thermal and oxidative stress imposed during cooking inevitably induces losses on this vitamin. From an initial amount of 6.8 mg/100g in raw potatoes, the values decreased significantly, ranging from 0.9 to 3.2 mg/100g (Table 10.7). Regarding the frying processes, DF induced an average loss of 85%, significantly higher on all occasions (p<0.05) than the other two processes, but no significant differences were perceived between the two low-fat frying processes, with losses ranging from 53 to 78%. Aware that processing occurs faster with DF (6 min) than with the other two alternatives (15 min MWG and 30 min OV), processing time alone cannot be the main determinant for ascorbic acid loss, nor moisture, equivalent on all processes. Air exposure could also be regarded as inducing vitamin C degradation but the opposite occurred because air contact is smaller in DF in comparison with MWG and OV. Thermal transfer efficiency, higher in DF, can have contributed to this effect, as well as some potential leaching accompanying water loss in DF. Regarding the different oils, no differences were perceived in the vitamin content, except an apparent reduced loss with OO in OV, indicative that the potential contribution of the oil components to an oxidant/antioxidant effect ascorbic acid, if present, is reduced. The ascorbic acid values in this study are generally lower than those found for vitamin C in fried potatoes (Fillion and Henry, 1998), probably derived from the small dimensions of the cubes used, necessary to increase homogeneity between processes. Nevertheless, these values are slightly higher than those found in air-frying systems under similar processing conditions (Santos *et al*., 2017).

Vitamin E is almost absent in raw potatoes (<0.02mg/100g), but present in substantial amounts in the vegetable oils, with intrinsic differences between them (Table 7.2), increasing by lipid incorporation (Table 10.7). Within each process, a higher tocopherol content was quantified in SO, followed by SFO and CO, with OO having the lowest amounts (p<0.05). Between processes, DF had significantly higher amounts of tocopherols due to the higher fat content, except with OO, while the differences between the low-fat processes were reduced, imposed by the lower incorporated fat amounts.

Raw potatoes are a source of carotenoids in the diet, particularly *β*-carotene, in this case with 79 µg/100 g on a fresh basis (Table 10.7). The amounts increased on all the processing methods, ranging from 120 µg/100 g (SFO-OV) to 180 µg/100 g (OO-DF), influenced by both moisture loss and fat incorporation. However, no differences were perceived between oils (p>0.05), except for MWG (p<0.05), with superior values with SO and OO, despite the vegetable oil initial composition (Table 7.2). When the processes are compared, no statistical differences were also perceived, except for CO, with higher amounts in DF, followed by MWG and OV, but all within the values presented by the other processes.

Regarding total phenolics, here expressed in Gallic acid equivalents (Table 10.7), raw potatoes amounts (22 mg/100 g) were essentially preserved with all frying processes, with a slight enrichment after frying. These could again derive directly from moisture loss, but the oil type had also some influence, particularly with OO, which, due to its naturally higher content in phenolic compounds (Table 7.2) might have induced significantly higher amounts (p<0.05) on all processes. Aware that fat incorporation was reduced in the low-fat frying processes, this increase in phenolic content, more than doubling the initial raw potatoes content, could also derive from an increased protection during processing. Globally, no differences were perceived between the two low-fat processes, both presenting significantly higher amounts than DF potatoes (p<0.05), for all vegetable oils. Moreover, these values are in the same order than those found in air-frying systems (Santos *et al*., 2017).

The results for the antioxidant activity under the DPPH radical assay (Table 10.7) showed that CO imposed consistently higher results on all the processes, as already expected from the oil composition (Table 7.2), followed by SO, SFO, and OO. The process had only a small impact on the final results, but on all cases the potatoes increased their antioxidant activity.

Table 10.7 Effect of different frying processes and vegetable oils on potatoes composition.

		C18:1n-9	C18:2n-6	C18:3n-3	Ascorbic acid	Tocopherols	Carotenoids	Total Phenolics	DPPH
		g/100 g	g/100 g	g/100 g	mg/100 g	mg/100 g	µg/100 g	mg GAE/100 g	mg GAE/100 g
Raw		-	-	-	6.8±0.1	-	79±13	22.1±0.6	6.1±0.9
Deep-frying (Control)	SO	1.53±0.15^{a,C}	2.92±0.15^{c,C}	0.32±0.01^{b,C}	0.9±0.4^{A}	4.5±0.3^{c,C}	171±23	20.5±0.8^{b,A}	9.1±0.6^{a,AB}
	SFO	2.74±0.31^{b,B}	2.50±0.30^{c,B}	0.03±0.01^{a,B}	0.9±0.2^{A}	3.7±0.3^{b,B}	145±14	20.6±1.5^{b,A}	8.4±0.7^{a,B}
	CO	3.38±0.03^{bc,B}	1.10±0.03^{b,B}	0.48±0.02^{c,B}	1.2±0.3^{A}	2.9±0.4^{b,C}	167±13^{B}	13.2±0.4^{a,A}	15.5±0.3^{b,A}
	OO	4.23±0.45^{c,C}	0.36±0.03^{a,C}	0.04±0.00^{a,C}	0.9±0.2^{A}	0.5±0.1^{a}	180±36	45.4±1.6^{c,A}	7.8±0.6^{a,B}
Microwave-grill	SO	0.28±0.03^{a,B}	0.56±0.06^{c,B}	0.07±0.01^{b,B}	1.7±0.6^{AB}	1.2±0.1^{b,B}	177±11^{c}	29.0±0.8^{b,B}	9.3±0.4^{b,B}
	SFO	0.53±0.03^{b,A}	0.51±0.03^{bc,A}	0.01±0.00^{a,A}	2.4±0.3^{C}	1.1±0.1^{b,A}	130±19^{a}	26.6±1.5^{b,B}	7.2±0.7^{a,AB}
	CO	0.62±0.06^{b,A}	0.33±0.10^{ab,A}	0.08±0.04^{b,A}	2.2±0.6^{B}	1.0±0.0^{b,B}	143±6^{ab,AB}	20.2±0.4^{a,B}	15.6±0.3^{c,A}
	OO	0.80±0.17^{b,B}	0.09±0.01^{a,B}	0.02±0.00^{a,B}	2.2±0.8^{B}	0.5±0.0^{a}	166±3^{bc}	54.9±0.9^{c,B}	6.6±0.2^{a,A}
Oven	SO	0.19±0.02^{a,A}	0.39±0.04^{c,A}	0.05±0.01^{b,A}	2.7±0.9^{ab,B}	0.8±0.1^{b,A}	146±22	27.5±0.8^{b,B}	8.5±0.3^{b,A}
	SFO	0.40±0.09^{b,A}	0.39±0.09^{c,A}	0.01±0.00^{a,A}	1.5±0.3^{a,B}	0.8±0.2^{b,A}	120±27	28.1±1.5^{b,B}	6.4±0.8^{a,A}
	CO	0.50±0.10^{b,A}	0.19±0.03^{b,A}	0.08±0.02^{b,A}	1.5±0.1^{a,AB}	0.8±0.1^{b,A}	127±8^{A}	22.0±0.7^{a,C}	16.4±0.4^{c,B}
	OO	0.51±0.08^{b,A}	0.06±0.01^{a,A}	0.01±0.00^{a,A}	3.2±0.7^{b,B}	0.4±0.0^{a}	157±22	56.1±0.8^{c,B}	6.4±0.3^{a,A}

$^{a-d}$ Statistically significant differences (p<0.05) between vegetable oils for the same frying process or $^{A-C}$ between frying processes for the same vegetable oil.
C18:1n-9 - oleic acid; C18:2n-6 - linoleic acid; C18:3n-3 - linolenic acid; CO - canola oil; DPPH - 2,2,diphenyl-1-picrylhydrazyl radical; GAE - Gallic acid equivalents; OO - olive oil; SFO - sunflower oil; SO - soybean oil.

10.2.3.2.2 Impact on lipid degradation and acrylamide formation

During the thermal and oxidative stress induced by frying, the oils are gradually deteriorated. Several degradation indicators were evaluated, in order to understand the impact of the frying procedures on the lipids quality, enabling to estimate and compare the ingestion of degraded lipids through the fried potatoes. Therefore, the lipids extracted from the processed potatoes were evaluated in terms of classical degradation indexes, including *p*-anisidine value that gives an estimation of aldehydes formed as secondary oxidation products, potentially impacting on odour quality and intensity, together with triglycerides hydrolysis, oxidation and polymerization, by analysing each of these fractions by HPLC.

The lipid degradation indicators are presented on Table 10.8, on a lipid basis, enabling a direct association of fat degradation between processes and oils. For an impact on health derived from their ingestion, all values can be converted into a potato basis, based on the potatoes lipid content presented in Table 10.7. Regarding oxidation, PAV values were consistently higher in DF, without differences between the two low-fat processes. When the oils are compared within each process, OO had significantly lower PAV values, being therefore less oxidized, which could be a consequence of its higher phenolic content, although its lower polyunsaturated degree can also contribute for its stability. In the low-fat processes, PAV value was clearly lower, reducing the ability to distinguish the different oils, but SFO had clearly higher amounts of secondary aldehydes. This pattern is repeated in the OTG fraction (OTG), again with significantly higher amounts in DF, but here with some differences between the low-fat processes, with higher oxidation in MWG with SO and CO. The TPC are a general index for oil degradation, corresponding to the sum of the previously mentioned OTG, together with polymeric forms (DPTG), diglycerides (DG) and FFA, and with a consensus over a 22 to 25% limit for rejection in several European countries (Gertz, 2000a). TPC were almost double in the DF (5.9-7.8%) in comparison with the other two methods (3.1-3.9), without differences between them (Table 10.8). For the polymeric forms, significantly higher amounts in DF than in the two other processes were also observed, being only residual in the low-fat approaches.

Fatty acids isomerization is also an indicator of thermal and oxidative degradation, although occurring at relatively low rates and amounts (Table 10.8). Indeed, TFA were low with all extracted lipids, slightly higher in CO, and similar to those of the fresh oils (Table 7.2). As to the processes, higher TFA were quantified in the DF, but always below 0.05%, corresponding to very low amounts from a health point of view.

