Radiação gama e por feixe de eletrões na preservação de cogumelos silvestres: efeito em parâmetros físico-químicos, nutricionais e bioativos

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Tese de Doutoramento

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Dissertação de candidatura ao grau de Doutor em
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Orientação

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Resumo

As condições naturais de Trás-os-Montes, como o relevo e o clima, são propícias ao desenvolvimento de uma flora muito diversificada, que por sua vez influência o aparecimento de cogumelos silvestres.

Estes, devido às suas características organoléticas são considerados produtos "gourmet" de preço elevado; e dado o seu elevado valor nutricional (ricos em proteína, fibra, glúcidos, minerais, e baixo teor em lípidos) são uma boa opção para incluir em dietas hipocalóricas.

São constituídos por cerca de 90% de água e, por isso, têm uma elevada perecibilidade. A água que compõe a estrutura do cogumelo gera um ambiente favorável para aos sistemas biológicos, bioquímicos e biofísicos, levando à sua fácil degradação.

Assim, a preservação de cogumelos tornou-se um desafio para a indústria alimentar que tem de responder às necessidades do aumento populacional, bem como disponibilizar alimentos seguros.

Têm sido usadas muitas tecnologias de processamento para conservar os cogumelos; a radiação ionizante é bastante utilizada para aumentar o prazo de validade dos alimentos, interromper o processo de maturação, descontaminar, reduzir a presença de bactérias e fungos, ou para esterilizar produtos alimentares. Com base em dados científicos, o comité de Especialistas da *Organização das Nações Unidas para a Alimentação e a Agricultura* (FAO), a *Organização Mundial de Saúde* (OMS) e a *Agência Internacional de Energia Atómica* (AIEA) concluíram que a irradiação de qualquer alimento, até uma dose de 10 kGy, não apresenta riscos toxicológicos e não introduz problemas de caráter nutricional ou microbiológico.

O principal objetivo deste trabalho foi estudar os efeitos da irradiação gama e por feixe de eletrões em cogumelos silvestres (*Lactarius deliciosus* L., *Boletus edulis* Bull., *Hydnum repandum* L. Fr., *Boletus pinophilus* Pilát & Dermek, *Clitocybe subconnexa* Murrill, *Macrolepiota procera* (Scop.) Singer, *Russula delica* Fr., *Amanita ceasarea* (Scop.) Pers. e *Amanita curtipes* E.-J. Gilbert) colhidos no Nordeste de Portugal. O estudo desenvolvido focou principalmente os efeitos em parâmetros físico-químicos, nutricionais e bioativos.

A irradiação das amostras frescas foi efetuada numa câmara experimental com quatro fontes de ^{60}Co no Centro de Ciências e Tecnologias Nucleares, Instituto

Superior Técnico da Universidade de Lisboa. A irradiação por feixe de eletrões das amostras secas foi efetuada num acelerador de eletrões 10 Mev no Instituto Nuclear de Química e Tecnologia, em Varsóvia, na Polónia.

Durante a execução deste trabalho foram utilizadas diferentes condições de extração para obter, desta matriz, os diferentes compostos alvo. Foram aplicadas várias metodologias analíticas para atingir os objetivos propostos incluindo os procedimentos internacionais AOAC e diferentes métodos cromatográficos (HPLC, UFLC e GC) acoplados a diferentes detetores (RI, Fluorescência, PAD, ELSD e FID), um método de espetrometria de absorção atómica com chama de ar-acetileno e espetrofotometria UV-visível (em espetrofotómetro e leitor de microplacas).

De acordo com os resultados, apesar de detetadas algumas diferenças nos compostos individuais (açúcares, ácidos gordos, tocoferóis, ácidos orgânicos e compostos fenólicos), verificou-se que a composição nutricional das amostras irradiadas foi sempre muito semelhante à das amostras controlo. A irradiação minimiza os efeitos causados pelo tempo de armazenamento, secagem e congelação. A bioatividade das amostras irradiadas foi em geral elevada, comparativamente com as amostras controlo.

De um modo global, pode encarar-se a irradiação como uma possível tecnologia de conservação de cogumelos silvestres, independentemente da fonte de irradiação, espécie e tipo de processamento.

Palavras-chave: cogumelos silvestres; radiação ionizante; parâmetros nutricionais; bioatividade.

Abstract

The climatic and relief conditions of Trás-os-Montes region favor the development of a high diversity of flora that influences the appearance of wild mushrooms.

Due to their organoleptic characteristics, mushrooms are considered "gourmet" products with high price; and due to their high nutritional value, being rich in protein, fiber, carbohydrates, minerals, and low in fat, mushrooms are also a good option to include in hypocaloric diets.

Containing approximately 90% of water, mushrooms have a high perishability. The water present in their structures creates a favorable environment for biological, biochemical and biophysical systems, leading to mushrooms degradation.

Thus, mushrooms preservation is a challenging target for the food industry due to increasing populations and all the aspects related with food security and safety. Many processing technologies are currently used to preserve mushrooms; ionizing radiation is often used to increase food shelf life, interrupt the maturation process, decontaminate, reduce the presence of bacteria and fungi, or to sterilize food products, eliminating any microorganisms that may be present in food. Based on scientific background, the Organization Expert Committee on Food and Agriculture (FAO), the World Health Organization (WHO) and the International Atomic Energy Agency (IAEA) concluded that irradiation of any food up to a dose of 10 kGy does not present any toxicological risk and does not introduce problems of nutritional or microbiological character.

The main objective of this work was to study the effects of gamma irradiation and electron beam in wild mushrooms (*Lactarius deliciosus* L., *Boletus edulis* Bull., *Hydnum repandum* L. Fr., *Boletus pinophilus* Pilát & Dermek, *Clitocybe subconnexa* Murrill, *Macrolepiota procera* (Scop.), *Russula delica* Fr., *Amanita ceasarea* (Scop.) Pers. and *Amanita curtipes* E.-J. Gilbert) from the Northeast of Portugal. The conducted study was mainly focused in the effects on physicochemical, nutritional and bioactive parameters.

The irradiation of fresh samples was performed on an experimental chamber with four sources of ^{60}Co in the Center for Sciences and Nuclear Technology, IST, University of Lisbon. The electron beam irradiation of dried samples was performed

in a 10 MeV electron accelerator at the Nuclear Institute of Chemistry and Technology in Warsaw, Poland.

Different extraction conditions were applied to this matrix in order to obtain different target compounds. Various analytical methodologies were applied to achieve the proposed objectives including international AOAC procedures, different chromatographic methods (HPLC, GC and UFLC) coupled to different detectors (RI, Fluorescence, PAD, ELSD and FID), atomic absorption spectroscopy with air/acetylene flame and spectrophotometry UV-visible (using spectrophotometer and microplate reader).

According to the results obtained, although some differences observed in the individual compounds (sugars, fatty acids, tocopherols, organic acids and phenolic compounds), the nutritional composition of the irradiated samples was always very similar to the control samples. The irradiation minimizes the effects caused by storage time, drying and freezing. The bioactivity of the irradiated samples was generally high, in comparison with the control samples.

Overall, the irradiation could be a possible conservation technology for application in wild mushrooms, regardless of the source of radiation, species and type of processing.

Keywords: wild mushrooms; ionizing radiation; nutritional parameters; bioactivity.

Lista de Publicações

<u>Capítulo de livro</u>

1. Gamma and electron-beam for nutrients and bioactives conservation in chestnuts, mushrooms and dried plants. In Food Processing Technologies: Impact on Product Attributes.

Amilcar L. Antonio, João C.M. Barreira, Ângela Fernandes, Eliana Pereira, Isabel C.F.R. Ferreira.

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<u>Publicações de artigos em revistas de circulação internacional com arbitragem Científica referênciadas no Journal Citation Reports da ISI Web of Knowledge</u>:

1. Ângela Fernandes, Amilcar L. Antonio, M.Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Effect of gamma and electron-beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, 2012, *135*, 641-650. Fator de impacto: 3,391 (Q1- *Food Science*).

2. Ângela Fernandes, Amilcar L. Antonio, João C.M. Barreira, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushroom. *Postharvest Biology and Technology*, 2012, *74*, 79-84. Fator de impacto: 2,223 (Q1- *Food Science*).

3. Ângela Fernandes, Amilcar L. Antonio, João Barreira, M. Luisa Botelho, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, 2013, *6*, 2895-2903. Fator de impacto: 2,691 (Q1- *Food Science*).

4. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, Pedro M.P. Santos, Anabela Martins, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira. Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component

analysis. *Food Research International*, 2013, *54*, 18-25. Fator de impacto: 2,818 (Q1- *Food Science*).

5. Ângela Fernandes, Lillian Barros, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. LWT - *Food Science and Technology*, 2013, *54*, 493-499. Fator de impacto: 2,416 (Q1- *Food Science*).

6. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera*. *Food Chemistry*, 2014,*149*, 91-98. Fator de impacto: 3,391 (Q1- *Food Science*).

7. Ângela Fernandes, Lillian Barros, Amilcar L. Antonio, João C.M. Barreira, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Using gamma irradiation to attenuate the effects caused by drying or freezing in *Macrolepiota procera* organic acids and phenolic compounds. *Food and Bioprocess Tecnology*, 2014, *7*, 3012-3021. Fator de impacto: 2,691 (Q1- *Food Science*).

8. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Tecnology*, 2014, *7*, 1606-1617. Fator de impacto: 2,691 (Q1- *Food Science*).

9. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Feasibility of electron-beam irradiation to preserve wild dried mushrooms: effects on chemical composition and antioxidant activity. *Innovative Food Science and Emerging Technologies*, 2014, *22*, 158-166. Fator de impacto: 3,273 (Q1- *Food Science*).

10. Maria José Alves; Ângela Fernandes; João C.M. Barreira; Inês Lourenço; Dina Fernandes; Ana Moura; Ana Raquel Ribeiro; Julie Salgado; Amilcar Antonio; Isabel Ferreira. How gamma-rays and electron-beam irradiation would affect the antimicrobial activity of differently processed wild mushroom extracts? *Journal of Applied Microbiology*, 2014, *118*, 592-598. Fator de impacto: 2,479 (Q2- *Applied Microbiology & Biotechnology*).

11. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Triacylglycerol profiling as a chemical tool to identify mushrooms submitted to gamma or electron-beam irradiation. *Food Chemistry*, 2014, *15*, 399-406. Fator de impacto: 3,391 (Q1- *Food Science*).
12. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, Andrzej Rafalski, Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. How does electron beam irradiation dose affect the chemical and antioxidant profiles of wild dried Amanita mushrooms? *Food Chemistry*, 2015, *182*, 309-315. Fator de impacto: 3,391 (Q1- *Food Science*).
13. Ângela Fernandes João C.M. Barreira, Amilcar L. Antonio Patricia Morales, Virginia Férnandez-Ruiz, Anabela Martins, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira. Exquisite wild mushrooms as a source of dietary fiber: analysis in electron-beam irradiated samples. LWT - *Food Science and Technology*, 2015, *60*, 855-859. Fator de impacto: 2,416 (Q1- *Food Science*).
14. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Extended use of gamma irradiation in wild mushrooms conservation: validation of 2 kGy dose to preserve their chemical characteristics. *Innovative Food Science and Emerging Technologies*. Fator de impacto: 3,273 (Q1- *Food Science*). **Submetido**.
15. Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, Andrzej Rafalski, Patricia Morales, Virginia Férnandez-Ruiz, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Gamma and electron-beam irradiation as viable technologies for wild mushrooms conservation: effects on macro- and micro-elements. *Journal of Food Composition and Analysis*. Fator de impacto: 1,985 (Q1- *Food Science*). **Submetido**.

Publicações em atas de encontros científicos

1. Fernandes A., Oliveira M.B.P.P., Martins A., Ferreira, I.C.F.R. 2012. Add-value of *Lactarius deliciosus* and *Macrolepiota procera* wild mushrooms due to their nutritional and nutraceutical potencial.
   Congresso Internacional de Valorização de Produtos Tradicionais. Atas. Viana do Castelo. Portugal.

2. Ângela Fernandes, M. Beatriz P.P. Oliveira, Amilcar L. Antonio, Anabela Martins, Isabel C.F.R. Ferreira. 2012. Combined Effects of γ-irradiation and storage times on sugars composition of *Lactarius deliciosus*: comparison through linear discriminant analysis.

FoodSIM'2012. Atas. Freising, Alemanha.

3. Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Influência da radiação gama nas propriedades físico-químicas de *Lactarius deliciosus*.

11.º Encontro de Química dos Alimentos. Atas. Bragança, Portugal.

4. Ângela Fernandes, Amílcar Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira. Efeito da radiação gama nos níveis de tocoferóis em *Lactarius deliciosus*.

11.º Encontro de Química dos Alimentos. Atas. Bragança, Portugal.

<u>Comunicações orais</u>

1. Efeito da radiação gama nas propriedades físicas e químicas de amostras de *Lactarius deliciosus* L. silvestres.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

Encontro Luso Galego de Química. 28 a 30 de novembro de 2012. Vila Real, Portugal.

2. Efeitos da radiação nas propriedades físico-químicas e nutricionais de cogumelos.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira, Anabela Martins.

Conservação e preservação de cogumelos silvestres. *Conferência Internacional - O Mundo Oculto dos Cogumelos.* 11 de maio de 2013. Évora, Portugal.

3. Efeito da radiação gama nas propriedades químicas de *Boletus edulis* bull: Fr. silvestre.

Fernandes, Ângela, Barreira, João C.M., Antonio, Amilcar, Oliveira, M. Beatriz P.P., Martins, Anabela, Ferreira, Isabel C.F.R.

Encontro de Jovens Investigadores. 15 e 16 de novembro de 2013. Bragança, Portugal.

4. Protective effect of electron-beam irradiation in tocopherols integrity: evaluation by HPLC-fluorescence detection.

Ângela Fernandes, João C.M. Barreira, Amílcar L. António, Anabela Martins, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira.

8º Encontro Nacional de Cromatografia. 2 a 4 de dezembro de 2013. Covilhã, Portugal.

5. Efeito da irradiação por feixe de eletrões e do tempo de armazenamento na composição química de *Macrolepiota procera* (Scop.) Singer.

Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

XX Luso-Galego de Química. 26 a 28 de novembro de 2014. Porto, Portugal.

Comunicações em poster em encontros científicos nacionais

1. An overview of the nutrients and non-nutrients present in the wild mushroom species most appreciated in the Northeast of Portugal.

Ângela Fernandes, Beatriz Oliveira, Anabela Martins, Isabel C.F.R Ferreira.

7º Encontro Nacional de Cromatografia, 09 a 11 de janeiro de 2012. Porto. Portugal.

2. Effects of γ-irradiation on the fatty acids profile of *Lactarius deliciosus* wild mushroom.

Ângela Fernandes, M. Beatriz P.P. Oliveira, Amilcar L. Antonio, Lillian Barros, Anabela Martins, Isabel C.F.R. Ferreira.

First North European Congress on Food – NEEFood, 22 a 24 de abril de 2012. Saint Petersburg, Rússia.

3. Antioxidant activity of gamma irradiated *Lactarius deliciosus* L. from Northeast Portugal.

Ângela Fernandes, M. Beatriz P.P. Oliveira, Amilcar L. Antonio, Anabela Martins, Isabel C.F.R. Ferreira.

First North European Congress on Food – NEEFood, 22 a 24 de abril de 2012. Saint Petersburg, Rússia.

4. Gamma irradiation protects oleic acid from oxidation: an experiment in *Lactarius deliciosus* wild mushroom.

Ângela Fernandes, M. Beatriz P.P. Oliveira, Amilcar L. Antonio, Anabela Martins, Isabel C.F.R. Ferreira.

3rd Portuguese Young Chemists Meeting – 3PYCheM, 09 a 11 de maio de 2012. Porto. Portugal.

5. Combined Effects of γ-irradiation and storage times on sugars composition of *Lactarius deliciosus*: comparison through linear discriminant analysis.

Ângela Fernandes, M. Beatriz P.P. Oliveira, Amilcar L. Antonio, Anabela Martins, Isabel C.F.R. Ferreira.

*FoodSIM'*2012, 18 a 20 de junho de 2012. Freising, Alemanha.

6. Influência da radiação gama nas propriedades físico-químicas de *Lactarius deliciosus*.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

11.º Encontro de Química dos Alimentos, 17 a 19 de setembro de 2012. Bragança, Portugal.

7. Efeito da radiação gama nos níveis de tocoferóis em *Lactarius deliciosus.*

Ângela Fernandes, Amílcar Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

11.º Encontro de Química dos Alimentos, 17 a 19 de setembro de 2012. Bragança, Portugal.

8. Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushroom.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

Multiphysics, 16 a 19 de dezembro de 2012, Lisboa, Portugal.

9. Effects of gamma radiation on chemical composition of processed samples of the wild mushroom *Macrolepiota procera.*

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

EuroFoodChem XVII, 7 a 10 de maio de 2013. Istambul, Turquia.

10. Gamma irradiation preserves palmitoleic acid, a bioactive omega-7 unsaturated fatty acid, in *Macrolepiota procera* fresh samples.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

1º Simpósio de Química Medicinal da Universidade do Minho, 17 de maio de 2013. Braga, Portugal.

11. Gamma irradiation combined with freezing of *Macrolepiota procera* preserves tocopherols, one of the most powerful natural antioxidants.

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

1º Simpósio de Química Medicinal da Universidade do Minho, 17 de maio de 2013. Braga, Portugal.

12. Antioxidant activity of *Macrolepiota procera* wild mushroom submitted to different processing technologies.

Ângela Fernandes, Márcio Carocho, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

NEEFood-2013, 26 a 29 de maio de 2013. Kyiv, Ucrânia.

13. Efeitos do processamento em parâmetros nutricionais e químicos do cogumelo silvestre *Macrolepiota procera*.

Ângela Fernandes, Amilcar L. Antonio, João C.M. Barreira, Anabela Martins, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira.

XXIII Encontro Nacional da Sociedade Portuguesa de Química, 12 a 14 de junho de 2013. Aveiro, Portugal.

14. Chromatographic analysis of the effects of gamma irradiation on organic acids composition of wild *Boletus edulis* and *Hydnum repandum*.

Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, Anabela Martins, M. Beatriz P.P. Oliveira, Isabel C.F.R. Ferreira.

8º Encontro Nacional de Cromatografia, 2 a 4 de dezembro de 2013. Covilhã, Portugal.

15. Gamma irradiation preserves the nutritional profile of Boletus edulis Bull.: Fr. Â. Fernandes, A.L. Antonio, P. Morales, V. Fernández-Ruiz, M.B.P.P. Oliveira, A. Martins, I.C.F.R. Ferreira.

XIX Symposium of the Baltic Mycologists and Lichenologists, 22 a 26 de setembro de 2014. Latvia, Letónia.

16. Gamma irradiation improves the nutritional profile of dried and sliced wild *Boletus edulis* Bull.

Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, Tugba Günaydi, Hasan Alkan, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

5th Food Safety Congress, de 7 a 8 de maio de 2015. Istambul, Turkey.

17. Gamma irradiation preserves oleic acid in wild *Hydnum repandum* L.: Fr.

Ângela Fernandes, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

2nd Symposium on Medicinal Chemistry of the University of Minho, 8 de maio de 2015. Braga, Portugal.

18. Effects of gamma irradiation in the antimicrobial activity of wild mushroom extracts.

Ângela Fernandes, Maria José Alves, João C.M. Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

2nd Symposium on Medicinal Chemistry of the University of Minho, 8 de maio de 2015. Braga, Portugal.

Comunicações em poster em encontros científicos internacionais

1. Add-value of *Lactarius deliciosus* and *Macrolepiota procera* Wild mushrooms due to their nutritional and nutraceutical potential.

Fernandes A., Oliveira M.B.P.P., Martins A., Ferreira, I.C.F.R.

Co*ngresso Internacional de Valorização de Produtos Tradicionais,* 03 a 05 de maio de 2012. Viana do Castelo. Portugal.

2. Effects of gamma rays on sugars composition of wild mushrooms from the Northeast of Portugal.

Ângela Fernandes, Amílcar L. António, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

CIGR-Ageng 2012 – International Conference of Agricultural Engineering, 8 a 12 de julho de 2012. Valência. Espanha.

3. Effects of gamma radiation on physical and chemical parameters of wild *Lactarius deliciosus L.*

Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

The 7th International Workshop on Edible Mycorrhizal Mushrooms-IWEMM-7, 29 de julho a 3 de agosto de 2013. La Antigua, Guatemala.

4. Electron-beam irradiation at low doses preserves dietary fiber content in *Boletus edulis* Bull.: Fr. wild mushroom.

Â. Fernandes, J.C.M. Barreira, A.L. Antonio, P. Morales, V. Fernández-Ruiz, M.B.P.P. Oliveira, A. Martins, I.C.F.R. Ferreira.

62nd International Congress and Annual Meeting of the Society for medicinal Plant and Natural Product research – GA2014, 30 de agosto a 4 de setembro de 2014. Guimarães, Portugal.

5. Effects of gamma irradiation on macro and microelements of *Boletus edulis* Bull.: Fr. and *Hydnum repandum* L.: Fr.

Â. Fernandes, J.C.M. Barreira, A.L. Antonio, P. Morales, V. Fernández-Ruiz, M.B.P.P. Oliveira, A. Martins, I.C.F.R. Ferreira.

62nd International Congress and Annual Meeting of the Society for medicinal Plant and Natural Product research – GA2014, 30 de agosto a 4 de setembro de 2014. Guimarães, Portugal.

6. Chemical profile of *Macrolepiota procera* wild mushroom submitted to different processing technologies.

   Ângela Fernandes, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

   I International Symposium on Profiling – ISPROF, 2 a 4 de setembro de 2013. Costa da Caparica, Portugal.

7. Irradiation and storage time effects on chemical parameters of processed samples of wild *Macrolepiota procera* (Scop.) Singer.

   Ângela Fernandes, Amilcar L. Antonio, Andrzej Rafalski, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

   International Symposium on Food Safety and Quality: Applications of Nuclear and Related Techniques, 10 a 13 de novembro de 2014. Viena, Áustria.

8. Effects of electron beam irradiation on antioxidant activity of mushrooms *Amanita* spp.

   Ângela Fernandes, João C.M Barreira, Amilcar L. Antonio, M. Beatriz P.P. Oliveira, Anabela Martins, Isabel C.F.R. Ferreira.

   5th MoniQA International Conference "Food and Health - Risks and Benefits", de 16 a 18 de setembro de 2015. Porto, Portugal.

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Lista de Abreviaturas

2-ACBs	2-Alkylcyclobutanones
AESA	Autoridade Europeia para a Segurança dos Alimentos
AIEA	Agência Internacional de Energia Atómica
AMP	Adenosina 5'-Monofosfatase
ANOVA	Analysis of Variance
AOAC	Associação Oficial de Químicos Analistas, Association of Official Analytical Chemists
approx.	Approximately
APX	Ascorbato Peroxidase
AAS	Atomic Absorption Spectroscopy
CA	Columbia Agar
CAT	Catalase
CE, EC	Comunidade Europeia, European Community
CFU	Colony-Forming Unit
C-N	Carbon-Nitrogen Bond
^{60}CO	Cobalt 60
2-DCB	2-Dodecylcyclobutanone
DPPH	2,2,Difenil-1-Picril-Hidrazilo
dw	Dried Weight
EB	Electron-Beam
EC_{50}	Extract Concentration that provides 50% of antioxidant activity
e.g.	For Example
ELSD	Detector Evaporativo de Dispersão de Luz, Evaporative Light-Scattering Detector
EMM	Estimated Marginal Means
EPR	Electron Paramagnetic Resonance
ESR	Electron Spin Resonance
EU	European Union
FA	Fatty Acids
F-CA	Folin-Ciocalteau Assay
FAME	Fatty Acid Methyl Esters
FID	Detector por Ionização em Chama, Flame Ionization Detector

FAO	Organização das Nações Unidas para a Alimentação e Agricultura, Food and Agriculture Organization of the United Nations
fw	Fresh Weight
GAE	Gallic Acid Equivalents
GBq	γ-L-Glutaminil-3,4-Benzoquinona
GC	Cromatografia gasosa, Gas Chromatography
GC-FID	Gas Chromatography-Flame Ionization Detector
GC-MS	Gas Chromatography-Mass Spectrometry
GDHB	γ-L-Glutaminil-3,4-di-Hidroxibenzeno
GDP	Guanosina 5'-Difosfatase
GHB	γ-L-Glutaminil-4-Hidroxibenzeno
GID	Gamma Irradiation Dose
GLM	General Linear Model
GMP	Guanosina 5'-Monofosfatase
GR	Glutationa redutase
HCA	Hierarchical Cluster Analysis
HPLC	Cromatografia Liquída de Alta Eficiência, High-Performance Liquid Chromatography
HSD	Tukey's Honestly Significant Difference
I	Intermediate
IAEA	International Atomic Energy Agency
ID	Irradiation Dose
IDF	Insoluble Dietary Fiber
i.e	id est/ that is
INCT	Nuclear Chemistry and Technology
IS	International System of Units
INT	*p*-Iodonitrotetrazolium Chloride
L*, a*, b*	Colour Parameter
LDA	Linear Discriminant Analyses
LLL	1,2,3-Trilinoleoylglycerol
LnLnLn	1,2,3-Trilinolenoylglycerol
LLnLn	1-Linoleoyl-2,3-Dinoleoylglycerol
LOD	Limit of Detection
LOQ	Limit of Quantification

MCV	Means of the Canonical Variance
MDA-TBA	Malondialdehyde-Thiobarbituric Acid
MeV	Megaelectron Volt
MHB	Muller Hinton Broth
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus Aureus*
MSSA	Methicillin-Sensitive *Staphylococcus Aureus*
MUFA	Monounsaturated Fatty Acids
n-3	Ácido Gordo Omega-3
n.d.	Não detetável, Unidentified
OLL	1,2-Dilinoleoyl-3-Oleoyl-*rac*-Glycerol
OOL	1,2-dioleoyl-3-Linoleoylglycerol
OMS	Organização Mundial de Saúde
OOO	1,2,3- Trioleoylglycerol
PA	Pro Analysis
PAD	Detetor de fotodíodos, Photodiode Array Detector
PAL	Fenilalanina Amónia-liase
PBA	Prussian Blue Assay
PLL	1,2-Dilinoleoyl- 3-Palmitoyl-*rac*-Glycerol
PMMA	Poly(Methyl Methacrylate)
PoPoPo	1,2,3-Tripalmitoleoylglycerol
POD	Peroxidase
POL	1-Palmitoyl-2-Oleoyl-3-Linoleoylglycerol
POO	1,2-Dioleoyl-3-Palmitoyl-*rac*-Glycerol
PPO	Polifenoloxidase, Polyphenoloxidase
PPO	1,2-Dipalmitoyl-3-Oleoyl-*rac*-Glycerol (pp. 324, 329, 330, 331 e 335)
PPP	1,2,3-Tripalmitoylglycerol
PSL	Photostimulated Luminescence
PT	Processing Type
PTs	Processing Types
PUFA	Polyunsaturated Fatty Acids
R	Resistant
RI	Detector por Indíce de Refração, Refraction Index Detector
RP	Reducing Power

RDA	Recommended Dietary Allowance
Rpm	Revolutions per Minute
RSA	Radical Scavenging Activity
S	Susceptible
SDF	Soluble Dietary Fiber
SFA	Saturated Fatty Acids
SOO	1,2-Dioleoyl-3-Stearoyl-*rac*-Glycerol
SOD	Superóxido Dismutase
SPME	Solid Phase Microextraction
SPO	1-Stearoyl-2-Palmitoyl-3-oleoylglycerol
SPP	1-Stearoyl-2,3-dipalmitoylglycerol
SPSS	Statistical Package for the Social Sciences
SSS	1,2,3-Tristearoylglycerol
ST	Storage Time
TAC	Total Available Carbohydrates
TAG	Triacylglycerols
TBA	Thiobarbituric Acid
TBARS	Substâncias Reativas do Ácido Tiobarbitúrico, Thiobarbituric Acid reactive Substances
TBq	Terabecquerel
TL	Thermoluminescence
UFLC	Cromatografia Líquida Ultra-Rápida, Ultra Fast Liquid Chromatography
UV-A, B, C	Ultraviolet Radiation, Radiação Ultravioleta
VIS	Visible
WCB	Wilkins-Chalgren Broth
WHO	World Health Organization
Wi	Initial Weight
Ws	Weight at Sampling Period

Capítulo 1

Motivação, Objetivos e Estrutura da tese

1.1. Motivação da tese

Os cogumelos são dos alimentos mais populares do mundo. Além da sua importância ecológica na renovação dos ecossistemas, o seu valor e consumo tem crescido exponencialmente, principalmente devido às suas propriedades organoléticas, nutricionais, nutracêuticas e funcionais (Barros, Cruz, Baptista, Estevinho & Ferreira, 2008; Ferreira, Vaz, Vasconcelos & Martins, 2010).

Considerados produtos muito perecíveis, devido ao seu elevado teor em água, tendem a perder qualidade logo após a colheita, ficando o seu tempo de vida útil reduzido a 1-3 dias, à temperatura ambiente. Por outro lado, não têm uma cutícula para os proteger dos danos físicos ou do ataque microbiano. As elevadas perdas de água, as taxas de respiração e a atividade enzimática, tornam-nos muito suscetíveis à deterioração (Aguirre, Frias, Barry-Ryan & Grogan, 2008). As perdas durante a comercialização podem chegar aos 40% e deste modo, causam algumas dificuldades na distribuição pelos mercados. Uma das preocupações da indústria alimentar é garantir a segurança dos alimentos para consumo e, neste sentido, requerem melhores técnicas de preservação (Lacroix & Ouattara, 2000).

Um grande número de técnicas de preservação e conservação são utilizadas, nomeadamente tratamentos químicos, refrigeração, lavagem com água, revestimento com alginato, embalamento em atmosfera modificada/controlada, uso de humectantes, inibidores da tirosina, tratamentos com ozono, secagem e/ou congelação. Mas, a aplicação destes processos apresenta alguns inconvenientes, tais como descoloração dos produtos, presença de resíduos, poluição ambiental, produção de odores, contaminação com microrganismos patogénicos, custos elevados, além de não serem, muitas vezes, fáceis de implementar à escala industrial (Fernandes, Antonio, Oliveira, Martins & Ferreira, 2012).

Apesar da grande popularidade dos cogumelos e face ao aumento da exportação para outros países (especialmente Espanha, França e Itália), as tecnologias alternativas de preservação e conservação são escassas. Neste sentido, a procura de uma tecnologia mais adequada tem sido grande. A irradiação (gama e por feixe de eletrões), como técnica de conservação, tem sido testada com sucesso em diversos produtos alimentares, tais como frutas, vegetais, carne e peixe e encontra-se regulamentada pela União Europeia através da Diretiva (CE) nº 1999/2/CE.

As diferentes aplicações tecnológicas para a preservação de alimentos usam irradiação em diferentes doses e gamas. Assim, para inibir a germinação recomenda-se 0,05-0,15 kGy; na desinfestação de insetos 0,15-0,5 kGy; para retardar processos fisiológicos 0,25-1,0 kGy; na eliminação de microorganismos 1-10 kGy; e na esterilização de alimentos 10-50 kGy (ICGFI, 1999).

Contudo, doses elevadas de radiação podem alterar a permeabilidade da membrana, provocar oxidação e causar alterações indesejáveis (Beaulieu, D'Aprano & Lacroix, 2002). Pelo referido, é obrigatório a um estudo específico de adequabilidade para cada alimento.

Independentemente das tecnologias de preservação aplicadas, qualquer produto alimentar deve, idealmente, conservar as características nutricionais e químicas que tipificam a sua forma fresca.

Existem muitos estudos de avaliação dos efeitos da radiação em cogumelos, mas em espécies cultivadas, tais como *Agaricus bisporus* (J.E.Lange), *Lentinus edodes* (Berk.) e *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm., sendo os estudos em espécies silvestres muito mais raros.

O Nordeste de Portugal, devido às condições climáticas e à diversidade da flora é uma das Regiões Europeias com maior diversidade de cogumelos silvestres comestíveis, alguns com grande relevância na gastronomia.

O presente trabalho compreende um estudo abrangente dos efeitos da radiação gama e por feixe de eletrões, em alguns dos mais apreciados cogumelos silvestres do Nordeste de Portugal, focando os efeitos em caracteristicas físico-químicas, nutricionais e bioativas.

1.2. Objetivos da investigação

Neste contexto, o principal objetivo do trabalho foi avaliar o efeito da radiação gama e por feixe de eletrões, como potenciais ferramentas para aumentar o prazo de validade de cogumelos silvestres, analisando os seus efeitos sobre:

- Parâmetros físicos: cor, perda de massa, diâmetro do chapéu e temperatura de armazenamento.
- Parâmetros químicos e nutricionais: valor energético, composição em ácidos gordos, triacilgliceróis e tocoferóis, açúcares, ácidos orgânicos, minerais (macro e micro elementos) e fibra.

- Parâmetros de bioatividade: atividade captadora de radicais DPPH (2,2-difenil-1-picril-hidrazilo), poder redutor, inibição da descoloração do β-caroteno e inibição da peroxidação lipídica através do ensaio do ácido tiobarbitúrico (TBARS), fenóis totais e compostos fenólicos individuais.

Estes efeitos foram avaliados utlizando:

i) Diferentes espécies silvestres (*Lactarius deliciosus* L., *Boletus edulis* Bull, *Hydnum repandum* L. Fr., *Boletus pinophilus* Pilát & Dermek, *Clitocybe subconnexa* Murrill, *Macrolepiota procera* (Scop.) Singer, *Russula delica* Fr., *Amanita caesarea* (Scop.) Pers. e *Amanita curtipes* E.-J. Gilbert), como evidencia a **Figura 1.1**;

ii) Diferentes tempos de armazenamento (0, 4 e 8 dias em amostras frescas e 0, 6 e 12 meses em amostras secas);

iii) Diferentes tecnologias de processamento (congelação e secagem);

iv) Diferentes ferramentas estatísticas (Análise de Variância; Análise de Clusters; Modelo Geral Linear; Análise Discriminante Linear e Análise de Componentes Principais). Foi desenvolvendo um estudo abrangente do efeito das doses de irradiação, espécies de cogumelos, tempo de armazenamento e diferentes técnicas de processamento nos parâmetros físico-químicos, nutricionais e bioativos.

Nome científico	*Lactarius deliciosus* L.	*Boletus edulis* Bull.	*Hydnum repandum* L. Fr.	*Boletus pinophilus* Pilát & Dermek	*Clitocybe subconnexa* Murrill
Habitat	Solos ácidos de árvores coníferas	Pinhais, soutos, carvalhais e montados de sobro ou azinho	Florestas de coníferas e folhosas	Solos pobres, ácidos, arenosos e florestas de coníferas	Restos de folhas e árvores coníferas, restos de madeiras
Nome comum em Inglês	Saffron milk cap, red pine mushroom	Penny-bun, porcini, cep, king bolete	Sweet tooth, wood hedgehog	Pine bolete or pinewood king bolete	Unknown
Nome comum em Português	Sancha, pinheira, míscaro	Cepe de Bordéus, cepas, míscaro	Línguas-de gato, pé de carneiro	Boleto dos pinheiros, tortulhos	Desconhecido
Data de Recolha	Novembro de 2011	Novembro de 2012	Novembro de 2012	Novembro de 2012	Novembro de 2013
Ecologia	Micorrízico	Micorrízico	Micorrízico	Micorrízico	Micorrízico

Nome científico	*Macrolepiota procera* (Scop.) Singer	*Russula delica* Fr.	*Amanita ceasarea* (Scop.) Pers.	*Amanita curtipes* E.-J. Gilbert
Habitat	Solos bem drenados, florestas	Florestas de coníferas e folhosas	Bosques de carvalho misturados com coníferas	Pinhais com areia, carvalhos, cortiça
Nome comum em Inglês	Parasol	Milk-white brittle gill	Caesar's mushroom	Unknown
Nome comum em Português	Púcaras, frade, fradinhos	Desconhecido	Amanitas dos césares, laranjinha, amanita-real	Desconhecido
Data de Recolha	Novembro de 2011	Novembro de 2012	Outubro de 2013	Outubro de 2013
Ecologia	Saprófita	Micorrízico	Micorrízico	Micorrízico

Figura 1.1. Principais características das espécies de cogumelos silvestres estudadas. Fonte das imagens: www.wikipedia.pt.

1.3. Organização e estrutura da tese

De acordo com o principal objetivo deste trabalho, a apresentação da tese foi organizada em 7 capítulos diferentes. Neste primeiro capítulo descrevem-se a motivação, os objetivos da investigação, a organização e estrutura da tese.

O capítulo 2 apresenta uma revisão geral sobre os efeitos da irradiação gama, por feixe de eletrões e ultravioleta nas propriedades físico-químicas e nutricionais de cogumelos, discutindo-se os principais efeitos.

Do capítulo 3 até ao capítulo 6, os principais resultados experimentais são apresentados e distribuídos da seguinte forma: Efeitos da radiação gama nos parâmetros físico-químicos, nutricionais e bioativos de cogumelos silvestres (capítulo 3); Efeitos de diferentes tecnologias de processamento em parâmetros químicos, nutricionais e bioativos de cogumelos silvestres (capítulo 4); Efeitos da radiação por feixe de eletrões nos parâmetros químicos, nutricionais e bioativos de cogumelos silvestres (capítulo 5); Efeitos comparativos da radiação gama e por feixe de eletrões em parâmetros químicos e bioativos de cogumelos silvestres (capítulo 6).

No capítulo 7 apresentam-se as conclusões gerais do trabalho desenvolvido, referindo-se as principais contribuições conseguidas através desta tese de doutoramento e as perspetivas futuras.

1.4. Plano de trabalho da tese

Para alcançar o objetivo proposto nesta tese, o trabalho desenvolvido foi organizado em quatro fases, de acordo com a sequência apresentada na **Tabela 1.1.**

O trabalho foi desenvolvido em três Laboratórios de Investigação:

- Centro de Investigação de Montanha, da Escola Superior Agrária de Bragança do Instituto Politécnico de Bragança.

- Laboratório de Bromatologia e Hidrologia, do Departamento de Ciências Químicas da Faculdade de Farmácia da Universidade do Porto.

- Departamento de Nutrição e Bromatologia II, da Faculdade de Farmácia da Universidade Complutense de Madrid.

A irradiação das amostras, gama e por feixe de eletrões, foi efectuada no Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico da Universidade de Lisboa e no Instituto Nuclear de Química e Tecnologia, em Varsóvia, na Polónia, respetivamente.

Os resultados experimentais obtidos são apresentados na forma de artigos científicos compreendidos entre o capítulo 3 e o capítulo 6 desta tese.

Tabela 1.1. Plano de trabalho executado durante o desenvolvimento desta tese.

Fase 1: *Amostras*	➢ Receção das amostras no laboratório, de seguida procedeu-se à sua completa identificação. ➢ As amostras foram divididas em grupos, devidamente embaladas em sacos de plástico de polietileno e prontamente transportadas para os Centros de irradiação.
Fase 2: *Irradiação*	➢ A irradiação gama foi realizada no Centro de Ciências e Tecnologias Nucleares, Instituto Superior Técnico da Universidade de Lisboa. ➢ A irradiação por feixe de eletrões foi realizada no Instituto Nuclear de Química e Tecnologia, em Varsóvia, na Polónia.
Fase 2.1: *Amostras analisadas em fresco*	***Com irradiação gama*** ➢ *Lactarius deliciosus* (Doses 0,5 e 1 kGy e com tempo de armazenamento a 0, 4 e 8 dias) ➢ *Boleutus edulis e Hydnum repandum* (Doses 1 e 2 kGy) ➢ *Boletus pinophilus* e *Clytocibe subconnexa* (Dose 2 kGy)
Fase 2.2: *Amostras analisadas após processamento*	***Com irradiação gama*** ➢ *Macrolepiota procera* (Secas, congeladas e irradiadas em fresco com a dose 0,5 kGy) ➢ *Macrolepiota procera* (Frescas, secas e congeladas, todas irradiadas com as doses 0,5 e 1 kGy).
Fase 2.3: *Amostras analisadas em seco*	***Com feixe de eletrões*** ➢ *Macrolepiota procera* (Doses 0,5, 1 e 6 kGy e com tempo de armazenamento a 0, 6 e 12 meses) ➢ *Boletus edulis e Russula delica* (Doses 2, 6 e 10 kGy) *Amanita caesarea e Amanita curtipes* (Doses 2, 6 e 10 kGy)
Fase 3: *Parâmetros analisados*	➢ Parâmetros físicos: Cor, massa, diâmetro do chapéu, temperatura de armazenamento. ➢ Parâmetros químicos e nutricionais: Valor energético, ácidos gordos, açúcares, tocoferóis, triacilgliceróis, ácidos orgânicos, minerais e fibras. ➢ Parâmetros bioativos: Atividade antioxidante e compostos fenólicos individuais.
Fase 4:	**Artigo I:** Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* L. wild edible mushroom.

Resultados	**Artigo II:** Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. **Artigo III:** Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: comparative study through principal component analysis. **Artigo IV:** Extended use of gamma irradiation in wild mushrooms conservation: validation of 2 kGy dose to preserve their chemical characteristics. **Artigo V:** Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. **Artigo VI:** Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera.* **Artigo VII:** Using gamma irradiation to attenuate the effects caused by drying or freezing in *Macrolepiota procera* organic acids and phenolic compounds. **Artigo VIII:** Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild Macrolepiota procera dried samples. **Artigo IX:** Feasibility of electron-beam irradiation to preserve wild dried mushrooms: effects on chemical composition and antioxidant activity. **Artigo X:** How does electron-beam irradiation dose affect the chemical and antioxidant profiles of wild dried Amanita mushrooms? **Artigo XI:** Exquisite wild mushrooms as a source of dietary fiber: Analysis in electron-beam irradiated samples. **Artigo XII:** Triacylglycerols profile as a chemical tool to identify mushrooms submitted to gamma or electron beam irradiation. **Artigo XIII:** Gamma and electron-beam irradiation as viable technologies for wild mushrooms conservation: effects on macro- and micro-elements. **Artigo XIV:** How does electron beam irradiation dose affect the chemical and antioxidant profiles of wild dried *Amanita* mushrooms?

1.5. Bibliografia

Aguirre, L., Frias, J.M., Barry-Ryan, C., & Grogan, H. (2008). Assessing the effect of product variability on the management of the quality of mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology*, *49*, 247-254.

Barros, L., Cruz, T., Baptista, P., Estevinho L.M., & Ferreira, I.C.F.R. (2008). Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food and Chemical Toxicology*, *46*, 2742-2747.

Beaulieu, M., D'Aprano, G., & Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry*, *63*, 311-315.

Directive 1999/2/EC of the European Parliament and of the Council of 22 February 1999 on the establishment of a Community list of foods and food ingredients treated with ionizing radiation.http://eurlex.europa.eu/LexUriServ/LexUriServ.do?uri=CELEX:31999L0003:EN:NOT.

Fernandes, Â., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Ferreira, I.C.F.R., Vaz, J. A., Vasconcelos, M.H., & Martins, A. (2010). Compounds from Wild Mushrooms with Antitumor Potential. *Anti-Cancer Agents in Medicinal Chemistry, 10,* 424-436.

ICGFI (1999). Facts about Food Irradiation. Vienna, Austria, International Consultative Group on Food Irradiation. Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture International Atomic Energy Agency.

Lacroix, M., & Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products - a review. *Food Research International*, *33*, 719-724.

CAPÍTULO 2.

Estado da arte relativo ao efeito da irradiação nas propriedades físico-químicas e nutricionais de cogumelos

Este capítulo consiste numa revisão bibliográfica que foca os efeitos da irradiação (gama e por feixe de eletrões) nas propriedades físico-químicas e nutricionais de cogumelos.

2.1. Introdução

2.1.1. Singularidade das propriedades dos cogumelos

Os cogumelos têm um lugar bem demarcado na alimentação humana e os dados estatísticos assim o indicam. De acordo com dados da Organização para a Alimentação e Agricultura (FAO, 2011), a produção mundial de cogumelos e trufas em 2009 ultrapassou 6 milhões de toneladas. Por outro lado, os cogumelos silvestres comestíveis são colhidos, consumidos e comercializados em mais de 80 países em todo o mundo, com milhões de toneladas colhidas anualmente no valor de 1,4 milhões de euros, sendo que muitos são colhidos pela população local e não são contabilizados.

O consumo de cogumelos tem vindo a aumentar devido ao seu valor nutricional (Kalac, 2009), mas também às suas propriedades medicinais e nutracêuticas (Ferreira, Barros, & Abreu, 2009; Ferreira, Vaz, Vasconcelos, & Martins, 2010).

Na Europa, os cogumelos silvestres são considerados uma boa fonte de proteínas digeríveis, glúcidos, fibras e vitaminas (Kalač, 2009; Grangeia, Heleno, Barros, Martins, & Ferreira, 2011; Heleno et al., 2011; Ouzouni, Petridis, Koller, & Riganakos, 2009). A massa seca é, em geral, 100 g/kg, sendo os polissacáridos estruturais e as proteínas os principais componentes da matéria seca. São produtos com um teor em lípidos muito baixo. Quitina, glicogénio, manitol e trealose são os representantes típicos dos glúcidos. A proporção de aminoácidos é nutricionalmente favorável e o conteúdo de ácidos gordos n-3 é negligenciável (Kalač, 2009).

Os macrofungos têm uma história de uso tradicional em terapias orientais e em práticas clínicas que usam preparações derivadas de cogumelos, uma vez que acumulam uma variedade enorme de metabolitos bioativos (por exemplo, compostos fenólicos, policétidos, terpenos, esterois e polissacáridos) com diversas atividades nomeadamente, imunomodulatória, cardiovascular, hepatoprotetora, antifibrótica, anti-inflamatória, antidiabética, antiviral, antimicrobiana e antitumoral (Poucheret, Fons, & Rapior, 2006; Ferreira et al., 2010).

Há mais de 3000 espécies de cogumelos consideradas comestíveis. Destas, 100 são cultivadas e comercializadas (Chang & Miles, 2004) sendo apenas 20 à escala industrial (Sadler et al., 2003). Historicamente, a comercialização de cogumelos silvestres é dominada pela espécie *Agaricus bisporus* L.. No entanto,

mais recentemente, a produção de outros cogumelos comestíveis, tais como *Pleurotus* spp., *Lentinula edodes* (Berk.) Pegler*, Flammulina velutipes* Singer e *Volvariella volvacea* (Bulliard ex Fries) Singer tem crescido consideravelmente (Aida et al., 2009; Venturini et al., 2011). Tratam-se de espécies com uma enorme importância comercial e o seu cultivo tem emergido como uma indústria independente e muito promissora. Cerca de 45% dos cogumelos produzidos são consumidos frescos. Os outros 55% são processados (5% desidratados e 50% em conserva), uma vez que o seu prazo de validade na forma fresca é muito limitado (Singh, Langowski, Wanib, & Saengerlaub, 2010).

2.1.2. Prazo de validade dos cogumelos

Os cogumelos são um dos produtos mais perecíveis e tendem a perder qualidade imediatamente após colheita. O curto prazo de validade dos cogumelos (entre 1-3 dias à temperatura ambiente) é uma limitação para a distribuição e comercialização do produto em fresco. Sofrem de imediato alterações pós-colheita, tais como escurecimento, abertura do chapéu (píleo), alongamento do pé (estirpe), aumento do diâmetro do chapéu, perda de massa e mudanças na textura, devidas não só à sua elevada taxa de respiração e falta de proteção física que evite a perda de água ou o ataque microbiano (Akram & Kwon, 2010; Singh et al, 2010; Sommer, Schwartz, Solar & Sontag, 2010) (**Figura 2.1**).

O ataque por bactérias e fungos, a atividade enzimática e as alterações bioquímicas são a causa da deterioração dos cogumelos durante o armazenamento (**Figura 2.1**). O escurecimento dos cogumelos ocorre também quando estes são submetidos a forças que prejudicam a integridade celular, tais como oscilações, manuseamento e o envelhecimento (Beaulieu, D'Aprano & Lacroix, 2002; Jiang, Luo, Chen, Shen & Ying, 2010).

Os fatores mais importantes para a taxa de escurecimento enzimático são: as concentrações da polifenoloxidase (PPO) e os compostos fenólicos, pH, temperatura, atividade da água e disponibilidade de oxigénio. O acastanhamento enzimático resulta de dois mecanismos distintos de oxidação do grupo fenólico: (a) ativação da tirosinase, uma enzima pertencente à família da PPO; e/ou (b) oxidação espontânea (Martinez & Whitaker, 1995; Jolivet, Arpin, Wichers & Pellon, 1998; Singh et al., 2010).

A PPO, presente no chapéu e no pé do cogumelo, catalisa duas reações diferentes: (i) a hidroxilação de monofenóis para os correspondentes compostos *o*-di-hidroxi e (ii) a oxidação de fenóis *o*-di-hidroxi para *o*-quinonas, que se condensam para formar os pigmentos castanhos de melanina (Beaulieu et al., 2002).

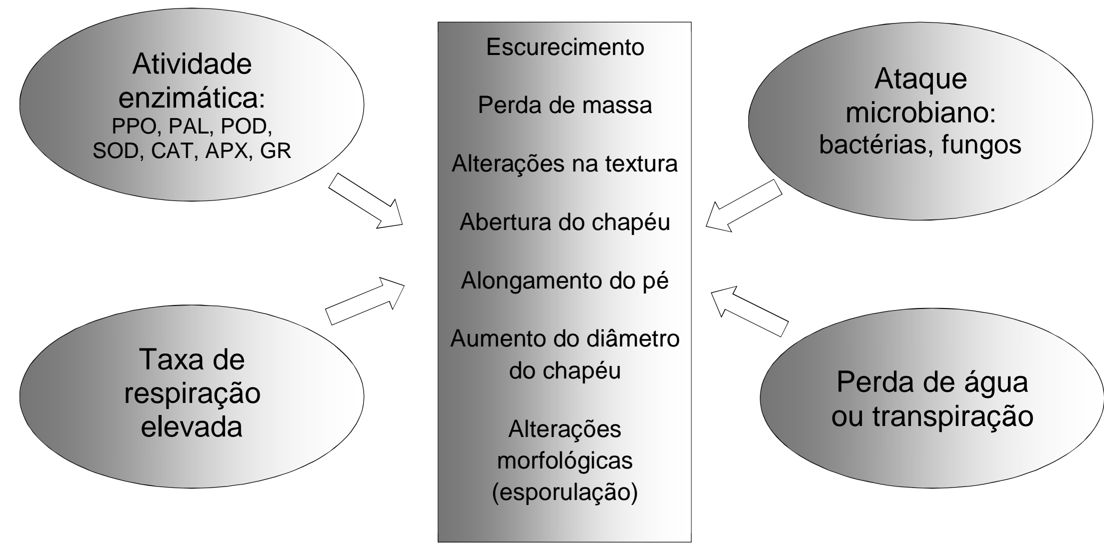

Figura 2.1. Alterações pós-colheita responsáveis pela curta vida de prateleira dos cogumelos (retângulo interno), e os principais fatores que contribuem para essas mudanças (elipses). PPO- polifenoloxidase, PAL- fenilalanina amónia-liase, POD- peroxidase, SOD- superóxido dismutase, CAT- catalase, APX- ascorbato peroxidase, GR- glutationa redutase.

Por exemplo, na presença de oxigénio e da tirosinase, o γ-L-glutaminil-4-hidroxibenzeno (GHB, o composto fenólico mais frequente nos cogumelos) é facilmente hidroxilado a γ-L-glutaminil-3,4-di-hidroxibenzeno (GDHB) e oxidado a γ-L-glutaminil-3,4-benzoquinona (GBq), o que leva ainda à formação de compostos de melanina polimerizados (Beaulieu, D'Aprano & Lacroix, 1999; Sommer et al, 2009) (**Figura 2.2**).

Além da PPO, a atividade de outras enzimas como a fenilalanina amónia-liase (PAL) e a peroxidase (POD) também tem sido relacionada com o desenvolvimento de pigmentos (escurecimento). A PAL é a enzima chave no metabolismo dos fenóis, catalisa a desaminação da L-fenilalanina, que por sua vez, vai produzir amoníaco e o ácido *trans*-cinâmico, a partir do qual são produzidos compostos fenólicos (Benoit, D'Aprano & Lacroix, 2000).

A superóxido dismutase (SOD) e a catalase (CAT) protegem as células contra os efeitos destrutivos das espécies reativas de oxigénio e constituem os principais componentes dos sistemas de defesa antioxidante da célula. A SOD converte

primeiro os aniões superóxido em peróxido de hidrogénio que, em seguida, são removidos pela CAT. Estas atividades combinadas aumentam a durabilidade dos cogumelos, protegendo a integridade das suas membranas celulares (Xiong, Xing, Feng, Tan & Bian, 2009).

Figura 2.2. Reações envolvidas no escurecimento dos cogumelos (adaptado a partir de Beaulieu et al., 1999). A hidroxilação de GHB (γ-glutaminil-4-hidroxibenzeno) mediada pela PPO monofenolase produz γ-L-glutaminil-3,4-di-hidroxibenzeno (GDHB); nesta reação, GHB atua tanto como substrato como co-fator. A oxidação de GDHB mediada pela PPO difenolase produz benzoquinona (γ-L-glutaminil-3,4-benzoquinona, GBQ) que, por sua vez, origina melanina por polimerização.

A perda de água ou a transpiração é outro processo fisiológico importante que afeta as principais características dos cogumelos frescos. Também a perda de massa, aparência e textura, podem ocorrer mas com a agravante de serem dependentes da temperatura e da humidade relativa circundantes. A temperatura de armazenamento é um dos principais fatores que afeta a maturação e qualidade dos cogumelos, tais como a respiração, transpiração, senescência e outras ações fisiológicas. A oscilação da temperatura durante o armazenamento pode desencadear a ação de vários tipos de enzimas, optimizar a atividade fisiológica, aumentando a senescência dos cogumelos armazenados (Pai, 2000; Singh et al., 2010).

As alterações morfológicas, que envolvem a exposição das lâminas e a esporulação, são suportadas pelos substratos da respiração que se encontram presentes nos basidiomas, aquando da colheita. Assim, o substrato dispendido no desenvolvimento dos basidiomas pós-colheita e a respiração, são fatores importantes que contribuem para a senescência; já a diminuição da atividade respiratória pós-colheita é devida ao esgotamento dos substratos e também à senescência dos tecidos (Singh et al., 2010).

Finalmente, a carga bacteriana em cogumelos frescos diminui significativamente a sua qualidade. A taxa de deterioração pós-colheita de cogumelos frescos foi diretamente relacionada com a carga microbiana inicial (Doores, Kramer & Beelman, 1987; Singh et al., 2010).

Alguns microrganismos Gram negativo, tais como *Pseudomonas fluorescens*, e leveduras nomeadamente, *Candida sake*, têm sido associados à deterioração dos cogumelos (Masson, Ainsworth, Fuller, Bozkurt & Ibanoglu, 2002; Jiang et al., 2010b). Os bolores podem também afetar a qualidade, e a contaminação por *Verticillium maltousei* provoca manchas castanhas nos cogumelos (Beaulieu et al., 1999; Beaulieu et al., 2002).

2.2. Irradiação de cogumelos

2.2.1. Aumento do tempo de vida útil dos cogumelos

Preservar a qualidade após a colheita e prolongar a sua conservação, é de extrema importância para a indústria de cogumelos, bem como para os consumidores destes (Akram & Kwon, 2010).

A procura de métodos de conservação é fundamental para o desenvolvimento de técnicas de conservação menos agressivas e menos prejudiciais para os produtos alimentares (Gould, 1989; Minnaar, Taylor & McGill, 1995). Hoje em dia, dada a preferência dos consumidores por alimentos frescos e minimamente processados, a indústria tem sido motivada para o desenvolvimento e aplicação de técnicas que ajudem a simplificar a forma de conservação e também a preparação dos alimentos (Ramos et al., 2011).

Tem havido uma intensa investigação no sentido de encontrar uma tecnologia de preservação mais adequada para os cogumelos. Vários tratamentos químicos (Sapers, Miller, Pilizota & Kamp, 2001), refrigeração (Murr & Morris, 1975), lavagem com água (Cliffe-Byrnes & O'Beirne, 2008), revestimento com alginato (Nussinovitch & Kampf, 1993), embalamento em atmosfera modificada/controlada (Lopez-Briones et al., 1992, Roy, Anantheswaran & Beelman, 1995), utilização de humectantes, inibidores da tirosinase (Singh et al., 2010) e tratamentos com ozono (Yuk, Yoo, Yoon, Marshall & Oh, 2007) são métodos frequentemente aplicados. No entanto, estes métodos apresentam algumas desvantagens, nomeadamente a descoloração. Podem deixar resíduos no alimento e levar à produção de odores, contaminação com microrganismos patogénicos, e alguns deles não são adequados para utilização à escala industrial (Duan, Xing, Shao & Zhao, 2010). Além disso, a maioria das técnicas de conservação de alimentos apenas retarda o crescimento de microrganismos. Em contraste, o processamento por radiação ionizante pode inibir ou inativar completamente o crescimento microbiano, resultando em produtos alimentares estéreis e com maior prazo de validade (Gould, 1989; Minnaar et al., 1995).

2.2.2. Técnicas de irradiação

A irradiação de alimentos pode ser considerada um segundo grande avanço após a pasteurização. Neste processo, o alimento é exposto a radiação ionizante (gama ou feixe de eletrões), a fim de aumentar o tempo de prateleira dos alimentos, bem como a sua segurança. A irradiação permite eliminar os microrganismos e

insetos que possam estar presentes nos alimentos, sem provocar alterações significativas na sua qualidade sensorial e nutricional (Akram & Kwon, 2010). É considerada um método físico não-térmico de conservação (pasteurização a frio) em que o processamento dos alimentos pode ser feito à temperatura ambiente (Duan et al., 2010).

A quantidade de alimentos irradiados em todo o mundo, em 2005, foi 405 000 toneladas, entre as quais 186 000 toneladas (46%) para a descontaminação de especiarias e produtos hortícolas secos, 82 000 toneladas (20%) para a desinfestação de grãos e frutas, 32 000 toneladas (8 %) para a descontaminação de carne e peixe, 88 000 toneladas (22%) para a inibição da germinação de alho e batata e 17 000 toneladas (4%) para outros alimentos onde se incluem os cogumelos (Kume, Furuta, Todoriki, Uenoyama, e Kobayashi, 2009). Nos países da UE, em 2013, foram tratados com radiação ionizante um total de 6 876,2 toneladas de produtos, 84% dos quais foram irradiados em 3 Estados-Membros: Bélgica (49,4%), Holanda (24,4%) e Espanha (12,7%). As categorias irradiadas em maior percentagem foram coxas de rã (46,4%) e ervas aromáticas e especiarias secas (24,4%) (Comissão Europeia, 2015).

A radiação gama (Beaulieu et al., 2002) e por feixe de eletrões (Koorapati, Foley, Pilling & Prakash, 2004) demonstraram ser ferramentas promissoras para aumentar o tempo de conservação pós-colheita dos cogumelos. A radiação gama inibe a abertura do chapéu e o alongamento do pé, bem como o escurecimento, reduz o nível de contaminação microbiana, permitindo aumentar o prazo de validade dos cogumelos frescos sem efeitos notórios no seu sabor (Lescano, 1994).