Table 10.8 Effect of different frying processes and vegetable oils on the degradation of incorporated fat.

g/100g of extracted lipids		PAV*	TPC	DPTG	OTG	FFA	TFA
Deep-frying (Control)	SO	$42\pm8^{b,B}$	$7.6\pm0.4^{b,B}$	$2.0\pm0.3^{b,C}$	$3.1\pm0.2^{b,B}$	0.5 ± 0.1^{a}	$0.39\pm0.003^{b,B}$
	SFO	$45\pm6^{b,B}$	$7.8\pm0.7^{b,B}$	$2.4\pm0.4^{b,B}$	$2.9\pm0.3^{b,B}$	0.6 ± 0.0^{b}	$0.19\pm0.002^{a,B}$
	CO	$53\pm1^{b,C}$	$6.4\pm0.7^{ab,B}$	$1.7\pm0.3^{b,C}$	$2.6\pm0.4^{ab,B}$	0.7 ± 0.0^{b}	$0.55\pm0.009^{c,B}$
	OO	$17\pm2^{a,B}$	$5.9\pm0.3^{a,B}$	$1.0\pm0.1^{a,B}$	$2.0\pm0.2^{a,B}$	$0.5\pm0.0^{a,A}$	$0.14\pm0.003^{a,B}$
Microwave-grill	SO	$2\pm1^{a,A}$	$3.9\pm0.1^{c,A}$	$0.13\pm0.00^{c,B}$	$1.3\pm0.0^{b,A}$	0.5 ± 0.1	$0.26\pm0.003^{c,A}$
	SFO	$6\pm1^{b,A}$	$3.5\pm0.0^{b,A}$	$0.12\pm0.01^{c,A}$	$1.1\pm0.2^{b,A}$	0.5 ± 0.2	$0.13\pm0.002^{b,A}$
	CO	$4\pm1^{a,A}$	$3.2\pm0.1^{a,A}$	$0.09\pm0.01^{b,B}$	$1.1\pm0.1^{b,A}$	0.6 ± 0.1	$0.30\pm0.007^{c,A}$
	OO	$2\pm1^{a,A}$	$3.1\pm0.2^{a,A}$	$0.04\pm0.01^{a,A}$	0.7 ± 0.1^{a}	0.5 ± 0.0^{A}	$0.08\pm0.002^{a,A}$
Oven	SO	$2\pm1^{a,A}$	$3.9\pm0.0^{c,A}$	$0.10\pm0.01^{b,A}$	$1.3\pm0.2^{c,A}$	0.6 ± 0.1	$0.21\pm0.002^{c,A}$
	SFO	$6\pm1^{b,A}$	$3.2\pm0.3^{ab,A}$	$0.11\pm0.02^{b,A}$	$1.0\pm0.2^{b,A}$	0.6 ± 0.1	$0.13\pm0.001^{b,A}$
	CO	$2\pm1^{a,A}$	$3.1\pm0.1^{a,A}$	$0.06\pm0.01^{a,A}$	$1.0\pm0.0^{b,A}$	0.7 ± 0.0	$0.34\pm0.004^{d,A}$
	OO	$3\pm2^{ab,A}$	$3.3\pm0.1^{b,A}$	$0.05\pm0.01^{a,A}$	$0.6\pm0.0^{a,A}$	0.7 ± 0.1^{B}	$0.06\pm0.000^{a,A}$

$^{a-d}$ Statistically significant differences ($p < 0.05$) between vegetable oils for the same frying process or $^{A-C}$ between frying processes for the same vegetable oil;
*expressed as 100 times the optical density measured at 350 nm (1 cm) of a solution containing 1.00 g of the oil in 100 mL, according to the method;
CO - canola oil; DPTG - dimeric and polymeric triglycerides; FFA - free fatty acids; OO - olive oil; OTG - oxidized triglycerides; PAV - *p*-anisidine value; SFO - sunflower oil; SO - soybean oil; TFA - *trans* fatty acids; TPC - total polar compounds.

Acrylamide was included in this section because it corresponds to a compound formed during heating, with potential deleterious effects on consumer's health. Acrylamide is a recognized processing contaminant in fried potatoes, being formed above 120°C and under low-moisture conditions (Molina-García *et al*., 2015). Mean values from 130 µg/kg (OV) to 550 µg/kg (DF) were detected in the fried samples, with a clear distinction between frying processes (Fig. 10.8).

When the two low-fat processes are compared, statistically significant differences (p<0.05) are observed, with lower amounts in the OV with all the oils [OV (207-SO, 144-CO, 95-SFO, 75-OO) < MWG (298-CO, 280-SFO, 248-OO, 186-SO) < DF (718-SFO, 606-SO, 510-OO, 369-CO), all in µg/kg]. When the oils are compared, a clear pattern was not perceived, with SFO presenting simultaneously among the highest average amounts in DF and MWG, with 718 µg/kg and 280 µg/kg, respectively, and being within the lowest range in OV, with 95 µg/kg. In opposition, CO presented the lower acrylamide content in DF (369 µg/kg) but was within the highest range in MWG (298 µg/kg). This high variability between oils shows that the processing technique effects superimpose those potentially derived from the oils composition.

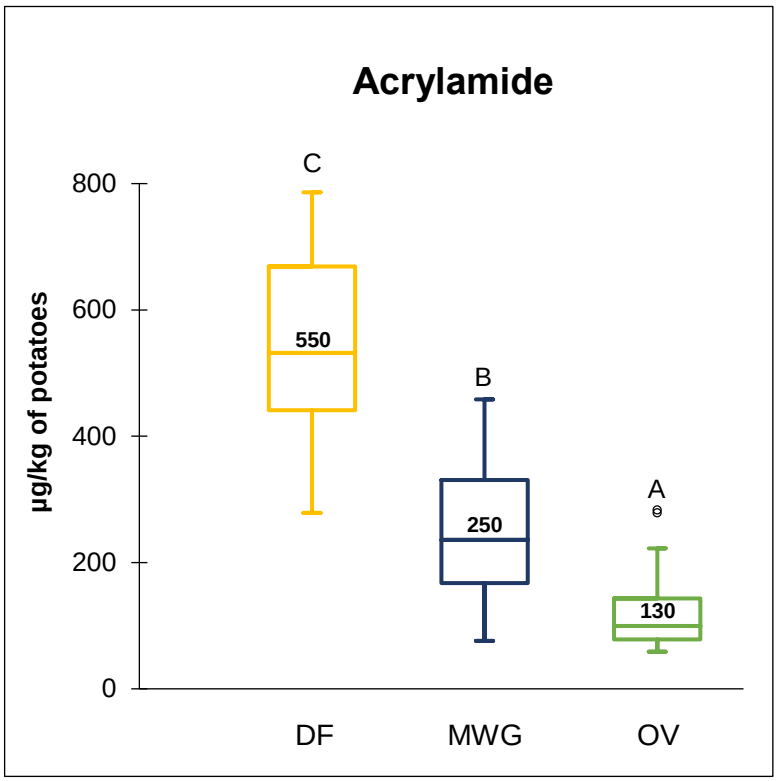


Figure 10.8 Potatoes acrylamide content ($^{A-C}$ for significant differences (p<0.05) between frying processes).

Also, the reductions observed with MWG and OV are interesting from a health point of view, while the differences between both, with lower amount in OV, can be explained by the direct contact with the crisp plate in MWG, consistent with the increased red tones in the instrumental colour reading and probable increased *Maillard* reactions extension on these darker spots. Indeed, colour intensity and redness (a*) are known to be associated with acrylamide content in fried potatoes (Pedreschi *et al*., 2006), being able to explain, respectively, 89 % and 85 % in the present study, when applying linear multiple regression models for each individual parameter.

In comparison with published data, superior average contents in MWG (376 µg/kg), and inferior in DF and OV (94 and 50 µg/kg, respectively) were found by Giovanelli *et al*. (2017).

10.2.3.3 Correlation between acceptability and composition

A PLSR1 model studied possible correlations between acceptability and the physicochemical parameters of fried potatoes (Chung *et al*., 2003), after standardization of the variables. The developed model is explained in four main components, with a cumulative R^2 of 0.98 and cumulative Q^2 of 0.84. These quality indices also show that the

model fits well and has good predictability, with an RMSE of 0.16. The first two main components are shown in Figure 10.9.

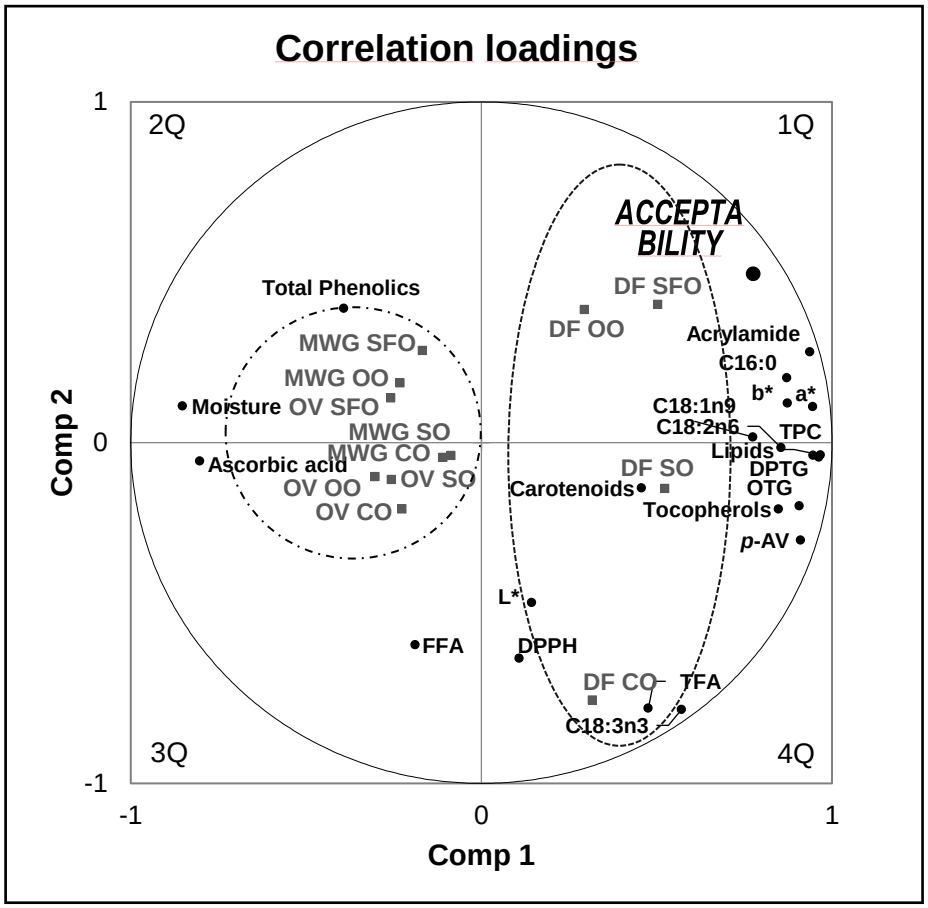


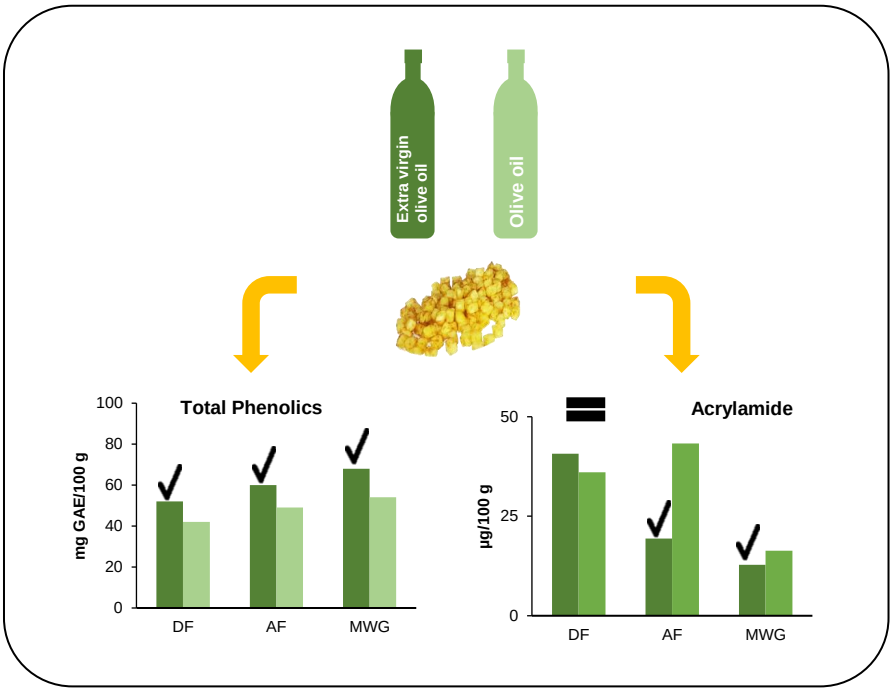
Figure 10.9 Correlation loading of partial least squares regression predicting fried potatoes acceptability and physicochemical parameters.

For processes, a distinction is made between the DF potatoes (positive region Comp1) and the remaining potatoes (negative region Comp1), without a clear distinction between MWG and OV. DF potatoes were characterized by higher lipid contents, and consequently their inherent fat-soluble constituents as tocopherols, fatty acids and lipid degradation components, but also acrylamide and colour coordinate values. MWG and OV are characterized by higher moisture contents, correlating negatively with acceptance, but with more water-soluble constituents, as ascorbic acid and total phenolics. In this model, acceptability is specifically associated with DF potatoes in SFO and OO. These results demonstrated the impact of the type of processing on the potatoes, corroborating the discussion carried out both in the sensorial analysis and in the physicochemical parameters.

10.2.4 Concluding remarks

The results of this work demonstrated that MWG and OV have potential to be implemented at domestic level as healthier alternatives to frying technologies. Its use with fresh potatoes, instead of pre-fried frozen ones, enables the consumer to choose the oil of its preference, while achieving true low-fat potatoes. Indeed, the amount of fat absorbed by the MWG and OV potatoes is around 80% lower than DF, corresponding to an average decrease of 50 kcal/100 g under the present conditions. However, the advantages are not restricted to the lipid amounts, with a direct ingestion of less amounts of degraded fat, higher preservation of ascorbic acid and total phenolics, particularly in OO, and smaller amounts of acrylamide. Despite the higher acceptability for DF potatoes, the vegetable oil had a strong influence. Regarding the two processes under study, MWG was sensory preferred to OV view being both similar from the chemical and nutritional points of view. To improve acceptability, clear instructions should be given by the equipment's producers, being also advisable not to change the vegetable oil that people are used to.

CHAPTER 11. Olive oil commercial categories: do they influence on the nutritional quality of white fried potatoes?



Parts of the text of this chapter were submitted to publication:

Santos CSP, Cunha SC, Casal S (2017) Olive oil commercial categories: do they influence on the nutritional quality of fried white potatoes? (Paper 9)

11.1 Background and aim of study

Virgin olive oil is increasingly recognized for its nutritional and health properties, with a growing consumption worldwide, both for dressing or cooking purposes. However, when used for thermal processing it will inevitably reduce its bioactive potential, in a direct dependence of its initial chemical composition and the characteristics of the processing method used, particularly temperature and length.

Olive oil is sold under several commercial categories (Commission Regulation (EEC) N.° 2568, 1991 and amendments), where extra-virgin olive oil (EVOO) stands has the highest quality one, usually also more expensive, followed by virgin olive oil (VOO), usually with higher acidity and lower sensorial quality. The third category, “olive oil” (OO), is a mixture of refined and virgin olive oil, the first resulting from virgin olive oil that did not fulfilled the quality attributes required for direct consumption and therefore requiring a refinement process with additional purification steps for the purpose. During refining, olive oil will inevitably loose some of its typical features (Roodaki *et al*., 2016), both chemical and sensorial. It is recognized that non-refined oils have better oxidative stability than refined ones (Gertz and Klostermann, 2000b), with the unsaponifiable compounds having determinant impact on the oils performance, as sterols and phenolics (Satue *et al*., 1995; Gutierrez *et al.*, 2001). By blending refined with virgin olive oil, a slight enrichment in bioactive compounds is granted, together with a partial recover of sensorial characteristics, being traded at a lower price than EVOO. Unfortunately, this commercial “olive oil” category is frequently sold with several adjectives on the label, as “pure”, “traditional” or “classical”, creating confusion to consumers, and at higher prices than one could expect from a lower grade product in terms of bioactivity, despite maintaining all the typical monounsaturated richness, a highly important nutritional feature.

Interestingly, few studies on the impact of the compositional differences between these olive oil categories are found, and even less under different cooking procedures, without a consistent knowledge on the impact and suitability of the various olive oil categories for thermal processing purposes, both in terms of losses and gains of nutrients and economic impact. Initial studies have shown that olive oil is among the high resistant unsaturated vegetable oils under real processing approaches, independently of the category (Casal *et al*., 2010; Zribi *et al*., 2014; Nieva-Echevarría *et al*., 2016; Santos *et al*., 2017). Also, when different EVOO oils are compared under frying, huge differences are found, in a direct proportion to the variability naturally found in EVOO composition, derived from the olive varieties used, maturation stage, and composition, particularly in terms of polyunsaturated content and phenolic compounds (Casal *et al*., 2010; Olivero-David *et al*., 2014; Abenoza *et al*., 2016; Hoffman and Gerber, 2015). It became also clear that certain monocultivar EVOO might be less resistant than EVOO blends and that the variations between years and

brands might superimpose to those of the olive oil category in terms of frying performance (Casal *et al*., 2010). In particular, microwave-heating induces fast quality losses on both EVOO and OO when heated alone (Cerretani *et al*., 2009), without studies dealing with the effects in the food. Indeed, studies performing direct comparisons under real food processing conditions are globally scarce, and even scarcer are the approaches that focus on the consumer's side – on the processed food.

Therefore, the aim of the present work was to determine the differences derived from the use of different olive oil categories for cooking. Aware that frying is among the most popular thermal processing methods, particularly potato frying, and that fried foods represent a health concern (Gadiraju *et al*., 2015), we have implemented a simultaneous comparison of potato frying using high and low-fat alternatives, focusing on the potato composition, bioactivity and sensorial impact.

11.2 Sampling

The cooking processes, including deep-frying, air-frying (ACT and AIR), and microwave-grill were performed according to Chapter 8. Each cooking process was performed in duplicate, on two different days. Two olive oil categories were chosen: EVOO and OO, using different bottles of each olive oil category on each day. Potatoes samples were immediately analysed by the sensory panel and for instrumental colour, followed by moisture and total ascorbic acid. Further analyses performed, namely lipid content, fatty acids composition, tocopherols, carotenoids, total phenolics, acrylamide, and degradation indicators (as *p*-anisidine value and polar compounds), all detailed in Chapter 6. Fresh vegetable oils were also analysed for some composition parameters (fatty acids composition, tocopherols, carotenoids, total phenolics, *p*-anisidine value, and polar compounds). All analytical determinations were performed in duplicate for each processing day (n = 4). For data discussion, both brands of each olive oil category were grouped, with n = 8 for each EVOO and OO.

11.3 Results and Discussion

The effect of using different olive oils categories can be compared from two perspectives. From a nutritional one, fried potatoes can be detailed for macro and micronutrients changes, while from a thermal degradation perspective, gains and losses from both potato and olive oil can be compared. This can be further enhanced by using diverse frying processes, from classical frying to more modern "low-fat" technologies and, for a wider comparison, different brands of each EVOO and OO should be used. On this basis, we have compared the results grouping two brands of each olive oil category, although their individual characteristics can be consulted in Table 7.2. We will discuss first

the nutritional effect of using different olive oil categories on diverse processing methods, including formation of processing hazardous (i.e. acrylamide), with a second part dedicated to lipids oxidation extension, and finally a sensorial perspective.

11.3.1 Effect of using different commercial olive oil categories on potatoes nutritional quality

Moisture and lipids are the main determinants for the final nutritional and sensorial properties in fried potatoes, strongly influencing the caloric value and the crust and taste sensations. After processing (Table 11.1), significant moisture lost was observed with all processing methods, with MWG presenting lower moisture contents (60 g/100g) than the other two processing methods (64 g/100g) (p<0.05). As to the olive oil categories, no differences were perceived (p>0.05).

For lipids (Table 11.1), DF potatoes presented higher lipid incorporation (p<0.05), with 6.4 g/100g on average, followed by AF with only 1.8 g/100g (-71%), and MWG with 1.6 g/100g (-76%). While these absolute amounts are determined by the processing methodology used for cooking (Fillion and Henry, 1998), the composition of the final lipids should reflect the incorporated fat because raw potatoes have a much reduced lipid content (0.14 g/100g). Therefore, consistent with the high relative MUFA content in the fresh olive oils, the MUFA in the lipids extracted from processed potatoes also varied from 75 to 79 g/100g (Table 7.2), with absolute amounts in a direct proportion to the total lipid content.

The results for vitamins, in particular for vitamin C (ascorbic acid), vitamin E (total tocopherols) and pro-vitamin A (total carotenoids) are also detailed in Table 11.1, together with total phenolic compounds, both for raw and processed potatoes. All results are expressed on a fresh basis, as consumed, detailing also the absolute retention (AR) in comparison with the amounts expected from the fresh products (raw potato plus olive oil), adapted from (Fillion and Henry, 1998).

The values obtained for raw potato and olive oil were all within the expected from the literature, particularly on potato richness in terms of vitamin C (Camire *et al*., 2009) and the typical features of olive oil, with interesting amounts of vitamin E, carotenoids and phenolic compounds, these last being among the most relevant bioactive compounds of virgin olive oil (Boskou *et al*., 2006; Servili *et al*., 2014). In addition, data form the two olive oil categories (EVOO and OO – Table 7.2) shows significant differences between and within categories, with EVOO being richer in phenolic compounds and carotenoids, mostly due to the absence of refining. Documented data shows that the variables behind the amounts of these bioactive compounds in EVOO are highly diverse, beginning already with the olive oil variety and growing condition, passing by the maturity and integrity of the fruits, the extracting method, and the preservation conditions and age (Servili *et al*., 2014).

For all the conditions tested, a huge decrease in ascorbic acid was verified, from 6.6 mg/100g in raw fresh potatoes to 1.9-3.9 mg/100g in processed ones, corresponding to low retentions, from 17 to 31%. For the same olive oil category, no differences were observed between processing methods but the differences between categories were remarkable, significant and consistent, with higher retention in EVOO than in OO (p<0.05). This is a strong indication that some EVOO components protect potatoes ascorbic acid from oxidation during the thermal process. From literature, AR values between 65 and 85% were found, showing that potatoes surface area has an important impact on ascorbic acid losses (Fillion and Henry, 1998). This is consistent with the high surface area on our study, as we have used small potatoes cubes to increase homogeneity between the different processing methods.

In opposition to ascorbic acid, there was a clear enrichment of potatoes in tocopherols, carotenoids and phenolic compounds because these are characteristic of the olive oil added. However, when the theoretical values are compared with the real ones, losses with processing are also perceived, particularly for tocopherols. Between categories, potatoes processed in EVOO had significantly more tocopherols than OO (p<0.05) in the DF, consistent with the heating studied reported by (Gharby *et al*., 2016), but the amounts were similar between categories in the other processing methods. When correcting these values for the initial olive oil characteristics, a higher retention was again observed with EVOO in DF (27% vs 8%), but no differences were observed between categories in the other processing methods. Still, these retentions are significantly lower than those observed with MWG (77-80%) and AF (77%). For carotenoids less variability was observed, with a general enrichment on all techniques and oil samples (152-169 µg/100g fresh weight), and consistent retentions, ranging from 64 to 71% in EVOO and from 72 to 75 % with OO, without differences between categories. Still, it is interesting no observe that despite the EVOO significantly higher amounts of carotenoids than OO (Table 7.2) before processing, these were not perceived in the processed potatoes.

Table 11.1 Impact of using different commercial olive oil categories on potato nutritional composition.