A radiação gama, por si só ou em combinação com a refrigeração, tem mostrado prolongar o tempo de validade através da redução da perda de humidade e da melhoria da cor e da aparência (Ajlouni, Beelman & Thompson, 1993).

A radiação por feixe de eletrões também é conhecida por ser altamente eficaz na redução de bactérias nocivas em frutas, legumes e outros alimentos, preservando o sabor, aroma, textura, salubridade e teor nutricional (Schmidt, Palekar, Maxim & Castillo, 2006; Duan et al., 2010).

A irradiação de alimentos com radiação ultravioleta foi testada com UV-A (400-315 nm) e UV-B (315-280 nm) e, principalmente, com UV-C (280-100 nm), uma vez que é mais energética. A radiação ultravioleta (UV-C) é amplamente utilizada como alternativa à esterilização química e redução microbiana em produtos alimentares e

a sua utilização foi aprovada como desinfetante para o tratamento superficial de alimentos (USFDA, 2002). Como tratamento pós-colheita de produtos frescos, a radiação UV-C mostrou ser benéfica na redução das taxas de respiração, controlo da decomposição, atraso da senescência e da maturação em diferentes frutas e legumes, como também em cogumelos (Guan, Fan & Yan, 2012). Além disso, as radiações UV-C, UV-B e UV-A têm a capacidade de converter o ergosterol dos cogumelos em vitamina D_2 (Teichmann, Dutta, Staffas & Jägerstad, 2007), sendo a UV-B a mais eficaz (Jasinghe & Perera, 2006; Ko, Lee, Lee & Park, 2008).

A irradiação de cogumelos pode ser um método seguro e de baixo custo para aumentar o tempo de vida útil, bem como para garantir a sua qualidade higiénica e sensorial (Akram & Kwon, 2010). O processo de amadurecimento de frutas e vegetais, pode ser retardado pela irradiação, preservando as características nutricionais e aumentando o seu prazo de validade (Akram & Kwon, 2010).

A aplicação da irradiação de produtos alimentares está aprovada pelas principais entidades reguladoras, incluindo a Comissão da União Europeia (Directiva 1999/2/CE), *Organização Mundial de Saúde* (WHO, 1981, 1994) e Comissão do *Codex Alimentarius* (CAC/RCP 19-1979, Rev. 2-2003). Estas entidades reguladoras asseguram que a irradiação de alimentos, no que se refere ao processamento de alimentos para consumo humano, é um processo seguro.

Em relação às doses máximas autorizadas para o processamento de alimentos, existe alguma discrepância entre países. Segundo as recomendações do relatório "*Integridade dos Alimentos Irradiados com doses acima de 10 kGy*", o comité de especialistas da FAO/IAEA/OMS considerou que não é tecnicamente necessário impor uma dose limite, indicando que "*alimentos irradiados com a dose adequada para atingir o objectivo técnico pretendido são seguros para consumo e nutricionalmente adequados*" (WHO, 1999). Além disso, o *Codex Alimentarius* transpôs esta conclusão, validando o uso de doses mais elevadas "*quando for necessário para atingir um objetivo tecnológico*" (Codex, 2003).

Contudo, o Comité Científico da Alimentação, da Autoridade Europeia para a Segurança dos Alimentos (AESA) mantém nos regulamentos o limite máximo de 10 kGy. No entanto, alguns produtos como especiarias, ervas secas e legumes utilizados como tempero podem necessitar de doses até 30 kGy para a descontaminação e "*para garantir um produto num estado de higiene satisfatório*" (EFSA, 2011).

A dose recomendada para aumentar o tempo de vida de cogumelos frescos em diferentes países como Argentina, China, Israel, Coreia, México, Polónia, Bélgica, República Checa e Reino Unido é 1 a 3 kGy, enquanto a dose recomendada para a descontaminação de cogumelos secos é 10 a 50 kGy (ICGFI, 1999; Jornal Oficial da União Europeia, 2009; Akram & Kwon, 2010).

A legislação de vários países impõe um rótulo para alimentos irradiados, de acordo com a Comissão do *Codex Alimentarius.* O uso do símbolo "*Radura*" (**Figura 2.3**) é considerado opcional, sendo obrigatória a menção escrita "*alimento tratado por radiações ionizantes*" (Codex, 1999, 2003). Outros países como os Estados Unidos da América, Canadá e China, incluíram na legislação a obrigatoriedade no rótulo, do símbolo e da menção escrita (Web, 2009). Na UE, só é necessária a menção "*irradiado*" ou "*tratado por radiação ionizante*" (EU, 1999).

De acordo com as conclusões e recomendações do relatório da OMS (WHO, 1994, 1999), a situação desejável seria a não utilização do símbolo "*Radura*", tendo em conta que as agências de segurança alimentar devem garantir a qualidade dos produtos alimentares, e não necessariamente indicar o tipo de processo utilizado para garantir a sua segurança, à semelhança do que acontece com outros processos de conservação. Desta forma, o rótulo com o símbolo pode induzir a uma informação incorreta e inibir os consumidores de adquirirem o produto assim rotulado.

Figura 2.3. Símbolo "*Radura*"

2.2.3. Espécies irradiadas

Estão disponíveis na literatura estudos de avaliação dos efeitos da radiação ionizante em espécies de cogumelos cultivados, com alto valor de produção, como por exemplo, *Agaricus bisporus* (J.E.Lange), *Lentinus edodes* (Berk.) e *Pleurotus ostreatus* (Jacq. ex Fr.) P.Kumm. Estudos com outras espécies tais como *Agaricus campestres* Linnaeus, *Cantharellus tubaeformis* (Bull.), Fr., *Hypsizygus marmoreus*

(Peck) H.E. Bigelow, *Inonotus obliquus* (Ach. ex Pers.) Pilát, *Pleurotus cystidiosus* O.K. Mill, *Pleurotus nebrodensis* (Inzenga) Quél., *Tuber aestivum* Vittad. e *Volvariella volvacea* (Bulliard ex Fries) Singer, estão também disponíveis.

As espécies estudadas provêm de todo o mundo, América (Argentina, Canadá e EUA), Ásia (Japão, China, Índia, Coreia, Filipinas e Singapura) e Europa (Dinamarca, Holanda, Espanha e Suécia) (**Tabela 2.1** e referências citadas).

A radiação gama (^{60}Co), radiação por feixe de eletrões e radiação UV são técnicas aplicadas, principalmente, em cogumelos frescos (**Tabela 2.1** e referências citadas), mas também em amostras liofilizadas (Teichmann et al., 2007), desidratadas ao ar (Rivera, Blanco, Marco, Oria & Venturini, 2011), e até mesmo em extratos aquosos secos (Kim et al., 2009). As doses aplicadas vão até 5 kGy e na maioria dos casos estão entre 1 e 2 kGy; as doses de UV são muito variáveis (**Tabela 2.1**).

Têm sido analisados vários parâmetros físico-químicos e microbiológicos, após diferentes períodos de armazenamento, a maioria 15 dias, e outros até 25 dias em amostras frescas, e até 42 dias em amostras desidratadas ao ar. Em geral, as análises das amostras frescas irradiadas são efetuadas diariamente ou a cada 2-4 dias (**Tabela 2.2**).

A **Tabela 2.2** compila os efeitos nos parâmetros físico-químicos, e a **Tabela 2.3** os verificados nos parâmetros nutricionais e bioativos relativos a trabalhos disponíveis na literatura.

2.3. Influência da irradiação na composição de cogumelos

2.3.1. Influência nos parâmetros físico-químicos

Os efeitos da irradiação sobre os parâmetros físico-químicos, tais como a perda de massa, cor, textura e pH, têm sido descritos por diferentes autores que analisaram diferentes espécies de cogumelos. Os resultados estão sumarizados na **Tabela 2.2**.

Tabela 2.1. Espécies de cogumelos submetidos a irradiação e condições de irradiação.

Espécies	Origem	Amostras	Fonte de radiação	Doses	Referência
Agaricus bisporus (J.E.Lange) Emil J. Imbach	Argentina	Frescas	Gama	3 kGy	Lescano (1994)
	Canadá	Frescas	Gama	2 kGy a 4,5 e 32 kGy/h	Beaulieu et al. (1999, 2002)
	Canadá	Frescas	Gama	0,5, 1,5 e 2,5 kGy	Benoit et al. (2000)
	Hungria	Frescas	Gama	1, 3 e 5 kGy; 34 ou 35 Gy/min	Sommer et al. (2009, 2010)
	India	Frescas	Gama	0,5, 1, 1,5, 2 e 2,5 kGy a 0,028 kGy/min	Gautan et al. (1998)
	India	Frescas	Gama	0,5 e 2 kGy a 200 Gy/h	Wani et al. (2009)
	USA	Frescas	Gama	0,1 a 2 kGy de 0,05 a 2 kGy/h	Gill et al. (1969)
	Dinamarca	Frescas	Gama e feixe de eletrões	10 MeV	Skou (1974)
	China	Frescas	Feixe de eletrões	1, 2, 3 e 4 kGy	Duan et al. (2010)
	USA	Frescas	Feixe de eletrões	0,5, 1, 3,1 e 5,2 kGy	Koorapati et al. (2004)
	Coreia	Frescas	UV-B	10, 20 e 30 kJ/m^2	Ko et al. (2008)
	China	Frescas	UV-C, UV-B	0,5, 1 e 2h	Mau et al. (1998)
	Singapura	Frescas	UV-C, UV-B, UV-A	23,0, 35,3 e 25,2 kJ/m^2	Jasinghe and Perera (2006)
	Holanda	Frescas	UV-C, UV-A	94,7, 189,5 e 379,0 J/cm^2	Teichmann et al. (2007)
	USA	Frescas	UV-C	0,225, 0,45 e 0,90 kJ/m^2	Guan et al. (2012)
Agaricus campestres Linnaeus	Argentina	Frescas	Gama	3 kGy	Narvaiz (1994)
Cantharellus tubaeformis (Bull.) Fr.	Suécia	Liofilizadas	UV-C, UV-A	94,7, 189,5 e 379,0 J/cm^2	Teichmann et al. (2007)
Hypsizygus marmoreus (Peck) H.E. Bigelow	China	Frescas	Gama	0,8 kGy a 0,2 kGy/h, 1,2 kGy a 0,3 kGy/h, 1,6 kGy a 0,4 kGy/h, 2,0 kGy a 0,5 kGy/h	Xing et al. (2007)
Inonotus obliquus (Ach. ex Pers.) Pilát	Coreia	Extratos aquosos secos	Gama	3, 5, 7 e 10 kGy a 10 kGy/h	Kim et al. (2009)
Lentinus edodes (Berk.)	China	Frescas/MAP	Gama	1, 1,5 e 2,0 kGy a 2,1 kGy/h	Jiang et al. (2010b)
	China	Frescas	UV-C, UV-B	0,5, 1 e 2h	Mau et al. (1998)
	Singapura	Frescas	UV-C, UV-B, UV-A	23,0, 35,3 e 25,2 kJ/m^2	Jasinghe and Perera (2006)
	Japão	Frescas	I UV-B	25, 50 e 75 kJ/m^2	Ko et al. (2008)
	China	Frescas/MAP	UV-C	4 kJ/m^2	Jiang et al. (2010a)
Pleurotus cystidiosus O.K. Mill	Singapura	Frescas	UV-C, UV-B, UV-A	23,0, 35,3 e 252 kJ/m^2	Jasinghe and Perera (2006)
Pleurotus nebrodensis (Inzenga) Quél.	China	Frescas	Gama	0,8 kGy a 0,2 kGy/h, 1,2 kGy a 0,3 kGy/h, 1,6 kGy a 0,4 kGy/h, 2,0 kGy a 0,5 kGy/h	Xiong et al. (2009)
Pleurotus ostreatus (Jacq. ex Fr.) P.Kumm	Singapura	Frescas	UV-C, UV-B, UV-A	23,0, 35,3 e 25,2 kJ/m^2	Jasinghe and Perera (2006)
Tuber aestivum Vittad	Espanha	Secas ao ar /MAP	Feixe de eletrões	1,5 e 2,5 kGy a 98 kGy/min	Rivera et al. (2011)
Volvariella volvacea (Bulliard ex Fries) Singer	China	Frescas	UV-C, UV-B	0,5, 1 e 2 h	Mau et al. (1998)
	Filipinas	Frescas	Gama	0,5 e 1,0 kGy a 2,57 e 2,60 kGy/h	Nayga-Mercado and Alabastro (1989)

Radiação gama: Fonte ^{60}Co; UV: Radiação ultravioleta; MAP- Embalamento em atmosfera modificada.

Tabela 2.2. Efeitos da irradiação em parâmetros físico-químicos de cogumelos.

Espécies	Dias de análise	Massa	Cor	Textura	Referência
Agaricus bisporus (J.E.Lange) Emil J. Imbach	Diariamente até 18 dias[a]	n.d.	Maior preservação da cor	Firmeza similar às amostras não irradiadas	Lescano (1994)
	0, 2, 4, 7, 9 e 11[a]	n.d.	Atraso no escurecimento	n.d.	Beaulieu et al. (1999, 2002)
	Diariamente até 12 dias[a]	n.d.	Atraso no escurecimento	n.d.	Benoit et al. (2000)
	A cada 2/3 dias até 15 dias[a]	Diminuiu a perda de massa	Atraso no escurecimento	Maior preservação da textura, mantendo a firmeza durante o armazenamento	Gautam et al. (1998)
	A cada 3 dias até 18 dias[a]	Diminuiu a perda de massa	Atraso no escurecimento	n.d.	Wani et al. (2009)
	A cada 3 dias até 12 dias[a,b]	Perda de massa similar às amostras não irradiadas	Maior preservação da cor	n.d.	Skou (1974)
	7 e 11 dias[a]	n.d.	Atraso escurecimento, Maior preservação da cor	Firmeza similar às amostras não irradiadas	Gill et al. (1969)
	A cada 3 dias até 16 dias[b]	Perda de massa similar às amostras não irradiadas	n.d.	Taxa de amolecimento inferior ao longo do tempo de armazenamento; Firmeza maior do que nas amostras não irradiadas	Duan et al. (2010)
	n.d.	n.d.	Preservação da cor[b]	Firmeza similar às amostras não irradiadas, exceto para as doses elevadas	Koorapati et al. (2004)
	1 , 7, 14 e 21 dias[c]	n.d.	Acastanhamento mais intenso com o aumento da dose	n.d.	Guan et al. (2012)
Agaricus campestres Linnaeus	A cada 4 dias até 16 dias[a]	Perda de massa similar às amostras não irradiadas	n.d.	Firmeza similar às amostras não irradiadas	Narvaiz (1994)[d]
Hypsizygus marmoreus (Peck) H.E. Bigelow	A cada 3 dias até 25 dias[a]	Perda de massa similar às amostras não irradiadas	n.d.	Firmeza similar às amostras não irradiadas	Xing et al (2007)
Inonotus obliquus (Ach. ex Pers.) Pilát	Imediatamente[a]	n.d.	Preservação da cor	n.d.	Kim et al. (2009)
Lentinus edodes (Berk.)	A cada 4 dias até 20 dias[a]	Diminuição na perda de massa	Atraso no escurecimento	Firmeza similar às amostras não irradiadas; diminuição da firmeza com doses maiores	Jiang et al. (2010b)
	A cada 3 dias até 15 dias[c]	n.d.	n.d.	Elevada firmeza ao longo do tempo de armazenamento	Jiang et al. (2010a)
Pleurotus nebrodensis (Inzenga) Quél.	A cada 3 dias até 22 dias[a]	n.d.	Atraso no escurecimento	Maior preservação da textura	Xiong et al. (2009)
Tuber aestivum Vittad	Semanalmente ate 42 dias[b]	n.d.	n.d.	Ligeiro amolecimento após o tratamento; Efeitos semelhantes após uma semana	Rivera et al. (2011)
Volvariella volvacea (Bulliard ex Fries) Singer	Diariamente até 5 dias[a]	Perda de massa similar às amostras não irradiadas	Melhoria da cor	Maior preservação da textura	Nayga-Mercado and Alabastro (1989)

[a]Radiação gama (^{60}Co); [b]Feixe de eletrões; [c]Radiação Ultravioleta (UV); [d]Estes autores também avaliaram a influência do pH e os resultados são similares aos das amostras não irradiadas; n.d.- não detetável.

Skou (1974), Nayga-Mercado & Alabastro (1989), Narvaiz (1994) e Xing et al. (2007) descreveram perdas de massa semelhantes em amostras irradiadas (radiação gama) e em amostras não irradiadas (controlo). Outros autores referiram uma ligeira diminuição (Gautam, Sharma, e Thomas, 1998) ou uma redução significativa da perda de massa em amostras irradiadas (radiação gama) (Wani, Hussain, Meena, Dar, & Mir, 2009) e com feixe de eletrões (Duan et al., 2010; Jiang et al., 2010b). A perda de massa em *A. bisporus* foi indiretamente afetada, uma vez que está diretamente relacionada com o efeito da irradiação no atraso do crescimento e na maturação (Skou, 1974).

Em relação aos efeitos da radiação gama ou por feixe de eletrões na cor, todos os autores descreveram um atraso no escurecimento e, portanto, um aumento do tempo de vida útil dos cogumelos (Gill, Markakis, & Markakis, 1969; Skou, 1974; Gautam et al., 1998; Beaulieu et al., 1999; Benoit et al., 2000; Beaulieu et al., 2002; Koorapati et al., 2004; Wani et al., 2009; Xiong et al., 2009; Jian et al., 2010a). Bennoit et al. (2000) também referiram uma diminuição na taxa de respiração em *A. bisporus* e um atraso no escurecimento, principalmente após o quarto dia.

Além do efeito da diminuição do escurecimento, uma dose de irradiação de 1,2 kGy retardou significativamente (por 6-9 dias) o amolecimento do corpo frutífero de *P. nebrodensis* em comparação com amostras não irradiadas (Xiong et al., 2009). Com boas condições de armazenamento, a irradiação melhorou a cor, embora tenha produzido a descoloração em amostras frescas de *A. bisporus* (Skou, 1974).

Amostras irradiadas de *A. bisporus* (Lescano, 1994) exibiram uma maior preservação da cor e um acentuado atraso na maturação e na abertura do chapéu, sendo o processo de irradiação adequado para a comercialização até 11 dias e ainda aceitável para a alimentação até 16 dias. Também foi confirmada uma maior preservação e melhoria da cor em amostras irradiadas de *A. bisporus* (Gill et al., 1969), *I. obliquus* (Kim et al., 2009) e *V. volvacea* (Nayga-Mercado & Alabastro, 1989). No entanto, com o aumento da dose de radiação UV-C verificou-se um escurecimento mais acentuado em amostras de *A. bisporus* (Guan et al., 2011).

A textura/firmeza permaneceu inalterada em amostras de *A. bisporus* (Gill et al, 1969; Lescano., 1994), *A. campestris* (Nairvaiz, 1994) e H*. marmoreus* (Xing et al., 2007) tratadas com radiação gama. No entanto, o mesmo não foi observado quando se usaram doses maiores de radiação gama (Jiang et al., 2010b) ou beta (Koorapati et al., 2004). Vários trabalhos descrevem uma melhoria da textura/firmeza e um

atraso no amolecimento de cogumelos irradiados com feixe de eletrões (Duan et al., 2010), UV-C (Jiang, Jahangir, Jiang, Lu, & Ying, 2010) e radiação gama (Nayga-Mercado & Alabastro, 1989; Guatam et al., 1998; Xiong et al., 2009). Apenas Rivera et al. (2011) referiram um ligeiro amolecimento em amostras de *T. aestivum* após o tratamento com feixe de eletrões e após uma semana.

A acidez observada em amostras de *A. campestris* tratadas com radiação gama foi semelhante à obtida em amostras não irradiadas (Narvaiz, 1994).

2.3.2. Influência nos parâmetros químicos, nutricionais e bioatividade

Os efeitos da irradiação nos parâmetros químicos, incluindo nutrientes (proteínas, açúcares, vitaminas), não-nutrientes (fenóis, flavonoides e compostos aromáticos) e na avaliação de bioatividade, tais como a atividade enzimática da PPO, PAL, CAT, SOD, APX (ascorbato peroxidase) e GR (glutationa redutase), estão descritos por diferentes autores, em várias espécies de cogumelos e sumarizam-se na **Tabela 2.3**.

Tabela 2.3. Efeitos da irradiação nos parâmetros químicos, nutricionais e na bioatividade de cogumelos.

Espécies	Proteínas	Açúcares	Vitaminas	Compostos aromáticos	Fenóis e Flavonoides	Atividade enzimática	Referências
Agaricus bisporus (J.E.Lange) Emil J. Imbach	n.d	n.d	n.d	n.d	Níveis de fenóis mais elevados nas amostras irradiadas do que nas amostras não irradiadas	Diminuição da atividade da PPO	Beaulieu et al. (1999, 2002)
	n.d	n.d	n.d	n.d	Níveis de fenóis mais elevados nas amostras irradiadas do que nas amostras não irradiadas; aumento em 1-3 dias e diminui em 3-12 dias[a]	Aumento da atividade da PPO; aumento da atividade da PAL em 1-4 dias e diminuiu em 4-12 dias;	Benoit et al. (2000)
	n.d	n.d	n.d	Diminuição dos níveis da GDP e da AMP; GMP, tirosina e fenilalanina não afetadas	Níveis similares de fenóis em amostras irradiadas e não irradiadas[a]	n.d	Sommer et al. (2009, 2010)
	n.d.	n.d	n.d	n.d	n.d	Diminuição da atividade da PPO[a]	Gautam et al. (1998)
	n.d	Diminuição ao longo do tempo de armazenamento[b]	n.d	n.d	n.d	Diminuição da atividade da PPO em 10 dias; diminuição da atividade da SOD; diminuição da atividade da CAT	Duan et al. (2010)
		n.d	n.d	n.d	n.d	Atividade similar da PPO[b]	Koorapati et al. (2004)
	n.d	n.d	Aumento da vitamina D_2[c]	n.d	n.d	n.d	Ko et al. (2008)
	n.d	n.d	Aumento da vitamina D_2[c]	n.d	n.d	n.d	Mau et al. (1998)
	n.d	n.d	Aumento da vitamina D_2[c]	n.d	n.d	n.d	Jasinghe and Perera (2006)
	n.d	n.d	Aumento da vitamina D_2[c]	n.d	n.d	n.d	Teichmann et al. (2007)
	n.d	n.d	Diminuição do ácido ascórbico após 7 dias; valores similares após 14 dias [c]	n.d	Diminuição dos teores de fenóis após 7 dias; valores similares após 14 dias	n.d	Guan et al. (2011)
Cantharellus tubaeformis	n.d	n.d	Aumento da vitamina D_2[c]	n.d	n.d	n.d	Teichmann et al. (2007)
Hypsizygus marmoreus (Peck) H.E. Bigelow	Pequena diminuição (com as doses mais baixas) ao logo dos 13 dias de armazenamento;	Pequena diminuição ao longo do tempo de armazenamento (com as doses mais baixas); diminuição similar a outras	n.d	n.d	n.d	Diminuição da atividade da PPO inversamente proporcional à dose; aumento da atividade da	Xing et al. (2007)

	valores similares após os 25 dias (com as doses mais baixas) e menor em outras amostras irradiadas[a]	amostras irradiadas				SOD e da CAT	
Inonotus obliquus (Ach. ex Pers.) Pilát	n.d	n.d	n.d	n.d	Aumento dos teores de fenóis totais[a]	n.d	Kim et al. (2009)
Lentinus edodes (Berk.)	Pequena diminuição ao longo do tempo de armazenamento[a]	Aumento ao longo do tempo de armazenamento	Valores similares de ácido ascórbico do que nas amostras controlo	n.d	Aumento dos teores de fenóis e flavonoides	n.d	Jiang et al. (2010b)
	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Ko et al. (2008)
	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Jasinghe and Perera (2006)
	n.d	n.d	Aumento do conteúdo em ácido ascórbico	n.d	Aumento nos teores de flavonoides; sem efeito nos fenóis	Aumento da atividade da CAT, SOD, APX e GR ao longo do tempo de armazenamento	Jiang et al. (2010a)
	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Mau et al. (1998)
Pleurotus cystidiosus O.K. Mill	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Jasinghe and Perera (2006)
Pleurotus nebrodensis (Inzenga) Quél.	Pequena diminuição ao longo do tempo de armazenamento[a]	n.d	n.d	n.d	n.d	Diminuição da atividade da PPO, SOD e CAT	Xiong et al. (2009)
Pleurotus ostreatus (Jacq. ex Fr.) P.Kumm	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Jasinghe and Perera (2006)
Volvariella volvacea (Bulliard ex Fries) Singer	n.d	n.d	Aumento da vitamina D2[c]	n.d	n.d	n.d	Mau et al. (1998)
	n.d	Valores similares às amostras controlo[a]	n.d	n.d	n.d	Diminuição da atividade da PPO ao longo do tempo de armazenamento	Nayga-Mercado and Alabastro (1989)

[a]Radiação gama (^{60}Co); [b]Feixe de eletrões; [c]Radiação Ultravioleta (UV); n.d.- não detetável; GDP- guanosina 5’-difosfatase; AMP- adenosina 5’-monofosfatase; GMP- guanosina 5’-monofosfatase; PPO- polifenoloxidase; PAL- fenilalanina amónia-liase, SOD- superóxido dismutase, CAT- catalase, APX- ascorbato peroxidase, GR- glutationa redutase.

Amostras de *H. marmoreus* tratadas com a dose de 0,8 kGy (Xing et al., 2007), *L. edodes* tratadas com 1,0 kGy (Jiang et al., 2010b) e *P. nebrodensis* tratadas com 1,2 e 1,6 kGy (Xiong et al., 2009) apresentaram inicialmente um menor declínio no teor em proteínas do que as amostras não irradiadas; doses mais elevadas apresentaram efeitos negativos (Xing et al, 2007; Jiang et al, 2010b).

H. marmoreus tratados com radiação gama (Xing et al., 2007) ou *A. bisporus* irradiado com feixe de eletrões (Duan at al., 2010) em doses baixas, apresentaram uma menor redução do teor de açúcares redutores durante o período de armazenamento. No entanto, *L. edodes* tratado com 1 kGy apresentou um aumento no teor em açúcares totais (Jiang et al., 2010b); já as amostras de *V. volvacea* irradiadas com 0,5 e 1 kGy, não sofreram alterações no teor em açúcares redutores (Nayga-Mercado & Alabastro, 1989).

De acordo com Ko et al. (2008), a exposição à luz UV-B mostroiu-se uma forma eficaz de aumentar a concentração de vitamina D_2 em cogumelos. À medida que aumentaram as doses de irradiação, a concentração de vitamina D_2 também aumentou em *A. bisporus* e *L. edodes*. A irradiação com luz UV-A afetou ligeiramente o teor de vitamina D_2. Em contraste, a irradiação com luz UV-C aumentou os teores de itamina D_2 até 9 vezes em amostras liofilizadas de *C. tubaeformis* e 14 vezes em amostras frescas de *A. bisporus* (Teichmann et al., 2007). Conclusão semelhante foi obtida por Mau, Chen, & Yang (1998) em *A. bisporus*, *L. edodes* e *V. volvacea* irradiados com UV-B e UV-C. Na verdade, quantidades extraordinariamente elevadas de vitamina D_2 podem ser obtidas com a irradiação de cogumelos com luz UV. A intensidade ou taxa de dose (W m^{-2}) da radiação UV e a dose de irradiação aplicada (J m^{-2}), também contribuem para a conversão do ergosterol, presente nos cogumelos, em vitamina D_2. Mesmo em condições normais, 5 g de shiitake (*Lentinus edodes*) fresco, submetido a irradiação durante 15 minutos com UV-A ou UV-B, garantem os valores diários recomendados de vitamina D para adultos (10 µg/dia) (Jasinghe & Perera, 2006).

No que diz respeito a outras vitaminas, nomeadamente para o ácido ascórbico, os resultados descritos são contraditórios. *A. bisporus* tratado com UV-C apresentou menor teor em ácido ascórbico em comparação com as amostras controlo (Guan et al., 2012), enquanto que *L. edodes* armazenado a frio com o mesmo tratamento, revelou um aumento nos teores de ácido ascórbico (Jiang et al, 2010a). Amostras de

L. edodes irradiadas com radiação gama apresentaram teores similares às amostras controlo (Jiang et al., 2010b).

Sommer et al. (2009, 2010) avaliaram o impacto da radiação gama em 5'-nucleótidos e aminoácidos livres (tirosina e fenilalanina) de amostras frescas de *A. bisporus.* Estes autores verificaram não haver uma influência significativa nos teores de GMP, tirosina e fenilalanina, mas verificaram uma diminuição ao nível da GDP e AMP, esta última apenas com a dose de 5 kGy.

Quanto ao teor em fenóis totais, os mesmos autores descreveram uma influência não significativa, tal como observado em cogumelos shiitake tratados com UV-C. No entanto, também foi referido um aumento em flavonoides e uma melhoria na atividade enzimática (Jiang et al., 2010a). A mesma espécie (*L. edodes*) tratada com radiação gama (1 kGy) acumulou mais fenóis e flavonoides (Jiang et al., 2010b). Níveis mais baixos e similares de fenóis foram registados em amostras de *A. bisporus* submetidas a radiação UV-C, após 7 e 14 dias de armazenamento, respetivamente (Guan et al., 2012). Kim et al. (2009) descreveram que a radiação gama poderia melhorar as propriedades antioxidantes de extratos de *I. obliquus*, em consequência do aumento do teor em fenóis totais.

Relativamente à atividade enzimática, a maioria dos estudos descrevem uma diminuição da atividade da PPO em diferentes espécies de cogumelos irradiados, em contraste com um aumento da atividade verificado nas amostras controlo, ao longo do tempo de armazenamento (Nayga-Mercado & Alabastro, 1989; Gautam et al., 1998; Beaulieu et al., 1999, 2002; Xing et al., 2007; Xiong et al., 2009; Duan et al., 2010). Beaulieu et al. (1999, 2002) referem que o tempo de vida útil de *A. bisporus* foi prolongado em mais 4 dias com uma dose de 4,5 kGy/h, e apenas em 2 dias com uma dose mais elevada, 32 kGy/h. Os autores explicaram que o aumento do prazo de validade com a dose de 4,5 kGy/h e a menor atividade da PPO levou a um aumento da concentração em fenóis e, consequentemente, a uma menor formação de melanina. O escurecimento observado com a dose de 32 kGy/h foi explicado pelas mudanças na permeabilidade da membrana (entrada de oxigénio molecular no citoplasma da célula) que favorece tanto a oxidação enzimática como a não enzimática de fenóis. Após aplicação de radiação por feixe de eletrões, Duan et al. (2010) referiram uma diminuição da atividade da PPO com doses de 1-4 kGy, comparativamente às amostras controlo, após 10 dias de armazenamento. Não foram descritos efeitos significativos na atividade da PPO em amostras de *A.*

bisporus também submetidas a radiação por feixe de eletrões (Koorapati et al., 2004). A atividade da SOD diminuiu, ao longo do tempo de armazenamento, nas amostras irradiadas e nas amostras controlo, mas não se verificou nenhuma correlação evidente entre a atividade enzimática e a dose de radiação por feixe de eletrões. A CAT diminuiu lentamente nos cogumelos expostos à dose de 1 kGy, em comparação com as amostras controlo e com as amostras irradiadas com outras doses (Duan et al., 2010). Verificou-se uma tendência semelhante na atividade da SOD e da CAT em amostras irradiadas de *P. nebrodensis* (Xiong et al., 2009). Por outro lado, Xing et al. (2007) descreveram níveis significativamente elevados da atividade da SOD em amostras de *H. marmoreus* expostas a 0,8 kGy em comparação com amostras controlo. Foi detetado um aumento inicial na atividade da CAT nas amostras irradiadas com 0,8, 1,2, e 1,6 kGy, embora os níveis da atividade enzimática tenham diminuído, gradualmente, em todas as amostras durante o armazenamento; os níveis residuais após 25 dias eram ainda superiores nas amostras irradiadas. No entanto, existe um estudo que descreve um aumento na atividade da PPO até ao 7º, 9º, e 12º dia em amostras de *A. bisporus* tratadas com as doses de 0,5, 1 e 2 kGy, respetivamente. Outros autores referem um aumento da atividade da PAL na fase inicial de armazenamento (1-4 dias). A PAL está diretamente ligada à síntese de fenóis, sendo que a radiação gama provocou um aumento significativo do teor de fenóis (1-3 dias). A partir dos 3-4 dias e no fim do período de armazenamento (12º dia), tanto a atividade da PAL como o teor de fenóis nas amostras irradiadas diminuíram (Benoit et al., 2000).

Ainda é necessária mais investigação sobre a irradiação de cogumelos, no sentido da obtenção de produtos microbiologicamente seguros, mantendo o seu valor nutricional e qualidade sensorial. O prazo de validade deve ser aumentado para permitir uma melhor distribuição e comercialização. Além disso, é necessária uma abordagem tecnológica que leve ao desenvolvimento de tecnologias de processamento e distribuição de cogumelos frescos, o que iria resolver algumas das limitações que os produtores e vendedores encontram atualmente para manter a qualidade dos cogumelos. A irradiação de alimentos é uma das melhores e mais seguras técnicas de conservação destinada a assegurar o fornecimento de cogumelos com qualidade e com um tempo de vida útil prolongado. Pode também

contribuir significativamente para a melhoria da saúde pública, uma vez que o risco de doenças de origem alimentar pode ser minimizado com o uso adequado desta tecnologia. A irradiação de alimentos é utilizada em diversos países, uma vez que os consumidores estão cada vez mais conscientes do papel da irradiação de alimentos em relação à sua segurança e aumento do prazo de validade. Particularmente, a dose recomendada para o aumento do tempo de vida útil de cogumelos frescos em diferentes países é entre 1-3 kGy. No entanto ainda há que ultrapassar alguns receios associados à irradiação de alimentos por parte dos consumidores e cabe à comunidade científica demonstrar a adequação desta metodologia à obtenção de alimentos saudáveis, seguros e com adequadas características organoléticas.

2.4. Bibliografia

Aida, F.M.N.A., Shuhaimi, M., Yazid, M., & Maaruf, A.G. (2009). Mushroom as a potential source of prebiotics: a review. *Trends in Food Science & Technol*ogy, *20*, 567-575.

Ajlouni, S.O., Beelman, R.B., & Thompson, D.B. (1993). Influence of gamma irradiation on quality characteristics, sugar content, and respiration rate of mushrooms during postharvest storage. In Food Flavors, Ingredients and Composition; Charalambous, C., Ed.; Elsevier Publishing Co.: Amsterdam, The Netherlands, 103-121.

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society for Applied Biological Chemistry*, *53*, 257-265.

Beaulieu, M., D'Aprano, G., & Lacroix, M. (1999). Dose rate effect of γ irradiation on phenolic compounds, polyphenol oxidase, and browning of mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry*, *47*, 2537-2543.

Beaulieu, M., D'Aprano, G., & Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry*, *63*, 311–315.

Benoit, M.A., D'Aprano G., & Lacroix M. (2000). Effect of γ-irradiation on phenylalanine ammonia-lyase activity, total phenolic content, and respiration of mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry*, *48*, 6312-6316.

Codex. (1999). General Standard for the Labeling of Prepackaged Foods *CODEX STAN 1-1985*, *Rev. 2-1999*: Codex Alimentarius Commission.

Codex. (2003). General Standard dor irradiated Foods *CODEX STAN 106-1983*, *Rev. 1-2003*: Codex Alimentarius Commission.

Chang, S.-T., & Miles, P.G. (2004). Mushrooms: Cultivation, nutritional value, medicinal effect, and environmental impact, 2nd ed.; CRC Press: Boca Raton.

Cliffe-Byrnes, V., & O'Beirne, D. (2008). Effects of washing treatment on microbial and sensory quality of modified atmosphere (MA) packaged fresh sliced mushroom (*Agaricus bisporus*). *Postharvest Biology and Technology*, *48*, 283–294.

Codex for Food Irradiation. Recommended International Code of Practice for Radiation Processing of Food (CAC/RCP 19-1979, Rev. 2-2003) http://www.codexalimentarius.net.

Comissão Europeia. 2015. Report from the commission to the european parliament and the council on food and food ingredients treated with ionising radiation for the year 2013. http://ec.europa.eu/transparency/regdoc/rep/1/2015/EN/1-2015-69-EN-F1-1.PDF.

Doores, S., Kramer, M., & Beelman, R. (1987). Evaluation and bacterial populations associated with fresh mushrooms (*Agaricus bisporus*), in Cultivating Edible Fungi: Developments in Crop Science, ed. by Wuest PJ, Royce DL and Beelman RB. Elsevier, Amsterdam, 283–294.

Duan, Z., Xing, Z., Shao, Y., & Zhao, X. (2010). Effect of electron-beam irradiation on postharvest quality and selected enzyme activities of the white button mushroom, *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*, *58*, 9617–9621.

EU (1999). On the approximation of the laws of the Member States concerning foods and food ingredientes treated with ionising radiation. *Directive 1999/2/EC, Official Journal of the European Communities* (p. 16-22): European Comission.

Ferreira, I.C.F.R., Barros, L., & Abreu, R.M.V. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, *16*, 1543-1560.

Ferreira, I.C.F.R., Vaz, J.A., Vasconcelos, M.H., & Martins, A. (2010). Compounds from wild mushrooms with antitumor potential. *Anti-cancer Agents in Medicinal Chemistry*, *10*, 424-436.

Gautam, S., Sharma, A., & Thomas, P. (1998). Gamma irradiation effects on shelf-life, texture, polyphenol oxidase ans microflora of mushroom *(Agaricus bisporus*). *International Journal of Food Science and Nutrition*, *49*, 5–10.

Gill, W.J., Markakis N., & Markakis, P. (1969). Irradiation of cultured mushrooms. *Food Technology*, *23*, 111–114.

Gould, G.W. (1989). Mechanisms of action of food preservation procedures. Elsevier Applied Science, London, 2, 4, 7, 9.

Grangeia, C., Heleno, S.A., Barros, L., Martins, A., & Ferreira, I.C.F.R. (2011). Effects of trophism on nutritional and nutraceutical potential of wild edible mushrooms. *Food Research International*, *44*, 1029-1035.

Guan, W., Fan, X., & Yan, R. (2012). Effects of UV-C treatment on inactivation of *Escherichia coli* O157:H7, microbial loads, and quality of button mushrooms. *Postharvest Biology and Technology*, *64*, 119-125.

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Santos-Buelga, B., & Ferreira, I.C.F.R. (2011). Targeted metabolites analysis in wild Boletus species. *LWT*, *44*, 1343-1348.

International Consultative Group on Food Irradiation (ICGFI). (1999). In Facts about Food Irradiation. Buckinghamshire, United Kingdom.

Jasinghe, V.J., & Perera, C.O. (2006) . Ultraviolet irradiation: The generator of Vitamin D2 in edible mushrooms. *Food Chemistry*, *95*, 638–643.

Jiang, T., Jahangir, M.M., Jiang, Z., Lu, X., & Ying, T. (2010a). Influence of UV-C treatment on antioxidant capacity, antioxidant enzyme activity and texture of postharvest shiitake (*Lentinus edodes*) mushrooms during storage. *Postharvest Biology and Technology*, *56*, 209-215.

Jiang, T., Luo, S., Chen, Q., Shen, L., & Ying, T. (2010b). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). *Food Chemistry*, *122*, 761–767.

Jolivet, S., Arpin, N., Wichers, H.J., & Pellon, G. (1998). *Agaricus bisporus* browning: a review. *Mycological Research*, *102*, 1459–1483.

Jornal Oficial da União Europeia (2009). Lista das autorizações dos Estados-Membros de alimentos e ingredientes alimentares que podem ser tratados por radiação ionizante. 24.11.2009, pp. C 283/5. ISSN 1725-2482. http://eur-lex.europa.eu/legal-content/PT/TXT/PDF/?uri=OJ:C:2009:283:FULL&from=PT.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9-16.

Kim, J.-H., Sung, N.-Y., Kwon, S.-K., Srinivasan, P., Song, B.-S., Choi, J., Yoon Y., Kim, J.K., Byun, M.-W., Kim M.-R., & Lee J.-W. (2009). γ-Irradiation improves the color and antioxidant properties of chaga mushroom (*Inonotus obliquus*) extract. *Journal of Medicinal Food*, *12*, 1343–1347.

Ko, J.A., Lee, B.H., Lee, J.S., & Park, H.J. (2008). Effect of UV-B exposure on the concentration of vitamin D2 in sliced shiitake mushroom (*Lentinus edodes*) and white button mushroom (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry*, *56*, 3671–3674.

Koorapati, A., Foley, D., Pilling, R., & Prakash, A. (2004). Electron-beam irradiation preserves the quality of white button mushrooms (*Agaricus bisporus*) slices. *Journal of Food Science*, *6*, 25–29.

Kume, T., Furuta, M., Todoriki, S., Uenoyama, N., & Kobayashi, Y. (2009). Status of food irradiation in the world. *Radiation Physics and Chemistry*, *78*, 222–226.

Lescano, G. (1994). Extension of mushroom (*Agaricus bisporus*) shelf life by gamma irradiation. *Postharvest Biology and Technology*, *4*, 255–60.

Lopez-Briones, G., Varoquaux, P., Chambroy, Y., Bouquant, J., Bureau, G., & Pascat, B. (1992). Storage of common mushroom under controlled atmospheres. *International Journal of Food Science and Technology*, *27*, 493–505.

Masson, Y., Ainsworth, P., Fuller, D., Bozkurt, H., & Ibanoglu, S. (2002). Growth of *Pseudomonas fluorescens* and *Candida sake* in homogenized mushrooms under modified atmosphere. *Journal of Food Engineer*, *54*, 125–131.

Martinez, M.V., & Whitaker, J.R. (1995). The biochemistry and control of enzymatic browning. *Trends in Food Science and Technology*, *6*, 195–200.

Mau, J.L., Chen, P.R., & Yang, J.H. (1998). Ultraviolet irradiation increased vitamin D_2 content in edible mushrooms. *Journal of Agricultural and Food Chemistry*, *46*, 5269–5272.

Minnaar, A., Taylor, J.R.N., & McGill, A.E.J. (1995). Heat-irradiation combination processing as an effective method of producing high quality shelf-stable, low-acid food products. *Food Control*, *6*, 165–170.

Murr, D. P., & Morris, L.L. (1975). Effect of storage temperature on postharvest changes in mushrooms. *Journal of American Society for Horticultural Science*, *100*, 16–19.

Narvaiz, P. (1994). Some Physicochemical measurements on Mushrooms (*Agaricus bisporus*) irradiated to extend shelf-life. *Lebensmittel-Wissenschaft & Technologie*, *27*, 7–10.

Nayga-Mercado, L., & Alabastrof, E.F. (1989). Effects of irradiation on the storage quality of fresh straw mushrooms *Volvariella volvacea. Food Quality and Preference*, *1*, 113–119.

Nussinovitch, A., & Kampf, N. (1993). Shelf life extension and conserved texture of alginate coated mushrooms (*Agaricus bisporus*). *Journal of Food Technology*, *26*, 469–475.

Organização para a Alimentação e Agricultura (FAO) (2011). Mushroom Industry Report. Economic Research Service, U.S. Department of Agriculture, USA. http://usda.mannlib.cornell.edu/MannUsda/viewDocumentInfo.do?documentID=1395 (accessed August 20, 2014).

Ouzouni, P.K., Petridis, D., Koller, W.-D., & Riganakos, K.A. (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, *115*, 1575–1580.

Pai, T. (2000). Effects of storage environmental conditions on weight loss whiteness change and microbial activity of mushrooms (*Agaricus bisporus*). *Agricultural Chemistry and Biotechnology*, *43*, 161–164.

Poucheret, P., Fons, F., & Rapior, S. (2006). Biological and pharmacological activity of higher fungi: 20-Year retrospective analysis. *Mycologie*, *27*, 311-333.

Ramos, C., Sapata, M., Ferreira, A., Andrada, L., & Candeias, M. (2011). Produção de três espécies de cogumelos *Pleurotus* e avaliação da qualidade em atmosfera modificada. *Revista de Ciências Agrárias*. 7-64.

Rivera, C.S., Blanco, D., Marco, P., Oria, R., & Venturini, M.E. (2011). Effects of electron-beam irradiation on the shelf life, microbial populations and sensory characteristics of summer truffles (*Tuber aestivum*) packaged under modified atmospheres. *Food Microbiology*, *28*, 141-148.

Roy, S., Anantheswaran, R.C., & Beelman, R.B. (1995). Fresh mushroom quality as affected by modified atmosphere packaging. *Journal of Food Science*, *60*, 334–340.

Sadler, M. (2003). Nutritional properties of edible fungi. *Nutrition Bulletin*, *28*, 305-308.

Sapers, G.M., Miller, R.L., Pilizota, V., & Kamp, F. (2001). Shelf-life extension of fresh mushrooms (*Agaricus bisporus*) by application of hydrogen peroxide and browning inhibitors. *Journal of Food Science*, *66*, 362–366.

Schmidt, H.M., Palekar, M.P., Maxim, J.E., & Castillo, A. (2006). Improving the microbiological quality and safety of fresh-cut tomatoes by low dose electron-beam irradiation. *Journal of Food Protection*, *69*, 575–581.

Singh, P., Langowski, H.-C., Wanib, A.A., & Saengerlaub, S. (2010). Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: a review. *Journal of Science and Food Agriculture*, *90*, 1393–1402.

Skou, J.P. (1974). Effects of ionizing irradiation in mushrooms as influenced by physiological and environmental conditions. *Radiation Botany*, *14*, 287-299.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2009). Effect of γ-irradiation on agaritine, γ-glutaminyl-4-hydroxybenzene (GHB), antioxidant capacity, and total phenolic content of mushrooms (*Agaricus bisporus*). *Journal of Agricultural and Food Chemistry, 57*, 5790–5794.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (*Agaricus bisporus*). *Food Chemistry*, *123*, 171–174.

Teichmann, A., Dutta, P.C., Staffas, A., & Jägerstad, M.J. (2007). Sterol and vitamin D2 concentrations in cultivated and wild grown mushrooms: Effects of UV irradiation. *LWT*, *40*, 815–822.

USFDA. (2002). United States Food and Drug Administration, Ultraviolet radiation for the processing and treatment of food, Code of Federal Regulations, 21 Part 179.39.

Venturini, M. E., Reyes, J. E., Rivera C. S., Oria, R., & Blanco, D. (2011). Microbiological quality and safety of fresh cultivated and wild mushrooms commercialized in Spain. *Food Microbiology*, *28*, 1492-1498.

Wani, A.M., Hussain, P.H., Meena, R.S., Dar, M.A., & Mir, M.A. (2009). Effect of gamma irradiation and sulphitation treatments on keeping quality of white button mushrooms *Agaricus bisporus* (J. Lge). *International Journal of Food Science and Technology*, *44*, 967–973.

Web (2009). Safety of Irradiated Food. *Risk Assessement Studies, Report No. 37. Centre for Food Safety, Food and Environmental Hygiene Department. The Government of the Hong Kong Special Administrative.* http://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_ft_01_03_irfood.html. Acesso em 20 de janeiro de 2015.

WHO, World Health Organization (1999). High-Dose Irradiation: Wholesomeness of food irradiated with doses above 10kGy. *Technical Report Series No. 890*, Geneva, Switzerland: World Health Organization.

WHO (World Health Organisation). (1994). Safety and nutritional adequacy of irradiated food. WHO, Geneva.

WHO (World Health Organization) (1999). High-Dose Irradiation: Wholesomeness of food irradiated with doses above 10kGy. *Technical Report Series No. 890*, Geneva, Switzerland: World Health Organization.

Xing, Z., Wang, Y., Feng, Z., Zhao, Z., & Liu, X. (2007). Effect of ^{60}Co-irradiation on postharvest quality and selected enzyme activities of *Hypsizygus marmoreus* fruit bodies. *Journal of Agricultural and Food Chemistry*, *55*, 8126–8132.

Xiong, Q.-L., Xing, Z.-T., Feng, Z., Tan, Q., & Bian, Y.-B. (2009). Effect of ^{60}Co γ-irradiation on postharvest quality and selected enzyme activities of *Pleurotus nebrodensis*. *LWT*, *42*, 157-161.

Yuk, H.G., Yoo, M.Y., Yoon, J.W., Marshall, D.L., & Oh, D.H. (2007). Effect of combined ozone and organic acid treatment for control of *Escherichia coli* O157:H7 and *Listeria monocytogenes* on enoki mushroom. *Food Control*, *18*, 548–553.

CAPÍTULO 3.

Efeito da radiação gama nos parâmetros físico-químicos, nutricionais e bioativos de cogumelos silvestres

Este capítulo apresenta os efeitos da radiação gama nos parâmetros físico-químicos, nutricionais e bioativos de cogumelos silvestres Lactarius deliciosus *L.*, Boletus edulis *Bull. e* Hydnum repandum *L. Fr.*

3.1

Efeito da radiação gama nos parâmetros físicos de cogumelos silvestres *Lactarius deliciosus* L.

Este sub-capítulo apresenta os efeitos da radiação gama (doses 0, 0,5 e 1 kGy) e do tempo de armazenamento (0, 4 e 8 dias) nos parâmetros físicos, nomeadamente cor, diâmetro do chapéu, perda de massa e temperatura de armazenamento, em amostras de Lactarius deliciosus *L.*

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Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* L. wild edible mushroom

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Abstract

Studies evaluating the effects of ionizing radiation on mushrooms are mostly available in cultivated rather than with wild species. In the present work, the effects of gamma radiation dose (0, 0.5 and 1 kGy) and storage time (0-8 days at 5 ºC) on the physical parameters (colour, cap diameter and weight) of the wild edible mushroom *Lactarius deliciosus* were evaluated. A slight decrease in redness (*a*) with irradiation dose and a slight decrease in the cap diameter with storage time were observed. The results of weight loss profiles during 8 days of storage were very similar for irradiated and non-irradiated samples. Overall, this study demonstrated that up to 1 kGy, gamma irradiation and cold storage did not significantly affect the measured physical properties.

Keywords: Gamma irradiation; Wild mushrooms; *Lactarius deliciosus*; Colour; Cap diameter; Weight

3.1.1. Introduction

Mushrooms are highly perishable and have a limited shelf-life, frequently only 1-3 days at room temperature, and tend to lose quality immediately after harvest (Akram and Kwon, 2010; Sommer et al., 2010). This is an impediment to the distribution and marketing of the fresh product.

The high respiration rate, the lack of physical protection to avoid water loss and the changes due to microbial attack are often associated with mushrooms loss of quality, contributing to their deterioration through browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture changes (Akram and Kwon, 2010; Singh et al., 2010; Sommer et al., 2010).

Bacteria, moulds, enzyme activity (mainly polyphenoloxidase, PPO) and biochemical changes can cause spoilage during storage. Furthermore, browning of mushroom cells occurs when they are subjected to forces that can disrupt cellular integrity such as vibration, rough handling, and ageing (Beaulieu et al., 2002; Jiang et al., 2010). The most important factors determining the rate of enzymatic browning are tissue concentrations of active PPO, pH, temperature, water activity and oxygen availability. Water loss or transpiration are important physiological processes that affect the main quality characteristics of fresh mushrooms, such as weight, appearance and texture, and these processes are dependent on surrounding temperatures and relative humidity (Pai, 2000; Singh et al., 2010).

Morphological changes, which involve exposure of the gills and sporulation, are supported by substrates which are present in the sporophore at harvest, rather than substrates of mycelial origin, as is the case of the growing sporophore. Thus the substrate expended in postharvest sporophore development, and hence respiration, is also an important factor in determining the onset of senescence; the overall decline in respiratory activity seen after harvest is due to the exhaustion of substrates and senescence of the tissues (Singh et al., 2010).

Extended shelf-life, therefore, is a key factor for making any food commodity more profitable and commercially available for long periods of time at the best possible quality. The producer will benefit from a longer shelf-life to develop markets

over greater distances (Akram and Kwon, 2010). A general trend in food preservation research is towards the development of preservation techniques that are less severe and therefore less damaging to food products (Gould, 1989; Minnaar et al., 1995). In this sense, there has been extensive research on finding the most appropriate technology for mushroom preservation.

Food irradiation appears as a possible alternative for stored mushrooms in order to enhance their shelf-life as well as their safety. Gamma irradiation has particularly been shown to be a potential tool in extending the postharvest shelf-life of fresh mushrooms (Beaulieu et al., 2002).

Studies evaluating the effects of ionizing radiation are mostly available in cultivated species with high production value such as *Agaricus bisporus* (Wani et al., 2009), *Lentinus edodes* (Jiang et al., 2010) and *Pleurotus ostreatus* (Jasinghe and Perera, 2006). Reports on wild species are scarce, and as far as we know, there are no studies evaluating the effects of irradiation on wild *Lactarius deliciosus* species. It should be highlighted that wild species are also considered as added-value foods for commercialization.

In the current research, the effects of gamma radiation doses and storage time on *L. deliciosus* physical parameters (colour, cap diameter and weight) were evaluated.

3.1.2. Materials and methods

Samples and samples irradiation

L. deliciosus fruiting bodies were collected in Trás-os-Montes (Northeast of Portugal) in November 2011, and divided into three groups: control (non-irradiated, 0 kGy), sample 1 (0.5 kGy), and sample 2 (1 kGy), with eighteen specimens per group. In each group, specimens at different maturity stages (distinguished by mushroom cap diameter) were included (**Figure 3.1.1**).

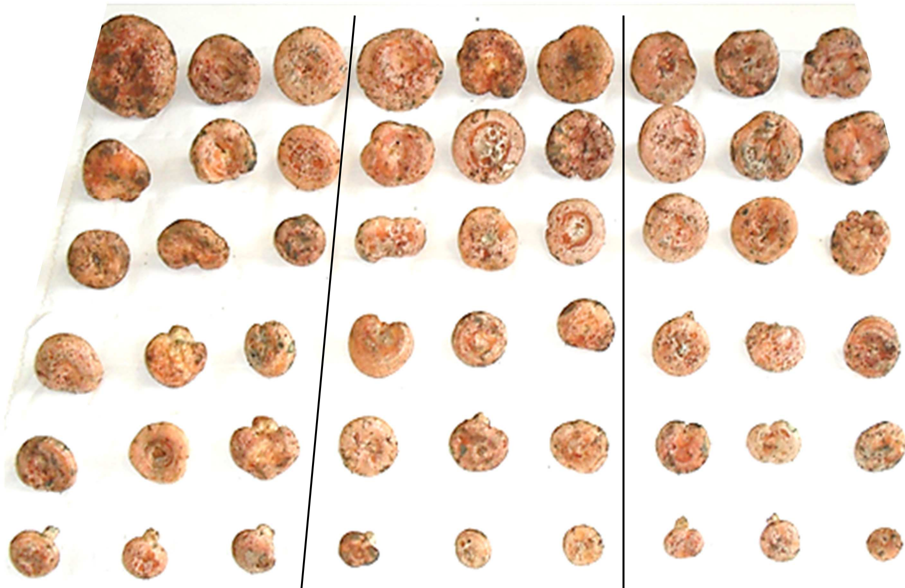


Figure 3.1.1. *Lactarius deliciosus* population divided into three groups: control (non-irradiated, 0 kGy), sample 1 (0.5 kGy) and sample 2 (1 kGy) with eighteen specimens per group (separated by lines). Several stages of maturity (distinguished by the cap diameter) may be found among each group. The cap diameter of the samples at day 0 of storage varied from 25 to 55 mm within each group.

The estimated dose rate for the irradiation position was obtained with a Fricke dosimeter, a reference standard that provides a reliable means of measurement of absorbed doses in water, based on an oxidation process of ferrous to ferric ions in an acidic aqueous solution by ionizing radiation. The acid aqueous Fricke dosimeter solution was prepared and read following the standard procedure (ASTM, 1992). The irradiation of groups 1 and 2 was performed immediately after harvest, in a Co-60 experimental chamber with four sources, total activity 267 TBq (6.35 kCi; Precisa 22, Graviner Manufacturing Company Ltd., U.K.).

After irradiation geometry dose rate estimation, using the Fricke dosimeter and the procedure described in the standards (ASTM, 1992), groups 2 and 3 were placed in poly(methyl methacrylate) (PMMA) box or acrylic glass, and irradiated at ambient atmosphere and temperature (15 ^{o}C). To monitor the process during irradiation, 4 routine dosimeters were used for the highest dose (1 kGy) in the corners of the irradiation box (Amber Perspex dosimeters, batch V, from Harwell company, U.K.). The samples were rotated upside down (180°) half of the time, to increase the dose

uniformity. The Amber Perspex dosimeters were twice read in a UV-VIS Spectrophotometer (Shimadzu mini UV 1240 spectrophotometer) at 603 nm, to estimate the dose according to a previous calibration curve. The estimated doses after irradiation were 0.6 ± 0.1 kGy and 1.1 ± 0.1 kGy for samples 2 and 3, respectively, at a dose rate of 2.3 ± 0.1 kGy h^{-1}. For simplicity, in the text, tables and graphs we used the values 0, 0.5 and 1 kGy, for non-irradiated and irradiated samples.

Colour measurement

A Minolta spectrophotometer (Konica Minolta Sensing, Inc., Chroma Meter CR-400, Japan) was used to measure daily the colour in three distinct zones of the mushroom surface, and these were considered the average value. Using illuminant C and the diaphragm opening of 8 mm, the Hunter colour *L*, *a* and *b* values were reported through the computerized system using a colour data software Spectra Magic Nx (version CM-S100W 2.03.0006, Konica Minolta company, Japan). The instrument was calibrated to standard white tiles before analysis (Spectra Magic NX Instruction Manual, Konica Minolta Sensing, Inc. (ver 2.0), 2009, Japan).

Cap diameter measurement

A digital caliper with precision of 0.01 mm was used to measure daily the diameter of the mushroom cap. Minimum and maximum diameters were obtained for each sample, to provide an average value.

Weight loss

An electronic balance Kern ABS (Type ABS 220-4, Germany) was used to daily weigh the samples. Weight loss was determined by periodical weighing, and was calculated as:

Weight loss (%) = $[(W_i - W_s)/ W_i] \times 100$.

where W_i - initial weight, W_s - weight at sampling period (Wani et al., 2009).

Temperature measurement

The samples were collected and transported in a thermal hermetic cage, with a USB data logger (model LASEL – USB-2+, from Lascar Electronics Ltd., U.K.) placed inside, using a software Easy-Log USB version 5.45, for temperature, dew point and humidity monitoring during storage.

3.1.3. Statistical analysis

For each of the storage times and irradiation doses, three samples were analysed, with all the assays being carried out in triplicate. An analysis of variance (ANOVA) with Type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software, version 18.0 (SPSS, Inc.). All dependent variables were analysed using a 2-way ANOVA, the main factors being the storage time (ST) (daily, 0-8 days) and the irradiation dose (ID) (0, 0.5 and 1 kGy).