		Fresh oils		Potatoes						
				Raw	Deep-frying		Air-frying		Microwave-grill	
		EVOO	OO		EVOO	OO	EVOO	OO	EVOO	OO
Moisture	g/100g	-	-	81.6±1.7	63.0±0.7^{B}	62.8±1.0^{B}	64.9±1.1^{b,C}	63.2±1.1^{a,B}	59.4±1.2^{A}	60.8±2.2^{A}
Lipids	g/100g	-	-	0.14±0.02	6.3±0.3^{C}	6.5±0.2^{C}	1.7±0.1^{a,B}	1.9±0.3^{b,B}	1.6±0.1^{A}	1.5±0.1^{A}
MUFA	g/100g	74.6±0.2^{a}	77.1±0.4^{b}	-	5.1±0.2^{B}	5.0±0.2^{C}	1.3±0.1^{a,A}	1.4±0.2^{b,B}	1.2±0.1^{A}	1.2±0.0^{A}
Ascorbic acid	mg/100g	-	-	6.6±0.5	3.4±0.8^{b,A}	1.9±0.8^{a,A}	3.3±0.4^{b,A}	2.7±0.6^{a,B}	3.9±0.7^{b,B}	2.9±0.5^{a,B}
	% AR	-	-	-	31±8	17±8	28±4	21±5	28±4	20±4
Tocopherols	mg/100g	36.1±3.4^{b}	32.4±2.2^{a}	0.02±0.00	0.6±0.1^{b,B}	0.2±0.0^{a,A}	0.5±0.1^{A}	0.5±0.1^{B}	0.5±0.1^{A}	0.4±0.1^{B}
	% AR	-	-	-	27±3	8±1	71±12	71±18	77±16	80±16
Carotenoids	µg/100g	1227±104^{b}	475±8^{a}	103±9	161±13	152±16	169±14^{b}	154±22^{a}	169±23	157±15
	% AR	-	-	-	64±5	75±9	81±6	75±11	71±11	72±5
Total Phenolics	mg GAE/100g	43±9^{b}	12±12^{a}	22±1	52±3^{b,A}	42±4^{a,A}	60±7^{b,B}	49±5^{a,B}	68±3^{b,C}	54±2^{a,C}
	% AR	-	-	-	131±7	111±11	148±20	115±13	143±10	118±9

$^{a-b}$ statistically significant differences between olive oil categories ($p < 0.05$); $^{A-C}$ statistically significant differences between cooking processing ($p < 0.05$);
AR -absolute retention; EVOO - extra virgin olive oil; GAE - Gallic acid equivalents; MUFA - monounsaturated fatty acids; OO - olive oil.

All potatoes were enriched in phenolic compounds, with a 2 to 3 fold increase on a wet basis (as consumed), and with significantly higher amounts when using EVOO than OO on all processing methods (p<0.05). Apart from this direct comparison of categories on the same sample basis, the results for total phenolic compounds should be interpreted with caution as no individual analysis was performed. Indeed, the amounts higher than 100 % are a direct consequence of it, probably with some degradation compounds formed during processing, as phenolic or sugar hydrolysis products being included. Still, the DF values were lower than the ones achieved with the other processing methods, and were also in agreement with the literature (Ramirez-Anaya *et al*., 2015), indicative again of higher thermal and oxidative stress with this procedure. In opposition, MWG showed the highest amounts of phenolic compounds for the three methods, which is in agreement with the microwave processing data (Goulas *et al*., 2015). On a retention basis, a higher preservation of the initial EVOO phenolics was also clear, with 20 to 33% more retention than with OO. No literature references were found for phenolic AR values.

On a dietary recommendation view, a 100g portion of fried potatoes provides between 2% (MWG-OO) to 9% (DF-OO) of fat, between 2% (DF-OO) and 5% (MWG-EVOO) of ascorbic acid daily recommendation (80 mg); and between 2% (DF-OO) to 5% (DF-EVOO) for vitamin E (12 mg) (Regulation (EU) N.°1169, 2011).

We have included the evaluation of acrylamide (Fig. 11.1), as this is a very important processing contaminant formed in fried potatoes, conditioned by potatoes composition and processing method (Friedman, 2015).

The average amounts found varied from 128 to 433 µg/kg, in agreement with published results for French fries obtained by different cooking (Santos *et al*., 2017, Molina-García *et al*., 2015, Giovanelli *et al*., 2017). Additionally, the significant impact of fried potatoes on the daily ingestion of acrylamide was reinforced (0.4 to 1.9 µg/kg body weight per day) (EFSA, 2015).

An influence of the olive oil category was only observed in the AF systems, with higher amounts formed with OO, but the differences in the other methods were less evident despite being significant (p<0.05). The AF systems presented also higher dispersion of acrylamide contents between replicates (Fig. 11.1B), influenced by the amounts determined on the AIR system (not shown). Between processing methods, acrylamide formation was higher in DF and AF, but lower in MWG. These lower amounts in MWG are consistent with the lower lipid degradation and preservation of bioactive components, as phenolics, previously discussed.

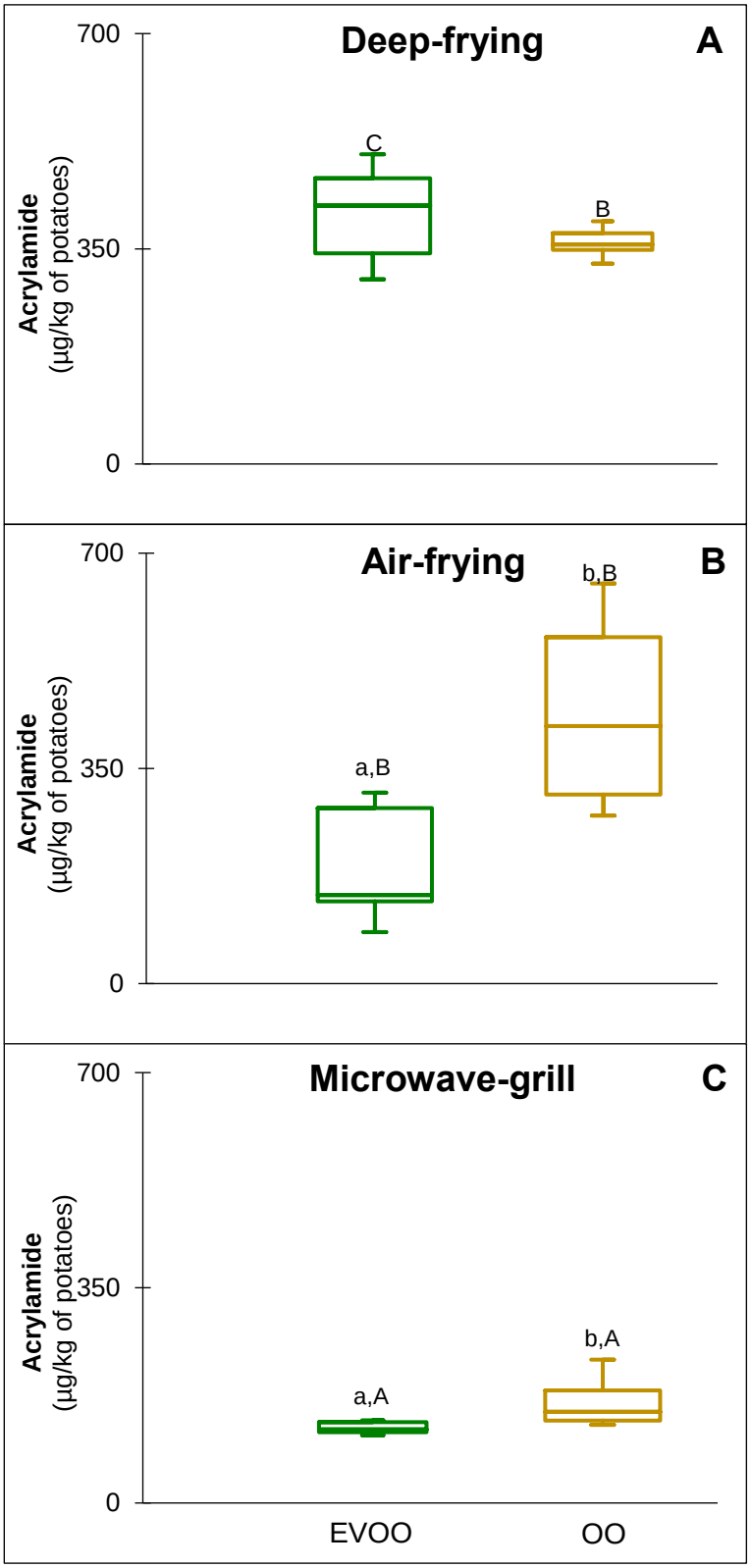


Figure 11.1 Acrylamide content in potatoes processed by: A) deep-frying; B) air-frying; and C) microwave-grill using different commercial olive oil categories. ($^{a-b}$ for significant differences (p<0.05) between olive oil categories and $^{A-C}$ for significant differences (p<0.05) between cooking techniques).

11.3.2 Effect of using different commercial olive oil categories on lipid oxidation indicators

We have compared several indicators of lipid degradation in the fat extracted from the processed potatoes, taking the fresh olive oils values as reference, and expressed on a lipid basis (*per* 100 g of extracted lipids). A general increase on all oxidation indicators was perceived with processing (Table 11.2), but differences between categories and processing methods were also observed.

Despite having lower oxidation degree before processing, EVOO showed higher oxidation than OO in the DF potatoes (p<0.05), while for the other low-fat processing methods the results were more consistent with the initial differences, with higher oxidation in OO. This is supported by significantly higher K_{232}, K_{270}, PAV and TPC values on EVOO-DF samples in comparison with OO ones. Also, lower PUFA/SAT ratios were observed in the DF-EVOO potatoes lipids in comparison with the fresh oils, indicative of fatty acids oxidation. Still, the higher initial PUFA amount in EVOO is not a typical parameter of this commercial category, but rather a consequence of using specific cultivars that might higher PUFA amounts. Indeed, EVOO1 had a higher PUFA/SAT ratio (Table 7.2) than EVOO2 (0.63 against 0.48), the later more similar to both OO, with 0.44 and 0.41. Therefore, the high PUFA/SAT deviation in EVOO ratios is a consequence of this variability, transversal to all processing methods. The DF-EVOO1 ratio was reduced from 0.63 to 0.62 and DF-EVOO2 from 0.48 to 0.46 after processing, corresponding to small variations. The increase in the PUFA/SAT ratio on all low-fat systems, independently of the category but proportional to the initial ratios, is a direct consequence of the low proportion of lipids incorporated in the potatoes, with potatoes natural lipids with a high PUFA content (Camire *et al*., 2009), influencing the final ratio. Indeed, this is an interesting health attribute for these low-fat processed potatoes. A general increase of TPC was also perceived in DF but not on the other processing methods. In DF, the increased TPC was sustained by higher OTG and FFA amounts. Again, the higher amount of PUFA previously discussed in EVOO1 can be the determinant for the high variation between the DF-EVOO replicates, with 8.1 for EVOO1 and only 5.5 for EVOO2, with 6.8 as average (Table 11.2). This is consistent with the expected higher amount of polar compounds in higher PUFA content oils, as observed by (Roodaki et al., 2016). Also, the higher amount of TPC in EVOO1 is supported by higher amount of oxidation products (OTG = 4.0 against 1.9 in EVOO2).

Table 11.2 Effect of using different commercial olive oil categories on the quality of the lipids incorporated in processed potatoes, expressed on a lipid basis.

		Fresh oils		Potatoes					
				Deep-frying		Air-frying		Microwave-grill	
		EVOO	OO	EVOO	OO	EVOO	OO	EVOO	OO
PUFA/SFA		0.56±0.08^{b}	0.43±0.02^{a}	0.54±0.08^{b,A}	0.43±0.02^{a,A}	0.66±0.08^{b,B}	0.54±0.03^{a,B}	0.65±0.05^{b,B}	0.53±0.02^{a,B}
TFA	g/100g	0.03±0.00^{a}	0.09±0.01^{b}	0.08±0.01^{a,B}	0.12±0.01^{b,B}	0.05±0.01^{a,A}	0.11±0.01^{b,A}	0.06±0.02^{a,A}	0.10±0.01^{b,A}
K_{232}		2.0±0.2	2.0±0.1	4.6±0.3^{b,B}	4.0±0.5^{a,B}	2.2±0.2^{a,A}	2.4±0.3^{b,A}	2.0±0.1^{a,A}	2.2±0.1^{b,A}
K_{270}		0.2±0.0^{a}	0.3±0.0^{b}	1.5±0.1^{b,B}	1.1±0.1^{a,B}	0.4±0.1^{a,A}	0.5±0.1^{b,A}	0.4±0.0^{a,A}	0.5±0.0^{b,A}
PAV*		9±1^{b}	3±0^{a}	23±4^{b,B}	18±2^{a,B}	5±1^{A}	5±1^{A}	6±1^{A}	6±1^{A}
TPC	g/100g	3.1±0.3^{a}	3.8±0.4^{b}	6.8±1.3^{b,B}	5.4±0.4^{a,B}	2.8±0.2^{a,A}	3.4±0.3^{b,A}	2.8±0.1^{a,A}	3.3±0.5^{b,A}
DPTG	g/100g	n.d.	n.d.	0.71±0.03	0.78±0.24	n.d.	n.d.	n.d.	n.d.
OTG	g/100g	0.7±0.1^{a}	1.0±0.3^{b}	2.9±1.0^{b,B}	1.8±0.3^{a,B}	0.6±0.1^{a,A}	0.8±0.2^{b,A}	0.6±0.1^{A}	0.8±0.3^{A}
DG	g/100g	1.4±0.4	1.5±0.1	1.6±0.2^{B}	1.7±0.1^{C}	1.1±0.2^{a,A}	1.5±0.1^{b,B}	1.2±0.0^{a,A}	1.3±0.1^{b,A}
FFA	g/100g	0.44±0.04^{a}	0.54±0.09^{b}	0.56±0.01^{b,B}	0.46±0.03^{a}	0.47±0.05^{A}	0.47±0.05	0.47±0.06^{A}	0.47±0.03

$^{a-b}$ statistically significant differences between olive oil categories ($p < 0.05$); $^{A-C}$ statistically significant differences between cooking processing ($p < 0.05$);
* expressed as 100 times the optical density measured at 350 nm in a 1 cm cuvette of a solution containing 1.00 g of the oil in 100 mL, according to method;
DG - diglycerides; DPTG - dimeric and polymeric triglycerides; EVOO - extra virgin olive oil; FFA - free fatty acids; OO - olive oil; OTG - oxidized triglycerides; PAV - *p*-anisidine value; PUFA/SFA - polyunsaturated fatty acids/saturated fatty acids ratio; TFA - trans fatty acids; TPC - total polar compounds.

For the low-fat systems, the differences between categories were less perceived, but still statistically different in some parameters, including the TFA content, K_{232}, K_{270}, TPC and OTG. It is worth mentioning that, independently of the oil category, DPTG were not detected in the low-fat systems, only in DF. In the particular case of MWG, the presence of food had a positive influence on fat oxidation, as was also observed with fish (Nieva-Echevarría *et al*., 2016), with lower oxidation than the one expected from studies without food (Cerretani *et al.*, 2009, Malheiro *et al*., 2009). Therefore, and independently of the category, DF was the most aggressive processing method, despite being the shorter one in terms of duration, with significantly higher oxidation in comparison with the other techniques (p<0.05). Indeed, the most determinant factor seems to be the temperature and its effective conduction to the food, in agreement with other studies simulating heating without food with diverse olive oil categories (Roodaki *et al*., 2016; Li *et al*., 2016). As previously mentioned, Table 11.2 results are expressed on a lipid basis, for an easier comparison of oils and methods, but the impact on processed potatoes will be proportional to the amount of incorporated lipids, significantly higher in DF, further increasing the impact of the oxidation differences observed with the diverse processing methods.

11.3.3 Effect of using different commercial olive oil categories on potatoes sensorial quality

A total of 32 (8 x 4) samples were evaluated for sensorial quality, aiming to perceive if the panellists were able to distinguish the olive oil categories and the most important attributes associated with their choices. Figure 11.2 resumes the average scores for each attribute, grouped by processing method, were a high consistency is observed for all categories and processing methods.

Although the comparison of thermal processes was not the focus of this work, it is clearly perceived that DF potatoes had the highest average scores on most attributes, particularly colour intensity and homogeneity, odour intensity and crispiness, important characteristics in high-quality fried products. Global acceptability was also higher in DF, close to AF, but superior to MWG. It can be perceived that adhesiveness is higher in AF and MWG, while crispness is reduced, both in comparison with DF. In addition, colour intensity is clearly lower in MWG, due to the inherent characteristics of this cooking method. Still, in DF (Fig. 11.2A) and AF potatoes (Fig. 11.2B) the panel was able to distinguish some attributes between categories (p<0.05), namely odour intensity (DF: EVOO > OO) and both colour attributes (AF: intensity: OO > EVOO and homogeneity: EVOO > OO), but for MWG potatoes (Fig. 11.2C) the panel was unable to make distinctions (p>0.05). Moreover, the panel was unable to distinguish the global acceptability between categories on all processing methods (p>0.05).

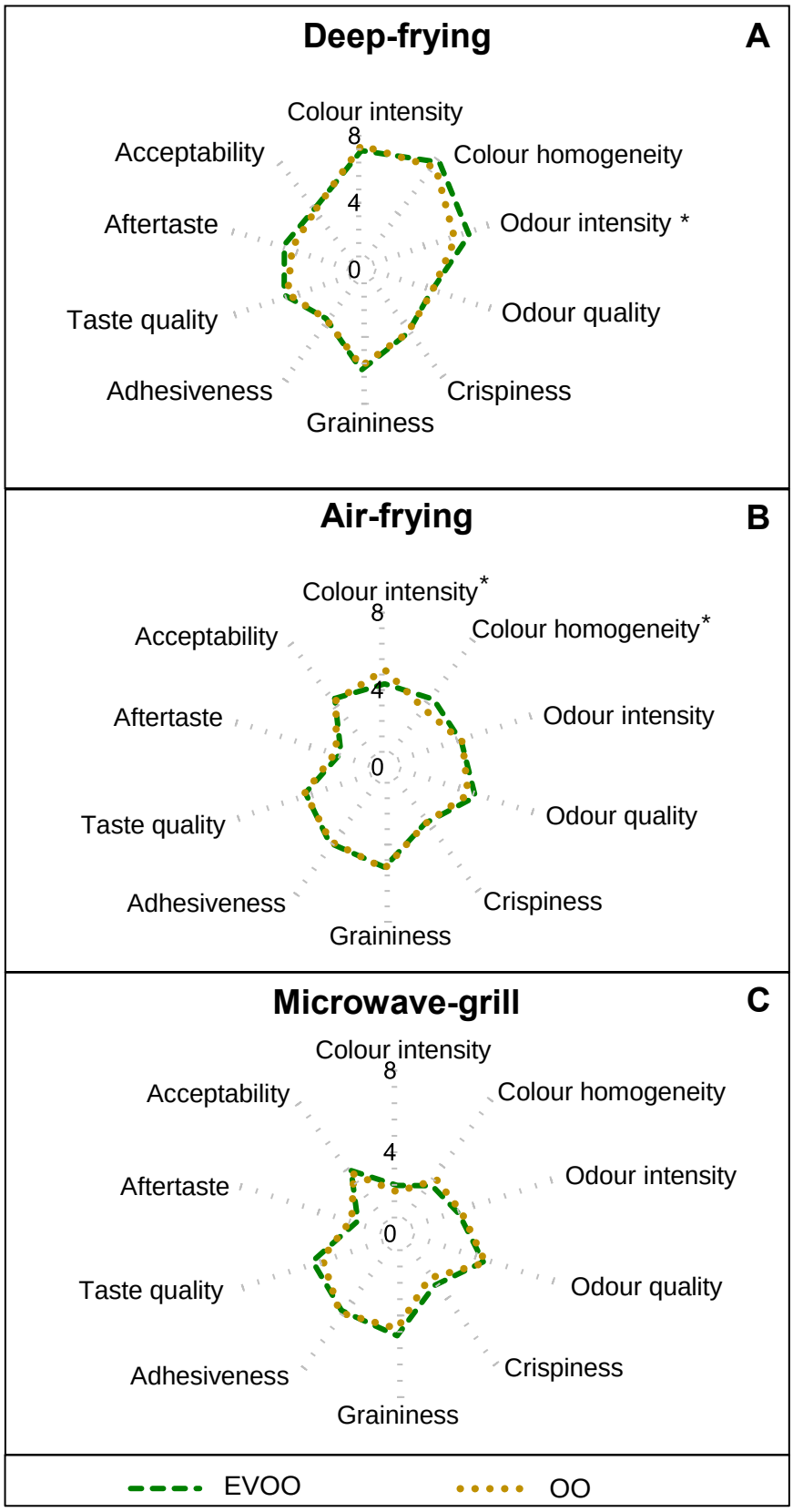


Figure 11.2 Potatoes sensorial profiles: A) deep-frying; B) air-frying; C) and microwave-grill using different commercial olive oil categories (*for significant differences (p<0.05) between olive oil categories).

11.4 Concluding remarks

The use of different olive oil categories influences the final composition of the processed food. Therefore, the choice between EVOO and OO should not be based solely on price as it impacts on important nutrients, including vitamins and antioxidants. EVOO, independently of being used in high or low-fat processes, protects more efficiently potatoes ascorbic acid than OO, while enriching potatoes also with phenolic compounds, despite the recognized losses imposed by the cooking process itself. When the methods are compared, MWG was the most protective one, followed by the air-frying devices, while deep-frying is more aggressive. In terms of olive oil degradation, the differences between EVOO and OO were only slightly perceptible, particularly in the low-fat devices, proportional to those presented by the fresh oils, but in deep-frying OO presented lower oxidation than EVOO. Aware that the panellists were unable to distinguish the two oil categories after all the processing methods, despite the clear preference for DF, the use of EVOO for low-fat frying is highly recommended, while for deep-frying, particularly if intended to be used on a prolonged basis, the choices could take also into account the economic impact.

PART IV

DISCUSSION AND CONCLUDING REMARKS

CHAPTER 12. General Discussion and Future Prospects

Both olive oil and potatoes are recognized sources of bioactive compounds in our diet. Their careful symbiosis can preserve the best of both, providing promising improvements in the nutritional quality and safety of fried potatoes, so appreciated worldwide.

In the Mediterranean countries there is a secular tradition of using olive oil as the main source of external fat, both for dressing and cooking purposes. However, there is a global consensus that, to preserve the most of its sensorial and nutritional properties, it should not be heated, questioning its adequacy for frying purposes, where high temperatures are used, sometimes for prolonged periods. Additionally, when using virgin olive oils, not subjected to refining, olive oil health effects are potentiated, but the same compounds that provide this bioactivity might be responsible for the formation of off-flavours under thermal stress. Furthermore, and for olive oil consumers, there is a generalized consensus that virgin olive oil has to be used at lower temperatures than other refined vegetable oils, due to its lower smoke point, but for non-conventional olive oil consumers, these issues raise a lot of discomfort and mistrust and, globally, they have been the driven force against the use of olive oil for frying purposes, demanding for clarification. Therefore, the main question developed through this thesis was:

Is olive oil adequate for frying?

Our assays focused on a direct comparison of extra-virgin olive oil with other vegetable oils and olive oil categories under real potato frying, converging mostly on the final potato composition, the food to be consumed. Additionally, in order to study the impact of cumulative heating time, as usual in restaurant frying, the assays were designed to simulate prolonged and intermittent potato frying. In order to avoid external interferences from other fat sources, only fresh potatoes were used. Indeed, and despite the generalized use by consumers and restaurants of frozen pre-fried potatoes, with a more convenient use and granted sensorial results, these potatoes contain fat that was added in a pre-fried step by the industries. Their release into the frying media during frying would interfere with the reactions taking place, and therefore add confounding factors to our assays.