When a statistical significant interaction effect (ID × ST) was found, the two factors were evaluated simultaneously by plotting the estimated marginal means for all levels of each factor. When the interaction was not found, data were compared by multiple comparisons, using Tukey's test, whenever the homoscedasticity requirement was fulfilled.

Hierarchical cluster analysis (HCA) was used as an unsupervised learning method. HCA was applied to standardized data to investigate similarities between samples stored for different periods or irradiated with different doses. HCA calculates the distances (or correlation) between all samples using a defined metric such as squared Euclidean distance or Chebychev distance. Hierarchical clustering is the most common approach in which clusters are formed sequentially. The most similar objects are first grouped, and these initial groups are merged according to their similarities. Eventually as the similarity decreases all subgroups are fused into a single cluster. The statistical tests were performed at a 5% significance level.

3.1.4. Results and discussion

Our previous studies assessing the potential of gamma irradiation as a suitable technique to increase natural product shelf-life were focused on nutritional and chemical parameters, or bioactive potential (Antonio et al., 2011; Fernandes et al., 2011a,b; Barreira et al., 2012), as well as the way these features changed with time. In general, the results obtained seemed to indicate that storage time caused more obvious modification of the evaluated parameters than the radiation dose. However, the application of gamma radiation, like any other conservation technique, demands the maintenance of the physical characteristics (such as colour, shape or weight) of the targeted food product.

The results of Hunter's colour *L* (lightness), *a* (redness) and *b* (yellowness) (Hunter and Harold, 1987) measurements obtained for the studied samples are presented in **Table 3.1.1.**

L, depending on reflectivity of the determined surface, was used to express luminosity of the sample surface. Lower *L* values indicate darkening of the mushroom, while increasing *a* values show increasing redness, and increasing *b* values suggests increasing yellowness of the samples (Du et al., 2009). The results are reported as mean values of each irradiation dose (ID) over the different storage times as well as mean values of all ID for each storage time (statistical treatment considering two factors). In this way, it is possible to evaluate the effect of irradiation without the potential influence of storage time, an essential feature in considering irradiation as a potential technique for increasing shelf-life. The applied multiple comparisons showed significant differences among 0 and 8 days, regarding storage time, and samples irradiated with 1 kGy and those treated with 0.5 kGy or non-irradiated. The differences induced by ID were clearer for *a* (**Figure 3.1.2a**) and *L* (**Figure 3.1.2b**) parameters.

Table 3.1.1. Hunter's colour *L* (lightness), *a* (redness) and *b* (yellowness) of non-irradiated and irradiated *Lactarius deliciosus* samples, after different storage times. The results are presented as mean ± SD[a] (n = 9, for each storage time, ST, n = 24 for each irradiation dose, ID).

		L value	*a* value	*b* value
ST	0 days	43±4	12±3 a	16±2 a
	1 day	42±4	11±2 ab	15±2 ab
	2 days	40±5	10±2 abc	15±2 abc
	3 days	41±4	11±2 abc	15±2 abc
	4 days	41±4	11±2 abc	15±2 abc
	5 days	40±4	10±3 abc	15±2 bc
	6 days	39±4	10±3 bc	15±2 bc
	7 days	39±4	10±3 bc	14±2 bc
	8 days	38±4	10±3 c	14±2 c
	p-value	<0.001	<0.001	<0.001
ID	0.0 kGy	42±4	11±3 a	15±2
	0.5 kGy	40±4	11±3 a	15±2
	1.0 kGy	39±4	10±3 b	15±2
	p-value	<0.001	0.001	0.471
ST×ID	*p*-value	0.044	0.978	0.552
Levene's test	*p*-value	0.304	0.768	0.609

[a] Results are reported as mean values of each irradiation dose (ID) over the different storage times (ST) as well as mean values of all ST within each ID. Therefore, SD reflects values in those samples (under different ID or ST).

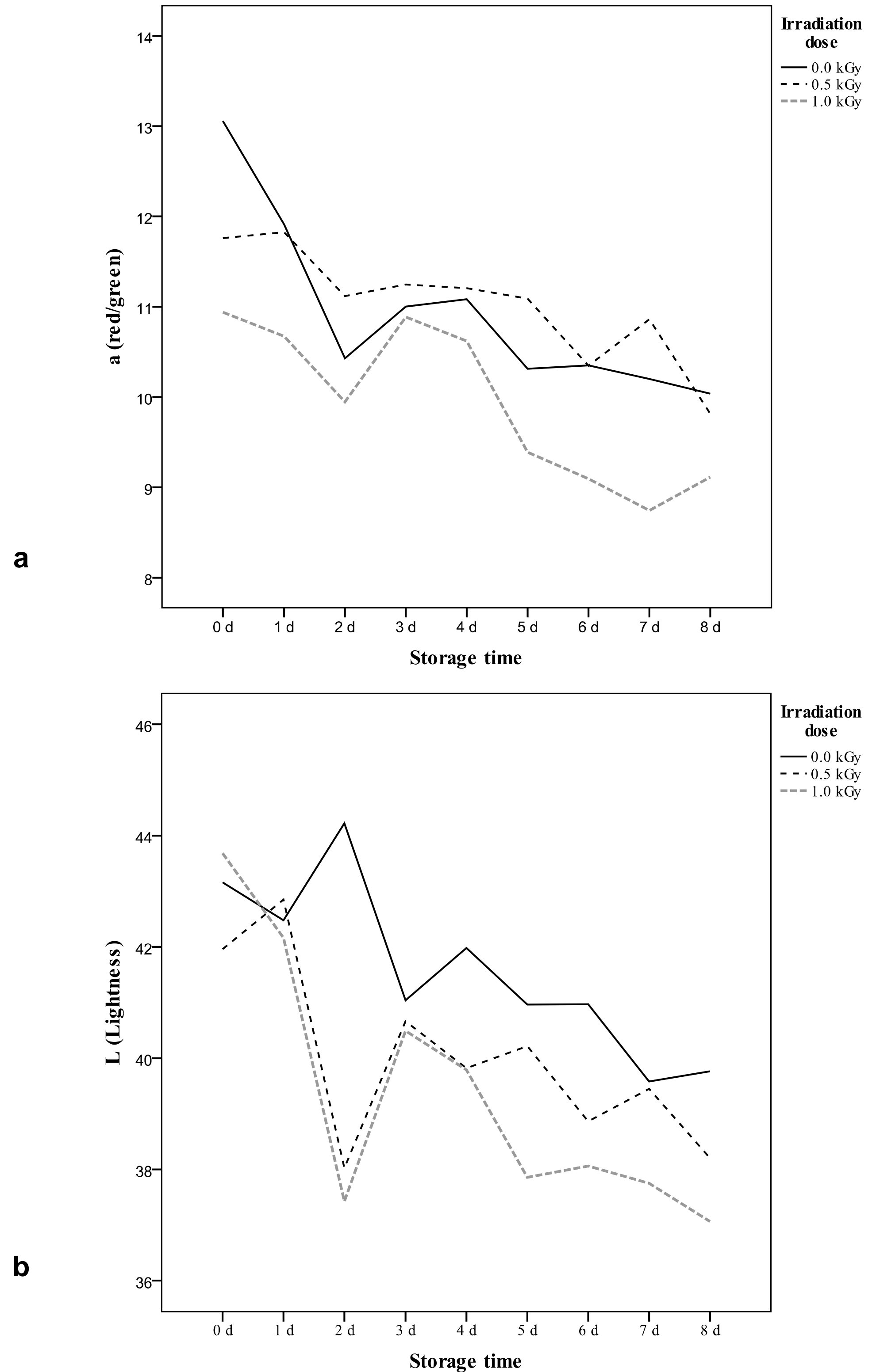


Figure 3.1.2. Interactions between ST (storage time) and ID (irradiation dose) effects on *Lactarius deliciosus* samples. Influence on redness (a) and lightness (b) parameters.

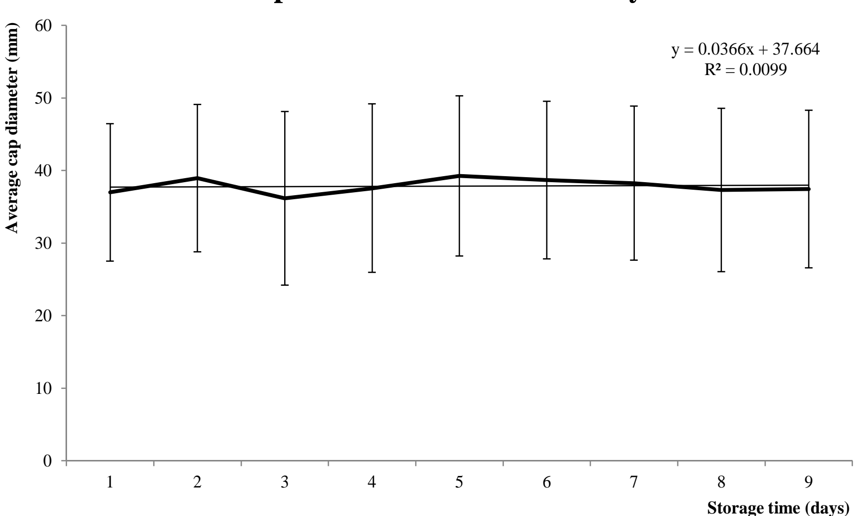
The decrease in redness (*a*) is in accordance with previous studies assessing the effect of irradiation on mushrooms (Kim et al., 2009) or plants (Jo et al., 2003a,b; Kim et al., 2006), and might be related to a secondary effect of water radiolysis. It is known that this process may result in the production of chemical species such as hydrated electrons, hydroxyl radicals or hydrogen atoms that might oxidize colour compounds such as carotenoids (Kim et al., 2009).

With regard to cap diameter, it was decided to analyse the results individually obtained for each ID. Despite the eighteen mushrooms selected for each ID being representative of different maturity stages (with high variation in cap diameter, **Figure 3.1.1**), there were differences among the samples selected for each group and corresponding to the same maturity stage, which makes discussion of the results difficult. In general, the cap diameter tended to decrease (as can be observed by the negative slope in the equations corresponding to 0 and 1 kGy, **Figure 3.1.3**), reaching the lowest values after 8 days of storage for all doses. The high standard deviations observed for each group are related to the presence of mushrooms in different maturity stages (distinguished by the cap diameter) which comprised a representative sample. The plotted values are the means of the eighteen mushrooms measured at each storage period and for each irradiation dose.

Non-irradiated samples

Average cap diameter (mm)
60
50
40
30
20
10
0
1 2 3 4 5 6 7 8 9
Storage time (days)
y = -0.4314x + 37.506
R² = 0.658

Samples irradiated with 0.5 kGy



Samples irradiated with 1.0 kGy

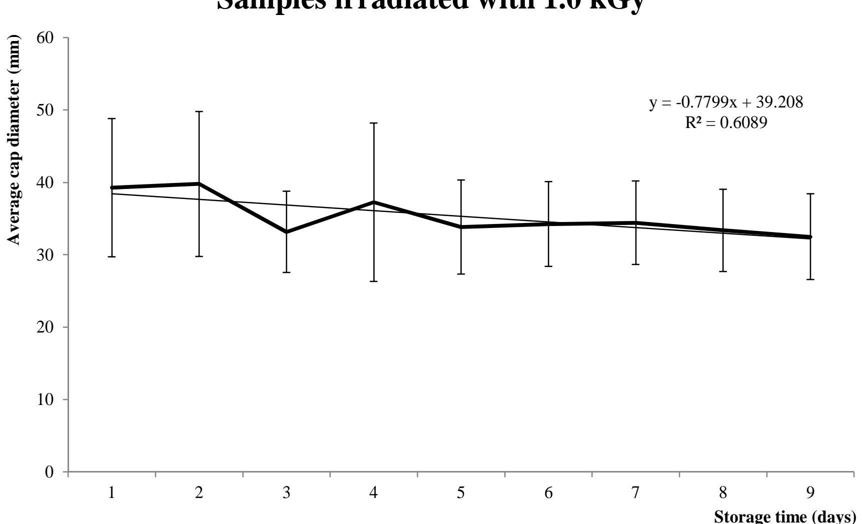


Figure 3.1.3. Variation in cap diameter during 8 days of storage.

With regard to weight loss profiles during the 8 days of storage, the results were very similar for irradiated and non-irradiated samples (**Figure 3.1.4**). The observed decrease would certainly be higher if samples were not refrigerated. The storage conditions were also controlled and can be checked in **Figure 3.1.5** in which temperature, dew point and relative humidity are plotted. The peaks in temperature and dew point, that overlap the valleys in relative humidity, correspond to the measurement periods in which samples were taken from the freezer to evaluate colour and cap diameter at room temperature. Except for these short periods, the analysis of **Figure 3.1.5** clearly indicates the homogeneity of storage temperature and humidity, allowing attribution of any observed change in the physical parameters to storage time and ID.

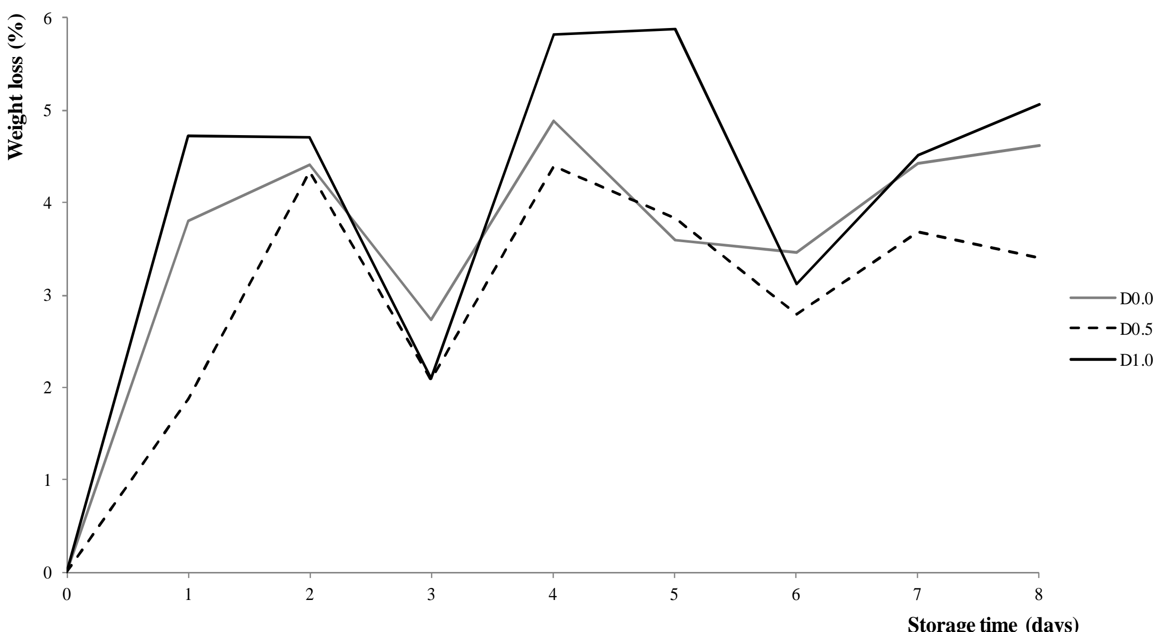


Figure 3.1.4. Weight loss profiles during 8 days of cold storage for non-irradiated samples and samples irradiated with 0.5 or 1.0 kGy.

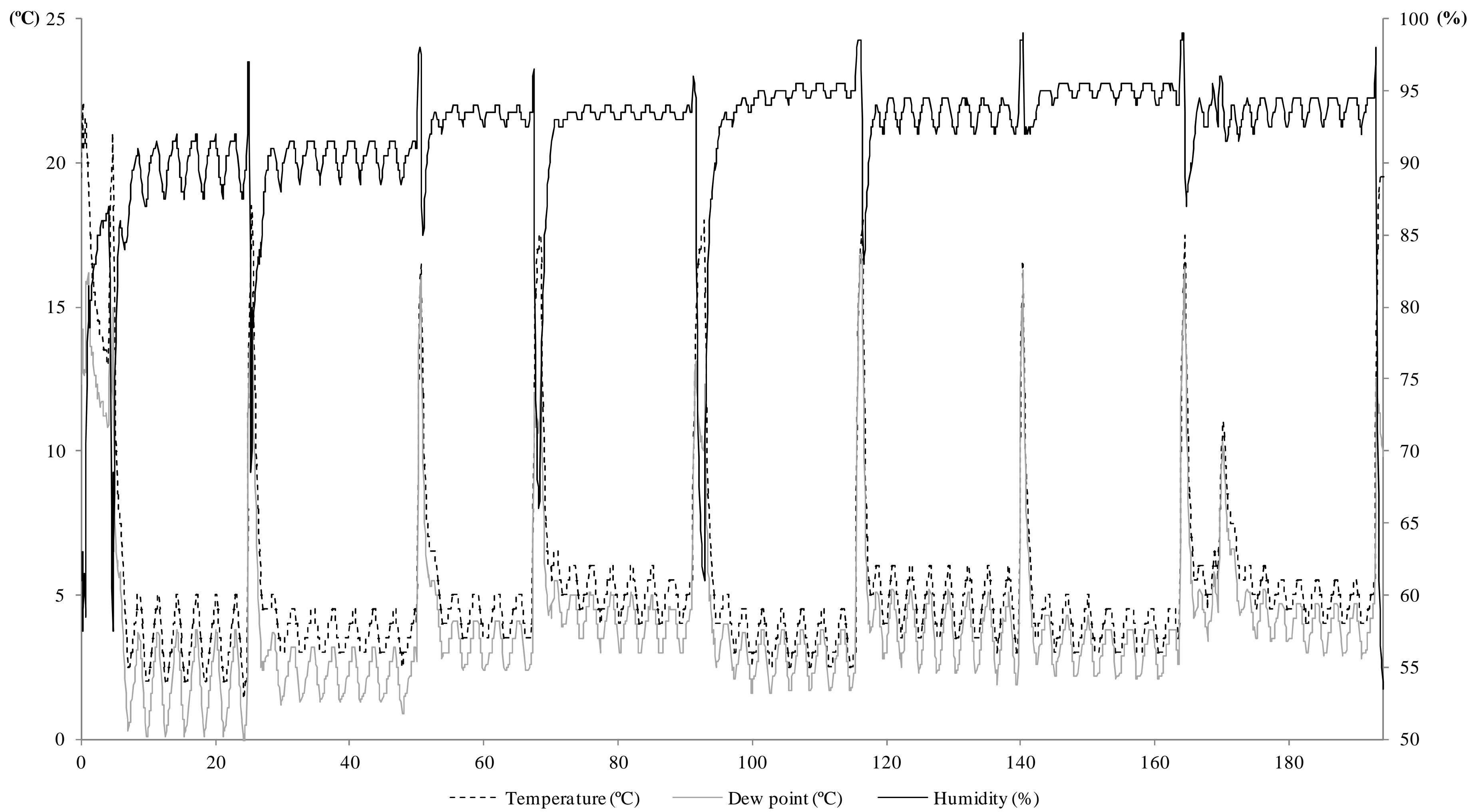


Figure 3.1.5. Over profile of temperature, dew point and humidity. Samples were kept in the refrigerator. Every 24 h, samples were removed to measure colour, cap diameter and weight.

Despite the particular tendencies described, the results obtained for the measured parameters indicated that neither irradiation nor cold storage exerted significant influence. To check this assumption, a hierarchical cluster analysis (HCA) was applied. The results obtained are shown as a dendrogram (**Figure 3.1.6**) in which well-defined clusters were not obtained. In this analysis, samples are grouped in clusters in terms of their nearness or similarity.

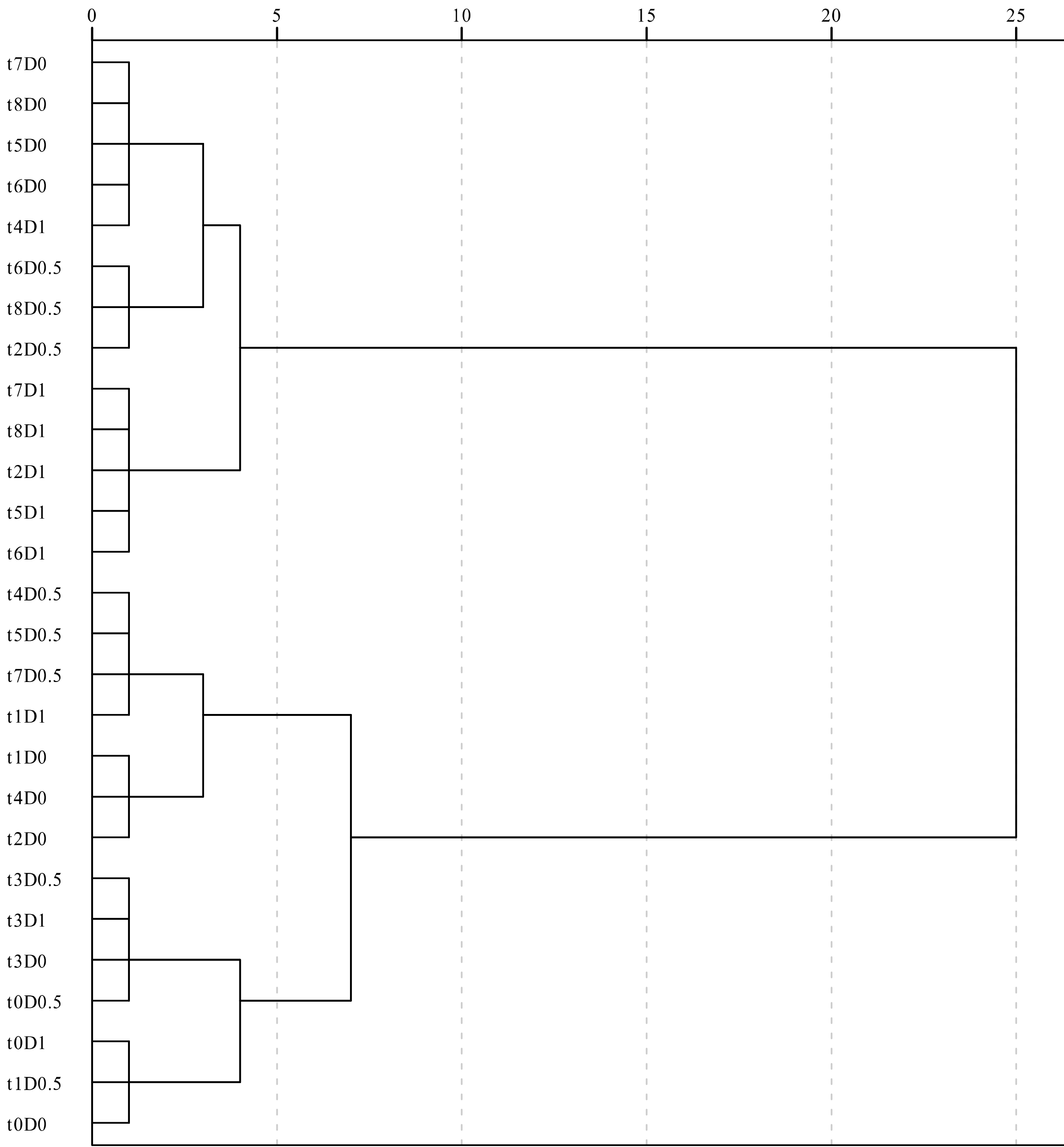


Figure 3.1.6. Dendrogram obtained with Z-scores standardization results after applying Ward linkage method.

If the differences for lightness (*L*), redness (*a*), yellowness (*b*), weight loss and cap diameter among samples irradiated with different doses or stored for different periods were significant, it would be expected that a number of naturally defined clusters (for instance, three clusters formed for ID) would be obtained. The dendrogram shows the opposite, indicating high similarity among the samples, since there are mixtures of different storage times or ID in each individualized cluster. Nevertheless, the well-defined changes that some Hunter colour parameters showed with irradiation dose should not be neglected.

Overall, this study demonstrated that up to 1 kGy, the combination of gamma irradiation and cold storage did not affect mushroom physical properties.

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3.1.5. References

Akram, K., Kwon, J.-H., 2010. Food Irradiation for Mushrooms: A Review. Journal of the Korean Society for Applied Biological Chemistry 53, 257-265.

Antonio, A.L., Fernandes, Â., Barreira, J.C.M., Bento, A., Botelho, M.L., Ferreira, I.C.F.R., 2011. Influence of gamma irradiation in the antioxidant potential of chestnuts (*Castanea sativa* Mill.) fruits and skins. Food and Chemical Toxicology 49, 1918-1923.

ASTM, American Society for Testing and Materials, 1992. Practice for using the Fricke Reference Standard Dosimetry System, ASTM E1026. Annual Book of ASTM Standards, vol. 12.02. ASTM, Philadelphia, PA.

Barreira, J.C.M., Antonio, A.L., Günaydi, T., Alkan, H., Bento, A., Botelho, M.L., Ferreira, I.C.F.R., 2012. Chemometric characterization of gamma irradiated

chestnuts from Turkey. Radiation Physics and Chemistry 81, 1520-1524, http://dx.doi.org/10.1016/j.radphyschem.2012.01.005.

Beaulieu, M., D'Aprano, G., Lacroix, M., 2002. Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. Radiation Physics and Chemistry 63, 311–315.

Du, J., Fu, Y., Wang, N., 2009. Effects of aqueous chlorine dioxide treatment on browning of fresh-cut lotus root. Lebensmittel-Wissenschaft und -Technologie 42, 654-659.

Fernandes, Â., Antonio, A.L., Barros, L., Barreira, J.C.M., Bento, A., Botelho, M. L., Ferreira, I.C.F.R., 2011a. Low dose γ-irradiation as a suitable solution for chestnut (*Castanea sativa* Miller) conservation: effects on sugars, fatty acids, and tocopherols. Journal of Agricultural and Food Chemistry 59, 10028-10033.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Bento, A., Botelho, M.L., Ferreira, I.C.F.R., 2011b. Assessing the effects of gamma irradiation and storage time in energetic value and in major individual nutrients of chestnuts. Food and Chemical Toxicology 49, 2429-2432.

Gould, G.W., 1989. Mechanisms of action of food preservation procedures. Elsevier Applied Science, London, p. 2, 4, 7, 9.

Hunter, R., Harold, R.W., 1987. The Measurement of Appearance. John Willey and Sons, New York, USA.

Jasinghe, V.J., Perera, C.O., 2006. Ultraviolet irradiation: the generator of vitamin D2 in edible mushrooms. Food Chemistry 95, 638–643.

Jiang, T., Luo, S., Chen, Q., Shen, L., Ying, T., 2010. Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). Food Chemistry 122, 761–767.

Jo, C., Son, J.H., Lee, H.J., Byun, M.-W., 2003a. Irradiation application of color removal and purification of green tea leave extract. Radiation Physics and Chemistry 66, 179-184.

Jo, C., Son, J.H., Shin, M.G., Byun, M.-W., 2003b. Irradiation effect on color and functional properties of persimmon (*Dyospyros kaki* L. *folium*) leaf extract and licorice (*Glycyrrhiza uralensis* Fischer) root extract during storage. Radiation Physics and Chemistry 67, 143-148.

Kim, J.K., Jo, C., Hwang, H.J., Park, H.J., Kim Y.J., Byun, M.-W., 2006. Color improvement by irradiation of *Curcuma aromatic* extract for industrial application. Radiation Physics and Chemistry 75, 449-452.

Kim, J.H., Sung, N.-Y., Kwon, S.-K., Srinivasan, P., Song, B.-S., Choi, J.-il, Yoon, Y., Kim, J.K., Byun, M.-W., Kim, M.-R., Lee, J.-W., 2009. γ-Irradiation improves the color and antioxidant properties of chaga mushroom (*Inonotus pbliquus*) extract. Journal of Medicinal Food 12, 1343-1347.

Minnaar, A., Taylor, J.R.N., McGill, A.E.J., 1995. Heat-irradiation combination processing as an effective method of producing high quality shelf-stable, low-acid food products. Food Control 6, 165–170.

Pai, T., 2000. Effects of storage environmental conditions on weight loss whiteness change and microbial activity of mushrooms (*Agaricus bisporus*). Agricultural Chemistry & Biotechnology 43, 161–164.

Singh, P., Langowski, H.-C., Wanib, A.A., Saengerlaub, S., 2010. Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: a review. Journal of the Science of Food and Agriculture 90, 1393–1402.

Sommer, I., Schwartz, H., Solar, S., Sontag, G., 2010. Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (Agaricus bisporus). Food Chemistry 123, 171–174.

Wani, A.M., Hussain, P.H., Meena, R.S., Dar, M.A., Mir, M.A., 2009. Effect of gamma irradiation and sulphitation treatments on keeping quality of white button mushrooms *Agaricus bisporus* (J. Lge). International Journal of Food Science & Technology 44, 967-973.

3.2.

Efeito da radiação gama na composição química e atividade antioxidante de cogumelos silvestres *Lactarius deliciosus* L.

Este sub-capítulo apresenta os efeitos da radiação gama (doses 0,5 e 1 kGy) sobre os parâmetros nutricionais (valor energético, ácidos gordos, açúcares livres e tocoferóis) e bioativos (atividade antioxidante) de amostras de Lactarius deliciosus *L.*

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ORIGINAL PAPER

Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom

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Abstract

The short shelf-life of mushrooms is an obstacle to the distribution and marketing of the fresh product. There has been extensive research on finding the most appropriate technology for mushrooms preservation and a particular interest arises for wild species. Treatment by irradiation emerges as a possible conservation technique that has been tested successfully in several food products. Herein, the effects of gamma irradiation on *Lactarius deliciosus* (L. ex Fr.) S. F. Gray chemical composition and antioxidant activity were evaluated in samples submitted to different storage periods (0, 4 and 8 days) at 4 ºC. The irradiation treatments were performed in aCo-60 experimental equipment. Nutritional value was accessed by macronutrients analysis and determination of energetic value; fatty acid, sugar and tocopherol

profiles were determined by gas chromatography-flame ionization detector, high-performance liquid chromatography (HPLC) refractive index and HPLC fluorescence, respectively. The antioxidant activity was evaluated through radical scavenging activity, reducing power, lipid peroxidation inhibition and phenolics content. The obtained data show that, until 1 kGy, gamma irradiation might provide a useful alternative to ensure the quality and extend the life of mushrooms, since its effects on macronutrients, energetic value, tocopherols and antioxidant activity EC_{50} values, were less significant than the changes caused by storage time. Moreover, the chemical and nutritional composition was similar in irradiated and non-irradiated *L. deliciosus* samples.

Keywords: Gamma irradiation; Wild mushrooms; *Lactarius deliciosus*; Chemical composition; Antioxidant activity

3.2.1. Introduction

Mushrooms are one of the most perishable products and tend to lose quality immediately after harvest. The short shelf-life (1-3 days at ambient temperature) is a drawback to the distribution and marketing of the fresh product. Their shelf-life is short due to postharvest changes, such as browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture changes, to their high respiration rate and lack of physical protection to avoid water loss or microbial attack (Akram and Kwon 2010; Singh et al. 2010; Sommer et al. 2010).

Extended shelf-life is a key factor for making any food commodity more profitable and commercially available for long periods of time at the best possible quality. The producer will benefit from the longer shelf-life to develop the market over greater distances (Akram and Kwon 2010).

Food irradiation appears as a possible alternative for stored mushrooms, by exposing food to ionizing radiation (such as gamma or electron-beam) in order to enhance its shelf-life as well as its safety. Particularly, gamma irradiation has been shown to be a potential tool in extending the postharvest shelf-life of fresh mushrooms (Beaulieu et al. 2002). Different regulatory agencies assure that food irradiation is a safe process with respect to food processing for humans (USFDA 1991; WHO 1994). Furthermore, the recommended dose for extending the shelf-life

of fresh mushrooms is 1-3 kGy, while the recommended dose regarding the decontamination of dried mushrooms (come under food additives with spices), used as seasonings, is 10-50 kGy (ICGFI 1999).

Studies evaluating the effects of ionizing radiation are mostly available in cultivated species with high production value such as *Agaricus bisporus* (Sommer et al. 2010), *Lentinus edodes* (Jiang et al. 2010) and *Pleurotus ostreatus* (Jasinghe and Perera 2006). Studies on wild species are scarce, and, as far as we know, there are no reports evaluating the effects of irradiation on wild *Lactarius deliciosus* species. Moreover, it should be highlighted that wild species are considered add-value foods for commercialization.

The Northeast of Portugal is one of the European regions with high wild edible mushrooms diversity, some of them with great gastronomic relevance. Within the local edible species, *L. deliciosus* (L.) Gray is one of the most important due to its high consumption by the rural population and its economic value in the markets of France and Spain (Ferreira et al. 2007; Martins et al. 2002). This species was previously studied by our research group regarding its nutritional value (Barros et al. 2007a), the effects of conservation (liophylization and freeze) and cooking on chemical composition and antioxidant activity (Barros et al. 2007b) and the effects of fruiting body maturity stage on chemical composition and antimicrobial activity (Barros et al. 2007c).

Herein, the effects of gamma irradiation on the chemical composition and antioxidant properties of the mentioned wild species were evaluated in order to see if this technique might be useful for increasing mushrooms shelf-life.

3.2.2. Materials and methods

Standards and reagents

To prepare the acid aqueous Fricke dosimeter solution, the following reagents were used: ferrous ammonium sulfate(II)hexahydrate, sodium chloride and sulphuric acid, all of them purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA).

For chemical analyses, acetonitrile 99.9 %, *n*-hexane 95 % and ethyl acetate 99.8 % were of high-performance liquid chromatography (HPLC) grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, tocopherols (α-, β-, γ-, and δ-isoforms) and sugars (D(-)-fructose, D-mannitol, D(+)-melezitose and D(+)-trehalose) standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA).

For antioxidant potential analysis, 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy- 2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were purchase from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

Samples and samples irradiation

Lactarius deliciosus fruiting bodies were obtained from Trás-os-Montes, in the Northeast of Portugal, in November 2011. They were divided in three groups: control (non-irradiated, 0 kGy); sample 1 (0.5 kGy) and sample 2 (1.0 kGy) with 18 specimens per group.

The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, a reference standard that provides a reliable means of absorbed doses measurement in water, based on an oxidation process of ferrous ions to ferric ions in acidic aqueous solution by ionizing radiation. The acid aqueous Fricke dosimeter solution was prepared and read following the standard procedure (ASTM 1992). The irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity of 267 TBq (6.35 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd, UK).

After irradiation geometry dose rate estimation, using the Fricke dosimeter and the procedure described in the standards (ASTM, 1992), groups 2 and 3 were placed in poly (methyl methacrylate) box, or acrylic glass, and irradiated at ambient atmosphere and temperature (15 ^{o}C). To monitor the process during the irradiation, four routine dosimeters in the corners of the irradiation box were used for the highest dose (1 kGy) (Amber Perspex dosimeters, batch V, from Harwell company, UK). The

samples were rotated upside down (180°) at half of the time to increase the dose uniformity. The Amber Perspex dosimeters were read in an ultraviolet-visible spectrophotometer (Shimadzu mini UV 1240 spectrophotometer) at 603 nm, two readings for each, to estimate the dose according to a previous calibration curve. The estimated doses after irradiation were 0.6 ± 0.1 kGy and 1.1 ± 0.1 kGy for samples 2 and 3, respectively, at a dose rate of 2.3 ± 0.1 kGy h^{-1}.

For simplicity, in the text, tables and graphs, we considered the values 0, 0.5 and 1 kGy for non-irradiated and irradiated samples.

From each group, three subgroups with six mushroom samples were randomly selected. Subgroup 1 was promptly analyzed, subgroup 2 was stored at 5 ºC (refrigerator) for 4 days and subgroup 3 was stored in the same conditions for 8 days. Prior to analysis, all the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenate samples.

Chemical composition

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the Association of Official Analytical Chemists (AOAC) procedures (AOAC 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method according to Léon-Guzmán et al. (1997); the crude fat was determined by extracting a known weight of powdered mushroom sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C; total carbohydrates were calculated by difference: total carbohydrates = 100 - (g moisture + g protein + g fat + g ash). Total energy was calculated according to the following equation: energy (kcal) = 4 × (g protein +g carbohydrate) + 9 × (g fat).

Fatty acids

Fatty acids were determined after a transesterification procedure as described previously by Heleno et al. (2009), using a gas chromatographer (DANI 1000) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty

acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7). The results were expressed in relative percentage of each fatty acid.

Free sugars

Free sugars were determined by a HPLC system consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco), coupled to a refraction index detector (RI detector Knauer Smartline 2300) as previously described by Heleno et al. (2009). Sugars identification was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, melezitose) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in grams per 100 g of dry weight (dw).

Tocopherols

Tocopherols were determined following a procedure previously optimized and described by Heleno et al. (2010). Analysis was performed by HPLC (equipment described above), and a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and using calibration curves obtained from commercial standards of each compound. The results were expressed in micrograms per 100 g of dw.

Antioxidant activity

Extraction procedure

The lyophilized powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman no. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Barros et al. 2011) to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

Total phenolics

Phenolics were determined by the Folin–Ciocalteu assay, measuring the absorbance at 765 nm. Gallic acid was used to obtain the standard curve (0.0094–0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per gram of extract.

DPPH radical scavenging activity

This methodology was performed using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, USA), and calculated as a percentage of DPPH discolouration using the formula: $[(A_{DPPH}-A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution containing the sample at 515 nm, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

This methodology evaluated the capacity to convert Fe^{3+} into Fe^{2+}, measuring the absorbance at 690 nm in the microplate reader mentioned above.

Inhibition of β-carotene bleaching

This capacity was evaluated through the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which was measured by the formula: β-carotene absorbance after 2 h of assay/initial absorbance) $\times$ 100.

TBARS assay

Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in TBARS; the colour intensity of the malondialdehyde-thiobarbituric acid (MDA-TBA) was measured by its absorbance at 532 nm; the inhibition ratio (%) was calculated using the following formula: $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

For each one of the storage times and irradiation doses three samples were analysed, with all the assays being also carried out in triplicate. An analysis of variance (ANOVA) with type III sums of squares was performed using the general linear model procedure of the SPSS software, version 18.0 (SPSS, Inc.). All dependent variables were analyzed using a two-way ANOVA, being the main factors the “storage time (ST)” (0, 4 and 8 days) and the “irradiation dose (ID)” (0.0, 0.50 and 1.0 kGy). Since a statistical significant interaction effect (“ID × ST) was found in all tests, the two factors were evaluated simultaneously by plotting the estimated marginal means for all levels of each factor.

In addition, a linear discriminant analysis (LDA) was used as a technique to classify the ID as well as the ST according to the evaluated chemical profiles and antioxidant activity assays. A stepwise technique, using the Wilks’ λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination procedures, where before selecting a new variable to be included, it is

verified whether all variables previously selected remain significant (López et al. 2008). To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance. The LDA statistical analysis and the other statistical tests were performed at a 5% significance level using the SPSS software mentioned above.

3.2.3. Results and discussion

Analysis of variance

The individual effects of gamma irradiation (0.0, 0.5 and 1.0 kGy) and storage time (0, 4 and 8 days), as well as the interaction of both effects, were assessed by evaluating changes in chemical composition and antioxidant activity of the wild mushroom *L. deliciosus*.

Nutritional value, fatty acids, free sugars, tocopherols (**Table 3.2.1**), phenolics and antioxidant activity EC_{50} values (**Table 3.2.2**), are reported as mean value of each irradiation dose (ID) over the different storage times (ST) as well as mean value of all irradiation doses for each ST. This approach allows the comprehension of irradiation effect independently of ST, an essential feature to consider irradiation as a shelf-life increasing technique. The results revealed a significant ($p < 0.001$) interaction among both factors (ID × ST) for all the evaluated parameters, making the application of multiple comparisons impossible. The individual factors (ID and ST) also showed a significant effect ($p \leq 0.015$). Nevertheless, the analysis of the estimated marginal mean plots (data presented only for selected parameters) conducted to specific conclusions that will be further discussed.

Table 3.2.1. Chemical composition, main fatty acids, sugars and tocopherols profile of non-irradiated and irradiated *Lactarius deliciosus* samples, after different times of storage.

	ST			ID		
	0 days	4 days	8 days	0.0 kGy	0.5 kGy	1.0 kGy
Dry matter (g/100 g fw)	9±1	12±1	15±1	12±2	12±3	12±2
Fat (g/100 g dw)	3.0±0.4	4±1	4±1	4±1	3±1	3±1
Protein (g/100 g dw)	16±4	18±3	20±5	16±5	20±4	17±3
Ash (g/100 g dw)	7±1	8±1	8.0±0.5	8±1	7±1	7±1
Carbohydrates (g/100 g dw)	75±4	70±4	69±5	72±6	70±5	72±3
Energetic value (kcal/100 g dw)	389±4	391±7	386±4	388±5	388±6	390±5
C16:0 (%)	5.7±0.5	7±2	6.1±0.4	5.6±0.5	6±1	7±2
C18:0 (%)	62±3	60±1	67±1	62±2	63±4	65±4
C18:1 (%)	9±1	10±2	6±1	8±2	8±1	10±2
C18:2 (%)	19±2	17±4	16±3	20±1	19±3	13±2
SFA (%)	72±2	73±2	78±2	72±2	73±4	77±3
MUFA (%)	9±1	10±2	6±1	8±2	8±1	10±2
PUFA (%)	19±2	18±4	16±3	20±1	19±3	14±2
Fructose (g/100 g dw)	0.18±0.03	0.15±0.04	0.06±0.03	0.13±0.05	0.11±0.05	0.15±0.04
Mannitol (g/100 g dw)	12±2	8±2	8±1	11±1	8±2	11±3
Trehalose (g/100 g dw)	1.4±0.5	1.1±0.2	1.2±0.3	0.77 ±0.05	1.8±0.5	1.1±0.2
α-tocopherol (μg/100 g dw)	52±66	10±7	2±3	47±69	9±3	7±7
β-tocopherol (μg/100 g dw)	31±45	nd	nd	31±45	nd	nd
γ-tocopherol (μg/100 g dw)	23±33	nd	nd	23±33	nd	nd
δ-tocopherol (μg/100 g dw)	58±50	9±13	nd	40±61	11±16	16±12
Total tocopherols (μg/100 g dw)	163±192	19±19	2±3	141±207	20±15	23±19

The results are present as mean ± SD (*n*=27, for each storage time, ST and each irradiation dose, ID) which are reported as mean value of each irradiation dose (ID) over the different storage times (ST) as well as mean value of all ST within each ID. Therefore, SD reflects values in those samples (under different ID or ST), and could be higher than mean values. For each parameter, *p* value of both factors (ST and ID) as well as their interaction (ST × ID) was <0.02. Palmitic acid (C16:0), stearic acid (C18:0), oleic acid (C18:1), linoleic acid (C18:2). *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids. The fatty acids results are expressed in percentage; the difference to 100 % corresponds to other 14 less abundant fatty acids (data not shown; only main fatty acids were presented).

Table 3.2.2. Phenolic content and antioxidant activity of non-irradiated and irradiated *Lactarius deliciosus* samples, after different times of storage.

	ST			ID		
	0 days	4 days	8 days	0.0 kGy	0.5 kGy	1.0 kGy
Phenolics (mg GAE/g extract)	24±10	15±6	12±6	18±11	21±9	12±5
DPPH scavenging activity (EC_{50},mg/mL)	11±7	11±4	16±2	14±6	12±5	12±4
Reducing power (EC_{50}, mg/mL)	3±2	2±1	4±3	3±3	2.0±0.5	3±2
β-Carotene bleaching inhibition (EC_{50}, mg/mL)	0.44±0.02	3±2	2±2	0.8±0.2	1±1	4±2
TBARS assay (EC_{50}, mg/mL)	1±1	3±1	6±3	4±4	1.7±0.5	4±2

The results are presented as mean ± SD[a] (*n*=27, for each storage time, ST, and each irradiation dose, ID), which are reported as mean value of each irradiation dose (ID) over the different storage times (ST) as well as mean value of all ST within each ID. Therefore, SD reflects values in those samples (under different ID or ST) and could be higher than mean values. For each parameter, *p* value of both factors (ST and ID) as well as their interaction (ST × ID) was <0.001.

Chemical composition

Regarding nutritional composition, the results are in agreement with former studies in non-irradiated samples of *L. deliciosus* (Barros et al. 2007b, c), with carbohydrates and proteins as major macronutrients. Dry matter was the only parameter with a significant response to the evaluated factors and, as expected, increased along ST (**Table 3.2.1**; **Figure 3.2.1A**). This apparent lack of defined effects caused by ID or ST was also reflected in the fatty acids profile, in which only the C_{18} molecules showed marked differences along ST, especially for the unsaturated compounds. This observation might be explained by the higher susceptibility of oleic (**Figure 3.2.1C**), linoleic and linolenic acids to oxidation.

Nevertheless, irradiation at 1.0 kGy seemed to protect some fatty acids from oxidation, such as oleic acid, which is higher in irradiated sample at the mentioned dose than in control sample, contributing to higher monounsaturated fat (MUFA) levels on the first case. Despite some qualitative differences in minor fatty acids, for example, the presence of C13:0 and the absence of C11:0 (data not shown), the obtained profiles are globally in accordance with a previous study in non-irradiated *Lactarius* species (Barros et al. 2007c), being C18:0 the most abundant fatty acid in all the studied samples. In fact, our research group has already reported in different studies stearic acid (C18:0) as the main fatty acid in *Lactarius* species: *L. deliciosus*, *L. piperatus* (Barros et al. 2007c) and *L. salmonicolor* (Heleno et al. 2009). Otherwise, other mushroom species present, in general, oleic and linoleic acids as main fatty acids and a prevalence of unsaturated over saturated fatty acids.

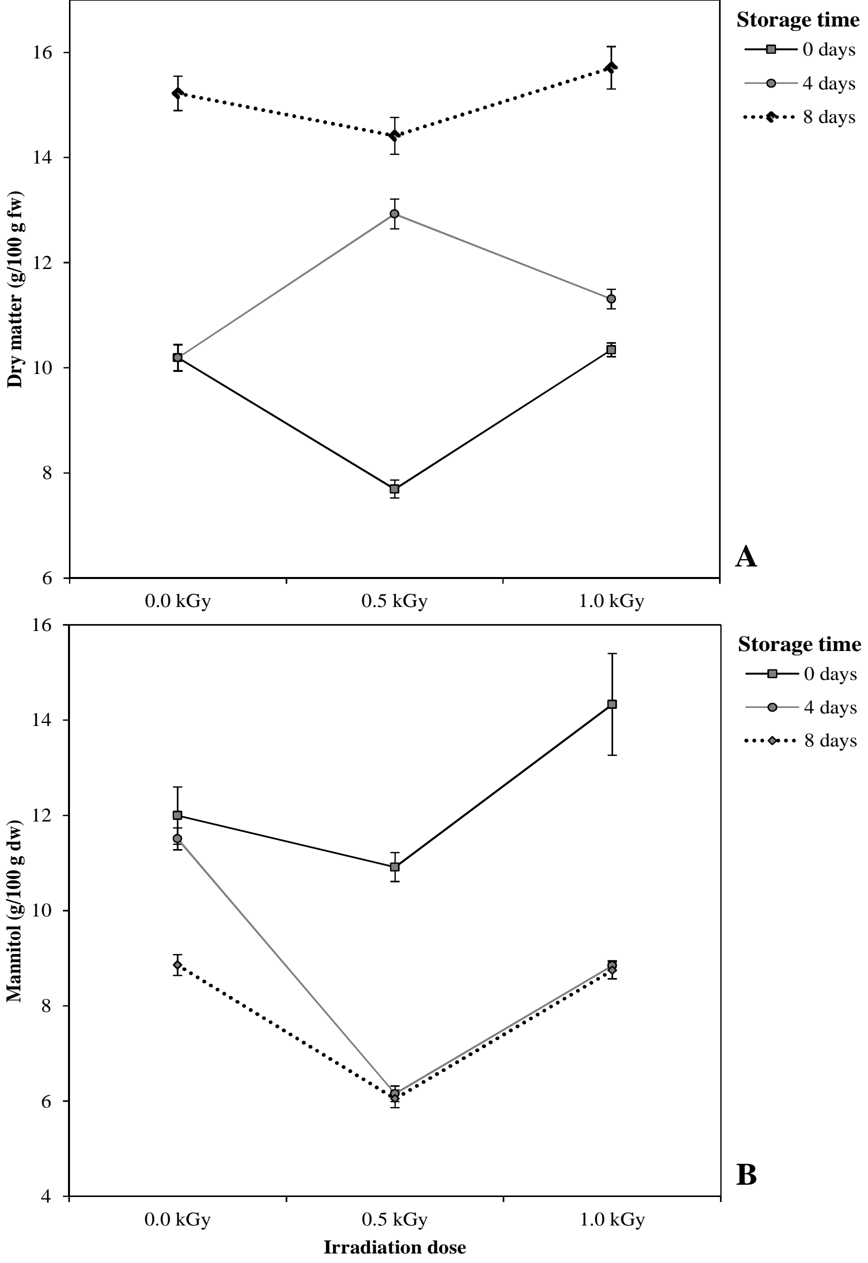
In the case of individual sugars, mannitol is the major compound followed by trehalose and fructose (**Table 3.2.1**), which is in agreement with the results reported by us in a previous study with non-irradiated samples (Barros et al. 2007b). Analyzing the effect of ST, mannitol (**Figure 3.2.1B**) and fructose decreased with storage. In the particular case of trehalose, a non-reducing sugar, the effect of ST is less observable due to its lower susceptibility to oxidation. Regarding ID effects, it seems that trehalose was preserved in irradiated samples (higher levels), while it decreased in non-irradiated samples (**Table 3.2.1**).

The results obtained for tocopherols content (**Table 3.2.1**) indicate the high sensibility of these compounds to ST or ID. In fact, non-irradiated and non stored samples were the only cases in which β-tocopherol (93±11 μg/100 g dw) and γ-

tocopherol (68±6 µg/100 g dw) were detected. Furthermore, the remaining vitamers decreased in irradiated or stored samples, probably due to oxidative processes. Tocopherols (vitamin E) were already reported as sensitive to radiation, being their losses during the irradiation often substantial (Dionísio et al. 2009).

Antioxidant activity

Regarding antioxidant activity, β-carotene bleaching inhibition assay, which exhibited higher EC_{50} values for irradiated samples, was the only assay that revealed a marked tendency (**Figure 3.2.1D**).



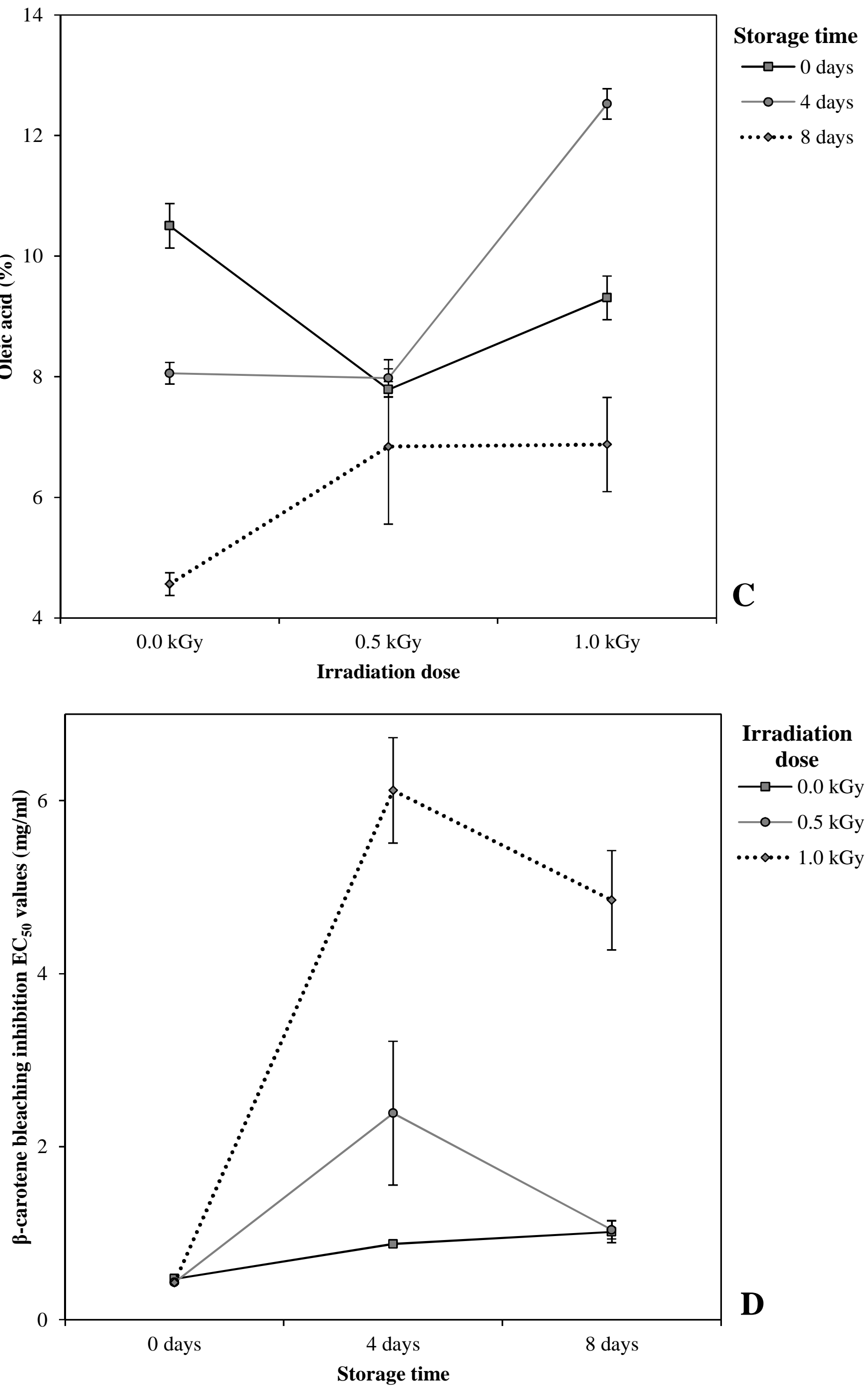


Figure 3.2.1. Interactions between ST and ID effects on *Lactarius deliciosus* extracts. Influence on (a) dry matter, (b) mannitol, (c) oleic acid, (d) β-carotene bleaching inhibition.

Nevertheless, the 0.50 kGy ID seemed to exert a protective effect on phenolics content, an outcome that is in agreement with the higher antioxidant activities, especially in the case of reducing power and TBARS formation inhibition, observed for this ID. In general, the obtained EC_{50} values are in accordance with previous

results using non-irradiated and non-stored samples (Ferreira et al. 2007). These results indicate different sensibilities among the performed antioxidant activity assays, which tended to be correlated with the phenolic contents (lower EC_{50} values correspond to higher phenolic contents).

Despite the low number of found tendencies according to each of the assayed considered factors, ST seemed to exert more evident changes in chemical profiles and antioxidant activity than ID dose. Nevertheless, considering only the results obtained with two-way ANOVA, the main conclusion would be that the assayed ST and ID did not affect the evaluated parameters in a great extent.

Linear Discriminant Analysis

In order to obtain a more comprehensive knowledge about the differences induced by ST and ID, different linear discriminant analyses (LDA) were performed. The obtained outputs indicated clearly that, diversely from what could be expected considering the two-way ANOVA results, the differences induced by ST and ID revealed strong discriminant ability. Actually, for ST, the classification was 100.0 % corrected (for original or cross-validated grouped cases) for all sets of assayed variables except antioxidant activity EC_{50} values (90.1%, for original grouped cases; 88.9%, for cross-validated grouped cases) and tocopherols profile (81.5%, for original grouped cases; 80.2%, for cross-validated grouped cases). The classification performance was lower when evaluating the effect of ID: the groups were classified with 100.0 % of effectiveness only when all the variables or the fatty acid results were included in the model. The classification for sugars (88.9 %, for original grouped cases and 85.2 %, for cross-validated grouped cases), antioxidant activity EC_{50} values (85.2 %, for original grouped cases and 84.0 %, for cross-validated grouped cases), nutritional parameters (71.6 %, for original grouped cases and 70.4 %, for cross-validated grouped cases) and tocopherols (59.3 %, for original grouped and cross-validated grouped cases) were always worse than those obtained for ST. In order to understand the effects of ST and ID in a general manner, the following discussion will be focused on the results obtained for LDA including all the assayed parameters. Concerning ST, the model defined two significant functions, which included 100.0 % of the observed variance (**Figure 3.2.2A**).

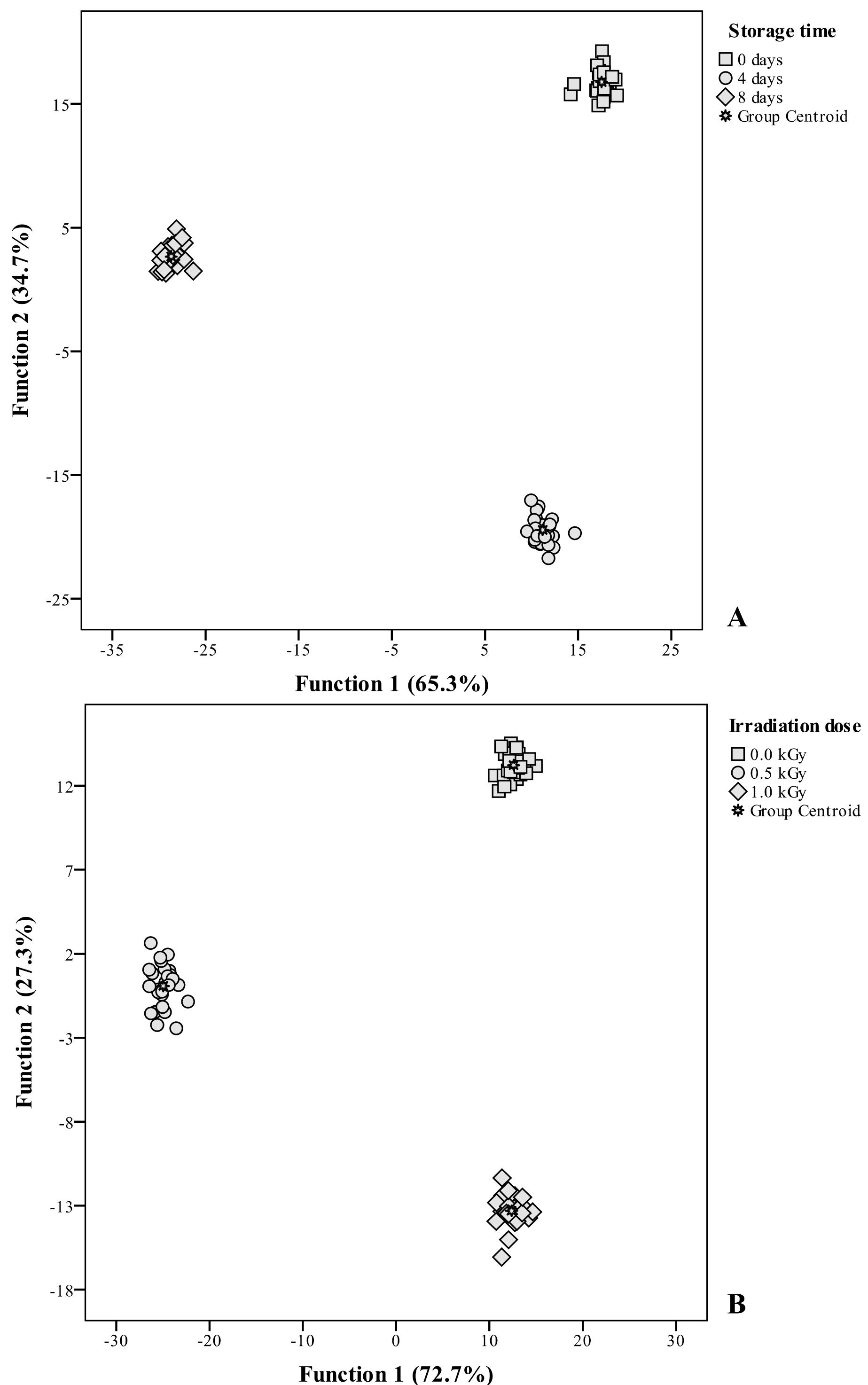


Figure 3.2.2. Score plots defined for the assayed parameters using ST (A) or ID (B) as grouping variables.