Soybean and sunflower are amongst the most common oils worldwide, chosen as a comparison in some of the assays. However, for a wider and clarifying comparison, we have also taken real frying assays against its most direct competitors from the chemical point of view: monounsaturated oils. Based on availability to consumers, we have chosen peanut and canola (rapeseed) oils for the purpose. Additionally, and refocusing on olive oil, we have also to address the different commercial olive oils categories, as they are not equivalent from the chemical point of view. Indeed, while both extra-virgin and virgin olive oils are commercialized without refining, there are other categories were refined olive oil is

included, as the category “olive oil”, or even the “refined olive oil” one, not commercialized in Portugal but regulated within the EU (Commission Regulation (EEC) N.° 2568, 1991 and amendments). As these products terminology can be misleading to the consumer, with price being usually the main determinant for acquisition, their different adequacies for frying purposes needs also to be clarified.

Based on all these premises, we are going to make an integrated discussion of the results achieved, attempting to support the following questions:

- *What nutritional gains and losses occur on both oils and potatoes with frying?*
- *How cumulative oil degradation with prolonged frying does affects the consumer?*

It should be emphasized that all the comparisons under deep-frying conditions were made using equivalent processing conditions, in order to grant that the differences observed were due to the oils alone and their interaction with potatoes. Heating and cooling cycles were included, as in restaurant frying, due to the recognized effect that intermittent cycles have on oxygen solubility and hence oil degradation (Choe and Min, 2007). Additionally, the oil:potato ratio, known to influence frying performance, was kept similar on the three oils. We have adopted a high oil:food ratio (30:1) (Kalogianni *et al*., 2010), to grant an elevated number of frying hours. Under these conditions, we were able to use the oils for up to 28 h without a high reduction in this oil:potato ratio due to lipid incorporation in the fried potatoes (24:1 in the end). Regarding oil replenishment, a controversial issue, some author’s state that it refreshes the oil in antioxidants while diluting degradation compounds (Romero *et al*., 1999), being therefore advisable, while others are more concerned with the disguised cumulative degradation with time under this process.

When considering the monounsaturated oils assayed, a closer look into their fatty acid compositions shows differences, particularly regarding the polyunsaturated amounts (PUFA), known to be more prone to oxidation: linoleic acid (PO>CO>EVOO) and linolenic acid (CO>EVOO>PO). Also, the amounts of minor compounds with potential activity under thermal stress were also different, namely vitamin E (CO>PO>EVOO), phenolic compounds (EVOO>PO, CO) and carotenoids (EVOO>PO, CO). Both PUFA and minor compounds are known to influence the oil’s chemical stability under frying (Aladedunye, 2015), being this particularly visible when olive oil is compared with soybean (Naz *et al*., 2005) or sunflower oil (Casal *et al*., 2010). Here, we have also demonstrated that the comparatively small differences between monounsaturated-rich oils are still sufficient to induce differences on the final products.

Several macromolecular differences are typically perceived under prolonged frying, resulting mostly from triglyceride degradation, with accumulation of the so-called “polar compounds” (TPC), a combination of hydrolysis, oxidation and polymerization compounds

(Warner, 2002). Accumulated TPC are presently the only restriction for used oil, with most countries limiting them to 25-27%. Regarding the monounsaturated oils studied, a similar degradation pattern was observed with PO and CO (Subchapter 9.1), allowing 18 h (PO) to 20 (CO) hours of frying under the frying conditions used in this experiment before reaching 25% of TPC. Under these same processing conditions, EVOO did not achieve this rejection point even at 28 h. With this first global approach we confirmed that EVOO can be used for more prolonged time when compared with PO or CO, based directly on the TPC. Aware that we have only used one brand of each oil in this assay, these numbers we should interpret on a relative basis because oils batches and brands can have very different compositional differences, both from the oil origin, together with alterations during refining and additivation. In the particular case of EVOO, despite the absence of refining and additivation the compositional differences between brands and batches are probably higher, with a strong influence from the olive varieties used, their maturation stages and growing conditions, together with extraction and preservations conditions.

In order to understand how these differences observed in the oils are transposed to the fried potatoes, we have implemented a deeper discussion of the fried product composition.

Figure 12.1 combines, as an example, two components that are recognized as indicatives of oil degradation, namely trans fatty acids and *E,E*-2,4-decadienal, on both oil and potatoes.

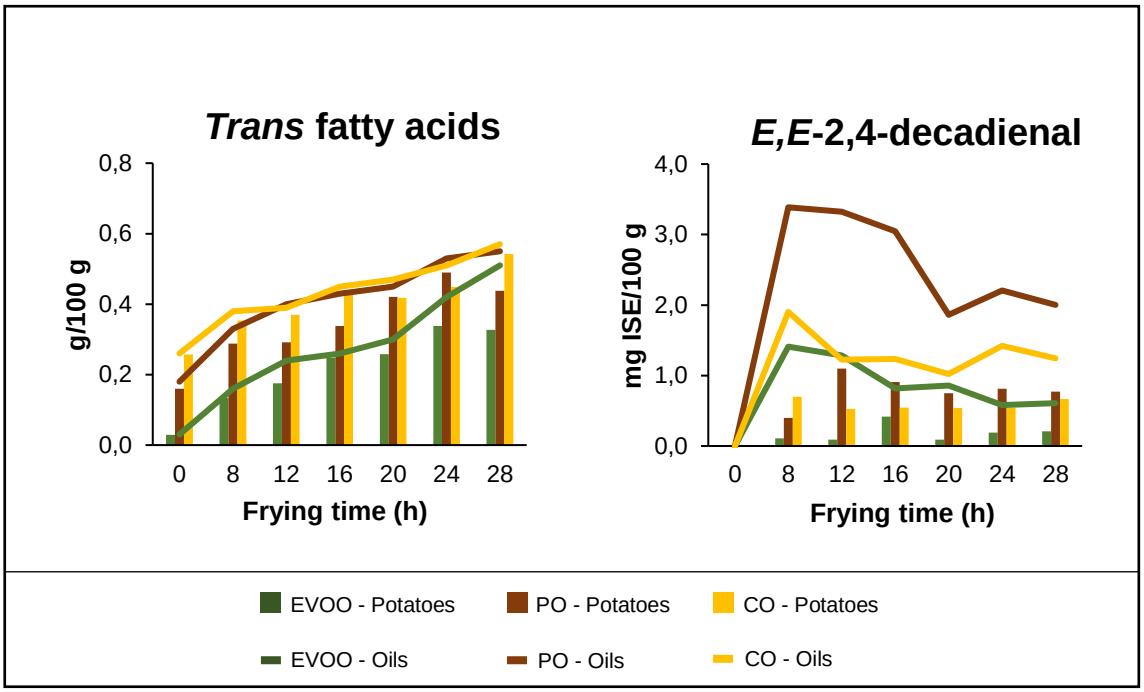


Figure 12.1 Results of *trans* fatty acids and *E,E*-2,4-decadienal on both vegetable oils and potatoes.

These compounds represent a measure of fatty acid degradation, due to isomerization and peroxidation, respectively. In the oils, *trans* fatty acids increased almost linearly with time ($p<0.05$), preserving the initial differences verified in the fresh oils (Table 7.2), where EVOO, due to absence of refining, has lower amounts. From the 20 hours forward, however, EVOO rate increased slightly, getting closer to the other oils. As to the potatoes, *trans* fatty acid amounts followed a similar and proportional trend to the oils. Therefore, and taken that the incorporated oils amounts was similar (CO>PO>EVOO) (Subchapter 9.2), even in the last sampling the amounts on the three oils are below 0.6% (on the extracted lipids), equivalent to less than 0.04% on a potato basis, therefore of no health concern (de Souza *et al*., 2015). As to the *E,E*-2,4-decadienal, derived from linoleic acid degradation, its amounts were significantly higher in PO than CO or EVOO, with a huge increased in the first 8 hours of frying, followed by a slight decrease with time, more noticeable in PO. Due to its volatile nature, we expect to have release of this compound during frying, and therefore the amounts can only be compared for a specific time and not on a cumulative basis. However, the differences between oils correlated well with the linoleic acid proportion in the oils, higher in PO (25.5%) against 19.0% and 8.6% in CO and EVOO, respectively. This increased amounts in PO are also reflected in the potato (PO>CO>EVOO), in a direct proportion to incorporated lipids, also peaking at 8-12 hours and then stabilizing up to the end. Despite being a typical frying aroma, it should be evaluated with caution due to its potential toxicity (Boskou *et al.*, 2006). However, and taken that there are no limitations or recommendations, and the generalized low amounts (<1.1mg/100g) in the fried potatoes, this compound represents only a health indicator as it is associated with LDL oxidation induction (Boskou *et al*., 2006).

Acrylamide is also a recognized toxic compound in foods, with fried potatoes representing one of the main sources in the diet (Vinci *et al*., 2012). Therefore, several studies dedicated to understand the mechanisms behind its formation have been published, supporting diverse mitigation strategies (Subchapter 10.1). Within these studies, the oil type issue, as well as the effect of accumulated frying time, have been addressed (Mestdagh *et al*., 2007; Zhang *et al*., 2015). However, no consistent results are found in the literature. Some apparent tendencies are probably masked by the high variability of results, even within replicates, with a generalized consensus that none of these parameters has a strong effect, particularly in comparison with free amino acids and reduced sugars on the potato side, or processing time and temperature of the oil.

Our studies (Subchapter 9.2) showed similar acrylamide amounts on the potatoes fried on the three oils at 8 h ($p>0.05$), corresponding also to the lowest amounts in this study. Acrylamide amounts increased with accumulated frying time (Fig. 12.2), without clear differences between oils. Therefore, the individual thermal characteristics of each oil, as

heat transfers characteristics or viscosity, do not seem to influence acrylamide formation is a significant extension. Regarding the increased with frying time, it should be discussed bearing in mind that frying time was kept constant during the whole assay (6 min), and that the oils colour exhibit progressively higher intensity (Subchapter 9.1). On the oil side, this is a typical degradation phenomenon, with oils microcomponents and leaching compounds from potatoes having a determinant effect in this browning effect, derived mainly from *Maillard* reactions (Aladedunye and Przybylski 2009). Additionally, potatoes colour also increased with time (Subchapter 9.2). Therefore, it seems feasible to conclude that frying efficiencies increased with time on the three oils, with 6 min in the final frying's corresponding to a slightly higher degree of doneness than in the first ones. This higher degree of doneness is corroborated by higher redness intensity and lower moisture content. It can be a consequence of both alterations in the oils with processing time, together with the alteration of the oil:potato ratios, as mentioned. Therefore, the higher acrylamide content with time is probably also associated with this doneness effect, rather than with accumulated processing time alone. If the potatoes were fried for less time, just to achieve a similar appearance, as usually performed in restaurants, the amounts could probably be more similar, corroborating the results of other authors with real restaurant frying (Zhang *et al*., 2015).

The formation of pyrazines, non-volatile aldehydes, is also described as being implicated in acrylamide formation, as its precursors, which could further contribute for the increased amount of acrylamide and its variability. We have observed an increase on the three oils with frying time, supporting the strong correlations presented in Table 9.8, but without a constant behaviour between oils. Additionally, a strong correlation between pyrazines e acrylamide was found in the potatoes fried in EVOO, further supporting a possible association between the maximum emission of this volatile compound and acrylamide content at 24 h of frying.

Together with free amino acids and reducing sugars, phenolic compounds are also known to interfere with acrylamide formation (Napolitano *et al*., 2008, Zhu *et al*., 2010; Vinci *et al*., 2012; Bethke, 2013). Therefore, together with potatoes phenolics, the additions of external phenolic compounds, particularly from EVOO, was expected to reduce acrylamide formation (Napolitano *et al*., 2008). However, our results (Fig. 12.2) showed no differences between the three oils. By having analysed only from the 8th hour forward, it is not to exclude the possibility of some differences in the initial times. Indeed, EVOO phenolics reduced very fast in these frying assays, with almost residual amounts at 8-12 h. Therefore, if EVOO phenolics are effective in the prevention of acrylamide formation as stated by several authors (Napolitano *et al*., 2008; Arribas-Lorenzo *et al*., 2009; Zhu *et al*., 2010), this will probably occur only with fresher oils. The only phenolics that remain in the potatoes

seems to be mainly derived from fresh potatoes, taking into account the 2.5 mass increase on a 100g basis derived from moisture loss (Subchapter 9.2). Indeed, in a latter assay attempting to compare low-fat frying systems, to be discussed below, we have implemented deep-frying assays for a reduced frying time (less than half an hour). Both CO and olive oil (OO) were used in this study, together with sunflower (SFO) and soybean oil (CO), corroboration this generalized preservation of oil phenolic compounds when used for a small period of time.

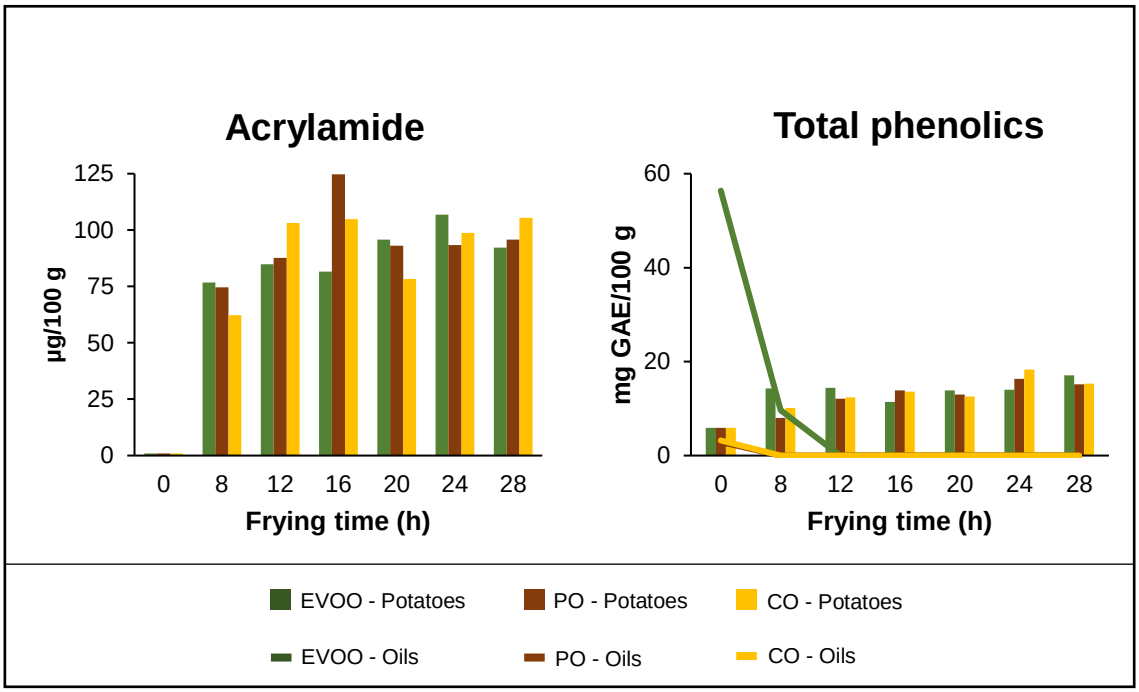


Figure 12.2 Results of acrylamide on potatoes, and total phenolics on both vegetable oils and potatoes.

Potatoes richness in ascorbic acid, not always recognized by consumers, was partially lost with frying, enhanced by the accumulated frying time. Again, no differences were observed between oils, except in the first frying hours, higher in EVOO. This is in accordance with the higher amounts of phenolic compounds in EVOO, probably with a protective effect regarding ascorbic acid. However, as mentioned, these EVOO phenolics are loss from the 12 h forward, without differences between oils perceived from this point up to the end of the assay, at 28 h. Vitamin E, an important lipid antioxidant, does not seem to have a protective effect regarding ascorbic acid, with the significantly higher vitamin E amounts in CO not supporting higher ascorbic acid protection.

Another interesting observation concerns antioxidant activity assays in the fried potatoes. Aware that these assays are just an *in vitro* estimation, only advised for comparative purposes (Prior *et al*., 2005), and without a straight association to what occurs

in our body, it is interesting to denote the absence of antioxidant capacity in the potatoes from the 12 h forward. Indeed, despite the quantified amounts of antioxidants in the potatoes, namely phenolics and ascorbic acid, when the global pool of extracted compounds was tested for antioxidant activity, no activity was observed. These results show that these antioxidant compounds, despite present, are not free to react under the tested conditions. It could be a consequence of the simultaneous presence of pro-oxidants in the same reaction media, namely radicals derived from lipid degradation. Therefore, and transposing this observation to what might happen after ingestion, instead of being free to act as antioxidants in our body, these compounds are probably lost in the protection of the food itself, reducing the expected bioactivity.

We have also addressed the sensory issue, as it influenced consumer's choices. Clearly, choices were highly influenced by cultural habits, but the panel was able to distinguish the progressive degradation with time on both EVOO and CO, but not on PO. When looking into potatoes volatiles, EVOO had lower total amounts, but a higher proportion of volatiles recognized as "off-flavours" (36-56%) which can support the distaste showed by some consumers. Considering the off-flavours highlighted in subchapter 9.2, the average nonanal content was superior in the potatoes fried in EVOO (398µg/100g), than PO (223µg/100g) or CO (251µg/100g), all superior to the retronasal odour threshold in oil (26 µg/100g) (Belitz *et al*., 2009). Similarly, the average contents of 2-undecenal were superior in the potatoes fried in PO (343µg/100g) and EVOO (337µg/100g) vs CO (268µg/100g), again superior to its retronasal odour threshold in oil (15 µg/100g) (Belitz *et al*., 2009). In this regard, each volatile threshold is also important, because even low amounts of specific volatiles can induce higher sensory perceptions. On the other hand, PO richness in the typical "frying-like" aromas (32-63% of total volatile fraction) could mask other degradation compounds. Again, these off-flavour and degradation compounds point into the advantages of being less permissive with abused oils.

Globally, and tentatively answering the questions raised, despite similar, the three monounsaturated rich oils tested do not have equivalent performances: EVOO is more resistant to frying cycles under potatoes frying, at 175ºC, while a distinction between CO and PO is more difficult to perceive. However, for consumer's protection, all oils should be used for shorter times than those recommended on the basis of the TPC. Indeed, up to the 8 h of frying potatoes were still being enriched in interesting compounds, as tocopherols and more polar phenolics, with better preservation of ascorbic acid. Acrylamide, also, was present in lower amounts in the first frying's. When the frying times increased, despite not being perceptible acceleration in the formation rate of typical oxidation markers, as TPC or *p*-anisidine values, when these "protective" agents are loss, the fact is that these minor bioactive compounds seem to be lost in interaction with other potato compounds, probably

lipid oxidation products, reducing their availability. These observations deserve further exploration, with a deeper study of antioxidants interactions taking place in the fried potato matrix, as well as a closer simulation to what happens in the body after ingestion.

These frying issues also raised a concurrent one, which is the health concern associated with the lipid amounts in fried potatoes (Gadiraju *et al*., 2015; Falade *et al*., 2017). We have been seeing that consumers are increasingly concerned regarding this issue, seeking for alternative frying methods: less fat with equivalent sensory attributes. In this field, the industry is developing equipment's for "low-fat frying" while consumers also attempt to use baking and microwave heating of pre-fried potatoes as cheaper and available alternatives. Being relevant issues these days, the previous question on olive oil adequacy for frying should not be restricted to classical deep-frying, but also to these modern alternatives.

Therefore, a comparison between alternative processed was implemented, including commercial equipment's for domestic use (Actifry e Airfryer), and more common ones, as microwave-grill and domestic convection oven. The doubts that influenced our study design were:

- *Are all alternative processing healthy and true low-fat?*
- *Which is the most adequate one?*
- *Do the compositional differences between oils influence the final low-fat fried potatoes composition and their sensory attributes?*

For this purpose, we have opened our field of comparisons to include sunflower (SFO) and soybean (SO), again with canola (CO) and olive oil (OO). We have adopted a constant oil:potato ratio, here of 1.2 g of fat *per* 100 g of potatoes, adapted to the size of the equipment and manufacturer recommendations. For deep-frying, an 8:1 ratio was used. Also, we have used an olive oil of a lower grade category in terms of bioactivity, the olive oil (OO), constituted mainly by refined olive, with small amounts of virgin olive oil. For microwave-grill and oven "fryings" we have previously optimized processing condition that could results in acceptable potatoes from a sensory point of view, and with similar degree of processing in comparison with the commercial equipment's. Again, despite being common to use on all these equipment's pre-fried frozen potatoes, not only their use would disable comparison between oils, while the final content in lipids, despite lower than in deep-frying, would be still relatively high - 7.4-11.1g/100g (Giovanelli *et al*., 2017).

Figure 12.3 compiles the results for total lipids, together with degradation indicators (trans fatty acids, *p*-anisidine, total polar compounds and acrylamide) and important bioactive components (ascorbic acid and phenolic compounds), all on a potato basis, together with sensory acceptability.

All ”frying” approaches were effective in the reduction of the fat content to true low-fat potatoes, with less than 2% of fat on a fresh basis, slightly lower in the oven option. This is significantly different from the deep-frying figures, with 15 to 45% of fat incorporation described (Kita, 2014), and even with the deep-fried used as control in our study, using the same oils and potatoes, with 7% incorporated fat. In contract to what was previously observed in the deep-frying assays, although aware that all lipid degradation compounds should be present in a direct proportion to the amount of incorporated lipids and their degradation extension, the differences were not so clear in the low-fat approaches as in deep-frying, despite being clearly lower. This is truly a consequence of the low amount of fat incorporated, where fresh potatoes lipids, despite reduced (0.1%) can also influence the final composition. However, and based on the discussion above, these degradation compounds are also directly associated with the oils chemical composition (Table 7.2).

Potatoes were enriched in tocopherols, carotenoids and phenolic compounds on all processing (deep-frying included), once again in a direct proportion to the fresh oils composition: higher phenolic amounts when using OO, tocopherols and carotenoids with SO (Table 7.2), and higher antioxidant activity with CO. Being observed also in deep-frying, it confirms that if used for short periods, deep-frying can still enrich potatoes in bioactive compounds. Regarding ascorbic acids, totally dependent on the potatoes initial composition, comparisons are made difficult to perceive. The Actifry system gave equivalent amounts to deep-frying, very close to Airfryer, followed by microwave and oven. Differences between oils were only perceived in oven “frying”, with higher preservation of ascorbic acid with SO and OO. Additionally, it should be explained that the variety of potatoes used in this assay was not the same as in the previous prolonged deep-frying ones, and the amounts of ascorbic acid were clearly different (33 *vs* 7 mg/100g, on a fresh basis). Therefore, no direct comparisons can be made, only relative ones. And these show that microwave-grill and oven preserved ascorbic acids better than the other methods, probably derived from the lower oxidation induced in the lipids with these processes, noticed from the lower TPC amounts or *p*-anisidine values. With similar processing times on all methods, ranging from 15-25 min in air-frying sessions and microwave-grill to 30 min in oven (except for the 6 min deep-frying) these differences indicate that the thermal and oxidative stress imposed by the different methods is not similar.