The first function separated mainly samples stored for 8 days (means of the canonical variance, (MCV): 0 days = 17.518; 4 days = 11.187; 8 days = -28.705), being more correlated with dry matter, C18:0, SFA and C18:1n9c. The second

function separated non stored samples from those with 4 days of storage (MCV: 0 days = 17.518; 4 days = 11.187; 8 days = -28.705) and was more correlated with mannitol, C16:0, fat and C18:3n3. From the 40 (fatty acids, 21; nutritional, 6; antioxidant activity, 5; tocopherols, 5; sugars, 3) analyzed variables, the model selected 17, whose standardised coefficients might be seen in **Table 3.2.3**.

Regarding ID, the model defined also two significant functions, which comprised 100.0 % of the observed variance (**Figure 3.2.2B**). The first function separated primarily samples irradiated with 0.5 kGy (MCV: 0.0 kGy = 12.596; 0.5 kGy = -24.968; 1.0 kGy = 12.372), and revealed to be more effectively correlated with trehalose, mannitol, TBARS and C18:3n3. The second function separated non irradiated samples from those irradiated with 1.0 kGy (MCV: 0.0 kGy = 13.225; 0.5 kGy = 0.079; 1.0 kGy = -13.304) and showed to be more correlated with C18:2n6c, C16:0, C15:0 and C22:0. From the 40 analyzed variables, the model selected 16, whose standardized coefficients might be seen in **Table 3.2.3**.

Table 3.2.3. Standardised canonical discriminant function coefficients.

Storage time			**Irradiation dose**		
Variables	Function		Variables	Function	
	1	2		1	2
dry matter	-8.303	-0.382	DPPH	0.164	1.671
Fat	3.274	-0.563	TBARS	1.772	0.887
Kcal	-2.749	-0.725	Dry matter	-4.250	2.689
C6:0	2.859	5.616	Ash	6.262	0.050
C13:0	-2.264	10.879	C6:0	7.376	-4.799
C15:0	1.162	4.117	C10:0	-3.602	4.085
C16:0	22.822	-0.982	C13:0	2.263	5.645
C18:0	11.985	19.052	C15:0	-3.079	4.778
C18:1n9c	0.183	1.810	C16:0	0.064	-13.786
C18:3n3	0.110	-13.632	C18:1n9c	-4.202	0.474
C20:0	-0.662	2.984	C18:2n6c	-5.803	4.930
C22:0	-8.572	-7.240	C18:3n3	10.162	2.921
C23:0	2.091	19.669	C22:0	-0.148	6.531
SFA	-15.587	-21.771	C23:0	-5.476	-1.779
Mannitol	1.791	4.834	Fructose	0.598	-3.106
Trehalose	-4.296	4.650	Mannitol	6.494	0.172
			Trehalose	-6.418	-1.442

3.2.4. Conclusions

The potential of low-dose gamma irradiation as a suitable technique to increase natural products shelf-life was previously evaluated in our laboratory using chestnut samples (António et al. 2011; Fernandes et al. 2011a, b). Overall, the obtained results indicate that storage time had higher influence over the evaluated parameters. However, the effect of gamma irradiation (especially in association with storage time effect) in matrixes with different chemical profiles remains unknown and demands additional studies. Another study on *L. deliciosus* demonstrated that up to 1 kGy, gamma irradiation did not affect significantly physical properties such as colour, cap diameter and weight (Fernandes et al. 2012).

The present study demonstrated, until the maximal assayed ID (1 kGy), that gamma irradiation might provide a useful alternative to ensure the quality and extend the life of mushrooms, since its effects on sugars, antioxidant activity EC_{50} values, nutritional parameters and tocopherols, were less significant than the changes caused by storage time. Sugar profile (known for being a reliable indicator of adequate conservation technology) and nutritional profile (which should be kept after any conservation method) revealed high resemblance among irradiated and non-irradiated *L. deliciosus* samples. In fact, the results seemed to indicate that the effect provoked by ST overcame the influence of ID, emphasizing this technique as a potential conservation method in mushrooms. Nevertheless, further studies are necessary to validate the use of gamma irradiation in wild mushrooms.

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3.2.5. References

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society of Applied Biological Chemistry*, *53*, 257-265.

Antonio, A. L., Fernandes, A., Barreira, J. C. M., Bento, A., Botelho, M. L., & Ferreira I. C. F. R. (2011). Influence of gamma irradiation in the antioxidant potential of chestnuts (*Castanea sativa* Mill.) fruits and skins. *Food and Chemical Toxicology*, *49*, 1918-1923.

AOAC. (1995). *Official methods of analysis* (16th ed.). Arlington: Association of Official Analytical Chemists.

ASTM. (1992). *Practice for using the Fricke Reference Standard Dosimetry System*, ASTM E1026, Annual Book of ASTM Standards, 12.02. Philadelphia: American Society for Testing and Materials.

Barros, L., Baptista, P., Correia, D. M., Casal, S., Oliveira, B., & Ferreira, I. C. F. R. (2007a). Fatty acid and sugar compositions, and nutritional value of five wild edible mushrooms from Northeast Portugal. *Food Chemistry*, *105*, 140-145.

Barros, L., Baptista, P., Correia, D. M., Morais, J. S., & Ferreira, I. C. F. R. (2007b). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, *55*, 4781-4788.

Barros, L., Baptista, P., Estevinho, L. M., & Ferreira, I. C. F. R. (2007c). Effects of fruiting body maturity stage on chemical composition and activity of *Lactarius* sp. mushrooms. *Journal of Agricultural and Food Chemistry*, *55*, 8766-8771.

Barros, L., Cabrita, L., Vilas Boas, M., Carvalho, A. M., & Ferreira, I. C. F. R. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry*, *127*, 1600-1608.

Beaulieu, M., D'Aprano, G., & Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry*, *63*, 311–315.

Dionísio, A. P., Gomes, R. T., & Oetterer, M. (2009). Ionizing radiation effects on food vitamins – A review. *Brazilian Archives of Biology and Technology*, *52*, 5, 1267-1278.

Fernandes, Â., Antonio, A. L., Barros, L., Barreira, J. C. M., Bento, A., Botelho, M. L., & Ferreira, I. C. F. R. (2011a). Low dose γ-irradiation as a suitable solution for

chestnut (*Castanea sativa* Miller) conservation: Effects on sugars, fatty acids, and tocopherols. *Journal of Agricultural and Food Chemistry*, *59*, 10028-10033.

Fernandes, Â., Barreira, J. C. M., Antonio, A. L., Bento, A., Botelho, M. L., & Ferreira, I. C. F. R. (2011b). Assessing the effects of gamma irradiation and storage time in energetic value and in major individual nutrients of chestnuts. *Food and Chemical Toxicology*, 49, 2429-2432.

Ferreira, I. C. F. R., Baptista, P., Vilas-Boas, M., & Barros, L. (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chemistry*, 100, 1511–1516.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchemical Journal*, 93, 195–199.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, 119, 1443–1450.

ICGFI. (1999). *In facts about food irradiation*. Buckinghamshire: International Consultative Group on Food Irradiation.

Jasinghe, V. J., & Perera, C. O. (2006). Ultraviolet irradiation: The generator of Vitamin D2 in edible mushrooms. *Food Chemistry*, 95, 638–643.

Jiang, T., Luo, S., Chen, Q., Shen, L., & Ying, T. (2010). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). *Food Chemistry*, 122, 761–767.

Léon-Guzmán, M. F., Silva, I., & López, M. G. (1997). Proximate chemical composition, free amino acid contents, and free fatty acids contents of some wild edible mushrooms from Queretaro, México. *Journal of Agricultural and Food Chemistry*, 45, 4329–4332.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, 106, 369-378.

Martins, A., Baptista, P., Sousa, M. J., Meireles, T., & Pais, M. S. (2002). Edible mycorrhizal fungi associated with *Castanea sativa* Mill trees in the Northeast of

Portugal. In I. Hall, Wang Yun, E. Danell, A. Zambonelli (Eds.). Proceedings of the second international workshop on edible mycorrhizal fungi. ISBN 0-478-10828-X.

Singh, P., Langowski, H.-C., Wanib, A. A., & Saengerlaub, S. (2010). Recent advances in extending the shelf life of fresh *Agaricus* mushrooms: A review. *Journal of the Science of Food and Agriculture*, 90, 1393–1402.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (*Agaricus bisporus*). *Food Chemistry*, 123, 171–174.

USFDA. (1991). Irradiation in the production, processing and handling of food. Code Federal Register, Title 21, part 179.

WHO. (1994). *Safety and nutritional adequacy of irradiated food*. Geneva, Switzerland: World Health Organization.

3.3

Estudo de alterações químicas e variação da atividade antioxidante induzidas pela irradiação gama em cogumelos silvestres: estudo comparativo através de análise de componentes principais

Este sub-capítulo apresenta os efeitos da radiação gama (doses 1 e 2 kGy) n*os parâmetros nutricionais (valor energético, açúcares livres, tocoferóis, ácidos gordos, ácidos orgânicos) e parâmetros bioativos (atividade antioxidante) de amostras de* Boletus edulis *Bull. e* Hydnum repandum *L. Fr.*

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Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: comparative study through principal component analysis

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Abstract

Mushrooms are especially sensitive to senescence, browning, water loss and microbial attack. Furthermore, wild species are characterized by their seasonality, demanding the development of suitable preservation technology. Gamma-irradiation was previously tested in wild *Lactarius deliciosus*, being verified that its application up to 1 kGy did not imply significant changes in chemical parameters. Herein, the effects of higher gamma-irradiation doses, typically used in natural food matrices like fruits or vegetables, were assessed in *Boletus edulis* Bull.: Fr. and *Hydnum repandum* L.: Fr.

by checking for changes in nutritional parameters, free sugars, tocopherols, fatty acids, organic acids and antioxidant activity indicators. To have representative samples, the used carpophores were collected in different maturity stages, using the same number of specimens for each stage and also for each mushroom species. The specific effects of each tested irradiation were evaluated in an integrated manner through principal component analysis. The correspondent biplots indicate that differences caused by gamma-irradiation are enough to separate irradiated and non-irradiated samples of both mushrooms. Nevertheless, nutritional profiles were not affected in high extension, indicating that gamma-irradiation, up to the doses used in this work, might represent a useful mushroom conservation technology.

Keywords: Wild mushrooms; Gamma irradiation; Chemical composition; Antioxidant activity; Principal component analysis

3.3.1. Introduction

Mushrooms are highly perishable food matrices mainly due to their high water content (approx. 90%), easily deteriorating due to senescence, browning, water loss and microbial attack (Jolivet et al., 1998). Furthermore, wild mushrooms such as *Boletus edulis* Bull.:Fr. and *Hydnum repandum* L.: Fr. are strictly seasonal, causing difficulties in their distribution and marketing as fresh products; the level of loss in similar matrices during marketing could be as high as 40% (Lacroix & Ouattara, 2000). Therefore, mushrooms need special care to keep quality and freshness. The northeast of Portugal, due to its climatic conditions and flora diversity, is one of the European regions with a high variety of wild mushrooms, some of them with great gastronomic significance (Martins, Baptista, Sousa, Meireles, & Pais, 2002). *B. edulis* and *H. repandum* are among the most commonly consumed wild mushrooms. Their popularity is mainly due to sensory qualities, in particular aroma, taste and texture. Moreover, it should be highlighted that wild species are considered add-value foods for commercialization in the markets of France and Spain (Martins et al., 2002).

Irradiation is recognized as a safe and effective method of preservation used worldwide to extend the shelf life of raw foods (*e.g.* fruits and vegetables, spices, grains, meat or seafood) (Andrews et al., 1998; Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). In fact, more than 26 countries are using the process on a

commercial scale (Lacroix & Ouattara, 2000; Stevenson, 1994). Different cultivated mushrooms (mainly from *Agaricus*, *Lentinula* and *Pleurotus* genera) were previously studied using gamma-irradiation as a potential conservation technology (Fernandes, Antonio, Oliveira, et al., 2012). Nevertheless, few studies were published regarding the application of gamma-irradiation in wild mushrooms. In a recent investigation studying the effects of gamma-irradiation on the chemical composition, antioxidant activity and physical parameters of fresh *Lactarius deliciosus* L. wild edible mushroom, we conclude that up to 1 kGy this technology was effective in maintaining chemical composition and controlling the deterioration of fresh samples (Fernandes, Antonio, Barreira, Botelho, et al., 2012; Fernandes, Antonio, Barreira, Oliveira, et al., 2012).

Herein, gamma-irradiation doses up to 2 kGy were applied to *B. edulis* and *H. repandum*. In fact, a dose of 2.0 kGy is usually the optimal dose that fruits and vegetables may tolerate to keep their quality intact (without suffering loss of firmness, change in flavor/taste, physiological breakage or accelerated ripening) (Lacroix & Ouattara, 2000). In addition, the elimination of mold and pathogenic bacteria from mushrooms can be achieved with 2.0 kGy dose, increasing their shelf life from 2 to 8 days when stored at 10ºC (Lacroix & Ouattara, 2000; Skou, Beett, & Lundsten, 1974). Accordingly, the effects on nutritional parameters, free sugars, tocopherols, fatty acids, organic acids and antioxidant activity were evaluated individually in each assayed mushroom. Furthermore, principal component analysis was applied to verify which specific parameters were more affected by each assayed gamma-irradiation dose.

3.3.2. Materials and methods

Standards and reagents

For irradiation: To estimate the dose and dose rate of irradiation it was used with a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM, 1992) and Amber Perspex dosimeters (batch V, from Harwell Co., UK). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with

purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA).

For chemical analyses: Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as were other individual fatty acid isomers, organic acids, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA).

For antioxidant potential analysis: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Samples and samples irradiation

B. edulis and *H. repandum* fruiting bodies (carpophores) were collected in different maturity stages; however, the same number of specimens belonging to each maturity stage was selected for both mushrooms. All mushrooms were collected in Trás-os-Montes (Northeast of Portugal) in November 2012.

B. edulis and *H. repandum* fresh samples were divided into three groups (each species) with three mushrooms per group. Each group corresponds to: control (non-irradiated, 0 kGy); sample 1 (1 kGy) and sample 2 (2 kGy).

The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, and the irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity 267 TBq (6.35 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd, U.K.), following the procedure previously described by the authors (Fernandes et al., 2012). The estimated doses, dose rates and dose uniformity ratios (Dmax/Dmin) were: 1.14±0.23 kGy, 1.71 kGy/h, 1.72 and 1.99±0.32 kGy, 1.49 kGy/h, 1.44, for *B. edulis*; and 1.02±0.04 kGy, 1.53 kGy/h, 1.08 and 1.66±0.46 kGy, 1.24 kGy/h, 1.98, *H. repandum*. For simplicity, in the text, tables and graphs we considered the values 0, 1 and 2 kGy, for non-irradiated and irradiated samples of both mushroom species.

All the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples for subsequent analysis.

Chemical parameters

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). Moisture content was evaluated by lyophilization (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method according to Léon-Guzmán,Silva, and Lopez (1997); the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ^{o}C using a Chamber furnace (Lenton Thermal Designs Ltd, model ECF 12/22). Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = $4 \times (g_{protein}) + 3.75 \times (g_{carbohydrate}) + 9 \times (g_{fat})$.

Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) after the extraction procedure described by Heleno et al. (2011), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures described previously (Heleno et al., 2011). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID at 260 ºC and a Macherey-Nagel column 50% cyanopropylmeythyl 50% phenylmethylpolysiloxane (30 m × 0.32 mm ID × 0.25 µm d*f*). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined after an extraction procedure previously described, using tocol as IS (Heleno, Barros, Sousa, Martins, & Ferreira, 2010). The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherol content was further expressed in µg per 100 g of dry weight (dw).

Organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira, & Ferreira, 2013). Analysis was performed by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PAD), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PAD, using 215 and 245 nm as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

Antioxidant parameters

Extraction procedure

Lyophilized powdered mushrooms samples (1 g) were stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution) for *B. edulis* and 40 mg/mL for *H. repandum*; and stored at 4ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Heleno et al., 2010) to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the

absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

Two different procedures were used to evaluate the reducing power:

A) The first methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm.

B) The second methodology followed the Folin-Ciocalteu assay. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for color development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution was pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was

calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

TBARS (thiobarbituric acid reactive substances) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 µL) of the supernatant was incubated with the different concentrations of the samples solutions (200 µL) in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1 mM; 100 µL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B) / A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

All analyses (extractions) were performed in triplicate; each replicate was quantified also three times. Data were expressed as means ± standard deviations.

The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro-Wilk's, and the Levene's tests, respectively. For each parameter, significant differences among mean values were checked by Welch's statistics ($p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others). In cases where statistical significance differences were identified, the dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method. PCA transforms the original, measured variables

into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on (Patras et al., 2011). The number of dimensions to keep for data analysis was evaluated by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected. The number of dimensions considered for PCA was chosen in order to allow meaningful interpretations, to ensure their reliability.

All statistical tests were performed at a 5% significance level using the SPSS software, version 18.0 (SPSS Inc).

3.4.3. Results and discussion

Results regarding the evaluated chemical parameters and antioxidant activity assays are presented in **Tables 3.3.1-3.3.6**.

Nutritionally (**Table 3.3.1**), *B. edulis* and *H. repandum* showed similar profiles, presenting water as predominant component (≈93% in *B. edulis*; ≈95% in *H. repandum*) and carbohydrates (≈70% in *B. edulis*; ≈75% in *H. repandum*) as major compounds in dry mass basis, in agreement with previous works (Ouzouni & Riganakos, 2007; Heleno et al. 2011). Fat contents were very low, highlighting the dietary interest of both species, especially in view of their low dry mass contents. Concerning the effects of gamma-irradiation, the most noticeable change was observed in protein content, which decreased with irradiation for both mushrooms. Actually, proteins are among the most reliable irradiation indicators, especially due to degradation reactions such as scission of the C-N bonds in the backbone of the polypeptide chain or splitting of the disulfide bonds, and physical changes like unfolding and aggregation (Molins, 2001). The remaining nutritional parameters showed very slight changes in response to the applied gamma-irradiation doses; dry mass, in particular did not reveal significant (p = 0.775 for *B. edulis*; p= 0.156 for *H. repandum*) variation among irradiated and non-irradiated samples.

Table 3.3.1. Proximate composition and corresponding energetic value of *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses. The results are presented as mean ± SD.[a]

		Dry matter (g/100 g fw)	Fat (g/100 g dw)	Proteins (g/100 g dw)	Carbohydrates (g/100 g dw)	Ash (g/100 g dw)	Energy (kcal/100 g dw)
Boletus edulis	0 kGy	7±1	4.3±0.3 b	23±2 a	65±2 b	7.9±0.1 b	390±2 ab
	1 kGy	7.3±0.4	4.6±0.1 a	15±1 b	72±1 a	8.6±0.2 a	389±1 b
	2 kGy	7±1	4.5±0.1 ab	16.0±0.4 b	71.5±0.5 a	7.9±0.2 b	391±1 a
Homoscedasticity[b]	*p*-value	0.004	<0.001	0.001	<0.001	0.003	0.022
One-way ANOVA[c]	*p*-value	0.775	0.002	<0.001	<0.001	<0.001	0.003
Hydnum repandum	0 kGy	6±2	4.6±0.1 a	14.1±0.2 a	72±1 b	9±1 c	385±4 a
	1 kGy	6±1	4.0±0.2 b	12.2±0.1 b	72.7±0.3 b	11.1±0.2 a	376±1 c
	2 kGy	4.5±0.1	4.0±0.1 b	8±1 c	77±1 a	10.4±0.1 b	378±1 b
Homoscedasticity[b]	*p*-value	0.001	0.894	0.003	0.051	<0.001	<0.001
One-way ANOVA[c]	*p*-value	0.156	<0.001	<0.001	<0.001	<0.001	<0.001

fw - fresh weight; dw - dry weight.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among cultivars was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Free sugar profiles (**Table 3.3.2**) were quite different among the assayed species: trehalose was the main (≈ 14 g/100 g dw) sugar in *B. edulis*, a value similar to that previously reported by our research group (Heleno et al., 2011), while mannitol predominated (≈ 14 g/100 g dw) in *H. repandum*. The identified sugars decreased with irradiation in all cases. Sugars are known as being good indicators of a suitable conservation technology due to their sensibility to technical practices (Barreira, Pereira, Oliveira, & Ferreira, 2010). Irradiation, in particular, is known for causing several changes in sugars, such as melting point decreases, reduction in optical rotation and browning. Furthermore, sugars may suffer degradation, producing a mixture of gases consisting primarily of H_2 and CO_2, together with traces of CH_4, CO and H_2O. The relative proportions depend on the type of sugar irradiated and the absorbed dose (Molins, 2001), as verified in this case, where 1 kGy caused the minimization of sugar contents.

Tocopherol contents (**Table 3.3.3**) suffered the most marked effect in all quantified isoforms (α-, γ- and δ-tocopherol) presenting maximum values in samples irradiated with 1 kGy. Interestingly, the same irradiation dose minimized free sugars and maximized γ- and δ-tocopherol, a result that might be explained by the packaging atmosphere changes as a result of sugar degradation, since degradation of tocopherols is highly related with the availability of free oxygen. α-Tocopherol, present in low amounts, was detected only in non-irradiated samples.

Table 3.3.2. Sugar composition of *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses. The results are presented as mean ± SD.[a]

		Fructose (g/100 g dw)	Glucose (g/100 g dw)	Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Total sugars (g/100 g dw)
Boletus edulis	0 kGy	0.41±0.01 b	1.57±0.03 a	1.13±0.03 a	17.7±0.1 a	20.9±0.1 a
	1 kGy	0.21±0.02 c	0.80±0.02 c	0.32±0.02 c	11.3±0.1 c	12.6±0.1 c
	2 kGy	0.45±0.02 a	1.29±0.03 b	0.67±0.01 b	14.1±0.1 b	16.5±0.1 b
Homoscedasticity[b]	*p*-value	0.080	0.424	0.016	0.067	0.036
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Hydnum repandum	0 kGy	nd	nd	13.0±0.2 a	4.4±0.1 a	17.4±0.2 a
	1 kGy	nd	nd	12.5±0.1 b	4.25±0.02 b	16.8±0.1 b
	2 kGy	nd	nd	12.6±0.1 b	4.3±0.1 b	16.9±0.1 b
Homoscedasticity[b]	*p*-value	-	-	0.007	<0.001	0.209
One-way ANOVA[c]	*p*-value	-	-	<0.001	<0.001	<0.001

dw - dry weight; nd - not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, p-value > 0.05; heteroscedasticity, p-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Table 3.3.3. Tocopherol composition of *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses. The results are presented as mean ± SD.[a]

		α-Tocopherol (μg/100 g dw)	γ-Tocopherol (μg/100 g dw)	δ-Tocopherol (μg /100 g dw)	Total tocopherols (μg /100 g dw)
Boletus edulis	0 kGy	1.5±0.1	25±2 b	33±4 b	59±2 b
	1 kGy	nd	85±3 a	58±1 a	143±3 a
	2 kGy	nd	nd	24±1 c	24±1 c
Homoscedasticity[b]	*p*-value	<0.001	<0.001	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001
Hydnum repandum	0 kGy	2.19±0.03	nd	8.9±0.2 c	11.1±0.2 c
	1 kGy	nd	nd	113±1 a	113±1 a
	2 kGy	nd	nd	80±2 b	80±1 b
Homoscedasticity[b]	*p*-value	<0.001	-	0.007	0.045
One-way ANOVA[c]	*p*-value	<0.001	-	<0.001	<0.001

dw - dry weight; nd - not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed).

In regard to fatty acids (FA), 25 individual molecules were quantified in both mushroom species. **Table 3.3.4** presents the individual FA quantified above 0.2% in each mushroom species (C8:0, C10:0, C12:0, C14:1, C15:0, C17:0, C18:3, C20:3, C20:5, C22:1, C23:0 and C24:1 in both mushrooms, besides C6:0, C17:1 and C22.0 in *B. edulis* and C14:0 in *H. repandum* were also quantified, but in percentages lower than 0.2%). The most abundant FA in both mushrooms were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), as it is common in these species (Heleno et al., 2011; Kalač, 2009). The high percentage of the latter is probably associated with the highly appreciated organoleptic characteristics of these mushrooms, since linoleic acid is the precursor of oct-1-en-3-ol, known as “fungi alcohol”, the main aromatic component in fungi (Maga, 1981). It became evident that irradiation caused a decrease in unsaturated fatty acids. Irradiation may change lipid profile by catalyzing their reaction with molecular oxygen (autoxidation) or by the action of high-energy radiation itself, more evidently in both cases in unsaturated molecules (Nawar, 1986). In fact, the general mechanism of lipid radiolysis is thought to involve primary ionization, followed by migration of the positive charge either toward the carboxyl carbonyl group or double bonds (Molins, 2001), thus enhancing unsaturated fatty acid degradation.

The profiles in organic acids (**Table 3.3.5**) were different among the two assayed mushrooms, particularly in regard to malic acid, which was absent in *B. edulis*. However, citric acid was the predominant organic acid, except in *H. repandum* samples irradiated with 2 kGy. Irradiated samples (excluding oxalic acid in *B. edulis*) presented higher organic acids values, as was previously verified for tocopherols.

Table 3.3.4. Fatty acids composition (relative percentages) of *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses. The results are presented as mean ± SD.[a]

		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C20:1	C20:2	C24:0	SFA	MUFA	PUFA
Boletus edulis	0 kGy	0.100±0.002 c	7.6±0.1 c	0.63±0.01 b	2.89±0.02 b	34.1±0.2 c	51.7±0.2 a	0.38±0.02 a	0.88±0.02 b	0.38±0.02 a	0.21±0.01 b	11.6±0.1 b	35.9±0.2 c	52.5±0.2 a
	1 kGy	0.118±0.001 b	7.8±0.1 b	0.71±0.01 a	2.59±0.03 c	37.4±0.1 a	48.1±0.2 b	0.30±0.01 b	1.07±0.02 a	0.37±0.02 a	0.34±0.01 a	11.7±0.1 b	39.5±0.1 a	48.8±0.2 c
	2 kGy	0.37±0.01 a	8.5±0.1 a	0.54±0.01 c	3.81±0.04 a	35.2±0.3 b	48.3±0.4 b	0.38±0.02 a	0.77±0.02 c	0.33±0.02 b	0.21±0.03 b	14.0±0.1 a	36.8±0.3 b	49.1±0.4 b
Homoscedasticity[b]	p-value	<0.001	0.004	0.010	0.243	0.023	0.015	0.490	0.392	0.751	0.04	0.237	0.018	0.027
One-way ANOVA[c]	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

		C6:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:0	C20:1	C20:2	C22:0	C24:0	SFA	MUFA	PUFA
Hydnum repandum	0 kGy	0.11±0.01 c	13.1±0.1 c	0.24±0.01 c	2.24±0.03 c	37.2±0.3 b	39.3±0.3 a	0.21±0.02 b	5.7±0.1 a	0.33±0.01 a	0.20±0.01 b	0.18±0.01 b	16.6±0.1 c	43.2±0.2 b	40.2±0.3 a
	1 kGy	0.74±0.01 b	16.4±0.1 b	0.27±0.01 b	2.8±0.1 b	40.9±0.4 a	33.3±0.3 b	0.26±0.01 a	2.9±0.1 b	0.32±0.01 a	0.32±0.01 a	0.40±0.01 a	21.6±0.1 b	44.2±0.3 a	34.1±0.3 b
	2 kGy	1.0±0.1 a	17±1 a	0.30±0.02 a	2.9±0.1 a	40±1 a	33±1 b	0.26±0.02 a	2.5±0.1 c	0.28±0.03 b	0.31±0.02 a	0.39±0.04 a	23±1 a	43±1 b	34±1 b
Homoscedasticity[b]	p-value	<0.001	<0.001	0.127	0.029	0.007	0.092	0.291	0.082	0.015	0.028	<0.001	<0.001	0.001	0.087
One-way ANOVA[c]	p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

dw - dry weight.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, p-value > 0.05; heteroscedasticity, p-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Table 3.3.5. Organic acid composition of *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses. The results are presented as mean ± SD.[a]

		Oxalic acid (g/100 g dw)	Malic acid (g/100 g dw)	Citric acid (g/100 g dw)	Fumaric acid (g/100 g dw)	Total organic acids (g/100 g dw)
Boletus edulis	0 kGy	0.485±0.004 a	nd	4.7±0.1 b	0.067±0.001 b	5.3±0.1 b
	1 kGy	0.36±0.01 b	nd	5.3±0.1 a	0.082±0.002 a	5.71±0.05 a
	2 kGy	0.18±0.1 c	nd	4.0±0.1 c	0.069±0.004 b	4.3±0.1 c
Homoscedasticity[b]	*p*-value	0.002	-	0.283	0.034	0.302
One-way ANOVA[c]	*p*-value	<0.001	-	<0.001	<0.001	<0.001
Hydnum repandum	0 kGy	0.0031±0.0002 c	3.47±0.03 b	3.82±0.03 b	0.61±0.01 b	7.90±0.04 b
	1 kGy	0.030±0.002 a	3.28±0.04 b	4.02±0.05 a	0.63±0.01 ab	7.96±0.05 b
	2 kGy	0.027±0.002 b	5.6±0.4 a	3.9±0.2 ab	0.65±0.04 a	10.2±0.4 a
Homoscedasticity[b]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	0.015	0.009	<0.001

dw - dry weight; nd- not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

The antioxidant potential of *B. edulis* and *H. repandum* was used as a measure of their bioactivity. Five *in vitro* chemical and biochemical assays were used: scavenging effects on DPPH radicals (measures the decrease in DPPH radical absorption after exposure to radical scavengers), reducing power (conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}, further denominated as Prussian blue assay, and Folin-Ciocalteau method), inhibition of β-carotene bleaching (measures the capacity to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models) and inhibition of lipid peroxidation in brain cell homogenates (measures the color intensity of MDA-TBA complex formed at the endpoint of the reaction). As mentioned in previous reports, *B. edulis* also gave higher antioxidant activity than *H. repandum* (Heleno et al., 2010; Heleno et al., 2011). Performed assays (**Table 3.3.6**) indicate that irradiated samples tended to have lower scavenging activity and reducing power, but higher lipid peroxidation inhibition. The observed decrease might be related with free radicals resulting from lipid radiolysis, which was higher in irradiated samples. On the other hand, the increase in lipid peroxidation inhibition is probably associated with the higher amount of tocopherols (powerful lipophilic antioxidants) detected in irradiated samples.

Table 3.3.6. *In vitro* antioxidant properties obtained for the extracts *B. edulis* and *H. repandum* samples submitted to different gamma irradiation doses (mean ± SD).[a] Values are presented as EC_{50} values (mg/mL) for all assays except Folin-Ciocalteau, expressed as mg GAE/g extract.

			Reducing power		Lipid peroxidation inhibition	
		DPPH scavenging activity	Ferricyanide/ Prussian blue assay	Folin-Ciocalteu assay	β-Carotene bleaching inhibition	TBARS formation inhibition
Boletus edulis	0 kGy	1.54±0.03 c	0.71±0.01 c	37±1 a	1.6±0.1 c	1.6±0.1 a
	1 kGy	2.22±0.03 a	0.96±0.02 a	30±1 b	2.5±0.3 a	0.53±0.03 b
	2 kGy	1.9±0.1 b	0.76±0.02 b	36±1 a	1.9±0.2 b	0.54±0.02 b
Homoscedasticity[b]	*p*-value	0.001	<0.001	0.018	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Hydnum repandum	0 kGy	31±1 c	2.47±0.05 c	6.8±0.2 b	3.8±0.1 b	0.9±0.1 b
	1 kGy	34±1 b	2.6±0.1 b	7.6±0.1 a	3.0±0.4 c	0.7±0.1 c
	2 kGy	39.6±0.5 a	2.8±0.1 a	6.9±0.2 b	8.3±0.4 a	1.1±0.1 a
Homoscedasticity[b]	*p*-value	0.022	0.051	0.069	0.197	0.002
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

dw - dry weight.
EC_{50} extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay. Concerning the Folin-Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, which means that higher values correspond to lower reducing power or antioxidant potential.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each gamma irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, p-value > 0.05; heteroscedasticity, p-value < 0.05.
[c] $p < 0.05$ meaning that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

To verify which parameters were more related with the detected differences, a principal components analysis (PCA) was applied to each mushroom species. The plot of component loadings (**Figure 3.3.1**) for *B. edulis*, indicates that the first two dimensions (first: Cronbach's α, 0.980; eigenvalue, 24.793; second: Cronbach's α, 0.962; eigenvalue, 17.447) account for most of the variance of all quantified variables (50.60% and 35.61%, respectively). Groups corresponding to each irradiation level (0 kGy, 1 kGy and 2 kGy) were clearly separated. Group corresponding to 0 kGy was more positively correlated to proteins, α-tocopherol, C6:0, C18:2, PUFA and TBARS (*i.e.*, it presented high contents in these parameters and was a weak inhibitor of TBARS formation); and more negatively correlated to fat, carbohydrates, C15:0, C17:1, C18:1, β-carotene and DPPH (*i.e.*, it presented low contents in these parameters and was a strong DPPH scavenger). 1 kGy was more positively correlated to ash, C23:0, C24:0, MUFA, fumaric acid and reducing power (Prussian blue assay, PBA); the highest negative correlations were associated with energy, fructose, C20:0, C20:5 and reducing power (Folin-Ciocalteau assay, F-CA); it should be noted that the correlations with reducing power are not conflicting since high values in PBA and low values in FC-A both mean lower reducing power. Objects corresponding to 2 kGy were mostly characterized by high contents in C10:0, C12:0, C14:0, C16:0, C17:0 and C20:3 and low contents in C20:2, C22:1, C24:1 and oxalic acid.

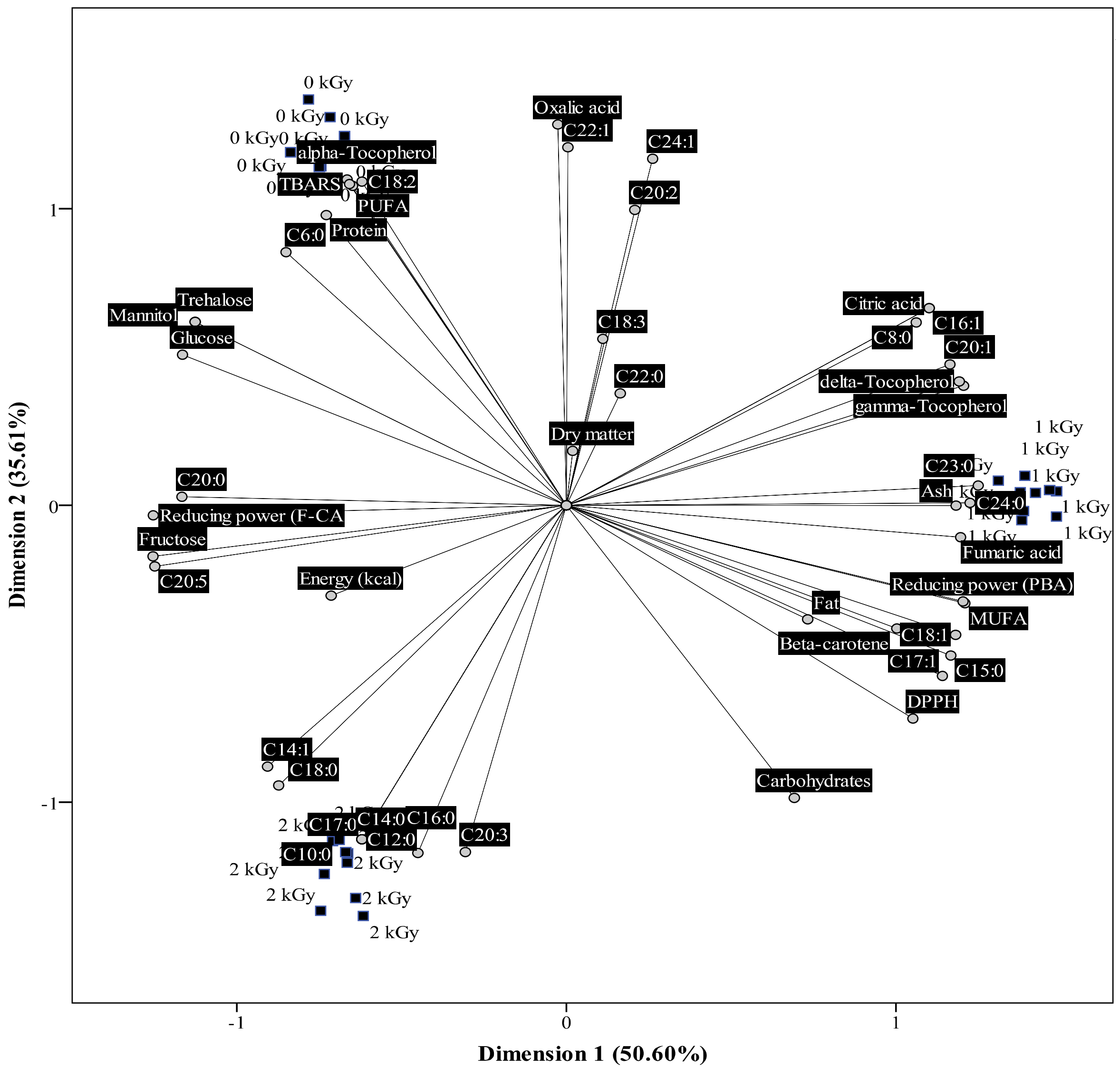

Figure 3.3.1. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *B. edulis*.

Concerning *H. repandum* (**Figure 3.3.2**) objects corresponding to each irradiation level were also clearly separated. The first two dimensions (first: Cronbach's α, 0.986; eigenvalue, 28.386; second: Cronbach's α, 0.907; eigenvalue, 8.855) include also most of the variance of all quantified variables (61.71% and 19.25%, respectively). In this case, the group corresponding to 0 kGy was more positively correlated to fat, energy, mannitol, trehalose, α-tocopherol, C18:2, C18:3, C20:1 and PUFA, and more negatively correlated to C6:0, C8:0, C14:1, C16:0, C17:0, C18:0, C18:1, C20:0, C22:0, C22:1, C24:0 and oxalic acid. Objects corresponding to 1 kGy were more positively correlated to C24:1, MUFA, citric acid and reducing power (F-CA); no negative correlations were detected. Finally, the 2

kGy group was mostly characterized by having high scores in carbohydrates, C10:0, C12:0, C14:0, C15:0, C16:1, fumaric acid, malic acid, β-carotene bleaching prevention, TBARS formation inhibition and reducing power (PBA) and low contents in dry matter, proteins, C20:2, C20:3 and C20:5.

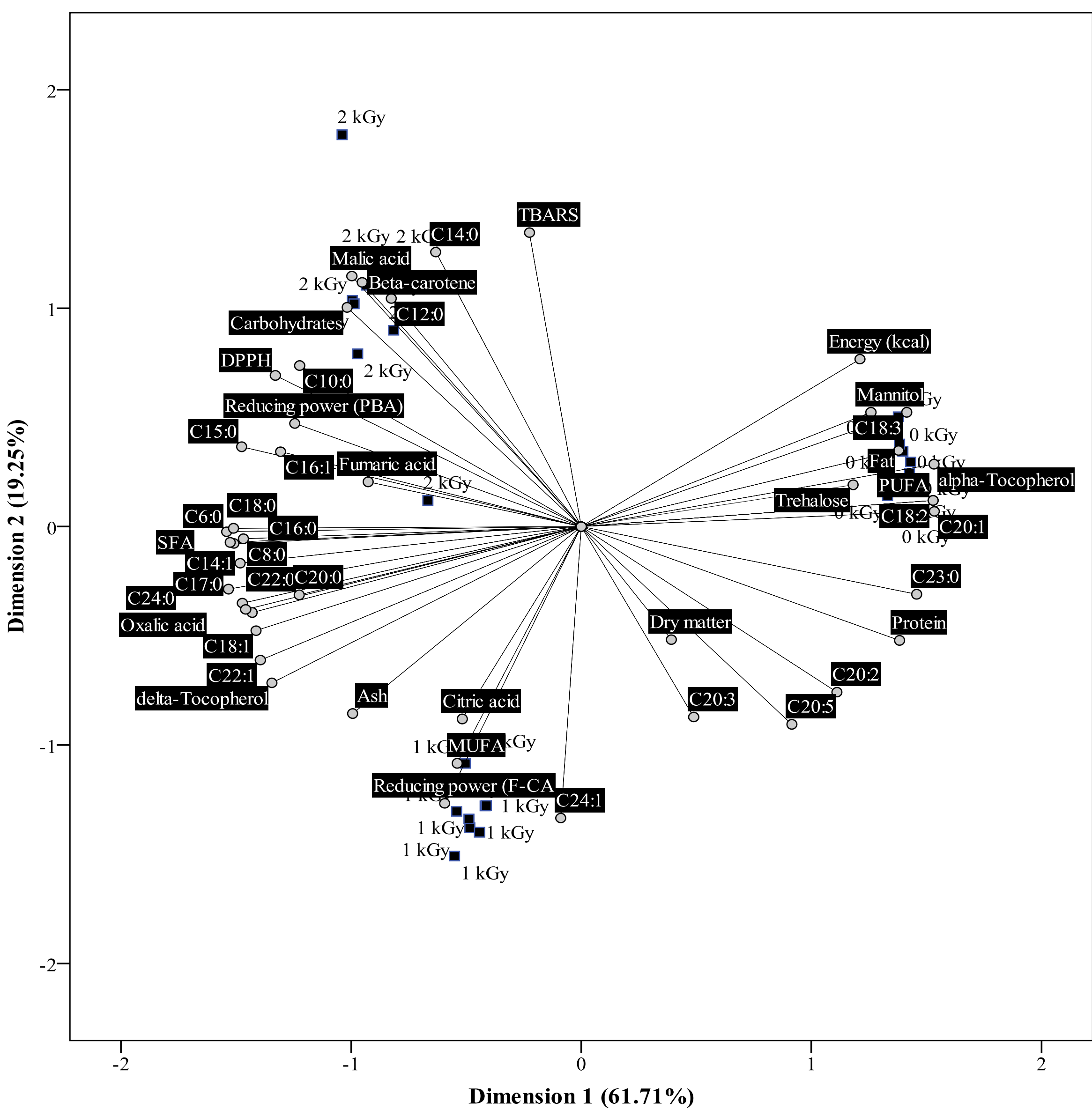

Figure 3.3.2. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *H. repandum*.

Overall, it might be concluded that irradiation caused changes in minor individual compounds. The spatial distribution of PCA biplot markers in different clusters (corresponding to each irradiation dose) confirmed that irradiation exerted marked effects over the assayed parameters. Fatty acids in particular, seemed to be the most affected components, since their component loadings were often correlated with the defined objects. Nevertheless, despite the detected differences in individual compounds, the results of nutritional parameters (the most relevant in terms of mushroom acceptability by consumers) were less affected, indicating an interesting potential of gamma-irradiation to be used as an effective conservation technology. Furthermore, considering previous research outcomes, irradiation minimizes the effects caused by storage time, being definitely indicated to be applied to mushrooms.

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3.3.4. References

Andrews, L. S., Ahmedna, M., Grodner, R. M., Liuzzo, J. A., Murano, P. S., & Murano, E. A. (1998). Food preservation using ionizing radiation. *Reviews of Environmental Contamination and Toxicology*, *154*, 1-53.

AOAC (1995). *Official methods of analysis* (16th ed.) Arlington VA, USA: Association of Official Analytical Chemists.

Barreira, J. C. M., Pereira, J. A., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2010). Sugars profiles of different chestnut (*Castanea sativa* Mill.) and almond (*Prunus dulcis*) Cultivars by HPLC-RI. *Plant Foods for Human Nutrition*, *65*, 38-43.

Barros, L., Pereira, C., & Ferreira, I. C. F. R. (2013). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. *Food Analytical Methods*, *6*, 309-316.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Botelho, L., Oliveira, M. B. P. P., Martins, A., et al. (2012a). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, http://dx.doi.org/10.1007/s11947-012-0931-5.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012b). Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushroom. *Postharvest Biology and Technology*, *74*, 79-84.

Fernandes, Â., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2012c). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*, 1443-1450.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., Santos-Buelga, C., & Ferreira, I. C. F. R. (2011). Targeted metabolites analysis in wild Boletus species. *LWT-Food Science and Technology*, *44*, 1343-1348.

Jolivet, S., Arpin, N., Wichers, H. J., & Pellon, G. (1998). Agaricus bisporus browning: a review. *Mycological Research*, *102*, 1459-1483.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9-16.

Lacroix, M., & Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products - a review. *Food Research International*, *33*, 719-724.

Léon-Guzmán, M. F., Silva, I., & Lopez, M. G. (1997). Proximate chemical composition, free amino acid contents, and free fatty acids contents of some wild edible mushrooms from Queretaro, Mexico. *Journal of Agricultural and Food Chemistry*, *45*, 4329-4332.

Maga J. A. (1981). Mushroom flavor. *Journal of Agricultural and Food Chemistry*, *29*, 4-7.

Martins, A., Baptista, P., Sousa, M. J., Meireles, T., & Pais, M. S. (2002). Edible mycorrhizal fungi associated with *Castanea sativa* Mill trees in the Northeast of Portugal. In I. Hall, Wan Yun, E. Danell, & A. Zambonelli (Eds.). *Proceedings of the second international workshop on edible mycorrhizal fungi* (ISBN 0-478-10828-X).

Molins, R. (2001). Food Irradiation. *Principles and applications*. USA: John Wiley & Sons, USA. 0-471-35634-4.

Nawar, W. W. (1986). Volatiles from food irradiation. *Food Reviews International*, *2*, 45-78.

Ouzouni, P. K., & Riganakos, K. A. (2007). Nutritional value and metal content of Greek wild edible fungi. *Acta Alimentaria*, *36*, 99-110.

Patras, A., Brunton, N. P., Downey, G., Rawson, A., Warriner, K., & Gernigon, G. (2011). Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *Journal of Food Composition and Analysis*, *24*, 250-256.

Skou, J. F., Beett, H., & Lundsten, K. (1974). Effects of ionizing radiation on mushrooms as influenced by physiological and environmental conditions. *Radiation Botany*, *14*, 287-299.

Stevenson, M. H. (1994). Nutritional and other implications of irradiating meat. *Proceedings of the Nutrition Society*, *53*, 317-325.

3.4

Uso generalizado da irradiação gama na conservação de cogumelos silvestres: validação da dose de 2 kGy para preservação de características químicas

Este sub-capítulo apresenta a validação da radiação gama com a dose de 2 kGy, analisando os parâmetros nutricionais (valor energético, açúcares livres, ácidos gordos, tocoferóis e ácidos orgânicos) e os parâmetros bioativos (atividade antioxidante e compostos fenólicos individuais) em amostras de Boletus pinophilus *Pilát & Dermek e* Clitocybe subconnexa *Murrill.*

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Extended use of gamma irradiation in wild mushrooms conservation: validation of 2 kGy dose to preserve their chemical characteristics

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Abstract

Irradiation is recognized by international organizations as a conservation technology, and its application to wild mushrooms has been tested in some species. Our research group evaluated the effectiveness of gamma irradiation to conserve different samples of highly appreciated species, particularly, *Lactarius deliciosus*, *Macrolepiota procera*, *Boletus edulis* and *Hydnum repandum*. From those results and considering also international recommendations on this subject, the 2 kGy dose was

chosen for further studies. Therefore, the application of gamma irradiation at 2 kGy dose was extended to *Boletus pinophilus* Pilát & Dermek and *Clitocybe subconnexa* Murrill to validate the proposed technology. Considering the obtained results, some of the analysed chemical parameters (specially sugars and fatty acids), as well as the antioxidant activity, showed significant changes after irradiation treatment, particularly in *B. pinophillus*, probably due to its higher water content. Nevertheless, the obtained differences did not seem to be sufficient to change the organoleptic characteristics of these mushrooms. Furthermore, the antioxidant activity was generally higher in irradiated samples. In conclusion, the detected chemical changes might be considered as acceptable, when considering the high advantages of gamma irradiation at decontamination and/or disinfestation level.

Keywords: Gamma irradiation; Wild mushrooms; Chemical composition; Antioxidant activity.

3.4.1. Introduction

Wild edible mushrooms, especially abundant in woods and forests, are natural resources of growing significance and market search. In recent years, mushrooms' picking ceased to be a family activity, of reduced size, to become a booming business, with annual trading indicators of thousands of tons of mushrooms in particular countries like Portugal (Koune, 2001; Marques, 2005).

The seasonal consumption of fresh mushrooms (mostly wild species that are highly consumed in fresh, being only available in specific periods) is mainly related to its high perishable nature. After harvest, the signs of deterioration include dehydration, loss of texture, enzymatic browning and bacterial lesions (Aguirre, Frias, Barry-Ryan & Grogan, 2008; Kulshreshtha, Singh & Deepti-Vipul, 2009). Mushroom' conservation and distribution are critical points, demanding appropriate methods of preservation once the level of losses during marketing achieves 40% (Lacroix & Ouattara, 2000).

Applying irradiation might be an alternative to minimize these losses, being recognized by international organizations as a valid conservation technology (WHO, 1991; Nagar, Hajare, Saroj & Bandekar, 2012), with recognized benefits such as extending the shelf life of many foods, stopping the maturation process,

decontaminating and lowering the presence of bacteria and fungi (Minnaar, Taylor & McGill, 1995).

Our research group evaluated the effectiveness of gamma irradiation in the maintenance of wild mushrooms' quality, using fresh and/or processed samples, with focus on *Lactarius deliciosus* (Fernandes et al., 2013a), *Macrolepiota procera* (Fernandes et al., 2014a), *Boletus edulis* and *Hydnum repandum* (Fernandes et al., 2013b). Gamma irradiated (up to 2 kGy) samples of *B. edulis* (fresh), maintain the amounts of fat, carbohydrates, ash, fructose, glucose, mannitol, trehalose, citric and fumaric saturated fatty acids as those observed in non-irradiated samples. Likewise, the antioxidant activity was preserved after irradiation (Fernandes et al., 2013b). The same dose had a similar effect on *H. repandum* fresh samples, as verified by the preservation of carbohydrates, mannitol, trehalose, ash, δ-tocopherol, saturated fatty acids, oxalic, malic, citric and fumaric acids, as well as its antioxidant activity (Fernandes et al., 2013b).

In different countries (Argentina, China, Croatia, Hungary, Israel, Korea, Poland, United Kingdom, Mexico) the recommended dose for extending the shelf life of fresh mushrooms is 1-3 kGy (Akram & Kwon, 2010). Moreover, ICGFI (1999) reported that irradiation of mushrooms at 2 to 3 kGy inhibits cap opening, stem elongation and has been shown to have minimal effect on flavour, aroma and colour (ICGFI, 1999, Sommer et al., 2009, 2010). The texture of mushroom is often the first of many quality attributes judged by the consumer and is, therefore, extremely important in overall product acceptance. However, many studies indicate that mushrooms' firmness is similar among irradiated and non-irradiated samples (Jiang et al., 2010; Fernandes et al., 2012).

According to Lacroix & Ouattara (2000), a dose of 2 kGy appears to be necessary for satisfactory prolongation of the shelf life of mushrooms at 10 ºC.

Therefore, the aim of the present work was validating the use of 2 kGy of gamma irradiation as a technology able to maintain chemical parameters of wild mushrooms, extending previous studies to unreported edible species: *Boletus pinophilus* Pilát & Dermek and *Clitocybe subconnexa* Murrill.

3.4.2. Materials and methods

Standards and Reagents

For irradiation: To estimate the dose and dose rate of irradiation it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM, 1992) and Amber Perspex dosimeters (batch V, from Harwell Co., UK). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA).

For chemical analyses: Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, organic acid, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA).

For antioxidant potential analysis: 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

Samples and samples irradiation

B. pinophilus wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012 and *C. subconnexa* in November 2013. Subsequently, the samples were divided in two groups with five mushrooms per group for *B. pinophilus* and seven mushrooms in each group, for *C. subconnexa*: control (non-irradiated, 0.0 kGy) and irradiated (2 kGy). The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, and the irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity 198 TBq (5.33 kCi) in November 2012 (Precisa 22, Graviner Manufacturing Company Ltd, U.K.), following the procedure previously described by the authors (Fernandes et al.,

2013a). The estimated doses, dose rates and dose uniformity ratios (Dmax/Dmin) were: 2.09±0.16 kGy, 1.56 kGy/h, 1.18 for *B. pinophilus*; and 1.95±0.22 kGy, 1.95 kGy/h, 1.33 and for *C. subconnexa*.

All the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples for subsequent analysis.

Chemical parameters

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). Moisture content was evaluated by lyophilization (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), crude protein content (N×4.38) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600±15 ^{o}C using a Chamber furnace Lenton Thermal Designs Ltd, model ECF 12/22. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × ($g_{protein}$) + 3.75 × ($g_{carbohydrate}$) + 9 × (g_{fat}).

Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) according to the extraction procedure described by Heleno et al., (2011), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analyzed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ^{o}C (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards.

Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), according to the extraction and derivatization procedures described previously (Heleno et al., 2011). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a flame ionization detector (FID) at 260 ºC and a Macherey-Nagel (Düren, Germany) column (50% cyanopropyl-methyl-50%phenylmethylpolysiloxane, 30 m × 0.32 mm i.d. × 0.25 μm d_f). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the Clarity DataApex 4.0 Software and expressed in relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined as previously described, using tocol as IS (Heleno, Barros, Sousa, Martins & Ferreira, 2010). The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols content was further expressed in μg per 100 g of dry weight (dw).

Organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira & Ferreira, 2013). Analysis was performed by ultra fast liquid chromatograph (UFLC) coupled to photodiode array detector (PAD), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PAD, using 215 and 245 nm as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

Phenolic compounds

Phenolic compounds were determined in the UFLC system mentioned above, as previously described by the authors (Barros et al. 2009). DAD detection was carried out using 280 nm and 370 nm as preferred wavelengths. The phenolic compounds were characterized according to their UV spectra and retention times, and comparison with authentic standards. For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in µg per 100 g dw.

Antioxidant parameters

Extraction procedure

Lyophilized powdered mushrooms samples (1 g) were stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution) for both mushrooms; and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Heleno, Barros, Sousa, Martins & Ferreira, 2010) to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of

antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek, Winooski, VT, USA). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μL) and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\% RSA = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

Two different procedures were used to evaluate the reducing power:

A) The first methodology was performed using the microplate reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm.

B) The second methodology followed the Folin-Ciocalteu assay. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for color development. Absorbance was then measured at 765 nm. Gallic acid was used

to obtain the standard curve (0.0094-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

TBARS (thiobarbituric acid reactive substances) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 µL) of the supernatant was incubated with the different concentrations of the samples solutions (200 µL) in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1 mM; 100 µL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

All extractions were performed in triplicate and each replicate was analyzed three times. Data were expressed as mean±standard deviation. The normal distribution of the residuals and the homogeneity of variance was tested by means of the Shapiro-Wilk and the Levene's tests, respectively. Results were classified using a *t*-test for equality of means. All statistical tests were performed at a 5% significance level using the SPSS software, version 20.0 (SPSS Inc).

3.4.3. Results and discussion

Effects on chemical parameters

As far as we know, there are no reports describing the nutritional and chemical composition or the antioxidant activity of *C. subconnexa*. Hence, comparisons with previous works could not be made.

The proximate composition and energetic value of *B. pinophilus* and *C. subconnexa* (**Table 3.4.1**) were quite distinct, which is an advantageous feature, considering the main purpose of validating gamma irradiation at 2 kGy as a suitable technology to increase wild mushrooms shelf life, preserving the wholesomeness of their chemical characteristics. Either way, water was the predominant component (≈93% in *B. pinophilus*; ≈70% in *C. subconnexa*).

In foods with high water percentages, irradiation might generate three primary free radicals (hydroxyl, hydrogen atoms and hydrated electrons). Therefore, several types of chemical compounds and quality attributes should be evaluated in irradiated foods, in order to understand the potential damages induced by irradiation treatment. Herein, a special attention was dedicated to those parameters more prone to suffer significant changes when treated with gamma irradiation. Other chemical substances, which typically occur in the same level as that observed in different heat treatments, were not considered at this stage (Institute of Food Science and Technology, 2015).

Regarding the composition in dry basis, *B. pinophilus* stood out for its content in proteins (≈55 g/100 g dw), indicating its adequacy to be included in vegetarian diets, carbohydrates (≈31 g/100 g dw), ash (≈8 g/100 g dw) and fat (≈5 g/100 g dw) contents. The detected values are generally in agreement with previous works (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004; Heleno et al., 2011; Fernandes et al.,

2014b). In *C. subconnexa*, carbohydrates were the major component (≈90 g/100 g dw), followed by ash (≈6 g/100 g dw), proteins (≈3 g/100 g dw) and fat (≈1 g/100 g dw) contents. In terms of gamma irradiation effects, dry matter, ash and carbohydrates revealed significant changes in both mushrooms. The effect on the protein content of *B. pinophillus* was also significant, as verified in other *Boletus* species submitted to gamma irradiation (Fernandes et al., 2013b).

Mannitol was the sugar detected in highest amount (**Table 3.4.2**) in *C. subconnexa*, while trehalose was the main sugar in *B. pinophillus*, in agreement with previous reports studying the *Boletus* genus (Heleno et al., 2011; Fernandes et al., 2013b, 2014b). All sugars showed a significant decrease in irradiated samples for both mushrooms, except for mannitol in *C. subconnexa*. The verified degradation may be explained by a reduction in the optical rotation of sugars, which is a common occurrence when sugars are irradiated (Molins, 2001).

Table 3.4.1. Proximate composition and energetic value of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD.

		Dry matter (g/100 g fw)	Fat (g/100 g dw)	Proteins (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
B. pinophilus	0 kGy	7±1	5.2±0.1	52±3	8.0±0.2	34±3	394±1
	2 kGy	6±1	5.2±0.2	59±3	8.3±0.2	28±3	393±2
Levene's test	*p*-value	0.623	0.082	0.438	0.642	0.454	0.249
t-test	*p*-value	0.022	0.820	<0.001	0.010	<0.001	0.102
C. subconnexa	0 kGy	34±3	1.4±0.1	2.9±0.2	5.5±0.3	90.2±03	385±2
	2 kGy	27±2	1.4±0.2	3.0±0.4	6.4±0.3	89.3±0.5	381±2
Levene's test	*p*-value	0.253	0.230	<0.001	0.963	0.052	0.730
t-test	*p*-value	<0.001	0.449	0.702	<0.001	<0.001	<0.001

Table 3.4.2. Free sugars (g/100 g dw) of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD.

		Fructose	Mannitol	Trehalose	Total sugars
B. pinophilus	0 kGy	0.085±0.05	0.82±0.05	6.6±0.1	7.6±0.1
	2 kGy	0.101±0.001	0.71±0.02	6.0±0.2	6.8±0.2
Levene's test	*p*-value	0.009	0.002	<0.001	<0.001
t-test	*p*-value	<0.001	0.002	<0.001	<0.001
C. subconnexa	0 kGy	0.37±0.03	9.5±0.3	1.56±0.03	11.4±0.4
	2 kGy	0.25±0.01	9.1±0.1	1.46±0.04	10.8±0.2
Levene's test	*p*-value	0.035	<0.001	<0.001	<0.001
t-test	*p*-value	<0.001	0.063	<0.001	<0.001

Regarding fatty acids profiles (**Table 3.4.3**), the studied mushrooms were surprisingly similar, with C18:1 (≈45% in *B. pinophilus*; ≈50% in *C. subconnexa*), C18:2 (≈42% in *B. pinophilus*; ≈36% in *C. subconnexa*) and C16:0 (≈7% in *B. pinophilus*; ≈8% in *C. subconnexa*) as major compounds. Due to the magnitude of their relative percentages, these results were ultimately reflected in the amounts of saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). The fatty acids profile of *B. pinophilus* showed percentages typically reported for *Boletus* genus (Hanuš, Shkrob & Dembitsky, 2008; Heleno et al., 2011). *B. pinophilus*, most likely because of its higher fat content, proved to be less sensitive to irradiation, since C18:3, C20:0 and C20:1 did not show significant changes. On the other hand, all fatty acids (except C16:1) quantified in *C. subconnexa* gave significant changes among unirradiated and irradiated samples. In most cases, these differences were characterized as slight decreases, which may result from oxidative or radiolytic processes. In fact, the observed changes were more likely produced by autoxidation, since the presence of oxygen accelerates the formation of free radicals and causes the breakdown of hydrogen peroxide and the destruction of antioxidants that would normally scavenge the free radicals formed (Nawar, 1977). The same reasoning might be applied to the α-tocopherol (in *C. subconnexa*) and δ-tocopherol (in *B. pinophilus*) contents, which showed the same decreasing effect (**Table 3.4.3**).

Table 3.4.3. Fatty acids (relative percentage)* and tocopherols (μg/g dw) of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD.

Analyte	*B. pinophilus*		Levene's test	*t*-test	*C. subconnexa*		Levene's test	*t*-test
	0 kGy	2 kGy	*p*-value	*p*-value	0 kGy	2 kGy	*p*-value	*p*-value
C14:0	0.134±0.002	0.125±0.002	0.602	<0.001	0.37±0.02	0.22±0.01	0.079	<0.001
C15:0	0.072±0.002	0.058±0.003	0.043	<0.001	0.40±0.01	0.34±0.01	0.673	<0.001
C16:0	7.3±0.1	6.9±0.1	0.003	<0.001	8.7±0.2	7.5±0.2	0.668	<0.001
C16:1	0.84±0.05	0.76±0.05	<0.001	0.010	0.63±0.05	0.64±0.02	0.014	0.691
C18:0	1.9±0.1	2.0±0.1	0.762	0.011	2.1±0.1	1.6±0.1	0.336	<0.001
C18:1	45.8±0.3	45.1±0.2	0.450	<0.001	47.9±0.3	52.5±0.3	0.993	<0.001
C18:2	41.0±0.2	42.3±0.2	0.548	<0.001	37.3±0.2	35.1±0.2	0.353	<0.001
C18:3	0.70±0.05	0.67±0.04	0.025	0.208	0.48±0.01	0.18±0.01	0.249	<0.001
C20:0	0.21±0.01	0.21±0.01	0.943	0.936	0.10±0.01	0.07±0.01	0.237	<0.001
C20:1	0.54±0.01	0.54±0.02	0.034	0.636	0.48±0.02	0.52±0.03	0.127	0.005
C20:2	0.23±0.01	0.21±0.01	0.022	0.001	0.09±0.01	0.10±0.01	0.911	0.031
C24:0	0.18±0.01	0.16±0.01	0.084	<0.001	0.38±0.02	0.27±0.02	0.584	<0.001
SFA	10.3±0.1	9.9±0.2	0.098	<0.001	12.7±0.1	10.6±0.1	0.329	<0.001
MUFA	47.5±0.3	46.7±0.2	0.238	<0.001	49.2±0.2	53.8±0.3	0.620	<0.001
PUFA	42.2±0.3	43.4±0.2	0.286	<0.001	38.0±0.2	35.6±0.2	0.413	<0.001
α-Tocopherol	nd	nd	-	-	5.2±0.5	4.5±0.4	0.495	0.004
δ-Tocopherol	70±7	29±5	0.251	<0.001	nd	nd	-	-

*Besides the tabled fatty acids, C6:0, C8:0, C10:0, C12:0, C14:1, C17:0, C20:3, C20:5, C22:0, C22:1, C23:0 and C24:1, were also quantified in amounts lower than 0.2% for both mushrooms.
nd - not detected.

The organic acids profile (**Table 3.4.4**) showed only oxalic acid and fumaric acid in both mushrooms, which revealed also similar amounts of each compound. Among the assayed parameters, the organic acids seemed to be the most resistant to gamma-irradiation, since only fumaric acid showed a significant increase in irradiated samples of *C. subconnexa*.

Table 3.4.4. Organic acids (g/100 g dw) of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD.

		Oxalic acid	Fumaric acid	Organic acids
B. pinophilus	0 kGy	4.0±0.2	0.35±0.03	4.3±0.2
	2 kGy	4.1±0.2	0.37±0.04	4.4±0.2
Levene's test	*p*-value	0.098	0.206	0.146
t-test	*p*-value	0.408	0.192	0.284
C. subconnexa	0 kGy	3.2±0.2	0.27±0.03	3.5±0.3
	2 kGy	3.4±0.2	0.31±0.04	3.7±0.2
Levene's test	*p*-value	0.604	0.472	0.038
t-test	*p*-value	0.141	0.023	0.053

Regarding phenolic acids composition (**Table 3.4.5**), *p*-hydroxybenzoic acid was the only compound detected in *B. pinophilus*, while *C. subconnexa* showed also gallic acid and protocatechuic acid, despite the lower quantified amounts in this species. Cinnamic acid was only detected in *B. pinophilus*, as it was previously reported for *B. reticulatus* (Heleno et al., 2011). Protocatechuic acid (in *C. subconnexa*) and cinnamic acid (in *B. pinophilus*) showed a significant decrease in irradiated samples, while *p*-hydroxybenzoic acid (in *C. subconnexa*) increased significantly with irradiation.

Table 3.4.5. Phenolic acids and related compounds (μg/100 g dw) of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD.

		Gallic acid	Protocatechuic acid	*p*-Hydroxybenzoic acid	Phenolic acids	Cinnamic acid
B. pinophilus	0 kGy	nd	nd	17.8±0.4	17.8±0.4	5.0±0.2
	2 kGy	nd	nd	17.9±0.3	17.9±0.3	4.7±0.2
Levene's test	*p*-value	-	-	0.618	0.618	0.415
t-test	*p*-value	-	-	0.434	0.434	<0.001
C. subconnexa	0 kGy	1.4±0.1	4.3±0.2	0.5±0.1	6.3±0.2	nd
	2 kGy	1.5±0.1	3.9±0.1	1.0±0.1	6.4±0.2	nd
Levene's test	*p*-value	0.061	0.159	0.015	0.954	-
t-test	*p*-value	0.139	<0.001	<0.001	0.198	-

Effects on antioxidant parameters

In order to know the effect of gamma irradiation in the antioxidant activity, five chemical and biochemical assays were tested (**Table 3.4.6**). *B. pinophilus* extracts showed to be more active as radical scavengers and reducing agents, while *C. subconnexa* showed higher activity as a lipid peroxidation inhibitor. Except for the results obtained from reducing power (as assessed through Folin-Ciocalteau assay) and β-carotene bleaching assays in *B. pinophilus* extracts, the antioxidant activity showed a significant increase in irradiated samples (except for TBARS formation inhibition in *C. subconnexa* extracts). Since phenolic acids and tocopherols decreased with irradiation, these results indicate that other antioxidant molecules (*e.g.*, ascorbic acid, flavonoids or carotenoids) might be present in higher amounts in mushroom samples submitted to irradiation.

Table 3.4.6. *In vitro* antioxidant properties obtained for the extracts of *Boletus pinophilus* and *Clitocybe subconnexa*. The results are presented as mean±SD. Values are presented as EC_{50} values (mg/mL) for all assays except Folin-Ciocalteau, expressed as mg GAE/g extract.

			Reducing power		Lipid peroxidation inhibition	
		DPPH scavenging activity	Ferricyanide/ Prussian blue assay	Folin-Ciocalteu assay	β-Carotene bleaching inhibition	TBARS formation inhibition
B. pinophilus	0 kGy	1.78±0.01	0.69±0.01	43±1	0.8±0.1	2.2±0.1
	2 kGy	1.71±0.03	0.62±0.01	44±1	0.9±0.2	1.1±0.1
Levene's test	*p*-value	0.022	<0.001	0.330	0.039	0.194
t-test	*p*-value	<0.001	<0.001	0.061	0.144	<0.001
C. subconnexa	0 kGy	2.9±0.1	1.05±0.02	32±1	0.61±0.02	0.7±0.1
	2 kGy	2.2±0.2	0.92±0.01	34±1	0.58±0.03	1.3±0.1
Levene's test	*p*-value	0.856	0.020	0.586	0.271	0.003
t-test	*p*-value	<0.001	<0.001	0.002	0.005	<0.001

3.4.3. Conclusions

Overall, some of the analysed chemical parameters showed significant changes after irradiation treatment. Nevertheless, the magnitude of the obtained differences did not seem to be sufficient to affect the chemical profiles of the assayed mushrooms. The higher effects observed in *B. pinophilus* might be related to its higher water content, which make it more prone to radiolysis and the consequent formation of oxidizing hydroxyl radicals and reducing aqueous electrons and hydrogen atoms that can cause several reactions such as addition to carboxylic acids, ketones, aldehydes, thiols, aromatic and olefinic compounds or abstracting hydrogen atoms from C-H bonds (Molins, 2001). The effect observed in the antioxidant activity of both mushrooms was quite interesting, since it showed an increased bioactivity in most cases, reflecting an additional advantage of irradiating mushrooms with the suggested dose. In conclusion, the detected chemical changes might be considered as allowable, in view of the high advantages offered by gamma irradiation at decontamination and/or disinfestation level.

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3.4.4. References

AOAC. (1995). *Official methods of analysis* (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Aguirre, L., Frias, J.M., Barry-Ryan, C., & Grogan, H. (2008). Assessing the effect of product variability on the management of the quality of mushrooms (*Agaricus bisporus*). *Postharvest Biology and Technology*, *49*, 247-254.

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society Applied Biology Chemistry*, *53*, 257-265.

ASTM, American Society for Testing and Materials. (1992). Practice for Using the Fricke Reference Standard Dosimetry System, ASTM E1026, Annual Book of ASTM Standards, 12.02, Philadelphia, PA.

Barros, L., Pereira, C., & Ferreira, I.C.F.R. (2013). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. *Food Analytical Methods*, *6*, 309-316.

Fernandes, Â., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Antonio, A.L., Barreira, J.C.M., Botelho, L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2013a). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, *6*, 2895-2903

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014a). Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera*. *Food Chemistry*, *149*, 91-98.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014b). Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. *Innovative Food Science and Emerging Technologies*, *22*, 158-166.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Santos, P.M.P., Martins, A., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2013b). Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Research International*, *54*, 18-25.

Hanuš, L.O., Shkrob I., & Dembitsky, V.M. (2008). Lipids and fatty acids of wild edible mushrooms of the genus *Boletus*. *Journal of Food Lipids*, *15*, 370-383.

Heleno, S.A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I.C.F.R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*, 1443-1450.

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Santos-Buelga, C., & Ferreira, I.C.F.R. (2011). Targeted metabolites analysis in wild *Boletus* species. *LWT-Food Science and Technology*, *44*, 1343-1348.

ICGFI. International Consultative Group on Food Irradiation. (1999). In Facts about Food Irradiation. Buckinghamshire, United Kingdom.

Institute of Food Science and Technology (2015) - Food Irradiation. http://www.ifst.org/knowledge-centre/information-statements/food-irradiation.

Jiang, T., Luo, S., Chen, Q., Shen, L., Ying, T. (2010). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (Lentinus edodes). Food Chemistry, 122, 761-767.Koune, J.-P. (2001). Threatened mushrooms in Europe. Convention on the Conservation of European Wildlife and Natural Habitats (Bern Convention). Nature and environment, nº 122. Conseil of Europe Publishing.

Kulshreshtha, M., Singh, A., & Deepti and Vipul. (2009). Effect of drying conditions on mushrooms quality. *Journal of Engineering Science and Technology*, *4*, 90-98.

Lacroix, M., & Ouattara, B. (2000). Combined industrial processes with irradiation to assure innocuity and preservation of food products - a review. *Food Research International*, *33*, 719-724.

Manzi, P., Marconi, S., Aguzzi, A., & Pizzoferrato, L. (2004). Commercial mushrooms: nutritional quality and effect of cooking. *Food Chemistry*, *84*, 201-206.

Minnaar, A., Taylor, J.R.N., & McGill, A.E.J. (1995). Heat-irradiation combination processing as an effective method of producing high quality shelf-stable, low-acid food products. *Food Control*, *6*, 165-170.

Marques, G. (2005). Cogumelos silvestres comestíveis: problemática actual e medidas para o aproveitamento sustentável. *In* V Congresso Florestal Nacional. Viseu, Portugal. Available at <http://www.esac.pt/cernas/cfn5/docs/t5-68.pdf>. Accessed on April, 30th, 2014.

Molins, R. (2001). Food Irradiation. Principles and applications. John Wiley & Sons, USA. ISBN 0-471-35634-4.

Nagar, V., Hajare, S.H., Saroj, S.D., & Bandekar, J.R. (2012). Radiation processing of minimally processed sprouts (dew gram and chick pea): effect on sensory,

nutritional and microbiological quality. *International Journal of Food Science and Technology*, *47*, 620-626.

Nawar, W.W. (1977). Radiation chemistry of lipids, in *Radiation Chemistry of Major Food Components*, Elias, P.S. and Cohen, A.J. (eds), Elsevier Scientific, Amsterdam, pp. 21-61.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2009). Effect of γ-irradiation on agaritine, γ-glutaminyl-4-hydroxybenzene (GHB), antioxidant capacity, and total phenolic content of mushrooms (Agaricus bisporus). Journal of Agricultural and Food Chemistry, 57, 5790–5794.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (Agaricus bisporus). Food Chemistry, 123, 171–174.WHO, World Health Organization (1999). High-Dose Irradiation: Wholesomeness of food irradiated with doses above 10kGy. *Technical Report Series No. 890*, Geneva, Switzerland: World Health Organization.

CAPÍTULO 4.

Efeito de diferentes tecnologias de processamento em parâmetros químicos, nutricionais e bioativos de cogumelos silvestres

Este capítulo apresenta os efeitos de diferentes tecnologias de processamento em parâmetros químicos, nutricionais e bioativos de cogumelos silvestres Macrolepiota procera *(Scop.) Singer.*

4.1.

Efeito de diferentes tecnologias de processamento em parâmetros químicos e antioxidantes de cogumelos silvestres Macrolepiota procera

Este sub-capítulo apresenta os efeitos de diferentes tecnologias de processamento, ou seja, congelamento, desidratação e irradiação gama (dose 0,5 kGy) nos parâmetros nutricionais (valor energético, ácidos gordos, açúcares livres e tocoferóis) e bioativos (atividade antioxidante) de amostras de Macrolepiota procera *(Scop.) Singer.*

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Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom

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Abstract

Mushrooms are very perishable foods, demanding for processing technologies that retain chemical and nutritional characteristics of fresh forms. In this work, the influence of freezing, drying and gamma irradiation on chemical parameters and antioxidant potential of *Macrolepiota procera* was assessed through one-way ANOVA complemented with principal component analysis. Proximate composition was evaluated by AOAC procedures, while fatty acids, tocopherols and free sugars were determined using chromatographic techniques. Antioxidant activity was measured using *in vitro* assays. *M. procera* samples have low energetic values, with moisture and carbohydrates as major nutrients. Linoleic, palmitic and oleic acids were the

major fatty acids; δ-tocopherol was the prevalent isoform in fresh, frozen and irradiated samples, while β-tocopherol predominated in dried samples. Trehalose was the most abundant sugar in fresh and irradiated samples, whereas mannitol predominated in frozen and dried samples. Dried samples gave higher DPPH scavenging activity and β-carotene bleaching inhibition; freeze and irradiated samples showed higher reducing power and TBARS formation inhibition, respectively. Overall, freezing and drying caused significant differences in chemical parameters. On the other hand, gamma irradiation revealed the highest capacity to retain chemical profile of fresh samples, which highlights its potential to be explored and validated as an alternative conservation methodology.

Keywords: Processing technology; *Macrolepiota procera*; Chemical composition; Antioxidant activity; Freezing/drying/gamma-irradiation

4.1.1. Introduction

Mushrooms are widely appreciated for their unique taste and flavor, but also for their nutritional (Kalač, 2009) and medicinal properties, such as anti-inflammatory, anti-diabetic, antibacterial and antitumor, attributed to the presence of bioactive metabolites (*e.g.* phenolic compounds, terpenes, steroids and polysaccharides) (Ferreira, Vaz, Vasconcelos, & Martins, 2010; Poucheret, Fons, & Rapior, 2006). In particular, edible mushrooms can be a source of nutraceuticals with important antioxidant properties, which can positively influence the oxidative stress in cells and related diseases (Ferreira, Barros, & Abreu, 2009).

Nevertheless, mushrooms are one of the most perishable food products and tend to lose quality immediately after harvest. The shelf life is reduced due to post-harvest changes, namely browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture damage, related to their high respiration rate and moisture, relatively high protein content, and lack of physical protection to avoid water loss or microbial attack (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Therefore, mushrooms are mainly used in the processed form (Jaworska & Bernás, 2009).

Extending shelf-life is an imperative factor to increase the profitability and availability of any food product, since it offers the possibility of developing markets at

a greater distance (Akram & Kwon, 2010), but the applied technology should not act itself as a source of chemical modifications.

Drying is the most common method for preserving mushrooms (Giri & Prasad, 2007) and freezing is becoming increasingly popular (Jaworska & Bernás, 2009, 2010). Drying is a comparatively cheaper method (Rama & Jacob, 2000; Walde, Velu, Jyothirmayi, & Math, 2006), while food freezing is among the most efficient and adequate preservation methods (Haiying, Shaozhi, & Guangming, 2007). The main advantage of freezing is that it allows the best retention of nutritional value as well as sensory qualities such as colour, aroma, taste and texture; during freezing most of the liquid water changes into ice, which greatly reduces microbial and enzymatic activities (Haiying et al. 2007).

Food irradiation has also been suggested as a safe and adequate process to maintain and increase the food shelf life (WHO, 1994). This physical method of conservation involves exposing a product to ionizing radiation, in a controlled dose and irradiation time (Akram & Kwon, 2010; Fernandes, Antonio, Oliveira, et al., 2012). The maximal recommended dose for extending the shelf-life of fresh mushrooms is 3 kGy (ICGFI, 1999).

Many studies have applied gamma irradiation to a range of mushrooms including cultivated (Jiang, Luo, Chen, Shen, & Ying, 2010; Sommer, Schwartz, Solar, & Sontag, 2010) and, more recently, wild species (Fernandes, Antonio, Barreira, Botelho, et al., 2012; Fernandes, Antonio, Barreira, Oliveira, et al., 2012). In those two studies of our research group, the effects of gamma irradiation on chemical composition, antioxidant activity and physical parameters of fresh *Lactarius deliciosus* wild edible mushroom were evaluated, being concluded that up to 1 kGy this technology was effective in maintaining chemical composition and controlling the deterioration of fresh samples.

The main objective of the present study was to assess the effects of different processing technologies (freezing, drying and gamma irradiation) on chemical and antioxidant parameters of the wild mushroom *Macrolepiota procera*, in order to select the most suitable solution to be applied in future studies related to its preserving ability.

4.1.2. Materials and methods

Standards and reagents

To estimate the dose and dose rate a Fricke dosimeter was used. This consists of a chemical solution sensitive to ionizing radiation, prepared in the laboratory following the standards and Amber Perspex dosimeters (batch V, from Harwell Dosimeters, Harwell, UK). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulfate(II)hexahydrate, sodium chloride and sulfuric acid, all of them purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis).

For chemical analyses: acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Pleasant Gap, PA, USA).

For antioxidant potential analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, SC, USA).

Samples and samples preparation

M. procera fruiting bodies were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011. The samples were divided into four groups with three mushrooms (whole fruiting bodies) per group with different stages of maturation, and then submitted to different processing technologies: freezing (at -20 ºC in a freezer), drying (at 30 ºC in an oven) and gamma irradiation (with a Co-60 source, following the procedure previously described by us; Fernandes, Antonio, Barreira,Botelho, et al., 2012). The estimated dose after irradiation was 0.6 ± 0.1 kGy, at a dose rate of 2.3 ± 0.1 kGy h^{-1}. The fourth group was kept fresh and promptly analyzed (control sample).

After each treatment, the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA) to prevent any further deterioration, reduced to a fine dried powder (0.85 mm) and mixed to obtain homogenized samples for subsequent analyses.

Chemical composition

Proximate composition

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content (N × 4.38) of the samples was estimated by the macroKjeldahl method; the crude fat was determined by extracting a known weight of the sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C; total carbohydrates were calculated by difference: total carbohydrates (g) = 100 - (g moisture + g protein + g fat + g ash). Total energetic value (100 g) was calculated according to the following equation: energetic value (kcal) = 4 × (g protein +g carbohydrate) + 9 × (g fat).

Fatty acids

Fatty acids were determined after a transesterification procedure as described previously by the authors (Heleno, Barros, Sousa, Martins, & Ferreira, 2009), using a gas chromatograph (DANI 1000, Contone, Switzerland) equipped with a split/splitless injector and a flame ionization detector (GC-FID). Fatty acids identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 software (DataApex 1.7). The results were expressed in relative percentage of each fatty acid (obtained after Soxhlet extraction).

Free sugars

Free sugars were determined by a High Performance Liquid Chromatography (HPLC) system consisted of an integrated system with a pump (Knauer, Smartline

system 1000, Berlin, Germany), degasser system (Smartline manager 5000) and auto-sampler (AS-2057 Jasco, MD, USA), coupled to a refraction index detector (RI; detector Knauer Smartline 2300, Berlin, Germany) as previously described by the authors (Heleno et al., 2009). Identification of sugars was made by comparing the relative retention times of sample peaks with standards. Data were analyzed using Clarity 2.4 Software (DataApex). Quantification was based on the RI signal response of each standard, using the internal standard (IS, raffinose) method and by using calibration curves obtained from commercial standards of each compound. The limits of detection (LOD), calculated as the concentration corresponding to three times the calibration error divided by the slope, were 0.05 mg/mL for fructose, and 0.07 mg/mL for mannitol, trehalose and melezitose. The limits of quantification (LOQ) were calculated using the concentration corresponding to ten times the calibration error divided by the slope, and were 0.18 mg/mL for fructose and melezitose, and 0.22 and 0.24 mg/mL for mannitol and trehalose respectively). The results were expressed in g per 100 g dry weight (dw).

Tocopherols

Tocopherols were determined following a procedure previously optimized and described by the authors (Barros, Correia, Ferreira, Baptista, & Santos-Buelga, 2008). Analysis was performed by HPLC (equipment described above), and a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) programmed for excitation at 290 nm and emission at 330 nm. The compounds were identified by chromatographic comparison with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. LOD values were 8.49, 20.03, 20.08, 20.09 ng/mL for α-, β-, γ- and δ-tocopherol, respectively; LOQ values were 28.29, 66.77, 66.93 and 66.95 ng/mL for α-, β-, γ- and δ-tocopherol, respectively. The results were expressed in μg per 100 g dw.

Antioxidant activity

Extraction procedure

The lyophilized powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Barros, Baptista, Correia, Morais, & Ferreira, 2007) to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

Total phenolics

Phenolics were determined by the Folin–Ciocalteu assay. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094–0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek, Winooski, VT, USA). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discolouration using the equation: $\% \text{ RSA} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

This methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1 g/100 mL, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10 g/100 mL, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionised water (0.8 mL) and ferric chloride (0.1 g/100 mL, 0.16 mL), and the absorbance was measured at 690 nm.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm β-Carotene bleaching inhibition was calculated using the following equation: (Absorbance after 2 h of assay/initial Absorbance) × 100.

TBARS assay

Porcine (*Sus scrofa*) brains were obtained from animals slaughtered at officially licensed premises, dissected, and homogenized with a Polytron in ice cold Tris–HCl buffer (20 mmol/L, pH 7.4) to produce a brain tissue homogenate (0.5 g/mL) which was centrifuged at 3000*g* for10 min. An aliquot (100 μL) of the supernatant was incubated with the different concentrations of the samples solutions (200 μL) in the presence of $FeSO_4$ (10 mmol/L; 100 μL) and ascorbic acid (0.1 mmol/L; 100 μL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28 g/100 mL, 500 μL), followed by thiobarbituric acid (TBA, 2 g/100 mL, 380 μL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000 *g* for 10 min

to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)–TBA complex in the supernatant was measured at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/ A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

For each processing technology three samples were analysed, with all the assays being also carried out in triplicate. Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS software, version 18.0.

Analysis of variance

The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro-Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Principal components analysis (PCA)

PCA was applied as the pattern recognition unsupervised classification method. PCA transforms the original, measured variables into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on (Patras et al., 2011). The number of dimensions considered for PCA was chosen in order to allow meaningful interpretations, and by ensuring their reliability.

4.1.3. Results and discussion

Chemical composition

Table 4.1.1 shows the mean values obtained for proximate composition of *M. procera* mushrooms submitted to different processing technologies. In general, the values are similar to those reported in previous studies (Barros et al., 2007; Ouzouni & Riganakos, 2007), except for higher protein and lower carbohydrates content than those observed by Barros et al. (2007). Moisture was the major component, followed by carbohydrates (60-70 g/ 100 g dw) and proteins (19-29 g/ 100 g dw). The mean values of all the assayed parameters revealed significant differences ($p < 0.05$) among the processing technologies, mainly for carbohydrates, ash and energetic value. Freezing prevented proteins degradation observed in the other samples, which could be related to cell wall disruption induced by low temperatures. Irradiation prevented the fat decrease verified in the other samples, probably due to a decrease in molecular oxygen, which is known to induce lipid auto-oxidation processes (Nawar, 1986).

Table 4.1.1. Moisture content (g/100 g fw), proximate composition (g/100 g dw) and energetic value (kcal/100 g dw) of fresh and processed *Macrolepiota procera* samples. The results are presented as mean ± SD (n = 36).[a]

		Moisture[d]	Fat	Protein	Carbohydrates	Ash	Energetic value
Samples	Fresh	85.9±0.3 b	2.9±0.1 b	19±1 c	70±1 a	8.0±0.2 c	383±1 b
	Frozen	87.7±0.3 a	2.2±0.1 c	28.6±0.5 a	60±1 d	9.0±0.2 b	375±1 c
	Dried	87.4±0.3 a	2.7±0.2 b	19.8±0.4 b	67±1 c	10.3±0.3 a	372±1 d
	Irradiated	85.8±0.3 b	3.6±0.5 a	20±1 b	69±1 b	7.6±0.3 d	388±3 a
Homocedasticity[b]	*p*-value	0.792	0.015	0.331	0.556	0.298	0.021
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

fw- Fresh weight; dw- Dry weight.

[a] Means within a column with different letters differ significantly ($p < 0.05$), evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.

[b] Homoscedasticity among processing technologies was tested by means of the Levene test.

[c] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case multiple comparison tests were performed).

[d] Moisture content was evaluated by lyophilization for fresh, frozen and irradiated samples; in the case of dry samples, water was removed using an oven.

Table 4.1.2 presents the mean values obtained for fatty acid profiles. Besides the tabled compounds, C6:0, C8:0, C10:0, C12:0, C14:1, C17:0, C18:3, C20:0, C20:1, C20:3, C21:0, C20:5, C22:0, C22:1, C23:0 and C24:1 were also detected, but in trace amounts (<0.3 g/100 g of fat). The statistical analysis showed homoscedastic distribution, except for C14:0 and C15:0. One-way ANOVA demonstrated significant statistical differences among the contents in fatty acids, especially for C15:0, C18:0, C18:1 and total monounsaturated fatty acids (MUFA). Dried and irradiated samples exhibited higher percentages of saturated fatty acids (SFA), while MUFA were higher in irradiated samples, and polyunsaturated fatty acids (PUFA) reached maximal values in frozen samples. Despite some differences regarding individual fatty acids, the global percentages obtained for SFA, MUFA and PUFA are similar to those presented in other studies (Kavishree et al., 2008).

The mean values obtained for tocopherol profiles are given in **Table 4.1.3**. Once again, the results of the Levene's test confirmed the homoscedasticity of distribution in all cases. At a 5% significance level, γ- and δ-tocopherols were the vitamers with the highest variability, but other statistical differences could be observed, proving that tocopherol profiles obtained for each technology were dissimilar. For instance, irradiated samples showed the highest contents of α- (5.2 μg/100 g dw) and γ-tocopherol (43 μg/100 g dw), while dried and fresh samples had the highest contents in β- (77 μg/100 g dw) and δ-tocopherol (91 μg/100 g of dw), respectively.

Regarding free sugars composition (**Table 4.1.4**), dried samples gave the highest total content (19.3 g/100 g dw), also presenting the highest levels of mannitol (11.4 g/100 g dw). The highest concentration of trehalose (10.2 g/100 g dw) and melezitose (1.42 g/100 g dw) was found in irradiated samples, while fructose (0.17 g/100 g dw) was higher in frozen samples. Fresh and irradiated samples presented trehalose as the main sugar, whereas mannitol predominated in frozen and dried samples. Trehalose, melezitose and total free sugars gave the most significant differences among processing technologies. The amounts quantified for the main sugars (trehalose and mannitol) are in agreement with previous results concerning different treatments applied to *M. procera* samples (Barros et al., 2007).

Table 4.1.2. Fatty acids composition (relative %) of fresh and processed *Macrolepiota procera* samples. The results are presented as mean ± SD (n = 36).[a]

		C14:0	C15:0	C16:0	C16:1	C18:0	C18:1	C18:2	C20:2	C24:0	SFA	MUFA	PUFA
Samples	Fresh	0.34±0.01 a	0.30±0.01 d	21.4±0.2 b	1.29±0.03 b	1.77±0.03 c	7.6±0.1 b	65.0±0.2 b	0.46±0.01 b	0.78±0.02 b	25.2±0.2 b	9.3±0.1 b	65.5±0.2 b
	Frozen	0.28±0.01 b	0.37±0.01 b	18.2±0.3 c	1.07±0.02 c	1.46±0.03 d	5.7±0.3 d	71.0±0.3 a	0.107±0.002 c	0.52±0.02 c	21.4±0.3 c	6.9±0.4 d	71.6±0.4 a
	Dried	0.35±0.01 a	0.41±0.01 a	22.7±0.5 a	1.23±0.03 b	2.32±0.05 a	6.8±0.1 c	63±1 c	0.76±0.02 a	0.85±0.02 a	28±1 a	8.5±0.2 c	64±1 c
	Irradiated	0.34±0.02 a	0.33±0.01 c	22±1 a	1.5±0.1 a	2.0±0.1 b	8.3±0.5 a	62±2 c	0.56±0.03 b	0.86±0.05 a	27±1 a	10±1 a	63±2 c
Homocedasticity[2]	*p*-value	0.186	0.086	0.001	0.001	0.042	0.006	0.001	<0.001	0.031	0.001	<0.001	<0.001
One-way ANOVA[3]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Means within a column with different letters differ significantly ($p < 0.05$), evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.
[b] Homoscedasticity among processing technologies was tested by means of the Levene test.
[c] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case multiple comparison tests were performed).

Table 4.1.3. Tocopherols composition (µg/100 g dw) of fresh and processed *Macrolepiota procera*. The results are presented as mean ± SD (n = 36).[a]

		α-Tocopherol	β-Tocopherol	γ-Tocopherol	δ-Tocopherol	Total tocopherols
Sample	Fresh	2.8±0.4 c	51±3 b	32±3 c	91±3 a	178±9 a
	Frozen	2.5±0.2 c	54±4 b	9±1 d	81±4 b	146±3 b
	Dried	4.5±0.3 b	77±8 a	38±1 b	65±1 d	185±8 a
	Irradiated	5.2±0.5 a	26±6 c	43±1 a	72±7 c	146±12 b
Homocedasticity[2]	*p*-value	<0.001	<0.001	<0.001	<0.001	0.001
One-way ANOVA[3]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

[1] Means within a column with different letters differ significantly ($p < 0.05$), evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.
[2] Homoscedasticity among processing technologies was tested by means of the Levene test.
[3] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case multiple comparison tests were performed).

Table 4.1.4. Free sugars composition (g/100 g dw) of fresh and processed *Macrolepiota procera*. The results are presented as mean ± SD (n = 36).[a]

		Fructose	Mannitol	Trehalose	Melezitose	Total sugars
Samples	Fresh	0.06±0.01 c	5.2±0.1 b	9.1±0.3 b	1.24±0.05 b	15.7±0.4 c
	Frozen	0.17±0.03 a	4.9±0.1 c	3.0±0.1 d	0.25±0.01 d	8.3±0.2 d
	Dried	0.10±0.01 b	11.4±0.2 a	6.8±0.2 c	1.02±0.04 c	19.3±0.4 a
	Irradiated	0.054±0.002 c	5.1±0.3 bc	10.2±0.2 a	1.42±0.05 a	16.7±0.5 b
Homocedasticity[2]	*p*-value	<0.001	0.043	0.008	0.014	0.011
One-way ANOVA[3]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Means within a column with different letters differ significantly ($p < 0.05$), evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.
[b] Homoscedasticity among processing technologies was tested by means of the Levene test.
[c] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case multiple comparison tests were performed).

Antioxidant activity

All *M. procera samples* proved to have antioxidant activity, but revealed different properties according to the applied processing technology. Dried samples had the highest antioxidant activity (**Table 4.1.5**), measured by DPPH scavenging activity (50% at 2.7 mg/mL) and β-carotene bleaching inhibition (50% at 1.10 mg/mL). Accordingly, these were also the samples with the highest phenolic content (19.2 mg GAE/g extract). However, the methanolic extracts obtained from frozen and irradiated samples presented the highest reducing power (0.5 absorbance at 1.27 mg/mL) and TBARS formation inhibition (50% at 0.78 mg/mL), respectively. Regarding frozen samples, the cell walls might be disrupted increasing the extractability of intracellular compounds, leading to the highest reducing power. The heat (30 ºC) applied to dried samples could inactivate endogenous oxidative enzymes (Barros et al., 2007), explaining the increased antioxidant activity.

In general, and independently of the processing technology, *M. procera* samples have low energetic values (372-388 kcal/100 g dw), with moisture and carbohydrates as major nutrients. The fatty acid profiles were similar for all the samples: linoleic, palmitic and oleic acids were the compounds present in major amounts. δ-Tocopherol was the prevalent vitamin E isoform in fresh, frozen and irradiated samples, while β-tocopherol predominated in dried samples. Regarding free sugars, trehalose was the most abundant compound in fresh and irradiated samples, whereas mannitol was the main sugar in frozen and dried samples. The processing technologies had specific effects on the antioxidant potential of *M. procera* extracts: dried samples gave higher DPPH scavenging activity and β-carotene bleaching inhibition; freeze samples showed higher reducing power and irradiated samples revealed higher TBARS formation inhibition.

Table 4.1.5. Antioxidant activity (EC_{50}; mg/mL) and total phenolics content (mg GAE/g extract) of fresh and processed *Macrolepiota procera*. The results are presented as mean ± SD (n = 36).[a]

		DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS formation inhibition	Phenolics
Samples	Fresh	4.9±0.4 b	1.44±0.02 b	6.7±0.5 b	1.97±0.03 c	18.2±0.1 b
	Frozen	3.7±0.2 c	1.27±0.01 d	8.3±0.2 a	3.5±0.2 b	15.3±0.1 d
	Dried	2.7±0.1 d	1.35±0.01 c	1.10±0.05 d	4.08±0.05 a	19.2±0.2 a
	Irradiated	7.9±0.5 a	1.74±0.01 a	2.5±0.4 c	0.78±0.05 d	17.1±0.4 c
Homocedasticity[2]	*p*-value	<0.001	<0.001	0.017	<0.001	<0.001
One-way ANOVA[3]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Means within a column with different letters differ significantly ($p < 0.05$), evaluated either using the multiple comparison Tukey's HSD or Tamhane's T2 tests, depending on the fulfilment or not of the homoscedasticity requirement.
[b] Homoscedasticity among processing technologies was tested by means of the Levene test.
[c] $p < 0.05$ indicates that the mean value of the evaluated parameter of at least one sample differs from the others (in this case multiple comparison tests were performed).

Principal Components Analysis (PCA)

For a better comprehension of the differences induced by each processing technology, data were evaluated through a PCA. The number of dimensions considered in the analysis was set in order to obtain meaningful outputs. The reliability of dimensions was ensured by Cronbach's alpha parameter (that must be positive) and eigenvalue (that should be higher than 1). The plot of object points (**Figure 4.1.1A**) indicates that the first two dimensions account for most of the variance of all quantified variables (57.19% and 27.46%, respectively). The parameters most correlated with the first dimension are highlighted (black dashed line) in **Figure 4.1.1B**. As it can be observed in **Figure 4.1.1**, these variables have a high impact especially within frozen samples, that are clearly separated mostly due to their high content of fructose, proteins, C18:2, PUFA, or the high EC_{50} values obtained for β-carotene bleaching inhibition. On the other hand, the variables most correlated with the second dimension are highlighted with dotted grey lines. These have a significant influence on dried samples, that were separated especially due to their high contents in mannitol, ash or β-tocopherol (**Figure 4.1.1B**).

Overall, irradiation was the processing technology with the highest ability to maintain the chemical profile of the fresh samples, indicating its high potential to be explored and validated as a conservation methodology for wild mushrooms. In fact, the spatial distribution of PCA markers (**Figure 4.1.1A**) indicates clearly that the processes of freezing and drying induced much higher differences in the evaluated chemical parameters.

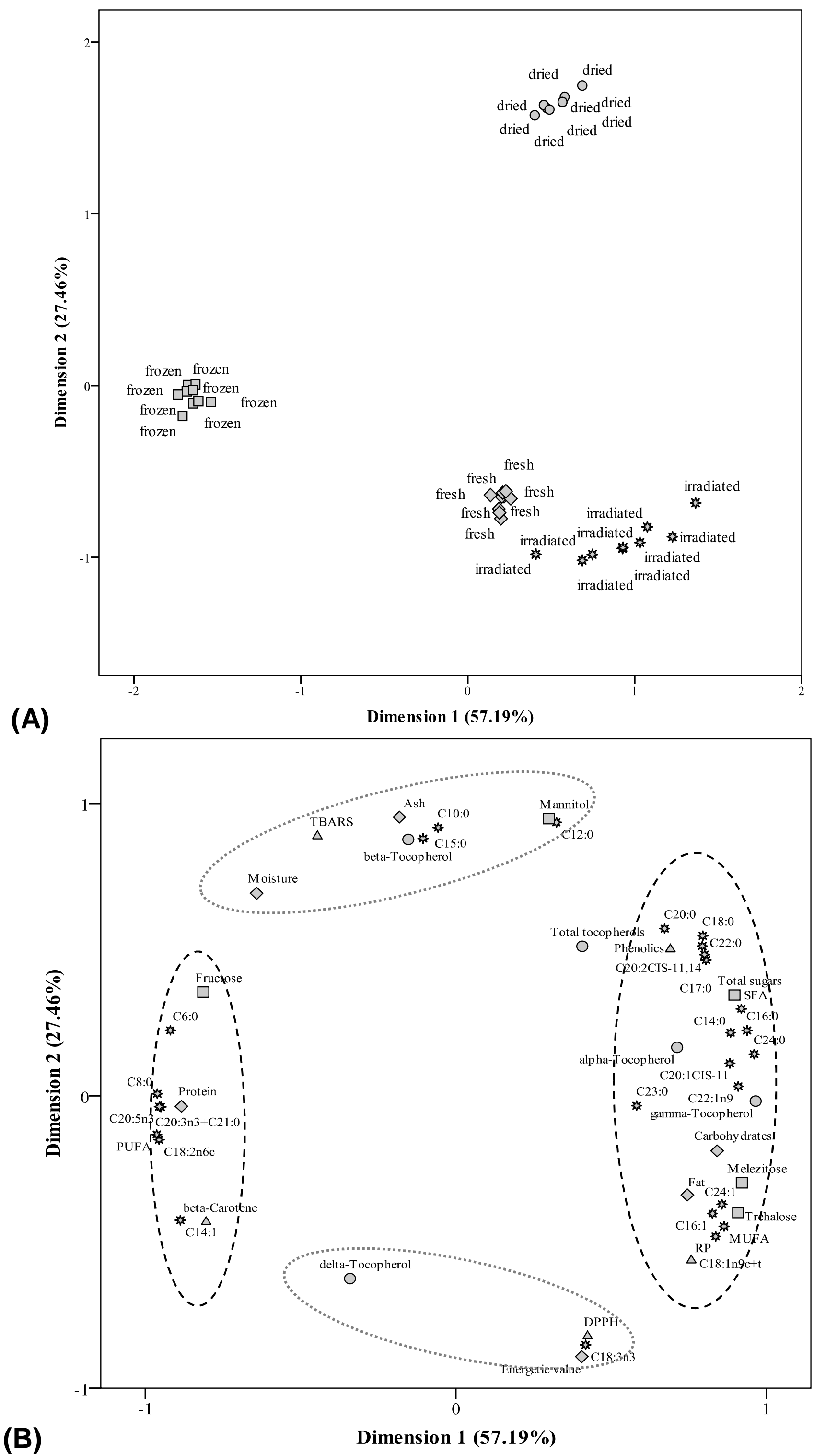

Figure 4.1.1. Object points labelled by processing technology (A) and component loadings (B) plot. Each group of assayed parameters (proximate composition, fatty acids, tocopherols, free sugars and antioxidant activity) is identified with a different symbol.

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4.1.4. References

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society for Applied Biological Chemistry*, *53*, 257-265.

AOAC. (1995). *Official methods of analysis* (16th ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Barros, L., Baptista, P., Correia, D. M., Sá Morais, J., & Ferreira, I. C. F. R. (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, *55*, 4781-4788.

Barros, L., Correia, D. M., Ferreira, I. C. F. R., Baptista, P., & Santos-Buelga, C. (2008). Optimization of the determination of tocopherols in Agaricus sp. edible mushrooms by a Normal Phase Liquid Chromatographic method. *Food Chemistry*, *110*, 1046-1050.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Botelho, L., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, http://dx.doi.org/10.1007/s11947-012-0931-5.

Fernandes, A., Antonio, A. L., Barreira, J. C. M., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effects of gamma irradiation on physical parameters of *Lactarius deliciosus* wild edible mushroom. *Postharvest Biology and Technology*, *74*, 79-84.

Fernandes, Â., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical

and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Ferreira, I. C. F. R., Barros, L., & Abreu, R. M. V. (2009). Antioxidants in wild mushrooms. *Current Medicinal Chemistry*, *16*, 1543-1560.

Ferreira, I. C. F. R., Vaz, J. A., Vasconcelos, M. H., & Martins, A. (2010). Compounds from wild mushrooms with antitumor potential. *Anti-cancer Agents in Medicinal Chemistry*, *10*, 424-436.

Giri, S. K., & Prasad, S. (2007). Drying kinetics and rehydration characteristics of microwave-vacuum and convective microwave-vacuum and convective hot-air dried mushrooms. *Journal of Food Engineering*, *78*, 512-552.

Haiying, W., Shaozhi, Z., & Guangming, C. (2007). Experimental study on the freezing characteristics of four kinds of vegetables. *LWT - Food Science and Technology*, *40*, 1112-1116.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchemical Journal*, *93*, 195-199.

ICGFI. International Consultative Group on Food Irradiation. (1999). In Facts about Food Irradiation. Buckinghamshire, United Kingdom.

Jaworska, G., & Bernás, E. (2009). The effect of preliminary processing and period of storage on the quality of frozen *Boletus edulis* (Bull: Fr.) mushrooms. *Food Chemistry*, *113*, 936-943.

Jaworska, G., & Bernás, E. (2010). Effects of pre-treatment, freezing and frozen storage on the texture of *Boletus edulis* (Bull: Fr.) mushrooms. *International Journal of refrigeration*, *33*, 877-885.

Jiang, T., Luo, S., Chen, Q., Shen, L., & Ying, T. (2010). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). *Food Chemistry*, *122*, 761-767.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9-16.

Kavishree, S., Hemavathy, J., Lokesh, B. R., Shashirekha, M. N., & Rajarathnam, S. (2008). Fat and fatty acids in Indian edible mushrooms. *Food Chemistry*, *106*, 597-602.

Nawar, W. W. (1986). Volatiles from food irradiation. *Food Reviews International*, *2*, 45-78.

Ouzouni, P. K., & Riganakos, K. A. (2007). Nutritional value and metal content of Greek wild edible fungi. *Acta Alimentaria*, *36*, 99-110.

Patras, A., Brunton, N. P., Downey, G., Rawson, A., Warriner, K., & Gernigon, G. (2011). Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *Journal of Food Composition and Analysis*, *24*, 250-256.

Poucheret, P., Fons, F., & Rapior, S. (2006). Biological and pharmacological activity of higher fungi: 20-Year retrospective analysis. *Mycologie*, *27*, 311-333.

Rama, V., & Jacob, J. P. (2000). Effects of methods of drying and pretreatments on quality of dehydrated mushroom. *Indian Food Packer*, *54*, 59-64.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (*Agaricus bisporus*). *Food Chemistry*, *123*, 171-174.

Walde S.G., Velu, V., Jyothirmayi, T., & Math, R.G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineering*, *74*, 108-115.

WHO (World Health Organisation). (1994). *Safety and nutritional adequacy of irradiated food*. Geneva: WHO.

4.2

Efeito da irradiação gama na composição química e atividade antioxidante de amostras processadas de cogumelos silvestres *Macrolepiota procera*

Este sub-capítulo apresenta os efeitos da radiação gama (doses 0,5 e 1 kGy) em amostras frescas e processadas (congeladas e desidratadas) de Macrolepiota procera *(Scop.), analisando os parâmetros nutricionais (valor energético, açúcares livres, ácidos gordos e tocoferóis) e bioativos (atividade antioxidante).*

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Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera*

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Abstract

It was previously demonstrated that gamma irradiation was the processing technology with the highest capacity to maintain the chemical profile of fresh *Macrolepiota procera* wild mushroom, when compared to freeze-dried or oven-dried samples. Herein, it was aimed to evaluate gamma irradiation effects on processed samples. Chemical composition and antioxidant potential of irradiated (0.5 and 1 kGy) fresh, frozen and dried samples were determined by chromatographic techniques and *in vitro* assays, respectively. *M. procera* irradiation attenuated the effects caused by oven-drying or freezing; combining freeze treatment with 0.5 kGy

dose preserved total tocopherols. Rather than a conservation methodology, gamma irradiation might act as a useful adjuvant to other conservation techniques (*e.g.*, freezing or oven-drying).

Keywords: Wild mushroom; *Macrolepiota procera*; Gamma irradiation; Drying; Freezing; Chemical parameters.

4.2.1. Introduction

Mushrooms perish rapidly and they start deteriorating within a day of harvest. In view of their highly perishable nature, fresh mushrooms have to be processed to extend their shelf life for off-season use (Walde, Velu, Jyothirmayi, & Math, 2006). Among the various methods employed for preservation, freezing and drying are the most used technologies. Blast freezing is the most common method used in mushroom freezing although, recently, cryogenic methods have gained in popularity. Cryogenic freezing provides a higher quality product; however, its application in the food industry is rather limited, due to its high cost (Jaworska & Bernás, 2009). Freezing allows a better retention of nutritional attributes, as well as sensory characteristics, such as colour, aroma, flavour and texture; during freezing most of the liquid water changes into ice, which reduces the microbial and enzymatic activities (Haiying, Shaozhi, & Guangming, 2007).

Dried mushrooms packed in airtight containers can have a shelf life of above one year (Bano, Rajarathnam, & Rekha, 1992; Walde et al., 2006). Different drying methods have been developed to preserve food, including mushrooms, such as drying by sun, hot air and oven-drying method (Ma, Haixia, Wenchai, & Zhaoshuai, 2013).

Food irradiation is a processing technique applied for decontamination and increasing shelf life of food, exposing food to ionising radiation in order to enhance its shelf-life as well as its safety. The aim is to destroy microorganisms or insects that could be present in the food, and sometimes to improve the functional properties of food or to eliminate toxins, with the least compromise on sensory and nutritive quality (Akram & Kwon, 2010; Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). According to several authors, irradiation decreases the normal changes associated with maturation, germination and ageing; it destroys insects and microorganisms that

cause food spoilage (Beaulieu, D'Aprano, & Lacroix, 2002; Jiang, Luo, Chen, Shen, & Ying, 2010) with minimum changes in nutritional and sensory quality (Akram & Kwon, 2010; Fernandes et al., 2012).

Gamma irradiation has been applied in extending the postharvest shelf-life of fresh mushrooms (Sommer, Schwartz, Solar, & Sontag, 2010). The recommended dose for extending the shelf-life of fresh mushroom in different countries (such as Argentina, China, Croatia, Hungary, Israel, Korea, Mexico, Poland and United Kingdom) is 1-3 kGy, while the recommended dose regarding the decontamination of dried mushrooms, used as seasonings, is 10-50 kGy (Akram & Kwon, 2010; ICGFI, 1999).

In a previous study, our research group reported the effects of gamma irradiation on chemical composition and antioxidant activity of *Lactarius deliciosus* fresh samples (Fernandes, Antonio, et al., 2013). The obtained data showed that, at or below 1 kGy, gamma irradiation might provide a useful alternative to ensure quality and extend shelf life, since its effects on the assayed parameters were less significant than the changes caused by storage time. In another study, the effects of different processing technologies (freezing, drying and gamma irradiation) on chemical and antioxidant parameters of the wild mushroom *Macrolepiota procera* were accessed, and irradiation was the processing technology with the highest ability to maintain the chemical profile characteristics of fresh samples (Fernandes, Barros, et al., 2013). *M. procera* is one of the most popular mushrooms, being considered an excellent edible species, highly appreciated for its culinary value (Polese, 2005) but so perishable that it is mostly used for self-consumption after harvest.

Therefore, in the present work, the study of gamma irradiation, already evaluated in fresh samples of *M. procera*, was extended to processed samples, comparing the chemical composition and antioxidant potential of irradiated fresh, frozen and dried mushrooms.

4.2.2. Materials and methods

Samples and samples irradiation

M. procera fruiting bodies were obtained from the region of Trás-os-Montes, in the Northeast of Portugal, in November 2011.

The samples were divided into three groups with nine mushrooms per group with different stages of maturation included in each sample, and further submitted to different processing technologies: freezing (at -20º C in a freezer) and drying (at 30 ºC in an oven); the third group was kept fresh (stored at 4 ºC in a refrigerator). Each group was further subdivided into three subgroups: control (non-irradiated, 0 kGy); sample 1 (0.5 kGy) and sample 2 (1.0 kGy).

The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, and the irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity 267 TBq (7.216 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd, U.K.), following the procedure previously described by the authors (Fernandes, Antonio, et al., 2013). The estimated doses after irradiation were 0.6±0.1 kGy and 1.1±0.1 kGy for samples 1 and 2, respectively, at a dose rate of 2.3 $kGyh^{-1}$. For simplicity, in the text, tables and graphs we considered the values 0, 0.5 and 1 kGy, for non-irradiated and irradiated samples, respectively.

After irradiation, all the samples were lyophilised (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh), mixed to obtain homogenate samples and promptly analysed.

Standards and reagents

For irradiation: to estimate the dose and dose rate of irradiation it was used a chemical solution sensitive to ionising radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM & Materials., 1992) and Amber Perspex dosimeters (batch V, from Harwell Co., UK). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulphate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, Billerica, MA).

For chemical analyses: acetonitrile 99.9%, *n*-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acid methyl ester (FAME) reference standard mixture 37 (standard 47,885-U) was purchased from Sigma (St. Louis, MO), as were other individual fatty acid isomers, tocopherol

and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (Plesant Gap, PA).

For antioxidant potential analysis: 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\cdot}$) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were purchased from Sigma. Methanol and all other chemicals were of analytical grade and obtained from common sources.

Chemical composition

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macroKjeldahl method; the crude fat was determined by extracting a known weight of the sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 °C using a chamber furnace (Lenton Thermal Designs Ltd., model ECF 12/22; total carbohydrates were calculated by difference: total carbohydrates = 100 - (g moisture + g protein + g fat + g ash). Total energy was calculated according to the following equation: energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refractive index detector (HPLC-RI) after the extraction procedure described by Reis, Barros, Martins, & Ferreira (2012), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000, Berlin, Germany), degasser system (Smartline manager 5000), autosampler (AS-2057; Jasco, Easton, MD) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionised water, 70:30 (v/v) at a flow rate of 1 mL/min. The

compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Fatty acids

Fatty acids were determined by gas chromatography with flame ionisation detection (GC-FID), after extraction and derivatisation procedures described previously (Reis, Barros, et al., 2012). The analysis was carried out with a DANI model GC 1000 instrument (Milan, Italy) equipped with a split/splitless injector, an FID at 260 ºC and a Macherey-Nagel column 50% cyanopropylmeythyl 50% phenylmethylpolysiloxane (30 m × 0.32 mm i.d. × 0.25 µm d*f*). The oven temperature programme was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using CSW 1.7 Software (DataApex 1.7) and expressed as relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined after an extraction procedure previously described, using tocol as IS (Reis, Barros, et al., 2012a). The analysis was carried out using the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The column used was a normal-phase 250 mm × 4.6 mm i.d., 5 µm, Polyamide II, with a 10 mm × 4 mm i.d., guard column of the same material (YMC Waters, Dinslaken, Germany), operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols content was further expressed in µg per 100 g of dry weight (dw).

Antioxidant parameters

Extraction preparation

The lyophilised powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays already described by the authors (Reis, Martins, Barros, & Ferreira, 2012) to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (BioTek Instruments, Inc., Winooski, VT). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 μL) and methanolic solution (270 μL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: % RSA = $[(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

The methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium

phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, alongside deionised water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distiled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

TBARS (thiobarbituric acid reactive substances) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected and homogenised with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 μL) of the supernatant was incubated with different concentrations of the sample solutions (200 μL) in the presence of $FeSO_4$ (10 mM; 100 μL) and ascorbic acid (0.1 mM; 100 μL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 μL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 μL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the colour intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = $[(A - B)/A] \times 100\%$, where A and B were the absorbance of the control and the sample solution, respectively.

Total phenolics measured by Folin-Ciocalteu assay

The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for colour development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Statistical analysis

An analysis of variance (ANOVA) with Type III sums of squares was performed using the GLM (General Linear Model) procedure of the SPSS software, Version 18.0. The dependent variables were analysed using 2-way ANOVA, with “processing type” (PT) and “gamma irradiation dose” (GID) as factors. As a significant interaction (PT×GID) was detected for all cases, the two factors were evaluated simultaneously by the estimated marginal means plots (EMM) for all levels of each single factor.

In addition, a linear discriminant analysis (LDA) was used to compare the effects of the PT and GID on nutritional value, free sugars, fatty acids, tocopherols and antioxidant parameters. A stepwise technique, using the Wilks' λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination processes, where the inclusion of a new variable is preceded by verifying if all variables previously selected remain significant (López, García, & Garrido, 2008; Maroco, 2003). With this approach, it is possible to identify the significant variables obtained for each sample. To verify the significance of canonical discriminant functions, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

All statistical tests were performed at a 5% significance level. For each GID and/or PT, three samples were analysed, with all the assays being also carried out in triplicate. The results are expressed as mean value ± standard deviation (SD).

4.2.3. Results and discussion

The tabled values obtained for each individual parameter are reported as mean value of each PT fixed with GID, along with the mean value of all PT doses within each GID. In this way, it is possible to define the PT that allows a better maintenance on any given component, independently of the applied GID, as well as the best GID to be applied without concerning the chosen PT. With no exceptions, PT $\times$ GID interaction was a significant ($p < 0.001$) source of variation for the results obtained in all the performed analytical assays. Accordingly, even though the least squares means are presented for both effects, no multiple comparisons could be performed. Nevertheless, from the analysis of the EMM plots (data generally not shown) some overall conclusions could be drawn.

Chemical composition

The values obtained for proximate composition (**Table 4.2.1**) of *M. procera* were similar to those reported in previous works (Fernandes, Barros, et al., 2013; Ouzouni & Riganakos, 2007), apart from higher protein and lower carbohydrates content (Barros, Baptista, Correia, Sá Morais, & Ferreira 2007). Moisture was the major component (~86 g/100 g fw), while carbohydrates predominated in the dehydrated material (66-70 g/100 g dw). Despite the similar nutritional profiles obtained within each PT or GID, the EMM plots showed that fat and carbohydrates tended to be higher in fresh samples; in addition, freeze treatment seemed to protect proteins, while ash contents were higher in dried samples. The effect of GID was less noticeable, with the higher content of ash in non-irradiated samples as the only marked change.

Free sugars are known for being good indicators of an adequate conservation technology, since their composition might be affected by technical practises (Barreira, Pereira, Oliveira, & Ferreira, 2010). Mannitol and trehalose were the major quantified sugars (**Table 4.2.1**). Dried samples presented the highest total free sugars content (19±1 g/100 g of dw) (**Figure 4.2.1A**), mainly due to the levels of mannitol (12±1 g/100 g of dw) detected in these samples. Fresh samples presented the highest contents in melezitose and trehalose, which might indicate that these sugars are more sensitive to the PT. The effect of GID was again less observable, showing

differences only in mannitol and total sugars, for which the 1.0 kGy dose gave higher contents. From a global point of view the results obtained for sugars profile were comparable to those reported for *M. procera* submitted to different processing actions (Barros et al., 2007; Fernandes, Barros, et al., 2013).