The external colour of the fried potatoes is associated with the characteristics of each thermal processing. In this way, traditional frying developed a more homogeneous colour, while the alternative methods developed more heterogeneous surfaces, probably due to conduction effect from the direct contact with hot surfaces. One could expect this minor colour homogeneity to be associated with acrylamide formation but there were significantly lower amounts in MWG (-57%) and OV (-87%) compared to deep-frying. Considering the

complexity of the acrylamide formation mechanism (Subchapter 10.1), these reductions are associated with the higher amounts of vitamins and antioxidants, which may hinder their formation.

However, one of the major concerns when developing an equipment or technique for low-fat purposes is the sensory aspect. In fact, oil absorption is determinant for the formation of crust and colour, as well as for improving palatability and flavour (Heredia *et al*., 2014). Inevitably, acceptability was higher in deep-frying, directly proportional to the lipid content. In the commercial systems and microwave-grill all potatoes showed equivalent or slightly lower acceptability to deep-fried ones, being therefore regarded as feasible alternatives, while the ones prepared in the oven received smaller scores. Interestingly, the vegetable oil effect was more visible in the deep-fried potatoes, ranging from 6.1/10 for SFO to 3.2/10 for CO, again motivated by tradition, but this effected was not perceived in the low-fat potatoes, possibly due to the low amounts of incorporated fat.

Giving sequence to an important question raised in the initial introduction of this thesis regarding microwave oil heating, where it was mentioned to be regarded as a highly aggressive technique for lipids in general (Caponio *et al*., 2003; Cerretani *et al*., 2009; Malheiro *et al*., 2009). However, we have also raised the issue that in the presence of food the effects might be totally different when using real food processing. In the present conditions were have not simulated deep-frying, only low-fat frying, with small amounts of fat. Still, its characteristics were preserved and lipids oxidation was even smaller than the one observed in the commercial devices. Therefore, this study should motivate further ones on microwave real cooking.

Tentatively answering to the questions raised regarding the low-fat devices, all tested devices proved to be healthy alternatives to traditional deep-frying in terms of incorporating fat. Regarding bioactivity, all devices were also able to enrich the potatoes in the oil components, while also preserving the potatoes, bearing in mind that his was also observe for deep-frying when using fresh oils. Aware that using deep-frying for a reduced pair of hours is not economically feasible on most restaurants, despite being at home, moderate use could be a better option. From the sensory point of view, the most adequate options were the commercial equipment's, being also easier to use, closely followed by microwave-grill. From a health point of view, the oven process was the most adequate one but it received less acceptability. As to the oil choice, OO was the most appropriate from both nutritional and safety points of view. Here, both oil and potatoes are preserved and therefore it is worth to use good quality products, as these will enrich the final product. When choosing the most adequate oil he consumer should focus on its main interests: bioactivity or frying resistance?

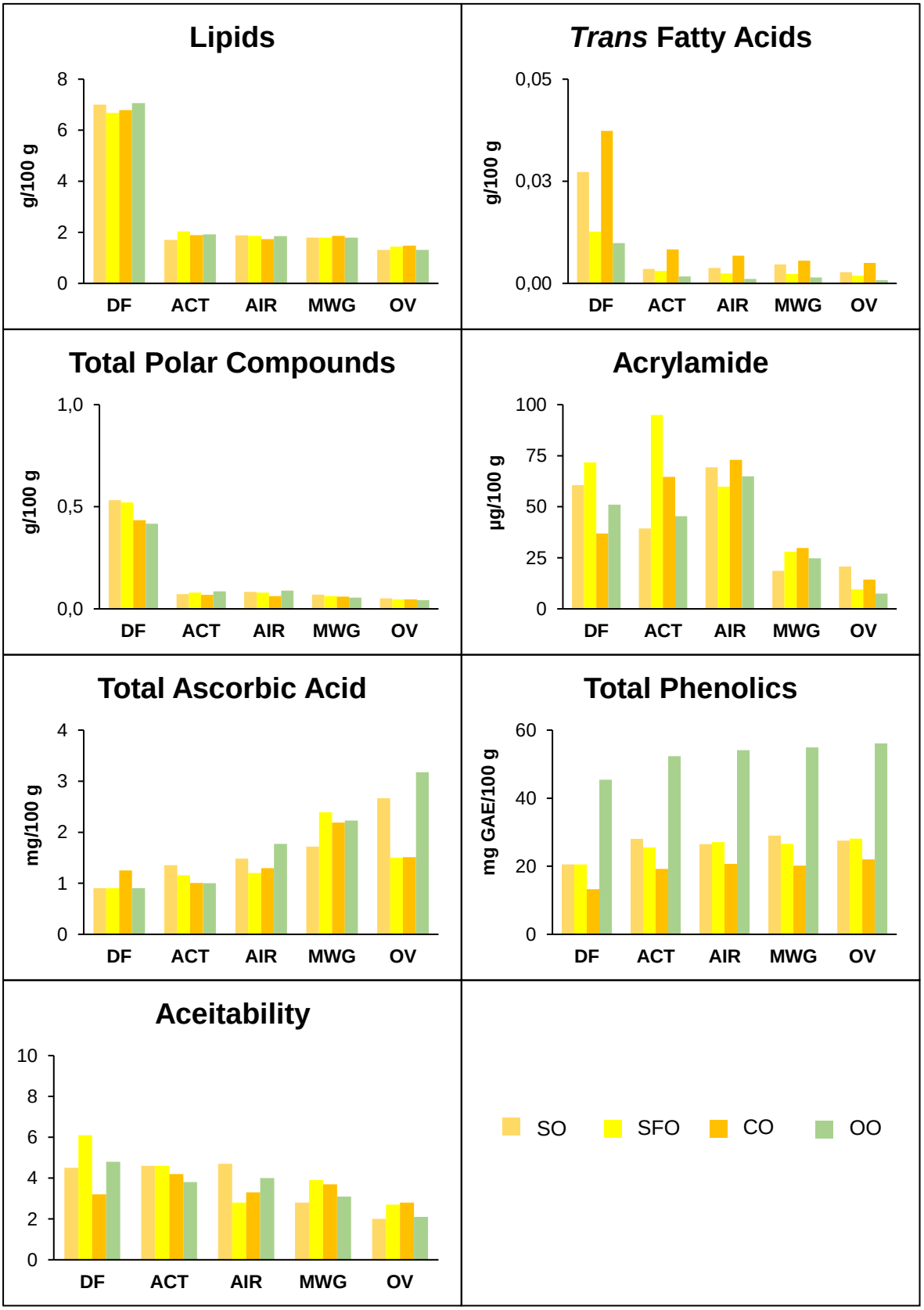


Figure 12.3 Results of lipids, *trans* fatty acids, *p*-anisidine, total polar compounds, total ascorbic acid, total phenolics, acrylamide, and acceptability on deep-frying and low-fat frying of potatoes.

Considering the previous discussions, and in the culmination of this thesis, to understand the relevance of using different olive oil categories for potato processing, a comparative study was implemented between the two more representative categories in the market: EVOO and OO. In particular, we were aiming to understand if one category is more adequate for deep-frying purposes, and how different choices would affect the consumer. Besides deep-frying, and based on the acceptability of the low-fat processes, we have also implemented a comparison using the commercial devices and microwave-grill. Oven was not chosen due to the reduced acceptability.

As previously mentioned, olive oil can present huge compositional differences, both on the fatty acid profile and minor compounds. Therefore, two commercial brands of each were tested, to support this natural variability. The compositional differences of the oils (Table 7.2) are expected to interfere with the alterations occurring with thermal processing, as will be discussed.

On one side, EVOO, with higher amounts of phenolic compounds and tocopherols, enriched the potatoes in these compounds, while also preserving potatoes bioactive compounds, namely ascorbic acid. This fact was observed on all processing techniques. However, we should have in consideration that the phenolic compounds evaluated in this thesis were only the results of an estimation based on the Folin-Ciocalteau method. Scientific evidences have demonstrated that olive oil phenolics, as hydroxitirosol, oleuropein and oleocantal, have positive effects on certain physiological parameters (Cicerale *et al*., 2010; Rodríguez-Morató *et al*., 2015; Parkinson and Cicerale, 2016). Therefore, it is on extreme importance to perform individual phenolic characterization by HPLC. Additionally, the ingestion of phenolic compounds from olive oil occurs as an integrated part of the diet, being in contact with other food matrix components, as potato in this case, where physical and chemical interaction can influence bioavailability. Therefore, it is important to develop further studies to fully understand the beneficial effects on human health on a more global biological approach, including in vivo antioxidant activity and cardiovascular risk factors.

We have observed lower lipid oxidation in OO than EVOO under deep-frying. This could be a direct consequence of their different fatty acids profiles, by chance with higher PUFA in the two EVOO chosen for the assays. For the other processing methods no differences were perceived. Therefore, these studied should be implemented on a wider range of brands with different compositions, particularly regarding PUFA and phenolic amounts and phenolics to fully understand the significance of the results achieved in this thesis. Globally, acrylamide was not associated with the olive oil category, but was influenced by the processing method, as expected from the previous studies (DF>AF>MWG). The sensory panel was not able to distinguish the categories after processing (p>0.05). However, a clear

preference for deep-fried potatoes was again observed, followed by the commercial devices and microwave-grill.

Only two potato varieties were tested in this thesis. Conscious that potatoes chemical composition induces different fat absorption, formation of acrylamide, and has different amounts of bioactive compounds, such as vitamin C and phenolics, it will be interesting to extend these studies to other varieties. In addition, potatoes are often associated with a high glycemic index, so studies on carbohydrate metabolism in different culinary applications should also be conducted.

CHAPTER 13. Conclusions

This doctoral thesis allowed to develop the current state of art on the chemistry and nutrition of potatoes processed in olive oil, focusing on the gains and losses associated to prolonged frying, to low-fat frying alternatives, and to the suitability of using different categories of olive oil in various culinary applications.

In this sense, it was possible to conclude that:

- Under deep-frying conditions, extra virgin olive oil is more resistant than other common oils, such as sunflower or soybean, and also in comparison to oils naturally rich in monounsaturated fatty acids, such as peanut or canola oil. This resistance was perceived by the increased preservation of both olive oil and potatoes quality, while giving rise to lower formation of toxic products such as oxidized lipids and acrylamide. However, prolonged frying can be regarded as a highly aggressive process, with deleterious effects on the processed potatoes long before reaching the legal limit of rejection: degraded oils are absorbed by the potatoes, while its degraded compounds reduce the availability of potato bioactive components, reducing their nutritional value and safety.

- All low-fat frying alternatives tested prove to be healthier than traditional frying, with effective production of low-fat potatoes (<3g/100g) and greater preservation of both potatoes and olive oil bioactive components. From the sensory point of view, the most accepted processes were the commercial equipment's, followed by microwave-grill, while "oven-frying" received less acceptability, all with a large cultural influence in the frying oil preference. Conversely, the greatest bioactive potential was found in potatoes processed in the oven, being lower in the commercial equipment's, but all clearly superior to classic deep-frying. In what concerns to the oils, olive oil was always the best choice, both from nutritional and food safety points of view.

- As regards to olive oil categories, the low amounts of fat spent in low-fat systems justifies the choice of higher olive oil categories, as their characteristics are preserved and protect potato bioactive compounds. In classical frying, oils with lower content of polyunsaturated fatty acids will be the most adequate choice, regardless of the category, favouring a higher resistance to oxidation. The use of mixtures with refined olive oil, under the commercial category of olive oil, may represent an economic advantage for prolonged frying, since in this option it is assumed that the focus is resistance to frying rather than bioactivity, clearly lost in the first few hours of heating.

With regard to the main objective proposed, it has allowed us to conclude that:

- *By careful selection and optimization of thermal processing, it is possible to preserve the most important bioactive compounds of potatoes and olive oil, providing improvements in the nutritional quality and food safety of potatoes processed in olive oil.*

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