Table 4.2.1. Proximate composition, energetic value and free sugars composition of *Macrolepiota procera* samples submitted to different processing types (PT) or gamma irradiation doses (GID). The results are presented as mean ± SD.

		Moisture (g/100 g fw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Carbohydrates (g/100 g dw)	Ash (g/100 g dw)	Energetic value (kcal/100 g dw)
PT	Fresh	86±1	3.3±0.5	19±1	70±1	7.8±0.3	385±2
	Frozen	85±2	1.9±0.3	27±3	63±4	8±1	377±3
	Dried	86±2	2.6±0.5	20±1	68±1	10±1	375±3
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
GID	0 kGy	87±1	2.7±0.4	22±5	66±4	9±1	377±4
	0.5 kGy	86±1	2.7±0.5	23±5	66±4	8±1	380±6
	1 kGy	85±2	2.5±0.5	21±3	69±2	8±1	380±6
	p-value (n=27)	<0.001	0.100	<0.001	<0.001	<0.001	<0.001
PT×GID	*p*-value (n=81)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

		Fructose (g/100 g dw)	Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Melezitose (g/100 g dw)	Total sugars (g/100 g dw)
PT	Fresh	0.052±0.005	6±1	9±1	1.4±0.2	17±1
	Frozen	0.17±0.04	6±1	5±1	0.21±0.03	11±2
	Dried	0.14±0.03	12±1	6±1	0.94±0.05	19±1
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
GID	0 kGy	0.11±0.05	7±3	6±2	0.8±0.4	14±4
	0.5 kGy	0.14±0.05	7±3	7±2	0.8±0.5	15±3
	1 kGy	0.11±0.05	10±3	7±1	0.9±0.5	17±3
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
PT × GID	*p*-value (n=81)	<0.001	<0.001	<0.001	<0.001	<0.001

fw- fresh weight; dw- dry weight.

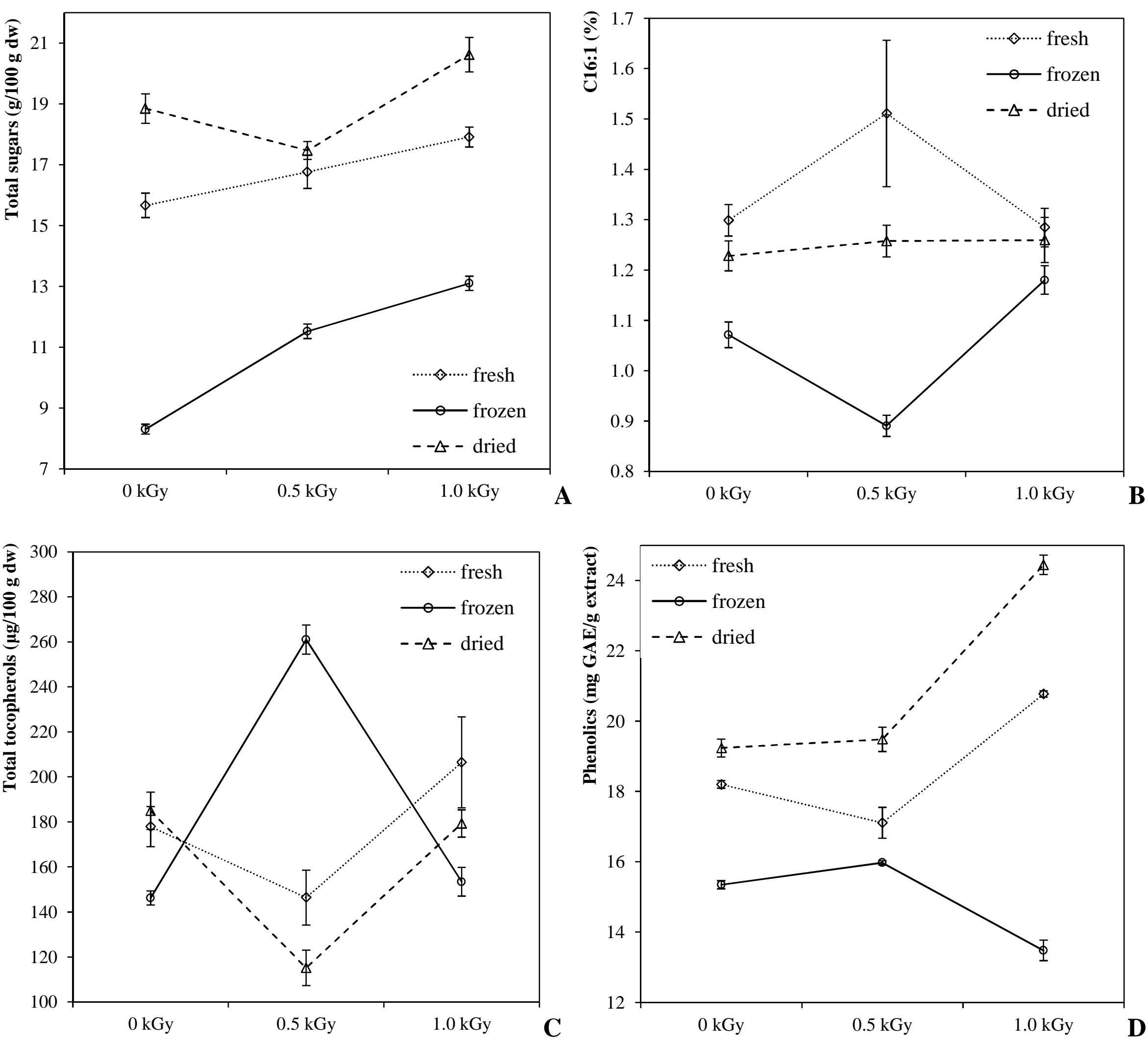

Figure 4.2.1. Interactions between processing technology (PT) and gamma irradiation dose (GID) effects on *M. procera* samples. Influence on total sugars (A), C16:1 (B), total tocopherols (C) and phenolics (D).

The fatty acids profile of *M. procera* included 24 compounds (the most abundant are presented in **Table 4.2.2**) with linoleic acid prevalent (65-68%). This fatty acid generally decreased in processed mushrooms, but often as a result of heat processing, which promotes the transformation of linoleic acid into 1-octen-3-ol (Maga, 1981); since the applied PT did not comprise high temperatures, the maintenance of C18:2 levels might be expected. In addition to the tabled fatty acids, C6:0, C8:0, C10:0, C12:0, C14:1, C17:0, C18:3, C20:0, C20:1, C22:0, C22:1, C23:0 and C24:1, were also detected in trace (< 0.3%) amounts (however, all the detected fatty acids were considered in the linear discriminant analysis presented further). Like in the previous results herein reported, the interaction among PT and GID was always significant, and the outcomes obtained in multiple comparison tests could not be presented. Nevertheless, from the analysis of the EMM, some general conclusions could be obtained. For instance, C14:0, C16:1 and C20:2 were lower in samples submitted to freeze conservation. C16:1 is a good example of the interaction among PT and GID; as can be seen in **Figure 4.2.1B**, the 0.5 kGy dose minimised the amount of C16:1 when combined with freeze treatment, but the same dose maximised the amount of C16:1 in fresh samples. Still concerning the PT influence, frozen samples presented higher quantities of C20:3 and C20:5. GID did not seem to cause remarkable changes, with the exception of the higher percentages of C14:0 and C20:5 obtained in non-irradiated samples and of C20:3 in samples irradiated with 1 kGy. Despite the indicated differences the percentages obtained for SFA, MUFA and PUFA did not revealed marked changes, and the percentages were in agreement with previous reports (Barros et al., 2007; Fernandes, Barros, et al., 2013; Kavishree, Hemavathy, Lokesh, Shashirekha, & Rajarathnam, 2008).

Table 4.2.2. Fatty acids composition of *Macrolepiota procera* samples submitted to different processing types (PT) or irradiation doses (GID). The results are presented as mean ± SD.

Fatty acid (relative %)	PT				GID				PT×GID
	Fresh	Frozen	Dried	*p*-value (n=27)	0 kGy	0.5 kGy	1 kGy	*p*-value (n=27)	*p*-value (n=81)
C14:0	0.33±0.02	0.25±0.03	0.31±0.03	<0.001	0.32±0.03	0.28±0.05	0.29±0.03	<0.001	<0.001
C15:0	0.35±0.05	0.35±0.03	0.39±0.03	<0.001	0.36±0.05	0.37±0.03	0.36±0.05	<0.001	<0.001
C16:0	21±1	20±1	20±2	<0.001	21±2	21±1	20±1	<0.001	<0.001
C16:1	1.4±0.1	1.0±0.1	1.25±0.04	<0.001	1.2±0.1	1.2±0.3	1.2±0.1	0.031	<0.001
C18:0	1.6±0.5	2.2±0.5	1.9±0.3	<0.001	1.8±0.4	2.2±0.5	1.6±0.5	<0.001	<0.001
C18:1	7±1	7±1	6±1	<0.001	7±1	7±1	6±1	<0.001	<0.001
C18:2	65±3	67±3	68±4	<0.001	66±3	66±4	68±3	<0.001	<0.001
C20:2	0.4±0.2	0.09±0.01	0.4±0.2	<0.001	0.4±0.2	0.3±0.2	0.13±0.05	<0.001	<0.001
C20:3	0.010±0.001	0.33±0.05	0.06±0.03	<0.001	0.023±0.002	0.048±0.005	0.10±0.02	<0.001	<0.001
C20:5	0.022±0.005	0.3±0.1	0.06±0.03	<0.001	0.10±0.04	0.09±0.05	0.2±0.1	<0.001	<0.001
C24:0	0.83±0.05	0.7±0.1	0.7±0.1	<0.001	0.7±0.1	0.8±0.1	0.7±0.1	<0.001	<0.001
SFA	25±1	24±2	24±3	<0.001	25±3	25±2	24±2	<0.001	<0.001
MUFA	9±1	8±1	7±1	<0.001	8±1	9±2	8±1	<0.001	<0.001
PUFA	66±3	67±3	69±4	<0.001	67±3	66±3	69±3	<0.001	<0.001

In a previous work from our research group (Fernandes, Barros, et al., 2013), it could be concluded that the application of a determined PT caused a decrease in total tocopherols content, when compared with fresh samples. However, as can be reasoned from **Table 4.2.3**, the combination of two different PT tended to preserve the level of total tocopherols. If we focused on this vitamin, combining the freeze treatment with a 0.5 kGy dose would optimise the amount of total tocopherols in *M. procera* samples (**Figure 4.2.1C**). Furthermore, frozen and dried samples presented lower amounts of γ-tocopherol and δ-tocopherol, respectively. Individually, the GID did not cause any noticeable change, either for individual vitamers or for total tocopherols.

Table 4.2.3. Tocopherols composition of *Macrolepiota procera* samples submitted to different processing types (PT) or irradiation doses (GID). The results are presented as mean ± SD.

		α-tocopherol (μg/100 g dw)	β-tocopherol (μg/100 g dw)	γ-tocopherol (μg/100 g dw)	δ-tocopherol (μg/100 g dw)	Total tocopherols (μg/100 g dw)
PT	Fresh	4±1	49±19	35±7	90±16	177±29
	Frozen	2±1	44±12	15±5	126±48	187±54
	Dried	3±1	53±28	41±3	63±6	160±33
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
GID	0 kGy	3±1	61±13	27±13	79±12	170±18
	0.5 kGy	3±1	30±15	35±10	106±61	174±64
	1 kGy	2.0±0.5	56±20	29±12	93±20	180±25
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	0.002
PT × GID	*p*-value (n=81)	<0.001	<0.001	<0.001	<0.001	<0.001

dw- dry weight.

Antioxidant parameters

The EC_{50} values calculated in each antioxidant activity evaluation assay remained nearly constant, independently of the applied GID (**Table 4.2.4**). Regarding PT, fresh samples presented lower DPPH scavenging activity, frozen samples were less effective inhibitors of β-carotene bleaching and dried samples had less activity

against TBARS formation. These results indicate that each PT might affect different chemical compounds, as endorsed by the results obtained for the changes in the chemical composition pointed out earlier, since the antioxidant activity was affected differently in each assay. Concerning phenolics content, the PT induced again higher changes than GID. Dried samples presented the highest amounts, while freeze treatment seemed to cause higher losses in phenolics. In fact, these compounds are unstable under heating, but at mild temperatures an increase in phenolics concentration may occur (Yen & Hung, 2000), which could explain the increase observed in dried mushrooms (**Figure 4.2.1D**). Furthermore, thermal treatment until 40 ºC (the temperature used to dry the mushrooms in the oven was 30 ºC) can inactivate endogenous oxidative enzymes (Dewanto, Wu, Adom, & Liu, 2002). In general, and despite the slightly higher antioxidant activity and phenolic compounds, tested samples showed stronger antioxidant activity (especially for TBARS formation inhibition) and higher phenolics amount, when compared with *M. procera* samples collected in a different season (Barros et al., 2007).

Table 4.2.4. Antioxidant activity and total phenolics content of *Macrolepiota procera* samples submitted to different processing types (PT) or irradiation doses (GID). The results are presented as mean ± SD.

		DPPH scavenging activity (EC_{50}; mg/mL)	Reducing power (EC_{50}; mg/mL)	β-carotene bleaching inhibition (EC_{50}; mg/mL)	TBARS formation inhibition (EC_{50}; mg/mL)	Phenolics (mg GAE/g extract)
PT	Fresh	6±2	1.5±0.2	4±2	1.7±0.5	19±2
	Frozen	4.0±0.3	1.4±0.1	8±1	3±1	15±1
	Dried	2.8±0.1	1.32±0.03	4±2	7±2	21±2
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
GID	0 kGy	4±1	1.4±0.1	5±3	3±1	18±2
	0.5 kGy	5±2	1.5±0.2	4±2	4±2	18±2
	1 kGy	4±1	1.4±0.2	6±3	4±2	20±4
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
PT × GID	*p*-value (n=81)	<0.001	<0.001	<0.001	<0.001	<0.001

Statistical analysis

In general, *M. procera* samples are characterised for having low caloric values (375-385 kcal/100 g of dw), presenting water and carbohydrates as major nutrients. Mannitol and trehalose were the predominant sugars, with special relevance of the sugar alcohol in samples dried or irradiated with 1.0 kGy, while the disaccharide presented higher amounts in fresh mushrooms. Linoleic, palmitic and oleic acids were, in this order, the main fatty acids; the most noticeable differences in these non-polar molecules were detected in unsaturated forms. Regarding tocopherols composition, δ-tocopherol was the prevalent vitamer reaching maximal values in samples submitted to freeze treatment or irradiated with 0.5 kGy. Neither PT nor GID seemed to affect greatly the antioxidant potential of *M. procera* extracts.

Despite the particular differences highlighted in the previous section, the global effect of each PT or GID still needs to be clarified. Accordingly, LDA was applied to fully understand the differences brought on by the two assayed factors. The discriminant ability of the differences obtained in the results for each assayed parameter is reflected in the classification performance, which can be assessed by evaluating the percentage of correctly classified groups. The parameters assembled in **Tables 4.2.1-4.2.4** were evaluated separately regarding their discriminant power, according to the differences induced either by PT or by GID. Despite the scarce number of well-defined changes in each assayed parameter herein described, it was clear that PT seemed to exert a higher influence, confirmed in the performed LDA assays. The plotted outputs are presented only for nutritional value, since it would be impractical presenting them in all cases.

For PT, the five obtained discriminant models were defined by two significant ($p < 0.001$ for the Wilks' λ test) discriminant functions, which included 100.0% of the variance of the experimental data in all cases. Regarding nutritional parameters (**Figure 4.2.2A**), function 1 (75.7%) and function 2 (24.3%) were mostly correlated with proteins content (higher in frozen samples) and energetic value (higher in fresh samples), respectively; moisture, fat and carbohydrates were the removed variables. In the case of free sugars, function 1 (88.0%) and function 2 (12.0%) presented the highest correlation with melezitose (higher in fresh samples) and mannitol (higher in dried samples), respectively; fructose and trehalose were the removed variables. Considering fatty acids, function 1 (90.8%) was mostly correlated with C14:0 (lower in

frozen samples) and C16:1 (higher in fresh samples), while function 2 (9.2%) presented the highest correlation with C12:0 (higher in dried samples, removed from **Table 4.2.2**) and C20:3 (higher in frozen samples); C8:0, C16:0, C18:0, C18:1, C18:2, SFA and MUFA were the removed variables. In what regards tocopherols, function 1 (97.8%) and function 2 (2.2%) were more strongly correlated with γ-tocopherol content (lower in frozen samples) and α-tocopherol, respectively; total tocopherols was the removed variable. In respect to antioxidant parameters, function 1 (76.7%) and function 2 (23.3%) were more correlated with phenolics content (higher in dried samples) and TBARS formation inhibition (higher EC_{50} values in dried samples), respectively; reducing power was the removed variable. In terms of classification performance, the differences resulting from the applied PT showed high discriminant power, since 100.0% of the samples were correctly classified, both for the original groups as well as for the cross-validation procedure for all the LDA analyses, except for the analyses performed with tocopherols (93.8% for the original groups and 92.6% for the cross-validation procedure) and antioxidant parameters (98.8% for the cross-validation procedure).

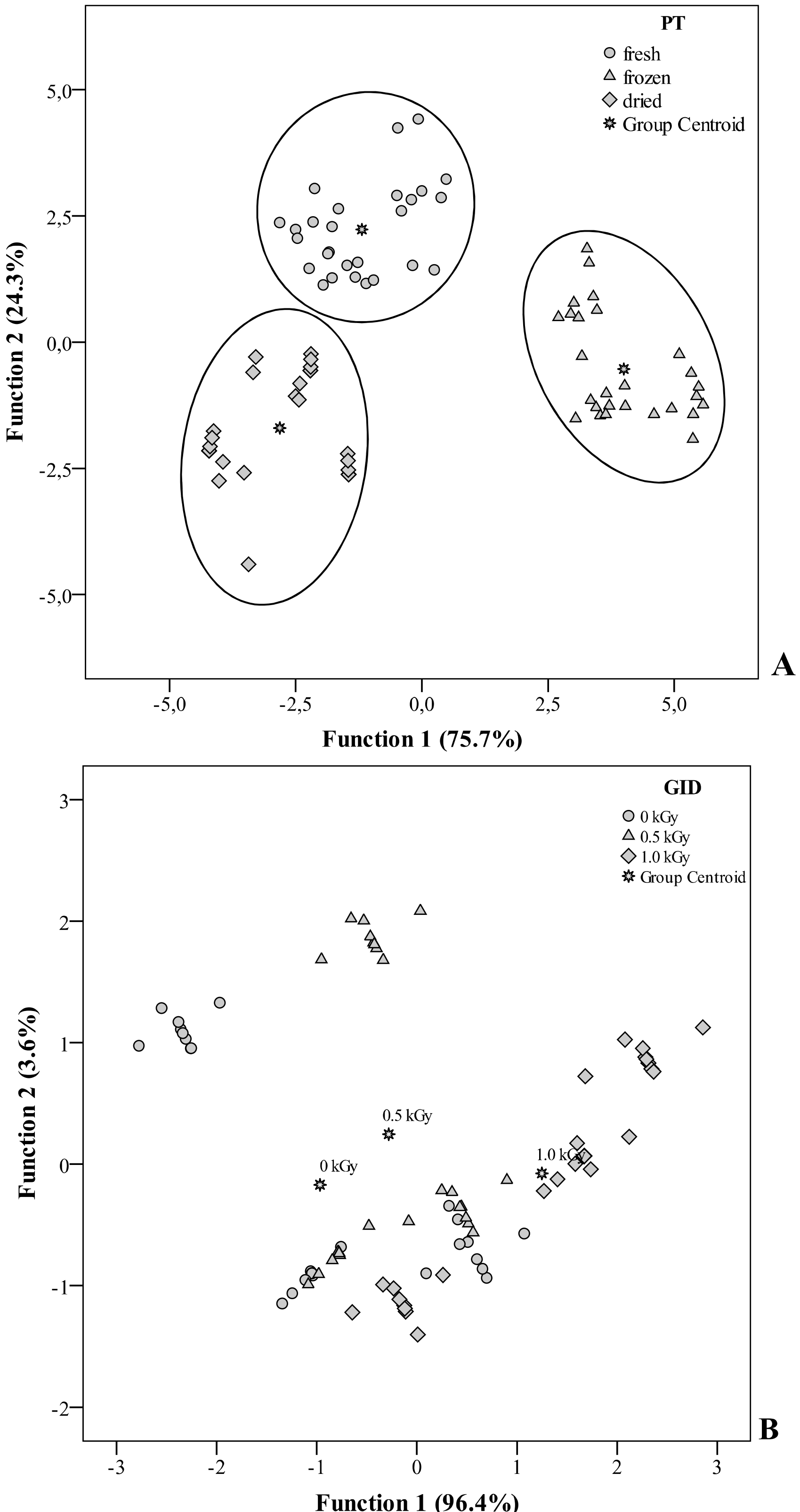

Figure 4.2.2. Discriminant scores scatter plot of the canonical functions defined for nutritional parameters results according with processing technology- PT (A) and gamma irradiation dose- GID (B).

Regarding GID, the five obtained discriminant models were also defined by two significant ($p < 0.001$ for the Wilks' λ test) discriminant functions in most cases except free sugars (one function), which included 100.0% of the variance of the experimental data. Concerning nutritional parameters, function 1 (96.4%) and function 2 (3.6%) were correlated with moisture and protein contents, respectively; moisture and proteins were the only selected variables. The classification performance was much lower, resulting in 59.3% of correctly classified cases for the original groups and for the cross-validation procedure. In respect to free sugars, only mannitol was selected as having some discriminant ability, associated with 33.3% of correctly classified cases for the original groups and 11.1% for the cross-validation procedure. For fatty acids, the LDA results identify differences that were not directly observable in **Table 4.2.2**, since all samples were correctly classified for the original groups and for the cross-validation procedure. Function 1 (89.9%) was mostly correlated with C14:0 (higher in non-irradiated samples) and C20:3 (higher in samples irradiated with 1.0 kGy), while function 2 (10.1%) presented the highest correlation with C22:0 (higher in samples irradiated with 0.5 kGy; removed from **Table 4.2.2**) and C20:5 (higher in non-irradiated samples); C8:0, C12:0, C18:1, C18:2, C20:0, C20:2, C24:0, C24:1, SFA, MUFA and PUFA were the removed variables. For tocopherols data, function 1 (91.9%) and function 2 (8.1%) were more strongly correlated with β-tocopherol content and α-tocopherol, respectively; γ-tocopherol was the removed variable. Concerning antioxidant parameters, function 1 (75.3%) and function 2 (24.7%) were more correlated with reducing power and phenolics content, respectively; DPPH scavenging activity was the removed variable. In terms of classification performance, the differences resulting from the applied GID showed low discriminant power: 69.1% of the samples were correctly classified for the original groups and 65.4% for the cross-validation procedure.

Overall, the chemical profiles obtained for each PT showed higher accuracy in defining the individual clusters corresponding to the naturally occurring groups, indicating that the tested GID caused weaker changes in *M. procera* samples. Furthermore, by treating *M. procera* with gamma irradiation, the differences caused by oven-drying or freeze conservation tended to be attenuated. In fact, the values obtained in *M. procera* samples submitted only to a single PT showed higher variability (Fernandes, Barros, at al., 2013), indicating that rather than a conservation

methodology *per se*, gamma irradiation might complement other conservation techniques.

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4.2.4. References

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of the Korean Society for Applied Biological Chemistry*, *53*, 257-265.

AOAC (1995). *Official methods of analysis* (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.

ASTM, American Society for Testing and Materials. (1992). *Practice for using the Fricke reference standard dosimetry system*. ASTM E1026, Annual Book of ASTM Standards, 12.02, Philadelphia, PA.

Bano, Z., Rajarathnam, S., & Rekha, M. N. S. (1992). Mushroom as the unconventional single cell protein for a conventional consumption. *Indian Food Packer*, *46*, 20-31.

Barreira, J. C. M., Pereira, J. A., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2010). Sugars profiles of different chestnut (*Castanea sativa* Mill.) and Almond (*Prunus dulcis*) Cultivars by HPLC-RI. *Plant Foods for Human Nutrition*, *65*, 38-43.

Barros, L., Baptista, P., Correia, D. M., Sá Morais, J., & Ferreira, I. C. F. R. (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, *55*, 4781-4788.

Beaulieu, M., D'Aprano, G., & Lacroix, M. (2002). Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry*, *63*, 311-315.

Dewanto, V., Wu, X., Adom, K. K., & Liu, R. H. (2002). Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. *Journal of Agricultural and Food Chemistry*, *50*, 3010-3014.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Botelho, L., Oliveira, M. P. P., Martins, A., et al., (2013). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Barros, L., Barreira, J. C. M., Antonio, A. L., Oliveira, M. P. P., Martins, A., et al. (2013). Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. *LWT - Food Science and Technology*, *54*, 493-499.

Haiying, W., Shaozhi, Z., & Guangming, C. (2007). Experimental study on the freezing characteristics of four kinds of vegetables. *LWT - Food Science and Technology*, *40*, 1112-1116.

ICGFI. International Consultative Group on Food Irradiation (1999). In Facts about Food Irradiation. United Kingdom: Buckinghamshire.

Jaworska, G., & Bernás, E. (2009). The effect of preliminary processing and period of storage on the quality of frozen *Boletus edulis* (Bull: Fr.) mushrooms. *Food Chemistry*, *113*, 936-943.

Jiang, T., Luo, S., Chen, Q., Shen, L., & Ying, T. (2010). Effect of integrated application of gamma irradiation and modified atmosphere packaging on physicochemical and microbiological properties of shiitake mushroom (*Lentinus edodes*). *Food Chemistry*, *122*, 761-767.

Kavishree, S., Hemavathy, J., Lokesh, B. R., Shashirekha, M. N., & Rajarathnam, S. (2008). Fat and fatty acids in Indian edible mushrooms. *Food Chemistry*, *106*, 597-602.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, *106*, 369-378.

Ma, L., Haixia, C., Wenchai, Z., & Zhaoshuai, W. (2013). Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom *Inonotus obliquus*. *Food Research International*, *50*, 633-640.

Maga J.A. (1981). Mushroom flavor. *Journal of Agricultural and Food Chemistry*, *29*, 4-7.

Maroco, J. (2003). *Análise Estatística*, *com utilização do SPSS*. Lisboa, Portugal: Edições Sílabo.

Ouzouni, P. K., & Riganakos, K. A. (2007). Nutritional value and metal content of Greek wild edible fungi. *Acta Alimentaria*, *36*, 99-110.

Polese, J.-M. (2005). *Mini Guía Setas*. Barcelona: Spanish Edition. ISBN: 3-8331-1810-5.

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. *Food and Chemical Toxicology*, *50*, 191-197.

Reis, F. S., Martins, A., Barros, L., & Ferreira, I. C. F. R. (2012). Antioxidant properties and phenolic profile of the most widely appreciated cultivated mushrooms: A comparative study between in vivo and in vitro samples. *Food and Chemical Toxicology*, *50*, 1201-1207.

Sommer, I., Schwartz, H., Solar, S., & Sontag, G. (2010). Effect of gamma-irradiation on flavour 50-nucleotides, tyrosine, and phenylalanine in mushrooms (*Agaricus bisporus*). *Food Chemistry*, *123*, 171-174.

Walde, S. G., Velu, V., Jyothirmayi, T., & Math, R. G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineering*, *74*, 108-115.

Yen, G.-C., & Hung, C.-Y. (2000). Effects of alkaline and heat treatment on antioxidative activity and total phenolics of extracts from Hsian-tsao (*Mesona procumbens* Hemsl.). *Food Research International*, *33*, 487-492.

4.3.

Utilização da irradiação gama para atenuar os efeitos nos ácidos orgânicos e compostos fenólicos causados pela desidratação e/ou congelamento de cogumelos silvestres *Macrolepiota procera*

Este sub-capítulo apresenta os efeitos da radiação gama (doses 0,5 e 1 kGy) em amostras frescas e processadas (congeladas e desidratadas) de Macrolepiota procera *(Scop.), analisando os ácidos orgânicos e compostos fenólicos.*

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ORIGINAL PAPER

Using gamma irradiation to attenuate the effects caused by drying or freezing in *Macrolepiota procera* organic acids and phenolic compounds

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Abstract

Macrolepiota procera (Scop.) Singer, commonly parasol mushroom, is an appreciated wild edible species. Due to its very perishable nature, *M. procera* must be processed to extend its shelf life. The chemical changes caused by common processing types (PTs) should be avoided to maintain the wholesomeness of organoleptic features. Irradiation might be used as a preservation methodology due to its safety, cost effectiveness and ability to ensure hygienic and sensory quality. Furthermore, when combined with other preservation technologies, irradiation

exhibits an attenuating effect over the chemical changes caused by some of those treatments per se. Herein, the effects of irradiation of *M. procera* processed samples (frozen, dried and fresh) were evaluated considering changes in organic acid and phenolic compound profiles. Detected contents of phenolic were much lower than those of organic acids. Differences caused by PT, specifically the lower levels of total organic acids and phenolic acids in dried and frozen samples, were larger than those observed for stronger irradiation doses, which did not cause remarkable changes, except for a slightly lower content of phenolic acids in nonirradiated samples. This larger effect was statistically confirmed in the performed linear discriminant analysis. Besides its slighter influence, irradiation showed potential usefulness to be used as complementary preservation technology since it attenuated the lowering effects of dehydration and freeze treatment over specific organic acid contents.

Keywords: Irradiation; Processed mushrooms; *Macrolepiota procera*; Organic acids; Phenolic compounds; LDA

4.3.1. Introduction

Macrolepiota procera (Scop.) Singer is one of the most popular mushrooms, being considered an excellent edible species, highly appreciated for its nutritional and culinary values (Polese 2005). In view of the very perishable nature, fresh mushrooms have to be processed to extend their shelf life. Among the various methods employed for preservation, canning is the most frequently adopted method in commercial scale (Walde et al. 2006), but drying is also a common method for preserving mushrooms (Giri and Prasad 2007) and freezing is becoming increasingly popular (Jaworska and Bernás, 2009, 2010). Drying is perhaps the oldest technique known by mankind for preservation of food commodities for long duration. It is a comparatively cheaper method (Rama and Jacob 2000; Walde et al. 2006), applied to decrease the moisture content of food to a level that can prevent the growth of mould and fungi and thus minimize microbial degradation. Food freezing is among the most efficient and adequate preservation methods, in which most of the liquid water changes into ice, which greatly reduces microbial and enzymatic activities (Haiying et al. 2007). Several studies indicate irradiation as a possible methodology to increase the shelf life of fresh mushrooms (Koorapati et al. 2004; Akram and Kwon

2010). It can be a safe and cost effective method to enhance shelf-life and ensure hygienic and sensory quality (Fernandes et al. 2012, 2013a).

Previously, our research group evaluated the effects of different processing technologies (freezing, drying and gamma irradiation) on chemical and antioxidant parameters of the wild mushroom *M. procera*, and irradiation was the processing technology with the highest ability to maintain the chemical characteristics of the fresh samples (Fernandes et al. 2013b). Moreover, *M. procera* gamma irradiation attenuated the effects caused by drying or freezing (*e.g.*, combining the freeze treatment with a 0.5 kGy dose preserved tocopherols). Rather than a preservation methodology, gamma irradiation emerged as a useful adjuvant for other preservation techniques such as freezing or drying (Fernandes et al. 2013c). Nevertheless, the mentioned reports did not assess the effects on organic acids or phenolic compounds, which are important molecules in mushrooms (Valentão et al. 2005; Ribeiro et al. 2006; Barros et al. 2009; Vaz et al. 2011; Barros et al. 2013).

Phenolic compounds might provide health benefits by reducing risk of chronic diseases due to their free radicals scavenging activity, singlet oxygen quenching or chelating effects. The antioxidant properties of phenolic compounds have also been related to the increased stability of food products, or to the antioxidant defense mechanisms of biological systems (Wright et al. 2001; Vaz et al. 2011).

Organic acids play a determinant role in maintaining fruit and vegetable quality and organoleptic characteristics and have also been used in their quality control (Cámara et al. 1994; Barros et al. 2013). Oxalic acid is very common in natural matrices, occurring also in animals; despite their biological functions, attention should be paid to the fact that calcium oxalate is the most common component of kidney stones. Quinic acid is a crystalline acid more common in plants, being often used as a versatile chiral-starting material for the synthesis of new pharmaceuticals; malic acid contributes to a pleasantly sour taste and is often used as a food additive. Citric acid is known to be very important in the prevention of mushroom browning and to extend its shelf life due to its antibacterial and antioxidant properties (Brennan et al. 2000). Fumaric acid is important because of its antioxidant, antimicrobial and acidifying properties (Ribeiro et al. 2008). The nature and concentration of these compounds are also important factors in mushrooms flavor (Valentão et al. 2005; Ribeiro et al. 2006).

Accordingly, the effects of irradiation of *M. procera* processed samples (frozen, dried and fresh mushrooms) were accessed regarding organic acids and phenolic compound profile and contents.

4.3.2. Materials and methods

Samples and samples irradiation

Macrolepiota procera fruiting bodies were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011.

The samples were divided in three groups with nine mushrooms per group with different stages of maturation and further submitted to different processing technologies: freezing (at -20º C in a freezer) and drying (at 30 ºC in an oven); the third group was kept fresh (stored at 4 ºC in a refrigerator). Each group was further subdivided in three subgroups: control (non-irradiated, 0 kGy); sample 1 (0.5 kGy) and sample 2 (1.0 kGy).

The estimated dose rate for the irradiation position was obtained with Fricke dosimeter, and the irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity 267 TBq (6.35 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd, U.K.), following the procedure previously described by the authors (Fernandes et al. 2013c). The estimated doses after irradiation were 0.6±0.1 kGy and 1.1±0.1 kGy for samples 1 and 2, respectively, at a dose rate of 2.3 $kGyh^{-1}$. For simplicity, in the text, tables and figures, we considered the values 0, 0.5 and 1 kGy, for nonirradiated and irradiated samples, respectively.

After irradiation, all the samples were freeze-dried (FreeZone 4.5 model 7750031, Labconco, KS, USA), reduced to a fine-dried powder (20 meshes), mixed to obtain homogenate samples, and promptly analyzed.

Standards and reagents

For irradiation: To estimate the dose and dose rate of irradiation a chemical solution sensitive to ionizing radiation was used, the Fricke dosimeter, prepared in the lab following the appropriate standard (American Society for Testing and

Materials 1992). To prepare the acid aqueous Fricke dosimeter solution the following reagents were used: ferrous ammonium sulfate(II) hexahydrate, sodium chloride and sulfuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water-treated in a Milli-Q water purification system (Millipore, model A10, USA).

For chemical analyses: Acetonitrile 99.9% was of hight-performance liquid chromatography (HPLC) grade from Lab-Scan (Lisbon, Portugal); other solvents were of analytical grade purity and were also supplied by Lab-Scan. Standards of phenolic compounds (protocatechuic, *p*-hydroxybenzoic, and *p*-coumaric acids), cinnamic acid and organic acids (oxalic acid, quinic acid, malic acid, citric acid and fumaric acid) were from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Organic acids identification and quantification

Samples (~1.5 g) were extracted by stirring with 25 mL of meta-phosphoric acid (25 °C at 150 rpm) for 25 min and subsequently filtered through Whatman No. 4 paper. Before analysis by ultrafast liquid chromatograph (UFLC) coupled to photodiode array detector (PAD), the sample was filtered through 0.2 µm nylon filters. Organic acids were determined following a procedure previously optimized and described by the authors (Barros et al. 2013).

Analysis was performed by UFLC coupled to a PAD, using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PAD, using 215 nm and 245 as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with those of calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight (dw; except for fumaric acid, expressed in µg per 100 g dw).

Phenolic compounds identification and quantification

Each sample (~1.5 g) was extracted with methanol:water (80:20, v/v; 30 mL) at -20 °C for 6 h. After sonication for 15 min and filtered through Whatman nº 4 paper. The residue was then extracted with two additional 30 mL portions of the

methanol:water mixture. Combined extracts were evaporated at 40 ºC under reduced pressure to remove methanol. The aqueous phase was submitted to a liquid-liquid extraction with diethyl ether (330 mL) and ethyl acetate (3 × 30 mL). The organic phases were evaporated at 40 °C to dryness, re-dissolved in water:methanol (80:20, v/v; 1 mL), followed by filtering through a 0.22 µm disposable LC filter disk for HPLC analysis.

Phenolic compounds were determined in the UFLC system mentioned above, as previously described by the authors (Barros et al. 2009). DAD detection was carried out using 280 nm and 370 nm as preferred wavelengths. The phenolic compounds were characterized according to their UV spectra and retention times, and comparison with authentic standards. For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in µg per 100 g dw.

Statistical analysis

Two samples from each subgroup (details in "*Samples and samples irradiation"* section) were extracted with *m*-phosphoric acid (for organic acids) or with acetone:water (80:20; for phenolic compounds and cinnamic acid extraction). Each purified extract was injected twice in the HPLC system.

The results were analyzed by means of an analysis of variance (ANOVA) with type III sums of squares performed using the general linear model (GLM) procedure of the SPSS software, version 18.0. The dependent variables were analyzed using two-way ANOVA, with "processing type" (PT) and "gamma irradiation dose" (ID) as factors. Since a significant interaction (PT × ID) was detected for all cases, the two factors were evaluated simultaneously by the estimated marginal means (EMM) for all levels of each single factor.

Further, a linear discriminant analysis (LDA) was used to compare the effect of the PT and ID on organic acids, phenolic compounds and cinnamic acid. A stepwise technique, using the Wilks' λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination processes, where the inclusion of a new variable is preceded by making sure that all variables previously selected remain significant (Maroco, 2003; López et al. 2008). With this approach, it

is possible to identify the significant variables obtained for each sample. To verify the significance of canonical discriminant functions, the Wilks' λ test was applied. A leaving-one-out cross-validation procedure was carried out to assess the model performance.

All statistical tests were performed at a 5% significance level. For each ID and or PT, three samples were analyzed, with all the assays being also carried out in triplicate. The results are expressed as mean value ± standard deviation (SD).

4.3.3. Results and discussion

The values for each individual parameter are presented as the mean value of each PT, considering different applied ID, and also the mean value of each ID, considering the results for all PT. This approach allows understanding the real influence of each factor, independently of the applied ID, as well as the most suitable ID to be applied, independently of the chosen PT. With no exception, PT × ID interaction was a significant ($p < 0.001$) source of variation for all the quantified compounds. Accordingly, and despite presenting the least squares means for both effects, no multiple comparisons could be performed. Nevertheless, from the analysis of the EMM plots (data shown only in specific cases) some overall conclusions can be outlined.

Organic acids

The UFLC-PAD analysis showed that all samples presented a profile composed of five organic acids: oxalic, quinic, malic, citric and fumaric acid, with malic acid as the main compound (**Table 4.3.1**). The obtained profiles were qualitatively similar to those reported previously (Barros et al. 2013), despite some quantitative differences, which might be related with the different collecting location. The interaction among PT×ID was a significant ($p < 0.001$) source of variation for all the quantified organic acids. Accordingly, the classification obtained by multiple comparisons tests could not be performed. Nevertheless, from the analysis of the EMM plots some particular tendencies could be identified.

Table 4.3.1. Organic acids composition of *Macrolepiota procera* samples submitted to different processing types (PTs) or gamma irradiation doses (IDs).

		Oxalic acid (mg/100g dw)	Quinic acid (mg/100g dw)	Mallic acid (mg/100g dw)	Citric acid (mg/100g dw)	Fumaric acid (µg/100g dw)	Total organic acids (mg/100g dw)
	Fresh	4.4±0.4	10±2	28±4	4±4	2±1	49±7
	Dried	7±1	11±7	13±6	2±1	5.1±0.5	38±2
PT	Frozen	8±3	3±1	21±1	6±1	1.6±0.1	41±6
	p-value (n=12)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	0 kGy	6±1	12±7	20±12	3±2	3±2	44±7
	0.5 kGy	8±4	7±2	19±4	4±3	3±2	41±5
GID	1 kGy	5±1	5±2	23±4	6±3	3±1	42±8
	p-value (n=12)	<0.001	<0.100	<0.001	<0.001	<0.001	<0.001
PT × GID	*p*-value (n=36)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± SD.
dw- dry weight.

For instance, quinic acid presented the lowest values in frozen samples and in samples irradiated with 1 kGy (**Figure 4.3.1A**); malic acid presented highest values in fresh samples (**Figure 4.3.1B**); fumaric acid (**Figure 4.3.1C)** like quinic acid showed minimal values in frozen samples. In terms of total organic acids, no particular tendency could be observed; the interaction among factors is evident, as it can be seen by the intersection of lines in **Figure 4.3.1D**. According to the identified tendencies, the variance caused by PT overcomes the effect of ID, but both factors induced only slight changes in organic acids. In fact, organic acids are known to have a lower susceptibility to change during processing than other components such as pigments and flavor compounds (Cámara et al. 1994). In order to obtain a clearer understanding of the effect of ID and PT on organic acids profiles, different LDA were applied. The discriminant ability of the differences obtained in those profiles can be inferred from the obtained classification performance, assessed by the percentage of correctly classified groups. The higher influence of PT was confirmed in the performed LDA assays, once 100.0% of the samples were correctly classified, both for the original groups and for the cross-validation procedure. The classification ability was quite lower for ID, resulting in 75.0% of accuracy for the original groups and 66.7% for the cross-validation procedure. In both cases, two significant ($p<0.001$ for the Wilks' λ test) discriminant functions, including 100.0% of the variance of the experimental data in all cases, were defined. Regarding PT (**Figure 4.3.2A**), function 1 (90.9%) and function 2 (9.1%) were mostly correlated with fumaric acid (dried>fresh>frozen) and malic acid (fresh>frozen>dried), respectively. Fumaric, malic and quinic acids were selected as discriminant variables. In the case of ID (**Figure 4.3.2B**), function 1 (80.2%) and function 2 (19.8%) were more highly correlated with citric acid (showing tendency to be higher in samples irradiated with 1 kGy) and oxalic acid (showing tendency to be higher in samples irradiated with 0.5 kGy), respectively. Besides these two, quinic and fumaric acids were also selected as discriminant variables.

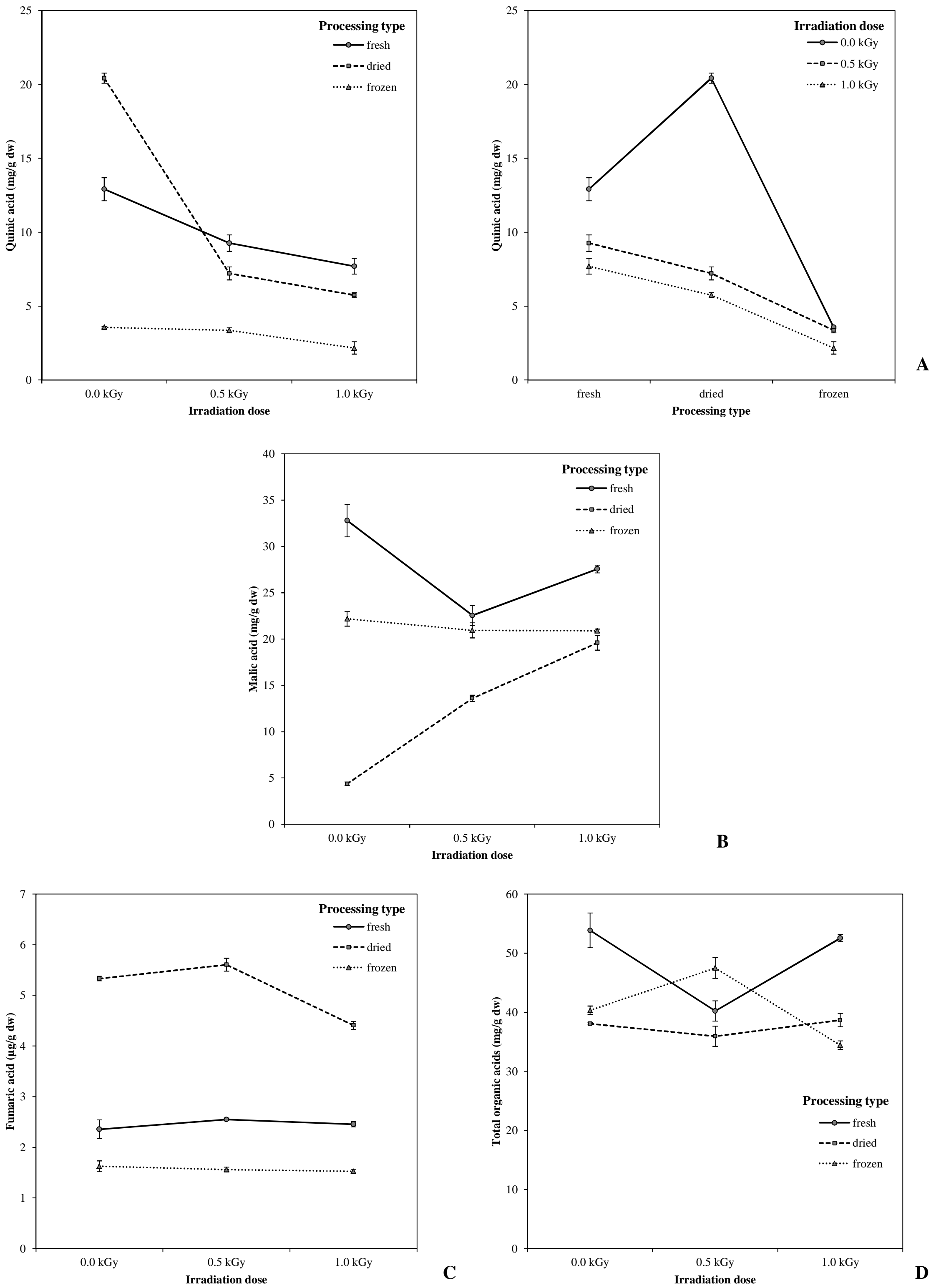

Figure 4.3.1. Interactions between processing type (PT) and/or gamma irradiation dose (ID) effects on the organic acids of *M. procera* samples. Quinic acid (A), malic acid (B), fumaric acid (C), total organic acids (D).

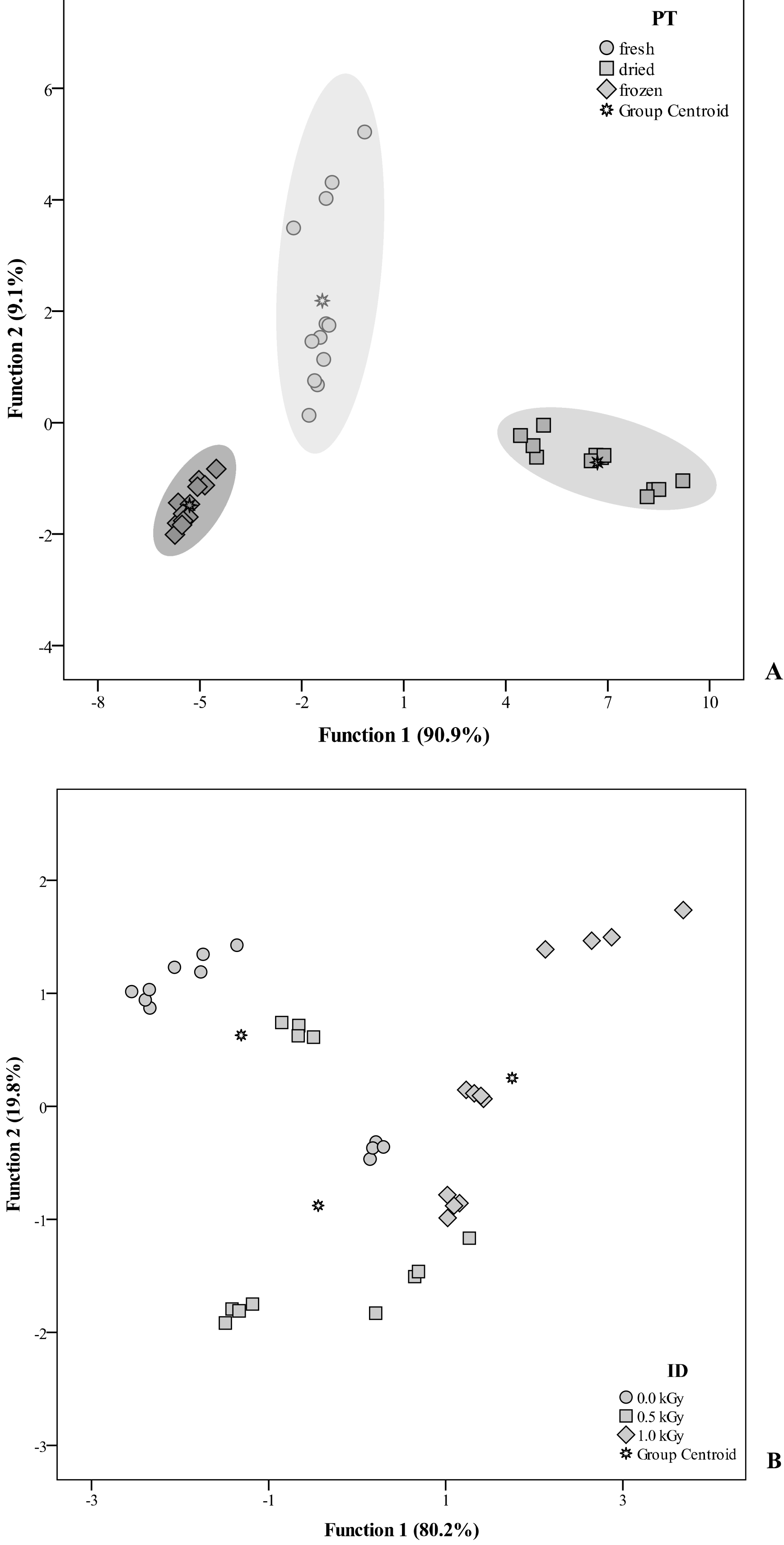

Figure 4.3.2. Discriminant scores scatter plot of the canonical functions defined for organic acids results according with PT (A) and ID (B).

Phenolic acids

The results obtained show that *M. procera* contain very small amounts of phenolic acids (**Table 4.3.2**), which are in agreement with values commonly found in mushrooms (Valentão et al. 2005).

The interaction among PT×ID was a significant ($p< 0.001$) source of variation for all the quantified phenolic acids. Accordingly, the results could not be classified by multiple comparisons tests. However, analyzing the estimated margins mean plots allowed identifying some general tendencies. For instance, protocatechuic acid had highest values on fresh samples and in nonirradiated samples (**Figure 4.3.3A**); *p*-hydroxybenzoic and *p*-coumaric acids presented highest values in fresh samples (**Figure 4.3.3B and C**); the results obtained for total phenolic acids are in line with the observed for each individual molecule. As it can be concluded from **Figure 4.3.3D**, dried samples tended to present lower amounts of phenolic acids; furthermore, irradiation (1.0 kGy dose, in particular) exerted a notorious protective effect on total phenolic acids content. The lowest value for cinnamic acid (**Figure 4.3.3E**), was also obtained in dried samples. Similarly to observations for organic acids, differences caused by PT were larger than those corresponding to ID. To clarify this conclusion, two additional LDA were applied. The higher influence of PT was confirmed, since 100.0% of the samples were correctly classified, both for the original groups as well as for the cross-validation procedure, regarding this factor. The classification ability was quite lower for ID; in fact, no qualifying variables were selected in this case. The discriminant model obtained for PT was defined by two significant ($p < 0.001$ for the Wilks' λ test) discriminant functions including 100.0% of the variance of the experimental data (**Figure 4.3.4**). Function 1 (88.9%) and function 2 (11.1%) were mostly correlated with *p*-coumaric acid (fresh>dried>frozen) and total phenolic acids (maximum value in fresh samples), respectively. Cinnamic acid was rejected as discriminant variable.

Consumer research is a key activity to evaluate the acceptance or liking of a determined product. This represents, in fact, important information regarding product decisions such as the development and marketing of new products, the reformulation of existing products, the acceptance of suppliers and processes, or the establishment of quality control specifications (Krishnamurthy et al. 2007). However, this type of descriptive tests requires a well-trained panel and tends to be expensive (Choi 2013).

Accordingly, we are conducting preliminary assays in several mushroom species, which are intended to be aggregated and submitted to sensory panels simultaneously. Nevertheless, we have assayed the effect on nutritional composition (Fernandes et al. 2012, 2013a, b, c), concluding that there were no significant differences among the assayed parameters.

Table 4.3.2. Phenolic and cinnamic acids composition of *Macrolepiota procera* samples submitted to different processing types (PT) or gamma irradiation doses (ID).

		Protocatechuic acid (µg/100g dw)	*p*-Hydroxybenzoic acid (µg/100g dw)	*p*-Coumaric acid (µg/100g dw)	Total phenolic acids (µg/100g dw)	Cinnamic acid (µg/100g dw)
PT	Fresh	8±3	1.1±0.4	1.9±0.2	11±3	3±1
	Dried	nd	0.5±0.1	1.5±0.1	2.0±0.1	1.5±0.2
	Frozen	2.0±0.5	0.2±0.1	nd	1.8±0.5	3.5±0.3
	p-value (*n*=12)	<0.001	<0.001	<0.001	<0.001	<0.001
GID	0 kGy	5±5	0.7±0.5	1±1	7±7	2±1
	0.5 kGy	3±3	0.4±0.2	1±1	4±4	2±1
	1 kGy	2±2	0.6±0.5	1±1	4±3	3±1
	p-value (*n*=12)	<0.001	<0.100	<0.001	<0.001	<0.001
PT×GID	*p*-value (*n*=36)	<0.001	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± SD.
dw- dry weight, nd - not detected

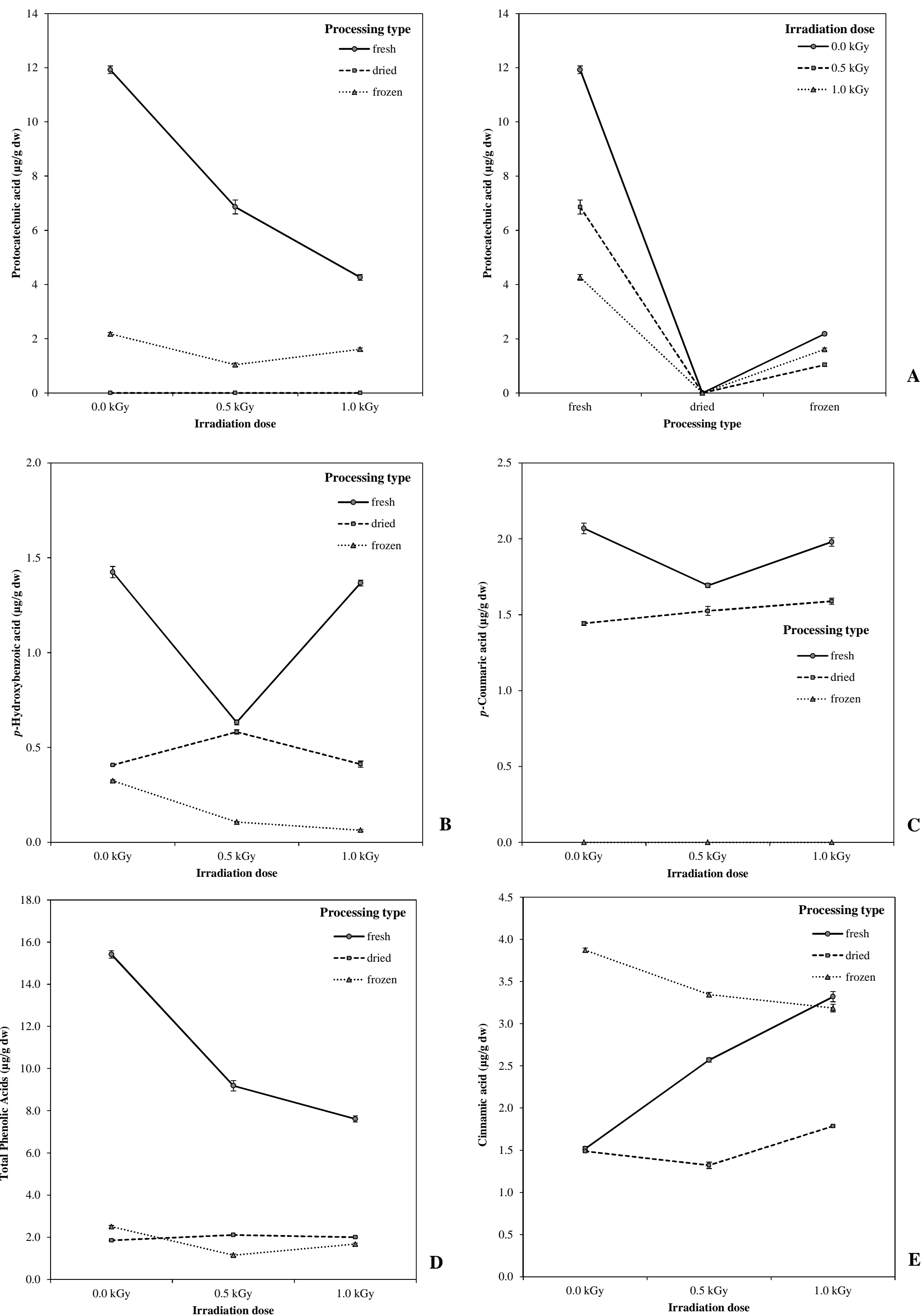

Table 4.3.3. Interactions between processing type (PT) and/or gamma irradiation dose (ID) effects on the phenolic compounds of *M. procera* samples. Protocatechuic acid (A), *p*-hydroxybenzoic acid (B), *p*-coumaric acid (C), total phenolic acids (D), cinnamic acid (E).

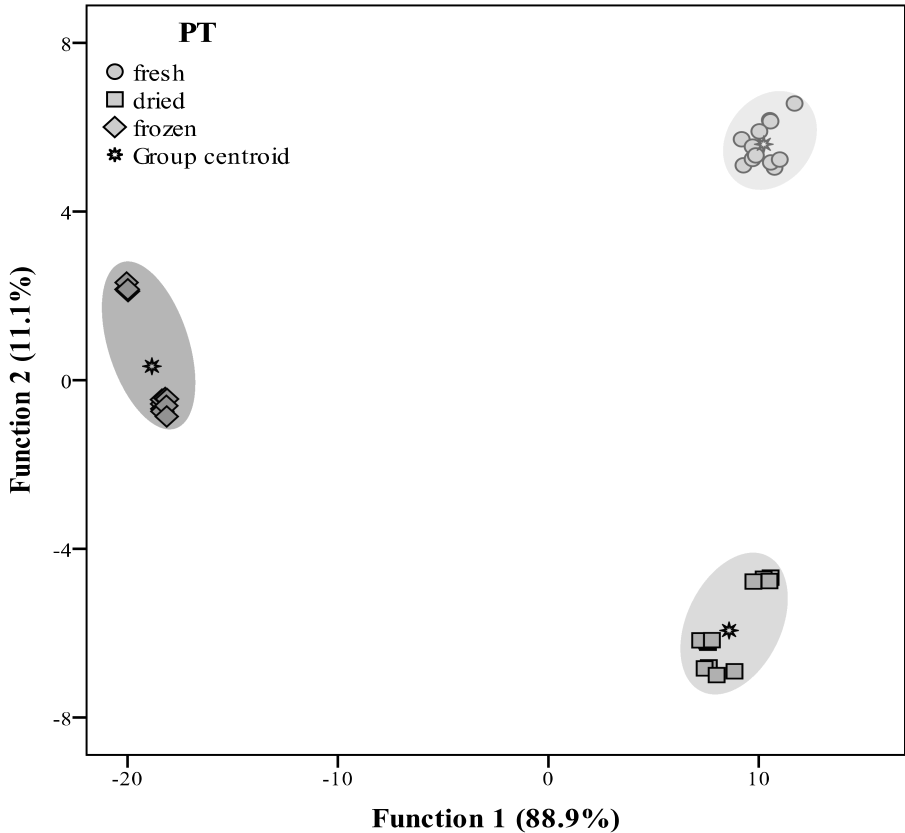

Figure 4.3.4. Discriminant scores scatter plot of the canonical functions defined for phenolic compounds results according with PT.

4.3.4. Conclusions

Herein, profiles of phenolic compounds and organic acids were characterized in samples submitted to different PTs and IDs. Comparing with organic acids, phenolics are present in notably lower contents. According to observed changes, irradiation might be a useful complementary preservation technology since it induced less significant effects when compared with common techniques like dehydration or freeze treatment. Furthermore, some pronounced effects of these PTs were attenuated by irradiation: the lower amounts of oxalic acid in fresh samples, malic acid in dried samples and citric acid in fresh and dried samples were significantly mitigated by irradiation treatment. In an overall perspective, it is possible to conclude that irradiation alone, especially 1.0 kGy dose, is the best option to preserve total organic acids and total phenolic acids.

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4.3.5. References

Akram, K., & Kwon, J.-H. (2010). Food irradiation for mushrooms: A review. *Journal of Korean Society for Applied Biological Chemistry*, *53*, 257-265.

American Society for Testing and Materials (1992). Practice for using the Fricke reference standard dosimetry system, ASTM E1026. *Annual book of ASTM Standards*, 12.02. Philadelphia.

Barros, L., Dueñas, M., Ferreira, I. C. F. R., Baptista, P., & Santos-Buelga, C. (2009). Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. *Food and Chemical Toxicology*, *47*, 1076-1079.

Barros, L., Pereira, C., & Ferreira, I. C. F. R. (2013). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. *Food Analytical Methods*, 6, 309-316.

Brennan, M., Le Port, G., & Gormley, R. (2000). Post-harvest treatment with citric acid or hydrogen peroxide to extend the shelf life of fresh sliced mushrooms. *Lebensmittel-Wissenschaft & Technologie*, *33*, 285-289.

Cámara, M. M., Díez, C., Torija, M. E., & Cano. M. P. (1994). HPLC determination of organic acids in pineapple juices and nectars. *Zeitschrift für Lebensmittel-Untersuchung und-Forschung*, *198*, 52-56.

Choi, E. C. (2014). Sensory Evaluation. In S. Edelstein (Ed.), *Food science - an ecological approach* (pp. 83-113). Burlington, Massachusetts: Jones Bartlett Learning.

Fernandes, Â., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Botelho, M. L., Oliveira, M. P. P., Martins, A., et al. (2013a). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Barros, L., Barreira, J. C. M., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2013b). Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. *LWT - Food Science and Technology,* 54, 493-499.

Fernandes, Â., Barreira, J. C. M., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2013c). Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera*. *Food Chemistry*. Doi:10.1016/j.foodchem.2013.10.050.

Giri, S. K., & Prasad, S. (2007). Drying kinetics and rehydration characteristics of microwave-vacuum and convective microwave-vacuum and convective hot-air dried mushrooms. *Journal of Food Engineering, 78*, 512-552.

Haiying, W., Shaozhi, Z., & Guangming, C. (2007). Experimental study on the freezing characteristics of four kinds of vegetables. *LWT - Food Science and Technology*, *40*, 1112-1116.

Jaworska, G., & Bernás, E. (2009). The effect of preliminary processing and period of storage on the quality of frozen *Boletus edulis* (Bull: Fr.) mushrooms. *Food Chemistry, 113*, 936-943.

Jaworska, G., & Bernás, E. (2010). Effects of pre-treatment, freezing and frozen storage on the texture of Boletus edulis (Bull: Fr.) mushrooms. *International Journal of Refrigeration*, *33*, 877-885.

Koorapati, A., Foley, D., Pilling, R., & Prakash, A. (2004). Electron-beam irradiation preserves the quality of white button mushroom (*Agaricus bisporus*) slices. *Journal of Food Sciences, 69*, SNQ25-SNQ29.

Krishnamurthy, R., Srivastava, A. K., Paton, J. E., Bell, G. A., & Levy, D. C. (2007). Prediction of consumer liking from trained sensory panel information: evaluation of neural networks. *Food Quality and Preference*, *18*, 275-285.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, *106*, 369-378.

Maroco, J. (2003). *Análise Estatística, com utilização do SPSS*. Lisboa: Edições Sílabo.

Polese, J.-M. (2005). Mini Guía Setas. ISBN: 3-8331-1810-5. Spanish Edition, Barcelona.

Rama, V., & Jacob, J. P. (2000). Effects of methods of drying and pretreatments on quality of dehydrated mushroom. *Indian Food Packer*, *54*, 59-64.

Ribeiro, B., Rangel, J., Valentão, P., Baptista, P., Seabra, R. M., & Andrade, P. B. (2006) Contents of carboxylic acids and two phenolics and antioxidant activity of dried Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry, 54*, 8530-8537.

Ribeiro, B., Andrade, P. B., Baptista, P., Barros, L., Ferreira, I. C. F. R., Seabra, R. M., et al. (2008). *Leucopaxillus giganteus* mycelium: effect of nitrogen source on organic acids and alkaloids. *Journal of Agricultural and Food Chemistry*, 56, 4769-4774.

Valentão, P., Lopes, G., Valente, M., Barbosa, P., Andrade, P.B., Silva, B.M., et al. (2005). Quantification of nine organic acids in wild mushrooms. *Journal of Agricultural and Food Chemistry, 53*, 3626-3630.

Vaz, J. A., Barros, L., Martins, A., Morais, J. S., Vasconcelos, M. H., & Ferreira, I. C. F. R. (2011). Phenolic profile of seventeen Portuguese wild mushrooms. *LWT - Food Science and Technology*, *44*, 343-346.

Walde, S. G., Velu, V., Jyothirmayi, T., & Math, R. G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineer*, *74*, 108-115.

Wright, J. S., Johnson, E. R., & DiLabio, G. A. (2001). Predicting the activity of phenolic antioxidants: theoretical method, analysis of substituent effects, and application to major families of antioxidants. *Journal of American Chemical Society*, *123*, 1173-1183.

CAPÍTULO 5.

Efeito da radiação por feixe de eletrões nos parâmetros químicos, nutricionais e bioativos de cogumelos silvestres

Este capítulo apresenta os efeito da radiação por feixe de eletrões nos parâmetros químicos, nutricionais e bioativos de cogumelos silvestres Macrolepiota procera *(Scop.) Singer*, Boletus edulis *Bull.*, Russula delica *Fr.*, Amanita caesarea (Scop.) *Pers. e* Amanita curtipes E.-J. Gilbert.

5.1

Efeito combinado da irradiação por feixe de eletrões e do tempo de armazenamento nos parâmetros químicos e antioxidantes de cogumelos silvestres desidratados de *Macrolepiota procera*

Este sub-capítulo apresenta os efeitos da radiação por feixe de eletrões (doses 0,5, 1 e 6 kGy) e do tempo de armazenamento (0, 6 e 12 meses) nos parâmetros nutricionais (valor energético, açúcares livres, ácidos gordos e tocoferóis) e bioativos (atividade antioxidante) de amostras desidratadas de Macrolepiota procera *(Scop.).*

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ORIGINAL PAPER

Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples

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Abstract

Mushrooms are very perishable foods due to their high susceptibility to moisture loss, changes in color and texture, or microbiological spoilage. Drying is considered as the most appropriate method to prevent these alterations, but it has some limitations, such as shrinkage, enzymatic and non-enzymatic browning reactions, and oxidation of lipids and vitamins. According to previous studies, irradiation might effectively attenuate the undesirable changes caused by drying process, ensuring also higher shelf-life of mushrooms and their decontamination.

Electron-beam irradiation presents some technological advantages, since it allows higher dose rates and the possibility to be used in most foods/or thin products in a short period. Herein, the combined effects of electron-beam irradiation (0, 0.5, 1 and 6 kGy) and storage time (0, 6 and 12 months) were evaluated by measuring changes in nutritional parameters, namely, free sugars, tocopherols, fatty acids and antioxidant activity. As indicated by linear discriminant analysis, storage time had a higher effect on all the evaluated parameters, except fatty acids, which suffer significant changes with both factors. Overall, the obtained results indicate that electron-beam irradiation might be considered as a suitable technique, allowing long-lasting conservation periods while reducing changes induced by drying treatment.

Keywords: Macrolepiota procera; Electron-beam; Drying; Chemical composition; Antioxidant activity

5.1.1. Introduction

Mushrooms are highly perishable products that undergo, after harvest, spoilage processes such as moisture loss, shrinkage and color and texture changes (Kulshreshtha et al. 2009). In addition, mushrooms are prone to the presence of parasites, insects and microorganisms. Accordingly, their safety and quality requirements call for better conservation techniques, urging finding alternatives to reduce losses in these food products and increase their shelf-life (Lacroix and Ouattara 2000).

Macrolepiota procera (Scop.) Singer (parasol mushroom) is an edible saprophytic fungus appreciated and consumed all over the world. It is a common species, but, like all mushrooms, when not consumed immediately, it requires some treatment to prevent its deterioration (Arora et al. 2003). Drying is a widely used postharvest technology, which overcomes problems related to overproduction and short shelf-life. In fact, dried mushrooms, packed in airtight containers can have a shelf-life of above 1 year (Walde et al. 2006). It is also expected that the decrease in moisture content may prevent the growth and development of mold and fungi, minimizing microbial degradation (Jangam et al. 2011). Furthermore, dried products occupy less space than fresh, frozen or canned products and can be stored at room

temperature and the nutritional value of the product is concentrated due to water loss (Cao et al. 2003; Celestino 2010).

Nevertheless, drying causes undesirable changes in the product such as loss of some nutrients, shrinkage, enzymatic and non-enzymatic browning reactions, and oxidation of lipids and vitamins (Celestino 2010). High temperatures and long drying periods may cause serious damage to the flavor, color and nutrients and reduce bulk density and rehydration capacity (Maskan 2000). Moreover, drying imparts the reduction of vegetative cells of microorganisms giving rise to a dominant flora of bacteria and mold with the ability to survive longer periods in dry foods (ICMSF 1985; Almeida 2006).

In this sense, the irradiation of mushrooms can be a safe and inexpensive method of ensuring hygienic and sensory quality (Akram and Kwon, 2010; Fernandes et al. 2012a), that could be applied to dry samples in order to prevent some of the disadvantages mentioned above. Gamma irradiation is commonly applied to fresh wild mushrooms (Fernandes et al. 2012b), but studies with electron-beam irradiation are scarcer. Electron-beam irradiation is known to be highly effective in reducing harmful bacteria in fruits, vegetables and other foods while preserving the taste, aroma, texture, wholesomeness and nutritional content (Schmidt et al. 2006; Duan et al. 2010; Fernandes et al. 2012a). Moreover, this technique has some advantages when compared to gamma irradiation; the electron-beam sources can be easily connected/disconnected, whereas the gamma sources are continually decaying and gamma irradiation needs to be applied for a larger period due to its lower dose rate. Nevertheless, both technologies are suitable for irradiating mushrooms and give reproducible dose sterilization in microorganisms; gamma irradiation is mainly used for large volumes/packages, while the electron-beam is used for most foods/or thin products of low density (International Atomic Energy Agency (IAEA), 2002).

Electron-beam irradiation was previously applied to fresh *Agaricus bisporus* (Koorapati et al. 2004; Duan et al. 2010) and air-dried *Tuber aestivum* (Rivera et al. 2011), but its effects on *M. procera* were not, as far as we know, studied. In the present work, the combined effects of electron-beam irradiation (0.5, 1 and 6 kGy) and storage time (0, 6 and 12 months) on chemical and antioxidant parameters of wild *M. procera* dried samples were studied in order to evaluate the feasibility of electron-beam irradiation as a complementary conservation technique.

5.1.2. Materials and methods

Standards and reagents

Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and were obtained from common sources. Water was treated in a Mili-Q water purification system (TGI Pure Water Systems, USA).

Samples and electron-beam irradiation

Macrolepiota procera wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011, and dried at 30 ºC in an oven. Subsequently, the samples were divided in four groups with three specimens in each group: control (non-irradiated, 0.0 kGy); sample 1 (0.5 kGy); sample 2 (1.0 kGy) and sample 3 (6.0 kGy).

The irradiation was performed at the Institute of Nuclear Chemistry and Technology (INCT, Warsaw, Poland). To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, a graphite calorimeter and two routine Gammachrome YR and Amber Perspex dosimeters from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 μs, pulse frequency of 440 Hz and average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min, and the scan frequency was 5 Hz. The absorbed dose was 0.53, 0.83 and 6.10 kGy, with an uncertainty of 20% for the two first doses and 10% for the last dose. To read the Amber and Gammachrome YR dosimeters, spectrophotometric methods were used. For the Graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a calibrated curve

(Carocho et al. 2012). For simplicity, we refer to the irradiation doses as: 0, 0.5, 1 and 6 kGy.

Before analysis, the samples were reduced to a fine-dried powder (20 mesh) and mixed to obtain homogenized samples. The analyses were performed after 0, 6 and 12 months of storage.

Chemical parameters

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method, the crude fat was determined by extracting a known weight of powdered sample with petroleum ether using a Soxhlet apparatus, and the ash content was determined by incineration at 600±15 °C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = $4 \times (g_{protein}) + 3.75 \times (g_{carbohydrate}) + 9 \times (g_{fat})$.

Free sugars

Free sugars were determined by HPLC coupled to a refraction index detector (HPLC-RI) following the extraction procedure described by Heleno et al. (2009), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analysed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6×250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the IS method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by Heleno et al. (2009). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID at 260 ºC and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d*f*). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC, held for 15 min. The carrier gas (hydrogen) flow rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined after an extraction procedure previously described by Heleno et al. (2010), using tocol as IS. The analysis was carried out in the above-described HPLC system that is connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 ml/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the IS method, and tocopherols content was further expressed in µg per 100 g of dw.

Extraction procedure

The lyophilized powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman no. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts

were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays to evaluate the antioxidant activity of the samples. The sample concentrations providing 50 % of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

Antioxidant activity

DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments; Winooski, VT, USA). The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\% \text{ RSA} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance (515 nm) of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution (Guimarães et al., 2013).

Two different procedures were used to evaluate the reducing power: (A) Ferricyanide/Prussian blue assay, based on the reduction of ferricyanide to ferrous form and measurement of the developed color at 690 nm in the microplate reader mentioned above; and (B) Folin-Ciocalteu assay, measuring the color development at 765 nm; the results were expressed as mg of gallic acid equivalents (GAE) per gram of extract (Barros et al. 2011).

Inhibition of β-carotene bleaching is based on the ability of different concentrations of the extracts to maintain the color of a β-carotene emulsion by neutralizing (50 ºC) of linoleate radicals and other free radicals formed in the system which attack the highly unsaturated β-carotene models). The zero time and endpoint (2 h) absorbances were measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100 (Guimarães et al. 2013).

Lipid peroxidation inhibition evaluates the capacity of different concentrations of the extracts to prevent the formation of the malondialdehyde (MDA)-TBA complex using brain homogenates. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the complex in the supernatant was

measured at 532 nm. The inhibition ratio (%) was calculated using the formula: Inhibition ratio (%) = $[(A - B)/A] \times 100$ %, where A and B were the absorbance of the control and the sample solution, respectively (Barros et al. 2011).

Statistical analysis

The values for each measured parameter will be presented as the mean value of each storage time (ST), for all applied electron-beam (EB), and the mean value of each ED, considering the results for all ST. In this way, the effect of each applied ED or ST is understood with higher accuracy, allowing the selection of the best ST independently of applied ED, as well as the most suitable ED independently of required ST.

An analysis of variance (ANOVA) with Type III sums of squares was performed using the general linear model (GLM) procedure of the SPSS software, version 18.0. The dependent variables were analyzed using 2-way ANOVA, with ST and ED as factors. As a significant interaction (ST × ED) was detected for all cases, the two factors were evaluated simultaneously by the estimated marginal means (EMM) plots for all levels of each factor.

In addition, a linear discriminant analysis (LDA) was used to assess the effect of the ST and ED on nutritional value, free sugars, fatty acids, tocopherols and antioxidant activity results. A stepwise technique, using the Wilks' λ method with the usual probabilities of F (3.84 to enter and 2.71 to remove), was applied for variable selection. This procedure uses a combination of forward selection and backward elimination processes, where each new included variable is preceded by the verification of significance of all previously selected variables (Maroco, 2003; López et al. 2008). With this approach, it is possible to identify the significant variables obtained for each sample. To verify the significance of canonical discriminant functions, the Wilks' λ test was applied. The classification accuracy of the model was assessed through a leaving-one-out cross-validation procedure.

All statistical tests were performed at a 5% significance level. For each ED and or ST, three samples were analyzed, with all the assays being also carried out in triplicate. The results are expressed as mean value ± standard deviation (SD).

5.1.3. Results and discussion

The fixed factors (ST and ED) showed significant (p < 0.001) interaction (ST×ED) in all assayed parameters. Accordingly, and despite the least squares means are presented for both effects, no multiple comparisons could be performed. Nevertheless, from the analysis of the EMM plots (data shown only in specific cases) some overall conclusions can be outlined.

The chemical parameters results are presented in **Tables 5.1.1 - 5.1.4**. Nutritionally, *M. procera* has high water content (≈90%), with carbohydrates (≈60%) and proteins (≈30%) as the main components in dry mass basis, in agreement with previous reports (Barros et al. 2007; Ouzouni and Riganakos 2007). Besides the low percentage in dry mass, *M. procera* presented low fat content, being a low caloric food. Concerning the effects of ST and ED, protein, fat and ash showed a decrease (**Table 5.1.1**) tendency with time, a result reflected in carbohydrates content (**Figure 5.1.1A**), which increased along time. On the hand, the applied irradiation did not cause any marked tendencies, except for lower protein value in samples irradiated with 0.5 kGy (**Table 5.1.1**).

Free sugars are good indicators of a suitable conservation technology due to their sensibility to technical practices (Barreira et al. 2010). Trehalose (≈9 g/100 g dw) and mannitol (≈5 g/100 g dw) were the main sugars (**Table 5.1.2**), presenting also low contents in fructose and melezitose in non-stored samples. The detected profiles are similar to the reported for *M. procera* submitted to different processing treatments (Barros et al. 2007). Once more, the applied irradiation did not cause particular changes, except for higher fructose values in samples irradiated with 0.5 kGy; on the other hand, trehalose (**Figure 5.1.1B**) and mannitol presented maximum values in nonstored samples.

δ-Tocopherol was the main vitamin E isoform (≈60 μg/100 g dw; **Table 5.1.3**). β-tocopherol and γ-tocopherol were also detected in significant amounts, but while δ-tocopherol was relatively stable along ST and among different applied ED, β- and γ-tocopherol were only detected in non-stored samples. α-Tocopherol tended to be lower in samples irradiated with higher ED (**Figure 5.1.1C**).

Table 5.1.1. Proximate composition and corresponding energetic value of dried *M. procera* submitted to different electron-beam irradiation doses (ED) and storage times (ST).

		Dry matter (g/100 g fw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Carbohydrates (g/100 g dw)	Ash (g/100 g dw)	Energy (kcal/100 g dw)
ED	0 kGy	10±1	2±1	29±6	63±8	6±1	388±2
	0.5 kGy	9±1	2±1	24±8	68±10	6±1	387±3
	1 kGy	9±1	2±1	28±8	64±10	6±1	385±2
	6 kGy	9±1	1.8±0.5	28±8	64±9	6±1	386±3
	p-value (n=36)	0.068	<0.001	<0.001	<0.001	<0.001	<0.001
ST	0 months	9±1	2.8±0.4	36±1	54±1	7.7±0.4	383±1
	6 months	na	2.0±0.2	28±4	64±4	5.2±0.3	389±1
	12 months	na	1.1±0.1	18±1	76±1	4.7±0.3	387±1
	p-value (n=27)	na	<0.001	<0.001	<0.001	<0.001	<0.001
ED × ST	*p*-value (n=108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± SD.
fw- fresh weight, dw- dry weight, na- not applicable.

Table 5.1.2. Sugars composition of dried *M. procera* submitted to different electron-beam irradiation doses (ED) and storage times (ST).

		Fructose (g/100 g dw)	Mannitol (g/100 g dw)	Trehalose (g/100 g dw)	Melezitose (g/100 g dw)	Total sugars (g/100 g dw)
ED	0 kGy	0.05±0.05	5±2	9±3	0.2±0.2	14±6
	0.5 kGy	0.1±0.1	4±1	7±1	0.2±0.2	12±2
	1 kGy	0.05±0.05	5±2	11±4	0.3±0.3	16±6
	6 kGy	0.05±0.05	4±1	10±2	0.2±0.2	14±4
	p-value (n=36)	<0.001	<0.001	<0.001	<0.001	<0.001
ST	0 months	0.15±0.05	7±1	13±3	0.7±0.1	20±4
	6 months	nd	3.3±0.3	7±1	nd	11±1
	12 months	nd	3.5±0.5	8±1	nd	11±2
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
ED × ST	*p*-value (n=108)	<0.001	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± SD.
fw- fresh weight, dw- dry weight, nd- not detected.

Table 5.1.3 Tocopherols composition of *M. procera* submitted to different electron-beam irradiation doses (ED) and storage times (ST).

		α-Tocopherol (μg/100 g dw)	β-Tocopherol (μg/100 g dw)	γ-Tocopherol (μg/100 g dw)	δ-Tocopherol (μg/100 g dw)	Total tocopherols (μg/100 g dw)
ED	0 kGy	8±5	23±33	8±12	64±10	103±43
	0.5 kGy	6±2	9±13	9±12	75±9	99±18
	1 kGy	2±1	4±6	19±27	51±10	77±38
	6 kGy	3±1	15±22	15±22	46±20	80±64
	p-value (n=36)	<0.001	<0.001	<0.001	<0.001	<0.001
ST	0 months	4±1	39±21	38±14	64±7	145±22
	6 months	7±5	nd	nd	56±20	63±21
	12 months	3±1	nd	nd	58±21	61±22
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001
ED × ST	*p*-value (n=108)	<0.001	<0.001	<0.001	<0.001	<0.001

The results are presented as mean ± SD.
fw- fresh weight, dw- dry weight, nd- not detected.

In what regards fatty acids (FA), 25 individual molecules were quantified; those detected in contents higher than 0.2% are presented in **Table 5.1.4** (in the LDS presented onwards all FA were included). Linoleic (≈60%), palmitic (≈25%) and oleic (≈9%) acids were the major FA in *M. procera*. The abundance of linoleic acid might be related with the organoleptic characteristics of this mushroom, since this FA is the precursor of 1-octen-3-ol, known as “fungi alcohol”, the main aromatic component in fungi (Maga 1981). In most cases, FA did not show marked tendencies caused by different ED or ST. Nevertheless, C6:0, C8:0, C10:0 and C23:0 were maximized after 6 months of storage. Non-stored samples had the highest C12:0 and the lowest C15:0 contents; whereas samples irradiated with 0.5 kGy presented the highest C8:0 and the lowest C14:0 levels, while the irradiation with 1 kGy minimizes the C15:0 content. C20:5, the well-known eicosapentaenoic acid (EPA), was the only FA with an increase along time (**Figure 5.1.1D**).

Table 5.1.4 Fatty acids composition (relative percentages)[a] of dried *M. procera* submitted to different electron-beam irradiation doses (ED) and storage times (ST).

		C6:0	C14:0	C15:0	C16:0	C16:1	C17:0	C18:0	C18:1	C18:2	C20:5	C22:0	C24:0	**SFA**	**MUFA**	**PUFA**
ED	0 kGy	0.73±0.05	0.29±0.01	0.64±0.05	27±1	1.5±0.3	0.23±0.02	3.4±0.2	7.6±0.1	56±2	0.22±0.05	0.25±0.04	1.0±0.2	**34±2**	**9.5±0.4**	**56±2**
	0.5 kGy	0.17±0.05	0.23±0.02	0.6±0.1	23±4	1.20±0.02	0.18±0.04	4±1	9.4±0.2	59±6	0.3±0.1	0.26±0.05	1.0±0.2	**30±5**	**10.8±0.2**	**59±6**
	1 kGy	0.5±0.2	0.35±0.04	0.5±0.1	22±2	1.3±0.2	0.19±0.05	2.4±0.4	7.9±0.5	62±3	0.4±0.3	0.23±0.03	0.8±0.1	**27±3**	**9.5±0.5**	**63±3**
	6 kGy	0.4±0.2	0.33±0.01	0.5±0.1	24±1	1.19±0.04	0.18±0.03	2.7±0.1	8.9±0.5	59±1	0.3±0.2	0.24±0.03	0.9±0.1	**29±1**	**10±1**	**60±1**
	p-value (n=36)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**	**<0.001**	**<0.001**
ST	0 months	0.3±0.3	0.28±0.04	0.4±0.1	22±4	1.3±0.3	0.16±0.05	2.6±0.5	9±1	62±5	0.10±0.05	0.21±0.02	0.9±0.2	**28±5**	**10±1**	**62±5**
	6 months	0.6±0.2	0.31±0.05	0.6±0.1	24±2	1.3±0.1	0.21±0.03	2.9±0.5	8±1	59±3	0.24±0.03	0.29±0.04	1.0±0.1	**31±3**	**10±1**	**59±4**
	12 months	0.4±0.2	0.31±0.04	0.63±0.03	25±1	1.3±0.1	0.23±0.02	4±1	9±1	57±2	0.5±0.2	0.24±0.02	0.9±0.1	**32±2**	**10±1**	**58±2**
	p-value (n=27)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**	**<0.001**	**<0.001**
ED × ST	*p*-value (n=108)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	**<0.001**	**<0.001**	**<0.001**

The results are presented as mean ± SD.
[a] Besides the tabled fatty acids, C8:0, C10:0, C12:0, C14:1, C18:3, C20:0, C20:1, C20:2, C20:3, C21:0, C22:1, C23:0 and C24:0 were also detected in vestigial amounts (<0.2%).

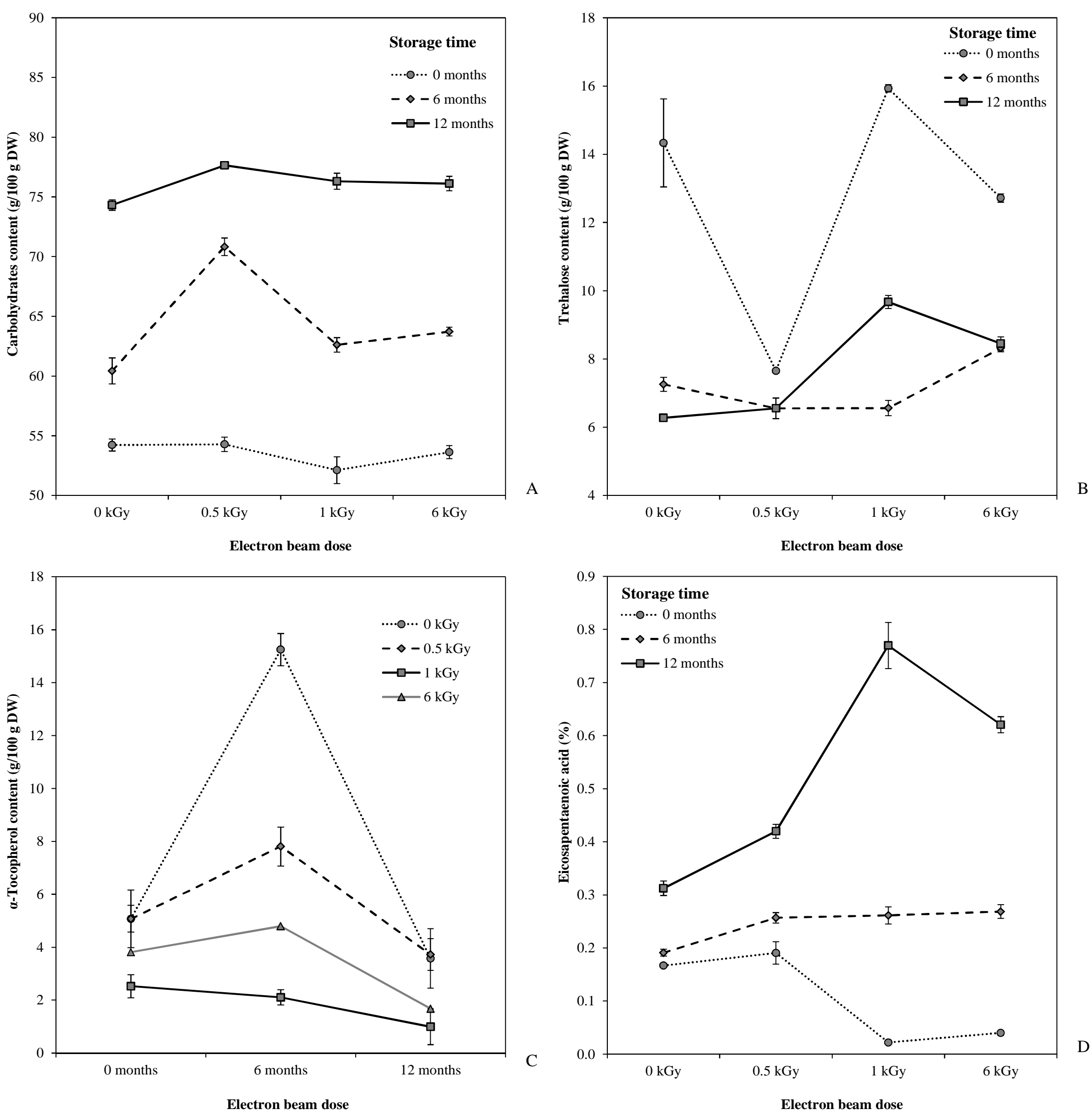

Figure 5.1.1. Interactions among storage time and electron-beam irradiation dose effects on the chemical parameters of *M. procera* dried samples. (A) Carbohydrates; (B) trehalose; (C) α-tocopherol; (D) eicosapentaenoic acid (C20:5).

The antioxidant potential of *M. procera* samples was used as a measure of their bioactivity (**Table 5.1.5)**. The results could not be classified by multiple comparison tests (the detected interactions were always significant), but ST seemed to cause a higher effect. This outcome was confirmed by the EMM plots, which clearly showed higher DPPH scavenging activity (**Figure 5.1.2A**), lower TBARS formation inhibition (**Figure 5.1.2B**) and higher reducing power (**Figure 5.1.2C** and **D**), for non-stored samples. Regarding different ED, the obtained results did not show to be correlated with the assayed doses.

Table 5.1.5. *In vitro* antioxidant properties obtained for the extracts of dried *M. procera* submitted to different electron-beam irradiation doses (ED) and storage times (ST) (mean ± SD).

			Reducing power		Lipid peroxidation inhibition	
		DPPH scavenging activity	Ferricyanide/ Prussian blue assay	Folin-Ciocalteu assay	β-Carotene bleaching inhibition	TBARS formation inhibition
ED	0 kGy	5±2	1.5±0.5	17±6	5±2	2±2
	0.5 kGy	6±2	1.6±0.2	14±5	4±1	3±3
	1 kGy	4±1	1.5±0.3	17±5	3±1	3±4
	6 kGy	5±2	1.8±0.4	17±5	4±2	6±8
	p-value (n=36)	0.068	<0.001	<0.001	<0.001	<0.001
ST	0 months	2.7±0.4	1.3±0.1	23±2	5±2	10±5
	6 months	4.1±0.5	1.5±0.2	12±2	4±1	0.5±0.1
	12 months	8±1	2.0±0.2	14±1	4±2	0.7±0.2
	p-value (n=27)	na	<0.001	<0.001	<0.001	<0.001
ED × ST	*p*-value (n=108)	<0.001	<0.001	<0.001	<0.001	<0.001

Values are presented as EC_{50} values (mg/ml) for all assays except Folin-Ciocalteau, expressed as mg GAE/g extract.
Concerning the Folin-Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential.

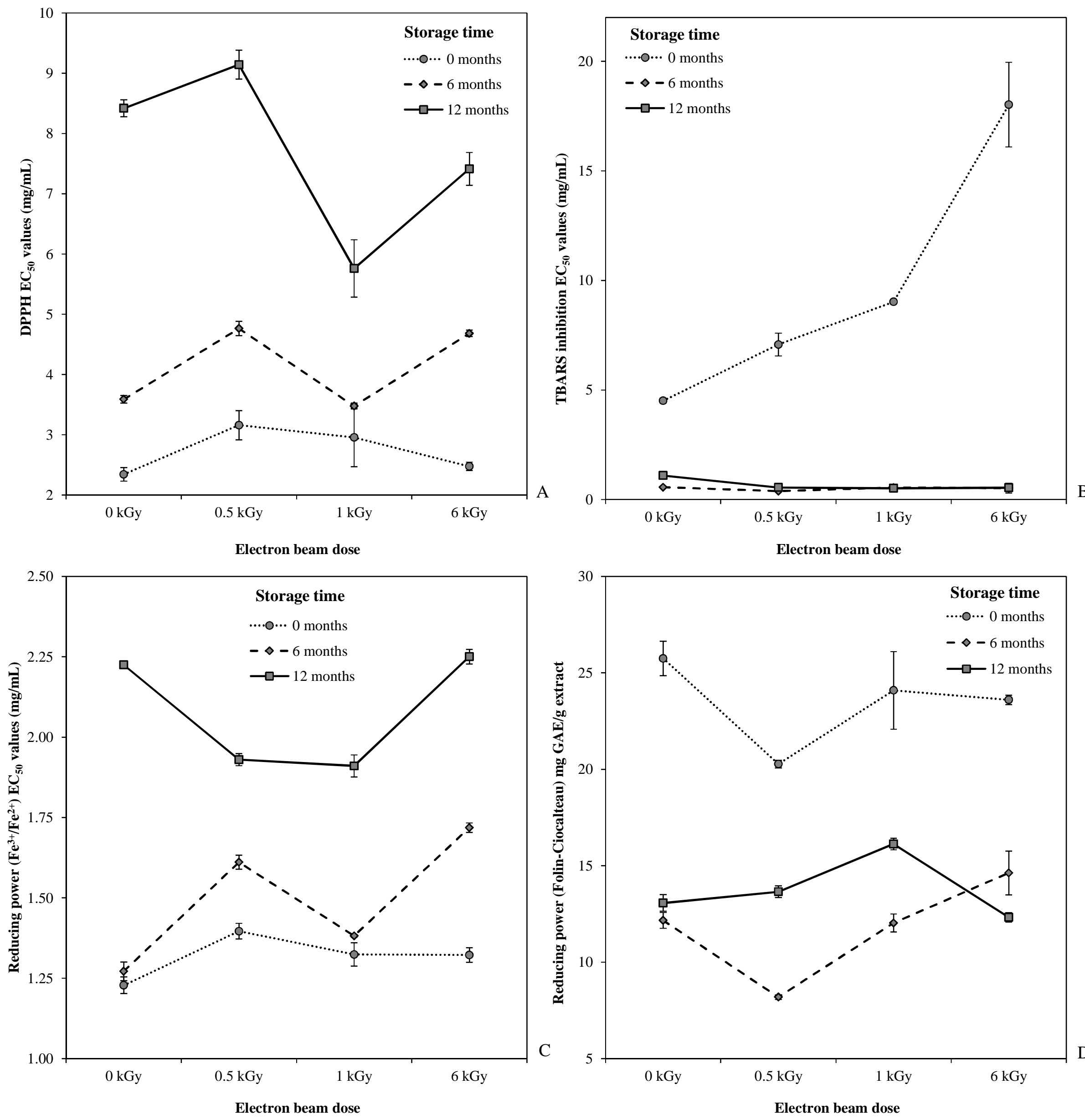

Figure 5.1.2. Interactions among storage time and electron-beam irradiation dose effects on the antioxidant activity of *M. procera* dried samples. (A) DPPH scavenging activtity; (B) TBARS inhibition; (C) reducing power (conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}); (D) reducing power (Folin-Ciocalteau).

Despite all the mentioned particular differences, the effect of both ST and ED was not yet well-defined. In order to acquire a better understanding of differences found in antioxidant activity and chemical parameters, several linear discriminant analyses were performed using different combinations of the studied variables (output plots are only presented in specific cases). In this analysis, higher differences among the results obtained for each defined group (0, 6 and 12 months or 0, 0.5, 1

and 6 kGy) will allow better classification performances, as it can be assessed by evaluating the percentage of correctly classified groups. Data presented in **Tables 5.1.1** - **5.1.5** were evaluated separately regarding differences induced by ST or by ED. Despite the similarity detected for some individual parameters, the classification performance was generally high, especially for ST.

The higher effect of ST was reflected in the percentages of correctly classified cases: all parameters, fatty acids, nutritional parameters and antioxidant activity results- 100 % for the original groups and for the cross-validation procedure; tocopherols- 83.8 % for the original groups and for the cross-validation procedure; free sugars- 66.7 % for the original groups and for the cross-validation procedure. All models defined two (except when using free sugars alone, for which only one significant function was defined) significant functions and included 100 % of the variance.

Regarding ED, the percentage of correctly classified groups was lower: all parameters and fatty acids- 100 % for the original groups and for the cross-validation procedure; antioxidant activity results- 66.7 % for the original groups and 61.1 % for the cross-validation procedure; free sugars- 65.7 % for the original groups and for the cross-validation procedure; tocopherols- 65.7 % for the original groups and 61.1 % for the cross-validation procedure; nutritional parameters- 56.5 % for the original groups and 40.7 % for the cross-validation procedure. All models defined three significant functions and included 100 % of the variance.

Further details regarding all performed LDA are presented in **Table 5.1.6**.

Since entering all variables, i.e. the results for all the assayed parameters, allows a better understanding of the effects caused by varying ST or ED, higher attention will now be given to the outputs obtained when all data were included in the LDA models.

Regarding ED (**Figure 5.1.3A**), function 1 [means of canonical variance (MCV), 0 kGy: -18.168; 0.5 kGy: -34.190; 1 kGy: 27.782 and 6 kGy: 24.577] and function 2 (MCV, 0 kGy: 19.436; 0.5 kGy: -14.209; 1 kGy: -1.322 and 6 kGy: -3.905) separated primarily 0 and 0.5 kGy from the remaining doses, indicating that fructose, δ-tocopherol, C14:0 C6:0, C15:0, C16:0, C16:1, C17:0, C18:1, C20:5, C22:1, trehalose and α-tocopherol are the parameters with highest variation among non-irradiated samples or samples irradiated with 0.5 kGy and those irradiated with higher doses (**Table 5.1.6**). Function 3 was effective to separate 1 and 6 kGy (MCV, 0 kGy: 0.719;

0.5 kGy: -1.120; 1 kGy: -10.961; 6 kGy: 11.363), with reducing power (RP) (Fe^{3+}/Fe^{2+}), β-tocopherol, C10:0, C12:0, C18:2, C20:0, C20:1 and C20:3 (**Table 5.1.6**) as the variables more affected by the increase from 1 to 6 kGy. On the other hand, nutritional parameters were the least affected by ED, as can be concluded from the low percentages of accurately classified groups (56.5 % for the original groups and 40.7 % for the cross-validation procedure) and verified on the plot of the MCV (**Figure 5.1.3B**), that do not individualize any specific group.

Regarding the effect of ST (**Figure 5.1.4**), function 1 separated primarily non-stored and stored samples (MCV 0 months: 69.213; 6 months: -31.094 and 12 months: -38.119), showing that melezitose, fructose, trehalose, β-tocopherol, C14:0, C16:0, C15:0, C17:0, C20:0 and C20:2 are the most affected parameters (**Table 5.1.6**). Function 2 separated 6 and 12 months (MCV 0 months: -1.917; 6 months: 29.283 and 12 months: -27.367), indicating that RP (Fe^{3+}/Fe^{2+}), C6:0, C10:0, C18:0, C20:3, C20:5, C23:0 and C24:0 (**Table 5.1.6**) are the most affected variables. On the other hand, free sugars revealed the lowest changes, as can be concluded from the low percentages of accurately classified groups (56.5 % for the original groups and 40.7 % for the cross-validation procedure). The results were not plotted because one single significant function was defined.

Table 5.1.6. Discriminant analysis features for the models obtained from the results of the applied assays.

		Selected variables	Most correlated variables with:		
			Function 1	Function 2	Function 3
ED	All parameters	reducing power (RP) (Fe^{3+}/Fe^{2+}), fructose, trehalose, α-tocopherol, β-tocopherol, δ-tocopherol, C6:0, C10:0, C12:0, C14:0, C15:0, C16:0, C16:1, C17:0, C18:1, C18:2, C20:0, C20:1, C20:3, C20:5, C22:1	fructose, δ-tocopherol, C14:0	C6:0, C15:0, C16:0, C16:1, C17:0, C18:1, C20:5, C22:1, trehalose, α-tocopherol	RP (Fe^{3+}/Fe^{2+}), β-tocopherol, C10:0, C12:0, C18:2, C20:0, C20:1, C20:3
	Antioxidant activity	β-carotene, DPPH, RP (Folin-Ciocalteau and Fe^{3+}/Fe^{2+}), TBARS	TBARS	RP (Folin-Ciocalteau)	β-carotene, DPPH, RP (Fe^{3+}/Fe^{2+})
	Nutritional	energetic value, carbohydrates and fat	energetic value	energetic value	carbohydrates, fat
	Free sugars	fructose, mannitol and trehalose	trehalose	trehalose, mannitol	fructose
	Tocopherols	α-tocopherol, β-tocopherol, γ-tocopherol and δ-tocopherol	α-tocopherol	β-tocopherol, δ-tocopherol	α-tocopherol, γ-tocopherol
	Fatty acids	C6:0, C10:0, C14:0, C16:1, C17:0, C18:1, C18:3, C20:1, C20:3 and C22:1	C14:0, C18:3, C20:2, C22:0	C6:0, C8:0, C16:1, C17:0, C18:1, C22:1, C24:1	C10:0, C12:0, C15:0, C16:0, C18:0, C20:1, C20:3, C20:5, C24:0,
ST	All parameters	RP (Fe^{3+}/Fe^{2+}), fructose, melezitose, trehalose, β-tocopherol, C6:0, C10:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C20:2, C20:3, C20:5, C24:0	melezitose, fructose, trehalose, β-tocopherol, C14:0, C16:0, C15:0, C17:0, C20:0, C20:2	RP (Fe^{3+}/Fe^{2+}), C6:0, C10:0, C18:0, C20:3, C20:5, C23:0, C24:0	-
	Antioxidant activity	β-carotene, DPPH, RP (Folin-Ciocalteau and Fe^{3+}/Fe^{2+}), TBARS	DPPH, reducing power (Fe^{3+}/Fe^{2+})	DPPH, RP (Folin-Ciocalteau)	-
	Nutritional	ash, carbohydrate and protein	ash, carbohydrate	ash	-
	Free sugars	fructose and melezitose	fructose, melezitose	-	-
	Tocopherols	α-tocopherol, β-tocopherol and γ-tocopherol	β-tocopherol, γ-tocopherol	α-tocopherol	-
	Fatty acids	C6:0, C10:0, C12:0, C14:1, C16:0, C16:1, C18:0, C20:3, C20:5, C22:0, C22:1, C23:0, C24:0	C12:0, C16:0, C20:3, C22:0	C6:0, C10:0, C14:1, C16:1, C18:0, C20:5, C22:1, C23:0, C24:0	-

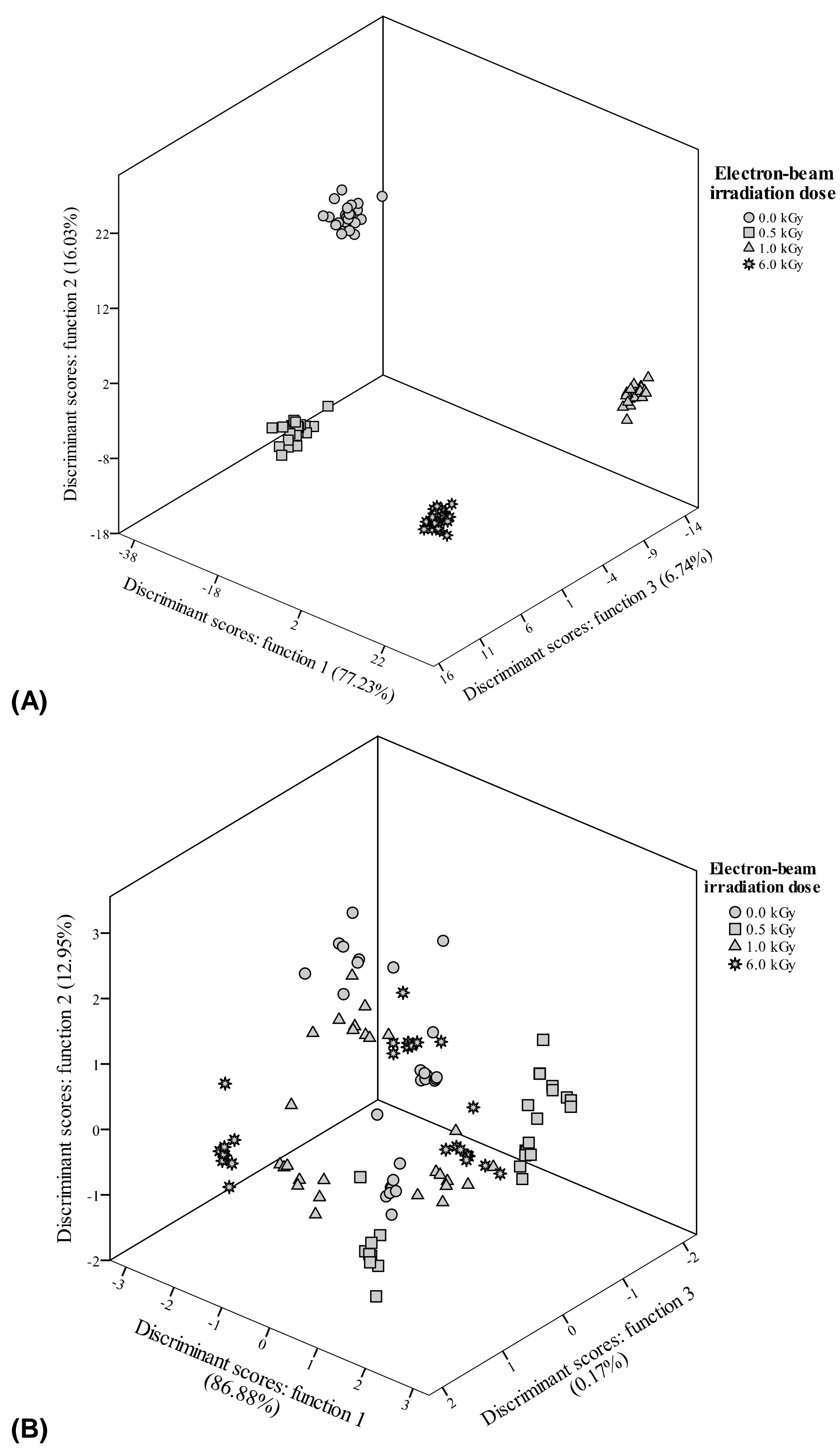

Figure 5.1.3. Discriminant scores scatter plot of the canonical functions defined for all assayed parameters (A) and the nutritional parameters alone (B) results according with electron-beam irradiation dose.

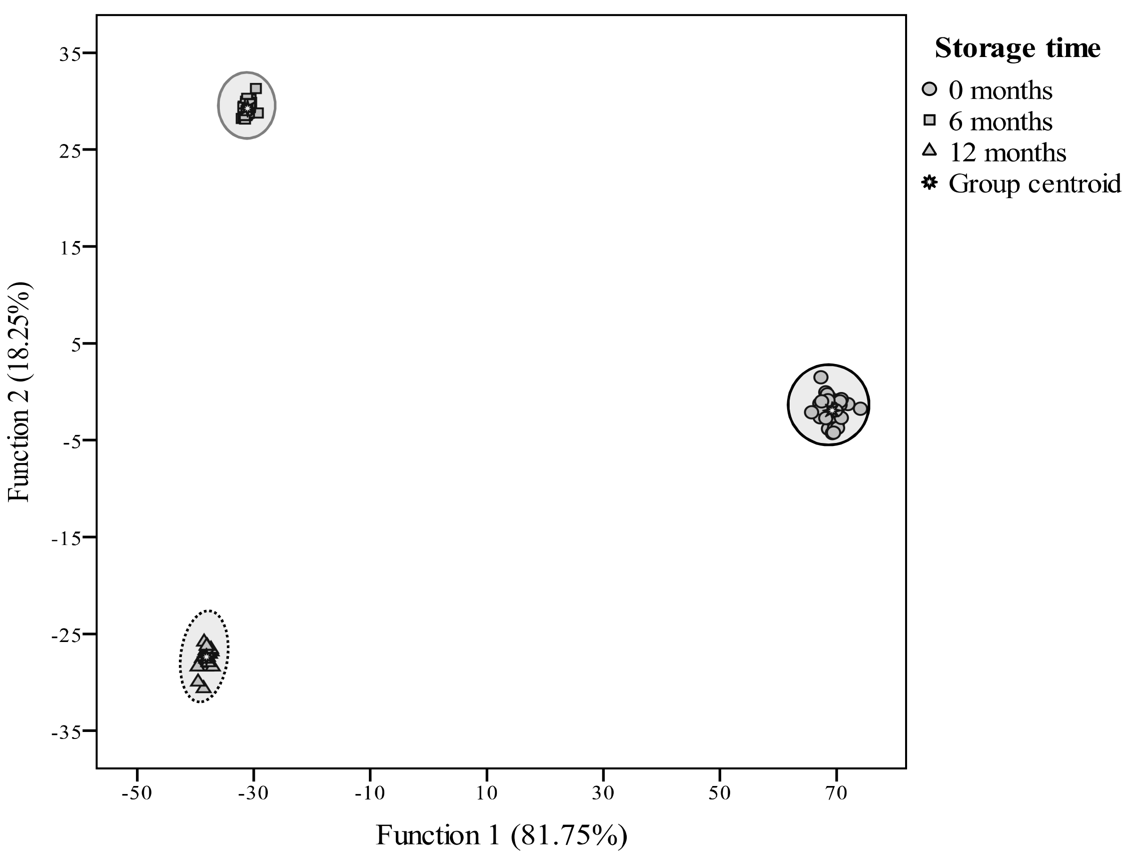

Figure 5.1.4. Discriminant scores scatter plot of the canonical functions defined for all assayed parameters according with storage time.

5.1.4. Conclusions

Overall, electron-beam irradiation did not impart additional changes to most chemical and antioxidant parameters of *M. procera* dried samples. Accordingly, irradiation might be applied as a potential complementary treatment, since it has decontaminating ability while maintaining the organoleptic characteristics of mushrooms. This is a very promising result, since electron-beam irradiation might attenuate most unwanted changes caused by drying, maintaining its long-term effectiveness.

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5.1.5. References

Almeida, A.P.G. (2006). Avaliação da influência do processo de irradiação em especiarias utilizando a técnica de difração de raios-X. Dissertação - Universidade Federal do Rio de Janeiro, COPPE, Brasil.

Arora, S., Shivhare, U. S., Ahmed, J., & Raghavan, G. S. V. (2003). Drying kinetics of *Agaricus bisporus* and *Pleurotus florida* mushrooms. *American Society of Agricultural Engineers*, *46*, 721-724.

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society of Applied Biological Chemistry*, *53*, 257-265.

AOAC. (1995). *Official methods of analysis* (16th ed.). Arlington, VA: Association of Official Analytical Chemists.

Barreira, J. C. M., Pereira, J.A., Oliveira, M. B. P. P., & Ferreira, I. C. F. R. (2010). Sugars profiles of different chestnut (*Castanea sativa* Mill.) and Almond (*Prunus dulcis*) Cultivars by HPLC-RI. *Plant Foods for Human Nutrition*, *65*, 38-43.

Barros, L., Baptista, P., Correia, D. M., Sá Morais, J., & Ferreira, I. C. F. R. (2007). Effects of conservation treatment and cooking on the chemical composition and antioxidant activity of Portuguese wild edible mushrooms. *Journal of Agricultural and Food Chemistry*, *55*, 4781-4788.

Barros, L., Cabrita, L., Vilas Boas, M., Carvalho, A. M., & Ferreira, I. C. F. R. (2011). Chemical, biochemical and electrochemical assays to evaluate phytochemicals and antioxidant activity of wild plants. *Food Chemistry*, *127*, 1600-1608.

Cao, W., Nishiyama, Y., & Koide, S. (2003). Thin-layer drying of Maitake Mushroom analysed with a simplified model. Biosystems Engineering, 85, 331-337.

Carocho, M., Barreira, J. C. M., Antonio, A. L., Bento, A., Kaluska, I., & Ferreira, I. C. F. R. (2012). Effects of Electron Beam Radiation on Nutritional parameters of Portuguese Chestnuts (Castanea sativa Mill). Journal of Agricultural and *Food Chemistry*, *60*, 7754-7760.

Celestino, S. M. C. (2010). Princípios de Secagem de Alimentos. Embrapa Cerrados, Planaltina. ISSN 1517-5111.

Duan, Z., Xing, Z., Shao, Y., & Zhao, X. (2010). Effect of electron-beam irradiation on postharvest quality and selected enzyme activities of the white button mushroom, *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*, *58*, 9617-9621.

Fernandes, Â., Antonio, A. L., Oliveira, M. P. P., Martins, A., & Ferreira, I. C. F. R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Antonio, A. L., Barreira, J. C. M., Botelho, L., Oliveira, M. B. P. P., Martins, A., & Ferreira, I. C. F. R. (2012b). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, doi 10.1007/s11947-012-0931-5.

Guimarães, R., Barros, L., Dueñas, M., Calhelha, R. C., Carvalho, A. M., Santos-Buelga, C., Queiroz, M. J. R. P., Ferreira, I. C. F. R. (2013). Nutrients, phytochemicals and bioactivity of wild Roman chamomile: A comparison between the herb and its preparations. *Food Chemistry*, *136*, 718-725.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchemical Journal*, *93*, 195-199.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I. C. F. R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*, 1443-1450.

International Atomic Energy Agency (IAEA). (2002). Dosimetry for food irradiation. Technical Report Series 490, Vienna.

ICMSF. (1985). *Ecologia microbiana de los alimentos 2. International Commission on Microbiological Specifications for Foods*. Zaragoza, Spain: Editorial Acribia.

Jangam, S. V., Law, C. L., & Mujumdar, A. S. (2011). *Drying of Foods, Vegetables and Fruits*, Vol 2. ISBN: 978-981-08-7985-3.

Koorapati, A., Foley, D., Pilling, R., & Prakash, A. (2004). Electron-beam irradiation preserves the quality of white button mushrooms (*Agaricus bisporus*) slices. *Journal of Food Science and Tecnology*, *6*, 25-29.

Kulshreshtha, M., Singh, A., & Deepti, & Vipul. (2009). Effect of drying conditions on mushrooms quality. *Journal of Engineering Science and Technology*, *4*, 90-98

Lacroix, M., & Ouattara, B. (2000) Combined industrial processes with irradiation to assure innocuity and preservation of food products - a review. *Food Research International*, *33*, 719-724.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, *106*, 369-378.

Maga J.A. (1981). Mushroom flavor. *Journal of Agricultural and Food Chemistry*, *29*, 4-7.

Maroco, J. (2003). *Análise Estatística, com utilização do SPSS*. Lisboa, Portugal: Edições Sílabo.

Maskan, M. (2000). Microwave/air and microwave finish drying of banana. *Journal of Food Engineering*, *44*, 71-78.

Ouzouni, P. K., & Riganakos, K. A. (2007). Nutritional value and metal content of Greek wild edible fungi. *Acta Alimentaria*, *36*, 99-110.

Rivera, C. S., Blanco, D., Marco, P., Oria, R., & Venturini, M. E. (2011). Effects of electron-beam irradiation on the shelf life, microbial populations and sensory characteristics of summer truffles (*Tuber aestivum*) packaged under modified atmospheres. *Food Microbioloy*, *28*, 141-148.

Schmidt, H. M., Palekar, M. P., Maxim, J. E., & Castillo, A. (2006). Improving the microbiological quality and safety of fresh-cut tomatoes by low dose electron-beam irradiation. *Journal of Food Protection*, *69*, 575-581.

Walde S. G., Velu, V., Jyothirmayi, T., & Math, R.G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineering*, *74*, 108-115.

5.2

Viabilidade da irradiação por feixe de eletrões na preservação de cogumelos silvestres desidratados: efeito sobre a composição química e atividade antioxidante

Este sub-capítulo apresenta os efeitos da radiação por feixe de eletrões (doses 2, 6 e 10 kGy) nos parâmetros nutricionais (valor energético, açúcares, ácidos orgânicos, tocoferóis e ácidos gordos) e bioativos (atividade antioxidante e compostos fenólicos individuais) de amostras desidratadas de Boletus edulis *Bull. e* Russula delica *Fr.*

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Feasibility of electron-beam irradiation to preserve wild dried mushrooms: effects on chemical composition and antioxidant activity

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Abstract

Mushrooms are highly perishable matrices and to extend time of consumption they need to be preserved. Since all the available conservation technologies present disadvantages, the combination of two different processes might minimize some of the limitations. Therefore, in the present work, electron-beam irradiation (up to 10 kGy) was applied to dried samples of *Boletus edulis* and *Russula delica*, extending

previous findings using gamma- and electron-beam irradiations at lower doses (up to 6 kGy) and different wild mushroom species. The effects on nutritional, chemical and antioxidant parameters were evaluated. In general, the applied irradiation, particularly at higher doses, had significant effects on chemical profiles (protein, sugar and organic acid levels tended to decrease, while unsaturated fatty acids, tocopherols and phenolic acids presented higher levels in irradiated samples) and antioxidant activity (increased in irradiated samples). Nevertheless, the assayed doses might be considered to enhance the conservation of *B. edulis*, allowing the simultaneous achievement of disinfestation and decontamination effects.
Industrial relevance: B. edulis is among the most commercialized mushrooms worldwide. However, as all mushrooms, suffers severe conservation problems. Electron-beam irradiation (specifically at 6 kGy) proved to be a suitable technology for mushrooms conservation, since it allows disinfestation and decontamination processes without causing high changes in the chemical profiles. In *Russula delica* case, differences caused by irradiation were higher, but it was also found that applying 6 kGy had the same effects of 2 kGy dose, which might be useful for disinfestation (insects elimination) and decontamination (elimination of bacteria and other microorganisms) purposes.

Keywords: Wild Mushrooms; Dried; Electron-beam; Chemical composition; Antioxidants.

5.2.1. Introduction

Mushrooms are usually eaten fresh but due to their high water content, they become highly perishable and need to be preserved (Ezekiel, Sulyok, Frisvad, Somorin, Warth, Houbraken et al., 2013). When compared to vegetables, the shelf-life of mushrooms is minor, requiring special attention in their postharvest chain (Iqbal, Rodrigues, Mahajan, & Kerry, 2009). In this sense, many technologies have been applied in order to increase mushrooms shelf-life, such as drying (Ma, Haixia, Wenchai, & Zhaoshuai, 2013), freezing and cryogenic freezing (Jaworska & Bernás, 2009), modified atmosphere packaging (MAP) (Oliveira, Sousa-Gallagher, Mahajan,

& Teixeira, 2012) and irradiation (Akram & Kwon, 2010; Fernandes, Barreira, Antonio, Martins, Oliveira & Ferreira, 2013a).

Drying is one of the most important processes used in preserving mushrooms fruiting bodies, removing water, so as to minimize biochemical and microbial activities (Ezekiel et al., 2013; Kumar, Singh, & Singh, 2011). Nevertheless, during the drying process, microorganisms may secrete potentially toxic metabolites and contaminate mushrooms (Ezekiel et al., 2013; Shephard, 2008).

The chemical sanitizing procedures have also inherent problems concerning residues and environmental pollution; several decontamination methods exist, but the most versatile treatment among them is the processing with ionizing radiation (Farkas, 1998). Being a cold process, food irradiation does not significantly alter physico-chemical characteristics of the treated product. It has the potential of disinfesting dried food to reduce storage losses and disinfesting fruits and vegetables to meet requirements for export trade (Loaharanu & Ahmed, 1991). Radiation decontamination of dry food, spices and herbs with doses of 3-10 kGy proved to be a viable alternative to fumigation with microbicidal gases (Farkas, 1998). The most common sources of ionizing radiation are gamma rays and electron-beam, being applied by many researchers in extending the postharvest shelf-life of mushrooms (Culleré, Ferreira, Venturini, Marco, & Blanco, 2012; Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012).

The safety of irradiated foods at specific doses applied for technological benefits is guaranteed by leading world health organizations (WHO, World Health Organization, 1999). Decontamination of food by ionizing radiation is a safe, efficient, environmentally clean and energy efficient process (Farkas, 1998). Many countries (Argentina, China, United Kingdom, Croatia, Belgium, Czech Republic, Poland, Serbia and Montenegro) allow the use of irradiation for fresh (1-3 kGy) and dried (1-10 kGy) mushrooms, for different technological purposes (Akram & Kwon, 2010).

In a recent study, our research group investigated and validated the effects of electron-beam irradiation (0, 0.5, 1 and 6 kGy) and storage time (0, 6 and 12 months) on nutritional and chemical parameters of dried wild *Macrolepiota procera*, concluding that this technology might act in cooperative manner, allowing benefiting from the long-lasting conservation period complied by a reduction in changes usually associated with drying treatment (Fernandes et al. 2013a). In the present work, the study was extended to different dried wild mushrooms (the worldwide appreciated

Boletus edulis Bull. and *Russula delica* Fr.), in order to confirm the effects of electron-beam irradiation at higher doses (2, 6 and 10 kGy) on nutritional, chemical and antioxidant parameters. Despite the effectiveness verified previously for lower irradiation doses, the advisory technological limits for good irradiation practices defines that the reduction of insects (disinfestation) in food might be achieved using 1-2 kGy doses, but the elimination of bacteria and other microorganisms requires doses up to 10 kGy (Molins, 2001).

5.2.2. Materials and methods

Standards and reagents

For chemical analyses: Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, organic acids, tocopherol and sugar standards. Racemic tocol, 50 mg/mL, was purchased from Matreya (PA, USA).

For antioxidant potential analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Standards of phenolic compounds (protocatechuic, *p*-hydroxybenzoic and *p*-coumaric acids), cinnamic acid and organic acids (oxalic acid, quinic acid, citric acid and fumaric acid) were from Sigma Chemical Co. (St. Louis, MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, USA).

Samples and electron-beam irradiation

B. edulis and *R. delica* wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012, and dried at 30 ºC in an oven. Subsequently, the samples were divided in four groups with six specimens of each

mushroom species: control (non-irradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy), kept in polyethylene bags.

The irradiation was performed at the INCT - Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, graphite calorimeter, and two routine dosimeters, Gammachrome YR and Amber Perspex, from Harwell Company (UK). The irradiation took place in e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 μs, a pulse frequency of 440 Hz, and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range of 20-100 cm/min and the scan frequency was 5 Hz. The estimated absorbed doses were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the Graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a previous calibration curve (Carocho, Barreira, Antonio, Bento, Kaluska & Ferreira, 2012). For simplicity, we refer to the irradiation doses as: 0, 2, 6 and 10 kGy.

Before analysis, the samples were reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples.

Chemical parameters

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content ($N \times 4.38$) of the samples was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 ^{o}C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = $4 \times (g_{protein}) + 3.75 \times (g_{carbohydrate}) + 9 \times (g_{fat})$.

Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI) after the extraction procedure described by Heleno, Barros, Sousa, Martins, and Ferreira (2009), using melezitose as internal standard (IS). The equipment consisted of an integrated system with a pump (Knauer, Smartline system 1000), degasser system (Smartline manager 5000), auto-sampler (AS-2057 Jasco) and a RI detector (Knauer Smartline 2300). Data were analyzed using Clarity 2.4 Software (DataApex). The chromatographic separation was achieved with a Eurospher 100-5 NH_2 column (4.6 × 250 mm, 5 mm, Knauer) operating at 30 ºC (7971 R Grace oven). The mobile phase was acetonitrile/deionized water, 70:30 (v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard method and sugar contents were further expressed in g per 100 g of dry weight (dw).

Fatty acids

Fatty acids were determined by gas chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by Heleno et al. (2009). The analysis was carried out with a DANI model GC 1000 instrument equipped with a split/splitless injector, a FID at 260 ºC and a Macherey-Nagel column (30 m × 0.32 mm ID × 0.25 µm d*f*). The oven temperature program was as follows: the initial temperature of the column was 50 ºC, held for 2 min, then a 30 ºC/min ramp to 125 ºC, 5 ºC/min ramp to 160 ºC, 20 ºC/min ramp to 180 ºC, 3 ºC/min ramp to 200 ºC, 20 ºC/min ramp to 220 ºC and held for 15 min. The carrier gas (hydrogen) flow-rate was 4.0 mL/min (0.61 bar), measured at 50 ºC. Split injection (1:40) was carried out at 250 ºC. Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using the CSW 1.7 Software (DataApex 1.7) and expressed in relative percentage of each fatty acid.

Tocopherols

Tocopherols were determined after an extraction procedure previously described by Heleno, Barros, Sousa, Martins, and Ferreira (2010), using tocol as IS.

The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco) programmed for excitation at 290 nm and emission at 330 nm. The chromatographic separation was achieved with a Polyamide II normal-phase column (250 × 4.6 mm; YMC Waters) operating at 30 ºC. The mobile phase used was a mixture of n-hexane and ethyl acetate (70:30, v/v) at a flow rate of 1 mL/min. The compounds were identified by chromatographic comparisons with authentic standards. Quantification was based on the fluorescence signal response, using the internal standard method, and tocopherols content was further expressed in µg per 100 g of dry weight (dw).

Organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira, & Ferreira, 2013). Analysis was performed by ultra-fast liquid chromatograph (UFLC) coupled to photodiode array detector (PAD), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PAD, using 215 and 245 nm as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. The results were expressed in g per 100 g of dry weight (dw).

Phenolic compounds

Phenolic compounds were determined in the UFLC system mentioned above, as previously described by the authors (Barros, Dueñas, Ferreira, Baptista, & Santos-Buelga, 2009). DAD detection was carried out using 280 nm and 370 nm as preferred wavelengths. The phenolic compounds were characterized according to their UV spectra and retention times, and comparison with authentic standards. For quantitative analysis, calibration curves were prepared from different standard compounds. The results were expressed in µg per 100 g dw.

Antioxidant parameters

Extraction procedure

The dried powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4 ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

DPPH radical scavenging activity

This methodology was performed using an ELX800 Microplate Reader (Bio-Tek). The reaction mixture in each one of the 96-wells consisted of one of the different concentrations of the extracts (30 µL) and methanolic solution (270 µL) containing DPPH radicals (6×10^{-5} mol/L). The mixture was left to stand for 60 min in the dark. The reduction of the DPPH radical was determined by measuring the absorption at 515 nm. The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation: $\% \text{ RSA} = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level, and A_{DPPH} is the absorbance of the DPPH solution.

Reducing power

Two different procedures were used to evaluate the reducing power:

A) The first methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was

added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm.

B) The second methodology followed the Folin-Ciocalteu assay. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for color development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution was pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

TBARS (thiobarbituric acid reactive substances) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 μL) of the supernatant was incubated with the different concentrations of the samples solutions (200 μL) in the presence of $FeSO_4$ (10 mM; 100 μL) and ascorbic acid (0.1 mM; 100 μL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 μL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 μL), and the mixture was then heated at 80 ºC

for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

All the analyses (extractions) were performed in triplicate; each replicate was quantified also three times. Data were expressed as means ± standard deviations.

The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro-Wilk's, and the Levene's tests, respectively. For each parameter, significant differences among mean values were checked by Welch's statistics ($p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others). In the cases where statistically significant differences were identified, the dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method. PCA transforms the original, measured variables into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on (Patras et al., 2011). The number of dimensions to keep for data analysis was evaluated by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected. The number of dimensions considered for PCA was chosen in order to allow meaningful interpretations, to ensure their reliability.

All statistical tests were performed at a 5% significance level using the SPSS software, version 18.0 (SPSS Inc).

5.2.3. Results and discussion

Effects on chemical parameters

The nutritional parameters of *B. edulis* and *R. delica* (**Table 5.2.1**) were similar, with water as predominant component (≈90% in *B. edulis*; ≈92% in *R. delica*) and carbohydrates (≈71% in *B. edulis*; ≈75% in *R. delica*) as major compound per dry weight, followed by proteins, ash and fat contents. The detected values are generally in agreement with previous works (Heleno et al., 2011), despite some differences in comparison with *R. delica* samples from different locations (Heleno et al., 2009; Ouzouni, Petridis, Koller, & Riganakos, 2009).

Analyzing the results obtained for each electron-beam irradiation level, it is noticeable that the 10 kGy dose exerted the most significant effect in *R. delica*, as it can be concluded from fat, proteins, carbohydrates and ash contents. For *B. edulis*, the most affected parameter was proteins, in line with the results obtained using gamma-irradiation in the same mushroom (Fernandes, Barreira, Antonio, Santos, Martins, Oliveira et al., 2013b). Likewise, proteins revealed the highest changes in *R. delica* samples, as it became evident from the different classifications for each irradiation dose. The higher sensitivity of proteins might be related to scission of the C-N bonds in the backbone of the polypeptide chain or splitting of the disulfide bonds, or physical changes like unfolding or aggregation (Molins, 2001). In a previous study conducted in our laboratory with *Macrolepiota procera* mushroom (Fernandes et al., 2013a), the effects of electron-beam irradiation were less pronounced; however, the assayed doses were lower and some of the putative changes might have been concealed due to the variation induced by different storage times, which was verified to exert, with no exception, a more relevant effect that irradiation on the chemical profiles of assayed samples.

Table 5.2.1. Proximate composition and corresponding energetic value of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		Dry matter (g/100 g fw)	Fat (g/100 g dw)	Proteins (g/100 g dw)	Carbohydrates (g/100 g dw)	Ash (g/100 g dw)	Energy (kcal/100 g dw)
Boletus edulis	0 kGy	9±1 c	5.0±0.4 ab	16.4±0.1 b	71±1 b	8.0±0.1 a	375±2 b
	2 kGy	11±1 a	4.8±0.4 b	17.0±0.5 a	71±1 ab	6.8±0.2 c	379±2 a
	6 kGy	9±1 bc	4.7±0.5 b	16.4±0.1 b	72±1 a	7.2±0.3 b	376±2 b
	10 kGy	10.1±0.2 ab	5.3±0.2 a	15.1±0.2 c	71.7±0.3 a	7.83±0.02 a	377±1 ab
Homoscedasticity[b]	*p*-value	0.003	0.218	<0.001	0.395	0.003	0.110
One-way ANOVA[c]	*p*-value	<0.001	0.005	<0.001	0.003	<0.001	0.001
Russula delica	0 kGy	8±1 a	3.4±0.2 b	13.8±0.5 b	74±1 b	8.8±0.4 a	363±2 c
	2 kGy	8±1 a	3.5±0.3 b	13.0±0.1 c	75.4±0.3 b	8.1±0.1 b	366±2 b
	6 kGy	6±1 b	3.8±0.4 a	14.8±0.1 a	74.0±0.3 a	7.5±0.1 c	370±2 a
	10 kGy	8±1 a	2.6±0.2 c	12.6±0.1 d	77.5±0.3	7.3±0.3 c	365±2 bc
Homoscedasticity[b]	*p*-value	0.687	0.053	<0.001	0.011	0.122	0.441
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

fw- fresh weight; dw- dry weight.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among cultivars was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Unlike nutritional parameters, free sugars composition (**Table 5.2.2**) had some important differences among *B. edulis* and *R. delica. B. edulis* presented lower amounts in total sugars when compared with previous studies (Fernandes et al., 2013b; Heleno et al., 2011), most likely because samples used in this study were in an earlier maturity stage. Despite these quantitative differences, trehalose was the main sugar in *B. edulis*, a common feature in this particular mushroom. On the other hand, mannitol was the top sugar in *R. delica*.

Except for sucrose in *B. edulis*, all sugars showed significant variations with the applied irradiation doses; nevertheless, the results are somehow surprising, since the most significant changes were not caused by the highest applied doses. Irradiation is known for causing sugars degradation mainly due to the production of a particular atmosphere consisting primarily of H_2 and CO_2, together with traces of CH_4, CO and H_2O (Molins, 2001). It is reasonable to assume that the gases proportion produced with the 10 kGy dose might be less harmful for the isolated sugars, attenuating the losses verified with the other doses.

Regarding phenolic acids composition (**Table 5.2.3**), *B. edulis* had interesting levels of *p*-coumaric acid and its content did not reveal a marked tendency with the increase in electron-beam irradiation. In *R. delica*, the only detected phenolic acid was gallic acid, which is in agreement with previous works in the same mushroom (Yaltirak, Aslim, Sahlan, & Alli, 2009). Both mushrooms presented cinnamic acid (the amounts in *B. edulis* were quite higher), which suffer the highest changes with the 6 kGy dose.

The effects over tocopherols contents (**Table 5.2.4**) were also significant for all quantified isoforms (except δ-tocopherol in *B. edulis*). Irradiated samples tended to present higher amounts, particularly for the 2 kGy dose in *B. edulis* and the 6 kGy in *R. delica.* Consistent with a previous study (Fernandes et al., 2013a), the electron-beam dose that allowed the highest tocopherols amount was the same causing the maximum loss in sugar content (except for trehalose in *B. edulis*). This result might be explained by differences in free oxygen availability inside the polyethylene bag, which may vary in result of sugars degradation.

Table 5.2.2. Sugars composition of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		Fructose (g/100 g dw)	Glucose (g/100 g dw)	Mannitol (g/100 g dw)	Sucrose (g/100 g dw)	Trehalose (g/100 g dw)	Total sugars (g/100 g dw)
Boletus edulis	0 kGy	0.08±0.01 a	nd	0.15±0.02 b	0.54±0.05	3.2±0.1 b	4.0±0.1 b
	2 kGy	0.06±0.02 b	nd	0.12±0.01 c	0.52±0.01	4.6±0.1 a	5.4±0.1 a
	6 kGy	nd	nd	0.19±0.03 a	0.56±0.05	3.5±0.b	4.2±0.b
	10 kGy	nd	nd	0.19±0.03 a	0.57±0.05	3.7±0.5 b	4.3±0.5 b
Homoscedasticity[b]	*p*-value	<0.001	-	0.006	<0.001	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	-	<0.001	0.347	<0.001	<0.001
Russula delica	0 kGy	nd	2.37±0.01 b	4.28±0.02 b	0.86±0.03 a	2.83±0.03 a	10.3±0.2 a
	2 kGy	nd	1.8±0.1 c	1.58±0.03 c	0.16±0.01 c	0.46±0.02 c	4.0±0.1 c
	6 kGy	nd	0.67±0.03 d	1.22±0.05 d	0.12±0.01 d	0.31±0.01 d	2.3±0.1 d
	10 kGy	nd	3.3±0.1 a	4.6±0.1 a	0.45±0.05 b	0.96±0.01 b	9.3±0.1 b
Homoscedasticity[b]	*p*-value	-	0.008	0.001	<0.001	0.002	0.024
One-way ANOVA[c]	*p*-value	-	<0.001	<0.001	<0.001	<0.001	<0.001

dw- dry weight; nd- not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] *p* < 0.05 means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Table 5.2.3. Phenolic and cinnamic acid composition of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		Gallic acid (μg/100 g dw)	*p*-Coumaric acid (μg/100 g dw)	Cinnamic acid (μg/100 g dw)
Boletus edulis	0 kGy	nd	339±8 c	997±2 c
	2 kGy	nd	559±3 a	1091±11 b
	6 kGy	nd	221±2 d	489±6 d
	10 kGy	nd	441±4 b	1113±12 a
Homoscedasticity[b]	*p*-value	-	<0.001	0.001
One-way ANOVA[c]	*p*-value	-	<0.001	<0.001
Russula delica	0 kGy	30.6±0.1 d	nd	0.77±0.01 d
	2 kGy	61±2 b	nd	0.89±0.01 b
	6 kGy	97±5 a	nd	0.92±0.01 a
	10 kGy	34.6±0.3 c	nd	0.79±0.01 c
Homoscedasticity[b]	*p*-value	<0.001	-	<0.001
One-way ANOVA[c]	*p*-value	<0.001	-	<0.001

dw- dry weight; nd- not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Table 5.2.4. Tocopherols composition of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		α-Tocopherol (μg/100 g dw)	γ-Tocopherol (μg/100 g dw)	δ-Tocopherol (μg /100 g dw)	Total tocopherols (μg /100 g dw)
Boletus edulis	0 kGy	17±1 b	20±5 b	57±13	94±12 b
	2 kGy	27±1 a	47±3 a	55±1	129±3 a
	6 kGy	18±1 b	46±3 a	57±1	121±3 a
	10 kGy	24±6 a	42±5 a	57±13	123±13 a
Homoscedasticity[b]	*p*-value	<0.001	0.118	0.001	0.022
One-way ANOVA[c]	*p*-value	<0.001	<0.001	0.929	<0.001
Russula delica	0 kGy	nd	10.7±0.2 c	15.3±0.3 c	26.0±0.3 c
	2 kGy	nd	7.6±0.3 d	4.3±0.2 d	11.9±0.5 d
	6 kGy	nd	26±1 a	61±6 a	87±6 a
	10 kGy	nd	15±1 b	34±2 b	50±2 b
Homoscedasticity[b]	*p*-value	-	<0.001	<0.001	<0.001
One-way ANOVA[c]	*p*-value	-	<0.001	<0.001	<0.001

dw- dry weight; nd- not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation dose differs from the others (in this case multiple comparison tests were performed).

Table 5.2.5 presents the individual fatty acids (FA) quantified above 0.2% in each mushroom species (C6:0, C8:0, C10:0, C12:0, C14:1, C15:0, C17:0, C20:0, C20:3, C20:5, C22:1 and C23:0 in both mushrooms, besides C18:3, C20:1 and C20:2 in *R. delica* were also quantified, but in percentages lower than 0.2%). The most abundant FA in both mushrooms were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), as it is commonly found in these mushrooms (Heleno et al., 2011; Kalaè, 2009). The higher electron-beam irradiation doses tended to cause more significant changes in *B. edulis* FA, while *R. delica* being the most affected by the 2 kGy dose. The electron-beam option seems to be a better choice when compared with gamma-radiation, since no decrease in unsaturated FA was noticeable as in the case of gamma-irradiation (Fernandes et al., 2013a). Nevertheless, the general mechanism of lipids radiolysis, involving primary ionization, followed by migration of the positive charge either toward the carboxyl carbonyl group or double bonds (Molins, 2001), is more likely to occur in fresh than in dried mushrooms, as is the case reported herein.

B. edulis presented a simpler profile in organic acids (**Table 5.2.6**), consisting of oxalic, citric and fumaric acids, which is in agreement with previous reports (Fernandes et al., 2013a). Besides the previous compounds, quinic and malic acid were also detected in *R. delica.* The 10 kGy dose caused the highest changes in both mushrooms; thereby, it does not seem to be a feasible solution in what concerns this particular parameter.

Table 5.2.5. Fatty acids composition (relative percentages) of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		C14:0	C16:0	C16:1	C17:1	C18:0	C18:1	C18:2	C18:3	C20:1	C20:2	C22:0	C24:0	SFA	MUFA	PUFA
Boletus edulis	0 kGy	0.35±0.01 b	11.0±0.1 b	1.01±0.01 a	0.41±0.01 b	0.92±0.02 d	5.7±0.2 c	77.2±0.1 b	1.76±0.01 b	0.12±0.01 b	0.27±0.01 b	0.13±0.01 d	0.19±0.01 d	13.2±0.1 c	7.4±0.2 c	79.3±0.1 b
	2 kGy	0.41±0.01 a	12.0±0.1 a	0.93±0.01 b	0.45±0.01 a	1.10±0.02 c	3.8±0.1 d	77.9±0.1 a	1.20±0.02 c	0.09±0.01 c	0.31±0.01 a	0.22±0.01 a	0.51±0.01 a	15.0±0.1 a	5.5±0.2 d	79.5±0.1 a
	6 kGy	0.34±0.01 b	11.0±0.1 b	1.02±0.01 a	0.33±0.01 c	1.30±0.01 a	10.6±0.1 b	72.7±0.1 c	0.61±0.01 d	0.22±0.01 a	0.27±0.01 b	0.16±0.01 b	0.35±0.01 b	13.9±0.1 b	12.4±0.1 b	73.7±0.1 d
	10 kGy	0.21±0.01 c	9.0±0.1 c	0.71±0.01 c	0.17±0.01 d	1.27±0.01 b	12.5±0.2 a	72.2±0.1 d	2.12±0.02 a	0.21±0.01 a	0.27±0.01 b	0.15±0.01 c	0.27±0.03 c	11.4±0.1 d	13.8±0.1 a	74.7±0.1 c
Homoscedasticity[b]	*p*-value	0.001	<0.001	<0.001	<0.001	0.012	<0.001	0.010	<0.001	<0.001	<0.001	0.001	<0.001	0.008	0.119	0.002
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

		C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C24:0	C24:1	SFA	MUFA	PUFA
Russula delica	0 kGy	0.23±0.01 c	12.2±0.1 b	0.49±0.01 b	1.50±0.01 a	16.3±0.1 d	67.5±0.1 a	0.35±0.02 a	0.20±0.01 c	15.0±0.1 c	17.2±0.1 d	67.9±0.1 a
	2 kGy	0.34±0.01 b	12.1±0.1 c	1.30±0.01 c	1.47±0.01 b	19.4±0.1 a	63.5±0.1 d	0.27±0.01 c	0.24±0.01 a	14.8±0.1 d	21.1±0.1 a	64.0±0.1 d
	6 kGy	0.36±0.01 a	12.2±0.1 b	1.47±0.01 a	1.50±0.01 a	17.6±0.3 b	64.9±0.3 c	0.30±0.01 b	0.21±0.01 b	15.2±0.1 b	19.4±0.3 b	65.4±0.3 c
	10 kGy	0.21±0.01 d	13.3±0.1 a	0.34±0.01 d	1.50±0.01 a	17.0±0.1 c	65.8±0.1 b	0.29±0.01 b	0.24±0.01 a	16.1±0.1 a	17.7±0.1 c	66.2±0.1 b
Homoscedasticity[b]	*p*-value	<0.001	0.002	0.003	<0.001	<0.001	<0.001	0.009	0.001	<0.001	0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

dw- dry weight.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Table 5.2.6. Organic acids composition of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a]

		Oxalic acid (g/100 g dw)	Quinic acid (g/100 g dw)	Malic acid (g/100 g dw)	Citric acid (g/100 g dw)	Fumaric acid (g/100 g dw)	Total organic acids (g/100 g dw)
Boletus edulis	0 kGy	0.65±0.05 a	nd	nd	4.1±0.2 a	0.022±0.002 c	4.8±0.2 a
	2 kGy	0.55±0.02 b	nd	nd	2.8±0.3 c	0.062±0.004 a	3.4±0.3 c
	6 kGy	0.56±0.04 b	nd	nd	3.5±0.1 b	0.037±0.002 b	4.1±0.1 b
	10 kGy	0.36±0.03 c	nd	nd	2.4±0.1 d	0.062±0.003 a	2.9±0.1 d
Homoscedasticity[b]	*p*-value	0.061	-	-	<0.001	0.012	<0.001
One-way ANOVA[c]	*p*-value	<0.001	-	-	<0.001	<0.001	<0.001
Russula delica	0 kGy	0.21±0.01 a	1.8±0.1 b	2.28±0.03 a	0.87±0.02 c	0.114±0.001 a	3.3±0.5 a
	2 kGy	0.20±0.01 a	1.90±0.04 a	2.11±0.01 c	1.06±0.05 a	0.099±0.002 b	3.5±0.1 a
	6 kGy	0.18±0.01 b	1.9±0.1 a	2.15±0.03 b	0.96±0.01 b	0.085±0.001 c	3.4±0.1 a
	10 kGy	0.17±0.01 c	1.43±0.03 c	1.83±0.05 d	0.76±0.01 d	0.061±0.002 d	2.8±0.1 b
Homoscedasticity[b]	*p*-value	<0.001	0.006	0.008	0.001	<0.001	0.013
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

dw- dry weight; nd- not detected.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Effects on antioxidant parameters

In order to compare the effects over antioxidant activity, five chemical and biochemical assays were used: scavenging effects on DPPH radicals (measures the decrease in DPPH radical absorption after exposure to radical scavengers), reducing power (conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}, further denominated as Prussian blue assay, and Folin-Ciocalteau method), inhibition of β-carotene bleaching (measures the capacity to neutralize the linoleate-free radical and other free radicals formed in the system which attack the highly unsaturated β-carotene models) and inhibition of lipid peroxidation in brain cells homogenates (measures the color intensity of MDA-TBA complex formed at the endpoint of the reaction); the results are expressed in **Table 5.2.7**. *R. delica* extracts showed to be more active as lipid peroxidation inhibitors and reducing agents. *B. edulis* was a stronger antioxidant only as DPPH radical scavenger. The measured activities were higher in *R. delica* (Heleno et al., 2010; Yaltirak et al., 2009) and slightly lower in *B. edulis* (Heleno et al., 2011), most likely due to seasonal variability or different geographical origins of the used samples. In all cases, except reducing power in *B. edulis* (Folin-Ciocalteau assay) and *R. delica* (ferricyanide/Prussian blue assay), the antioxidant activity was improved in irradiated samples; for *R. delica* it is even possible to point out 6 kGy as the most suitable dose enhance antioxidant activity. The increased lipid peroxidation inhibition verified in both mushroom species might probably be related to the high amount of tocopherols (powerful lipophilic antioxidants) detected in the irradiated samples.

Table 5.2.7. *In vitro* antioxidant properties obtained for the extracts of *B. edulis* and *R. delica* samples submitted to different electron-beam irradiation doses. The results are presented as mean ± SD.[a] Values are presented as EC_{50} values (mg/mL) for all assays except Folin-Ciocalteau, expressed as mg GAE/g extract.

			Reducing power		Lipid peroxidation inhibition	
		DPPH scavenging activity	Ferricyanide/Prussian blue assay	Folin Ciocalteu assay	β-Carotene bleaching inhibition	TBARS formation inhibition
Boletus edulis	0 kGy	2.0±0.2 b	0.62±0.02 a	57±1 a	2.0±0.3 b	3.3±0.1 a
	2 kGy	2.5±0.1 a	0.39±0.01 d	51±1 b	3.8±0.2 a	2.9±0.5 a
	6 kGy	1.8±0.1 c	0.48±0.02 b	40±1 d	0.8±0.1 c	3.0±0.4 a
	10 kGy	1.9±0.1 bc	0.46±0.01 c	48±1 c	0.9±2 c	0.7±0.1 b
Homoscedasticity[b]	*p*-value	0.001	<0.001	<0.001	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001
Russula delica	0 kGy	4.3±0.2 b	0.26±0.01 c	47±1 c	0.53±0.03 b	1.23±0.03 a
	2 kGy	4.4±0.3 b	0.32±0.01 b	50±1 b	1.6±0.1 a	1.0±0.1 b
	6 kGy	3.8±0.1 c	0.36±0.01 a	54±1 a	0.24±0.03 c	0.34±0.05 d
	10 kGy	4.7±0.1 a	0.32±0.02 b	45±1 d	0.5±0.1 b	0.5±0.1 c
Homoscedasticity[b]	*p*-value	<0.001	<0.001	0.048	<0.001	<0.001
One-way ANOVA[c]	*p*-value	<0.001	<0.001	<0.001	<0.001	<0.001

dw- dry weight.
EC_{50}- extract concentration corresponding to 50% of antioxidant activity or 0.5 of absorbance for the Ferricyanide/Prussian blue assay. Concerning the Folin-Ciocalteu assay, higher values mean higher reducing power; for the other assays, the results are presented in EC_{50} values, what means that higher values correspond to lower reducing power or antioxidant potential.
[a] Different letters in each column and for each mushroom indicate significant differences among mean values of each electron-beam irradiation level.
[b] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, *p*-value > 0.05; heteroscedasticity, *p*-value < 0.05.
[c] $p < 0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others (in this case multiple comparison tests were performed).

Principal component analysis

In order to verify all the parameters simultaneously, as well as inferring which irradiation allow obtaining samples that keep the most similar chemical profiles to non-irradiated samples, principal components analysis (PCA) was applied.

The plot of component loadings (**Figure 5.2.1**) for *B. edulis*, indicates that the first two dimensions (first: Cronbach's α, 0.969; eigenvalue, 20.471; second: Cronbach's α, 0.949; eigenvalue, 14.523) account for most of the variance of all quantified variables (37.91% and 26.90%, respectively). The included variance would ideally be higher, but the inclusion of additional dimensions, despite being significant, would not allow a meaningful interpretation. Groups corresponding to each electron-beam irradiation level (0 kGy, 2 kGy, 6 kGy and 10 kGy) were clearly separated, as it was indicated in **Tables 5.2.1-5.2.7**. Group corresponding to 0 kGy is mainly characterized by the high levels in total organic acids, citric acid, C8:0 and low contents in tocopherols, C22:1 and carbohydrates, besides presenting weak reducing power, as measured by ferricyanide/Prussian blue assay (RP-F/PB). The same reasoning might be applied to the remaining electron-beam doses, but the most interesting finding considering the defined objectives, was the resemblance among non-irradiated samples and those irradiated with 6 kGy, indicating that this should be the dose chosen to maintain as well as possible nutritional parameters, fatty acids, tocopherols, phenolic acids, organic acids, sugars and antioxidant profiles.

Concerning *R. delica* objects corresponding to each irradiation level were also clearly separated (**Figure 5.2.2**). The first two dimensions (first: Cronbach's α, 0.975; eigenvalue, 23.267; second: Cronbach's α, 0.951; eigenvalue, 15.027) included most of the variance of all quantified variables (43.09% and 27.83%, respectively), despite the obtained percentage would, once again, be preferably higher. Samples used as control in *R. delica* (0 kGy) were mainly characterized as having high amounts of trehalose, C24:0, C17:0, C18:2 and PUFA and low amounts of C10:0, C12:0, C18:1, C20:3, C20:5 and MUFA, besides showing weak reducing power (RP-F/PB assay). Once again, the same reasoning might be followed for the remaining assayed irradiation doses. Unlike *B. edulis* results, in this case it was not possible to indicate the optimal electron-beam irradiation dose, since it is noticeable that all assayed levels had significant effects on the profiles of the assayed parameters. Nevertheless, it is also evident that 2 and 6 kGy doses had similar effects, while the 10 kGy dose

caused new changes, especially in organic acids (lower) and fatty acids, particularly SFA, which tended to be higher with this dose.

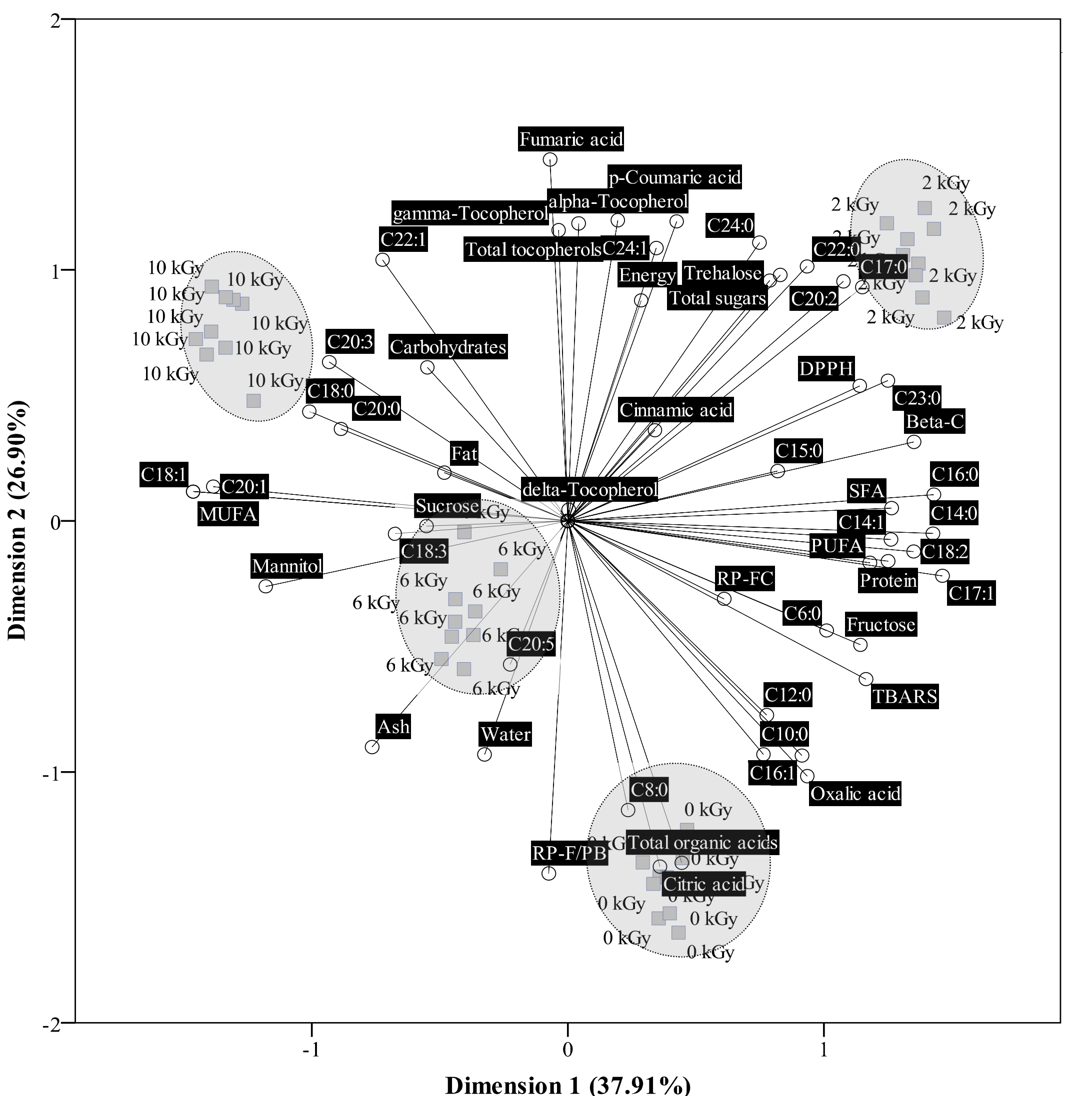

Figure 5.2.1. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *B. edulis*.

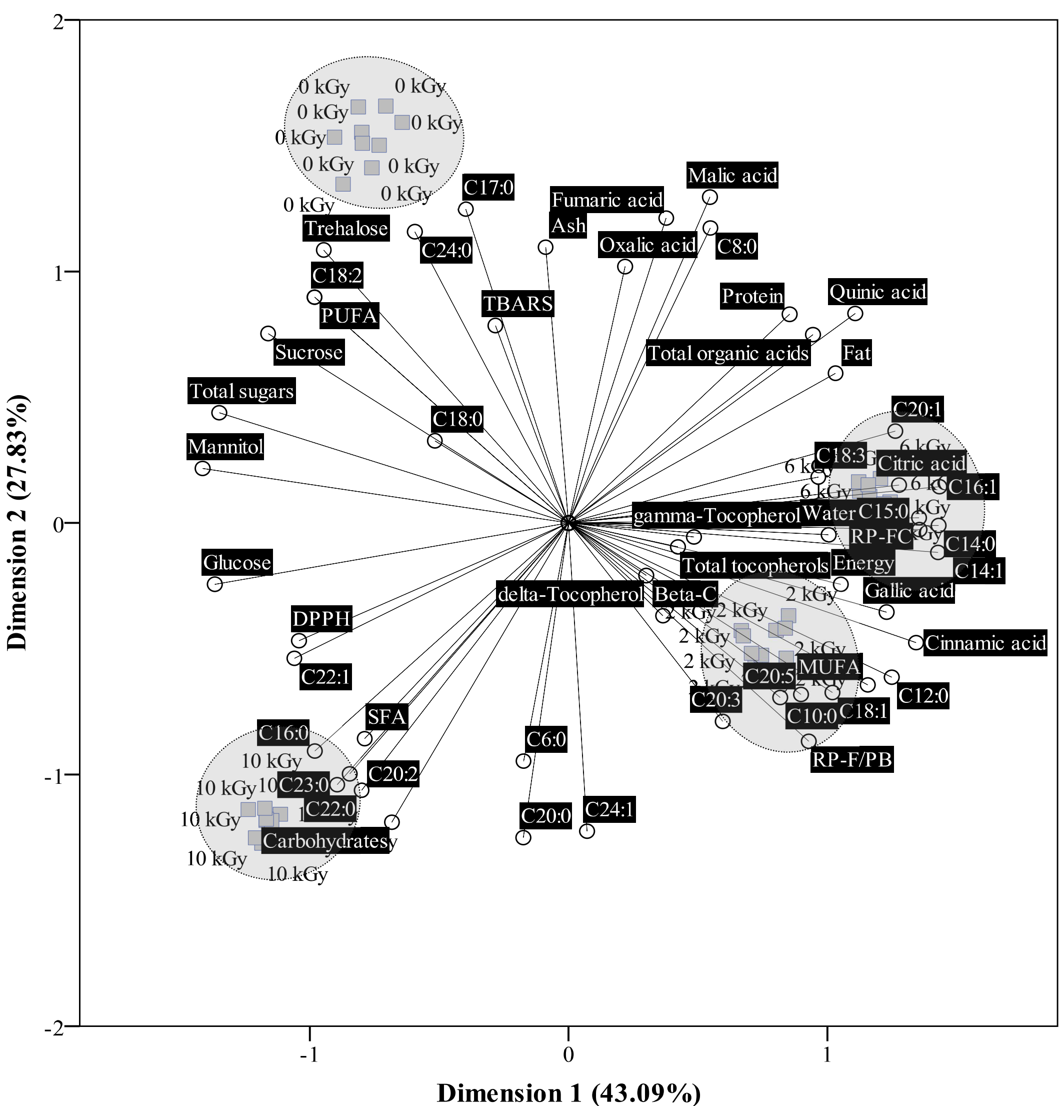

Figure 5.2.2. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *R. delica*.

5.2.4. Conclusions

The effects of gamma-irradiation up to 2 kGy in chemical parameters of fresh wild *B. edulis* were previously studied, indicating that gamma-irradiation, up to those doses, might represent a useful mushroom conservation technology (Fernandes et al. 2013b). Furthermore, electron-beam irradiation (up to 6 kGy) was also applied with success to dried wild *M. procera* (Fernandes et al. 2013a). In this work, it was intended to verify the effects of this irradiation applied at higher doses (up to 10 kGy) and to different mushroom species in order to extend the study and validate the technology.

Despite the 2 kGy dose proved to be effective in previous studies, using higher doses it is possible to achieve not only disinfestation purposes but also decontaminated samples. In this way, treated foods might be available for persons with particular food safety concerns like immunocompromised or elderly people (FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, 2010). Concerning nutritional parameters, the applied irradiation had significant effects, particularly in protein levels and when using 10 kGy. Free sugars were particularly affected by 6 kGy dose in *R. delica* and 2 kGy dose in *B. edulis*, while phenolic acids suffer most appreciable changes with 6 kGy dose in both mushrooms. Tocopherol contents were higher in irradiated samples, especially for the 2 kGy dose in *B. edulis* and the 6 kGy dose in *R. delica.* The decrease in unsaturated fatty acids commonly verified in mushroom samples treated with gamma-irradiation did not occur in this study, indicating that electron-beam irradiation might be a better choice concerning this parameter. Finally, organic acids were most sensitive to the 10 kGy dose. The antioxidant activity was improved in irradiated samples, especially the lipid peroxidation inhibition, probably due to the higher amounts of tocopherols retained by these samples.

The distribution of PCA biplot markers in different clusters (corresponding to each irradiation dose) confirmed the previous highlighted effects, but the obtained results should be considered under the scope of the included percentages of variance in each case. Nevertheless, applying electron-beam irradiation at 6 kGy seems to be the most suitable of those tested in order to keep the composition of this mushroom.

Acknowledgements

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5.2.5. References

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of Korean Society for Applied Biological Chemistry*, *53*, 257-265.

AOAC (1995). *Official methods of analysis* (16th ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Barros, L., Dueñas, M., Ferreira, I.C.F.R., Baptista, P., & Santos-Buelga, C. (2009). Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different Portuguese wild mushrooms species. *Food and Chemical Toxicology*, *47*, 1076-1079

Barros, L., Pereira, C., & Ferreira, I.C.F.R. (2013). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. *Food Analytical Methods*, *6*, 309-316.

Carocho, M., Barreira, J. C., Antonio, A. L., Bento, A., Kaluska, I., & Ferreira, I. C. F. R. (2012). Effects of electron beam radiation on nutritional parameters of Portuguese chestnuts (*Castanea sativa* Mill). *Journal of Agricultural and Food Chemistry*, *60*, 7754-7760.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P., & Blanco D. (2012). Evaluation of gamma and electron-beam irradiation on the aromatic profile of black truffle (*Tuber melanosporum*) and summer truffle (*Tuber aestivum*). *Innovative Food Science and Emerging Technologies*, *13*, 151-157.

Ezekiel, C. N., Sulyok, M., Frisvad, J. C, Somorin, Y. M., Warth, B., Houbraken, J., et al., (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology*, *162*, 231-236.

FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. (2010). The Development of Irradiated Foods for Immuno-Compromised Patients and other potential target groups, Vienna, Austria: IAEA.

Farkas, J. (1998). Irradiation as a method for decontaminating food - A review. *International Journal of Food Microbiology*, *44*, 189-204.

Fernandes, Â., Antonio, A.L., Oliveira, M.P.P., Martins, A., & Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Martins, A., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2013). Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Santos, P.M.P., Martins, A., Oliveira, M.B.P.P., et al., (2013). Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: comparative study through principal component analysis. *Food Research International*, *54*, 18-25.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I.C.F.R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchemical Journal*, *93*, 195-199.

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., & Ferreira, I.C.F.R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*, 1443-1450.

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Santos-Buelga, C., & Ferreira, I.C.F.R. (2011). Targeted metabolites analysis in wild Boletus species. *LWT-Food Science and Technology*, *44*, 1343-1348.

Iqbal, T., Rodrigues, F.A., Mahajan, P.V., & Kerry, J.P. (2009). Effect of time, temperature, and slicing on respiration rate of mushrooms. *Journal of Food Science*, *74*, 298-303.

Jaworska, G., & Bernás, E. (2009). The effect of preliminary processing and period of storage on the quality of frozen *Boletus edulis* (Bull: Fr.) mushrooms. *Food Chemistry*, *113*, 936-943.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9-16.

Kumar, A., Singh, M., & Singh, G. (2013). Effect of different pretreatments on the quality of mushrooms during solar drying. *Journal of Food Science and Technology*, *50*, 165-170.

Loaharanu, P., & Ahmed, M. (1991). Advantages and disadvantages of the use of irradiation for food preservation. *Journal of Agricultural and Environmental Ethics*, *4*, 14-30.

Ma, L., Haixia, C., Wenchai, Z., & Zhaoshuai, W. (2013). Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom *Inonotus obliquus*. *Food Research International*, *50*, 633-640.

Molins, R. (2001). *Food Irradiation. Principles and applications*. USA: John Wiley & Sons. 0-471-35634-4.

Oliveira, F., Sousa-Gallagher, M.J., Mahajan, P.V., & Teixeira, J.A. (2012). Evaluation of MAP engineering design parameters on quality of fresh-sliced mushrooms. *Journal of Food Engineering*, *108*, 507-514.

Ouzouni, P. K., Petridis, D., Koller, W.-D., & Riganakos, K. A. (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. *Food Chemistry*, *115*, 1575-1580.

Patras, A., Brunton, N.P., Downey, G., Rawson, A., Warriner, K., & Gernigon, G. (2011). Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *Journal of Food Composition and Analysis*, *24*, 250-256.

Shephard, G.S. (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants*: *Part A*, *25*, 146-151.

WHO, World Health Organization (1999). High-Dose Irradiation: Wholesomeness of food irradiated with doses above 10kGy. *Technical Report Series No. 890*, Geneva, Switzerland: World Health Organization.

Yaltirak, T., Aslim, B., Sahlan, O., & Alli, H. (2009). Antimicrobial and antioxidant activities of *Russula delica* Fr. *Food and Chemical Toxicology*, *47*, 2052-2056.

5.3.

De que forma a irradiação por feixe de eletrões em diferentes doses afeta o perfil químico e antioxidante de cogumelos silvestres desidratados do género *Amanita*?

Este sub-capítulo apresenta os efeitos da radiação por feixe de eletrões (doses 2, 6 e 10 kGy) no valor nutricional (valor energético, açúcares, ácidos orgânicos, ácidos gordos e tocoferóis) e nos parâmetros bioativos (atividade antioxidante) de amostras desidratadas de Amanita caesarea *(Scop.) Pers. e* Amanita curtipes *E.-J. Gilbert.*

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How does electron beam irradiation dose affect the chemical and antioxidant profiles of wild dried *Amanita* mushrooms?

Running title: Chemical and antioxidant profiles of electron beam irradiated Amanita

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Abstract

As all the mushrooms, Amanita species experiment several conservion problems, with a post-harvest life limited to a few days. Drying is one of the most used methods in mushrooms preservation. Food irradiation is another possible way to improve food quality and insure its security. Among the emerging irradiation

technologies, electron beam has wide application, allowing high throughput, wide flexibility and potential, without any negative effect on the environment. The effects of different electron beam irradiation doses in *Amanita* genus, were assessed by measuring the changes produced on a wide variety of nutritional, chemical and antioxidant indicators. The evaluated profiles indicated differences among non-irradiated and irradiated samples, but a high similarity among different doses. This finding advises the highest assayed dose (10 kGy), ensuring higher effectiveness from the decontamination and disinfestation point of view, without having stronger effects than those observed for the lower doses.

Keywords: Amanita spp.; Electron beam; Chemical composition; Antioxidant properties; Principal Component Analysis.

5.3.1. Introduction

The post-harvest life of mushrooms is limited to a few days, due to their fast quality devaluation (Lukasse, & Polderdijk, 2003). After harvesting, moisture loss, shrinkage and rapid spoilage in terms of color and texture occur. Normally, mushrooms are consumed in fresh, but in recent years their consumption in dried forms has been increasing (Şevik, Aktaş, Doğan, & Koçak, 2013). Drying is one of the oldest methods for preservation of food commodities for long duration and also one of the most used conservation methods employed in storage of mushrooms (Ma, Chen, Zhu, & Wang, 2013).

The drying process causes the reduction of vegetative cells of microorganisms, which gives rise to a flora of bacteria and fungi that have the ability to survive for long periods in dried foods and produce toxins harmful to human health (ICMSF, 1985); the high humidity that exists during storage also predisposes dried mushrooms to invasion by microorganisms (Shephard, 2008; Ezekiel et al., 2013).

The prevention of food deterioration and the control of infection by microorganisms have been a major preoccupation of man over the centuries (ICGFI, 1999). Accordingly, food irradiation is one of the possible ways to improve the quality, reduce the incidence of foodborne diseases caused by microorganisms, decontaminate pests, insects or parasites that inflict food spoilage and toxicity,

thereby replacing the chemical treatments (Supriya, Sridhar, Nareshkumar, & Ganesh, 2012; Culleré, Ferreira, Venturini, Marco, & Blanco, 2012). Among the emerging technologies of irradiation, electron beam (EB) has wide applications in improvement of food quality and safety; this technology is highly attractive due to its technological advantages in terms of high throughput, wide flexibility and potential, use of a non-thermal process, leaving no toxic residues and without any negative effect on the environment (Supriya, Sridhar, Nareshkumar, & Ganesh, 2012).

Our research group has been demonstrating that EB irradiation does not significantly alter the antioxidant, chemical and nutritional parameters of different mushrooms species namely, *Macrolepiota procera* (0.5, 1 and 6 kGy) (Fernandes et al., 2013), *Boletus edulis* and *Russula delica* (2, 6 and 10 kGy) (Fernandes et al., 2014). The aim of the present study was to validate the use of EB irradiation preservation to other mushrooms species, maintaining their nutritional and chemical quality, as also antioxidant potential. *Amanita caesarea* (Scop.) Pers. and *Amanita curtipes* E.-J. Gilbert were irradiated at 2, 6 and 10 kGy, doses typical used for mushrooms decontamination and conservation.

5.3.2. Materials and methods

Standards and reagents

For chemical analyses: Acetonitrile 99.9%, n-hexane 95% and ethyl acetate 99.8% were of HPLC grade from Lab-Scan (Lisbon, Portugal). The fatty acids methyl ester (FAME) reference standard mixture 37 (standard 47885-U) was purchased from Sigma (St. Louis, MO, USA), as also other individual fatty acid isomers, organic acids and sugar standards.

For antioxidant potential analysis: 2,2-diphenyl-1-picrylhydrazyl (DPPH) was obtained from Alfa Aesar (Ward Hill, MA, USA). Standards trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) and gallic acid were from Sigma (St. Louis, MO, USA). Methanol and all other chemicals were of analytical grade and obtained from common sources. Standards of phenolic compounds (protocatechuic, *p*-hydroxybenzoic and *p*-coumaric acids), cinnamic acid and organic acids (oxalic acid, quinic acid, citric acid and fumaric acid) were from Sigma Chemical Co. (St. Louis,

MO, USA). Water was treated in a Milli-Q water purification system (TGI Pure Water Systems, Greenville, South Carolina, USA).

Samples and electron beam irradiation

A. caesarea and *A. curtipes* samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in October 2013, and dried at 30 ºC in an oven. Subsequently, the samples were divided in four groups with five specimens of each mushroom species: control (non-irradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy), kept in polyethylene bags.

The irradiation was performed at the INCT - Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used a standard dosimeter, graphite calorimeter, and two routine dosimeters, Gammachrome YR and Amber Perspex, from Harwell Company (UK). The irradiation took place in e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 μs, a pulse frequency of 440 Hz, and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min and the scan frequency was 5 Hz. The estimated absorbed doses were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the Graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a previous calibration curve (Fernandes et al., 2013). For simplicity, we refer to the irradiation doses as: 0, 2, 6 and 10 kGy.

Before analysis, the samples were reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples.

Chemical parameters

Nutritional value

Moisture, protein, fat, carbohydrates and ash were determined following the AOAC procedures (AOAC, 1995). The crude protein content (N×4.38) of the samples

was estimated by the macro-Kjeldahl method; the crude fat was determined by extracting a known weight of powdered sample with petroleum ether, using a Soxhlet apparatus; the ash content was determined by incineration at 600 ± 15 ^{o}C. Total carbohydrates were calculated by difference. Energy was calculated according to the following equation: Energy (kcal) = 4 × ($g_{protein}$) + 3.75 × ($g_{carbohydrate}$) + 9 × (g_{fat}).

Free sugars

Free sugars were determined by high performance liquid chromatography coupled to a refraction index detector (HPLC-RI), using a previously described procedure (Heleno, Barros, Sousa, Martins, & Ferreira, 2009). Data were analysed using Clarity 2.4 Software (DataApex). The compounds were identified by chromatographic comparisons with authentic standards. Quantification was performed using the internal standard (raffinose) method.

Organic acids

Organic acids were determined following a procedure previously optimized and described by the authors (Barros, Pereira, & Ferreira, 2013). Analysis was performed by ultra-fast liquid chromatograph (UFLC) coupled to photodiode array detector (PAD), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PAD, using 215 and 245 nm as preferred wavelengths. The organic acids were quantified by comparison of the area of their peaks recorded at 215 nm with calibration curves obtained from commercial standards of each compound. Standards of organic acids (oxalic acid, quinic acid, citric acid and fumaric acid) were from Sigma Chemical Co. (St. Louis, MO, USA). The results were expressed in g per 100 g of dry weight (dw).

Phenolic compounds

Phenolic compounds were determined in the UFLC system mentioned above, as previously described by the authors (Barros, Dueñas, Ferreira, Baptista, & Santos-Buelga, 2009). DAD detection was carried out using 280 nm and 370 nm as preferred wavelengths. The phenolic compounds were characterized according to their UV

spectra and retention times, and comparison with authentic standards. For quantitative analysis, calibration curves were prepared from different standard compounds. Standards of phenolic compounds (protocatechuic, p-hydroxybenzoic and p-coumaric acids) and cinnamic acid were from Sigma Chemical Co. (St. Louis, MO, USA). The results were expressed in mg per 100 g dw.

Fatty acids

Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID)/capillary column as described previously by the authors (Heleno, Barros, Sousa, Martins, & Ferreira, 2009). Fatty acid identification was made by comparing the relative retention times of FAME peaks from samples with standards. The results were recorded and processed using Clarity DataApex 4.0 Software (DataApex 4.0, Prague, Czech Republic) and expressed in relative percentage of each fatty acid (obtained after Soxhlet extraction).

Tocopherols

Tocopherols were determined following a procedure previously optimized and described by the authors (Reis et al., 2012). The compounds were identified by chromatographic comparison with authentic standards. Quantification was based on the fluorescence signal response of each standard, using the IS (tocol) method and by using calibration curves obtained from commercial standards of each compound. The results were expressed in µg per 100 g dw.

Antioxidant parameters

Extraction procedure

The dried powder (1 g) was stirred with methanol (30 mL) at 25 ºC at 150 rpm for 1 h and filtered through Whatman No. 4 paper. The residue was then extracted with an additional portion of methanol. The combined methanolic extracts were evaporated under reduced pressure (rotary evaporator Büchi R-210; Flawil, Switzerland), re-dissolved in methanol at 20 mg/mL (stock solution), and stored at 4

ºC for further use. Successive dilutions were made from the stock solution and submitted to *in vitro* assays to evaluate the antioxidant activity of the samples. The sample concentrations providing 50% of antioxidant activity or 0.5 of absorbance (EC_{50}) were calculated from the graphs of antioxidant activity percentages (DPPH, β-carotene/linoleate and TBARS assays) or absorbance at 690 nm (reducing power assay) against sample concentrations. Trolox was used as standard.

DPPH radical scavenging activity

DPPH radical-scavenging activity was evaluated by using an ELX800 microplate reader (Bio-Tek Instruments, Inc; Winooski, VT, USA), and calculated as a percentage of DPPH discoloration using the formula: [(ADPPH-AS)/ADPPH] 100, where AS is the absorbance of the solution containing the sample at 515 nm, and ADPPH is the absorbance of the DPPH solution. Reducing power was evaluated by the capacity to convert Fe3+ into Fe2+, measuring the absorbance at 690 nm in the microplate reader mentioned above. Inhibition of β-carotene bleaching was evaluated though the β-carotene/linoleate assay; the neutralization of linoleate free radicals avoids β-carotene bleaching, which is measured by the formula: β-carotene absorbance after 2h of assay/initial absorbance) x 100% (Fernandes et al. 2014).

Reducing power

Two different procedures were used to evaluate the reducing power:

A) The first methodology was performed using the Microplate Reader described above. The different concentrations of the extracts (0.5 mL) were mixed with sodium phosphate buffer (200 mmol/L, pH 6.6, 0.5 mL) and potassium ferricyanide (1% w/v, 0.5 mL). For each concentration, the mixture was incubated at 50 ºC for 20 min, and trichloroacetic acid (10% w/v, 0.5 mL) was added. The mixture (0.8 mL) was poured in the 48-wells, as also deionized water (0.8 mL) and ferric chloride (0.1% w/v, 0.16 mL), and the absorbance was measured at 690 nm.

B) The second methodology followed the Folin-Ciocalteu assay. The extract solution (1 mL) was mixed with Folin-Ciocalteu reagent (5 mL, previously diluted with water 1:10, v/v) and sodium carbonate (75 g/L, 4 mL). The tubes were

vortex mixed for 15 s and allowed to stand for 30 min at 40 ºC for color development. Absorbance was then measured at 765 nm. Gallic acid was used to obtain the standard curve (0.0094-0.15 mg/mL), and the results were expressed as mg of gallic acid equivalents (GAE) per g of extract.

Inhibition of β-carotene bleaching

β-carotene (2 mg) was dissolved in chloroform (10 mL) and 2 mL of this solution were pipetted into a round-bottom flask. After the chloroform was removed at 40 ºC under vacuum, linoleic acid (40 mg), Tween 80 emulsifier (400 mg), and distilled water (100 mL) were added to the flask with vigorous shaking. Aliquots (4.8 mL) of this emulsion were transferred into different test tubes containing different concentrations of the extracts (0.2 mL). The tubes were shaken and incubated at 50 ºC in a water bath. As soon as the emulsion was added to each tube, the zero time absorbance was measured at 470 nm. β-Carotene bleaching inhibition was calculated using the following equation: (absorbance after 2 h of assay/initial absorbance) × 100.

TBARS (thiobarbituric acid reactive substances) assay

Porcine (*Sus scrofa*) brains were obtained from official slaughtering animals, dissected, and homogenized with a Polytron in ice cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1:2 w/v brain tissue homogenate which was centrifuged at 3000*g* for 10 min. An aliquot (100 µL) of the supernatant was incubated with the different concentrations of the samples solutions (200 µL) in the presence of $FeSO_4$ (10 mM; 100 µL) and ascorbic acid (0.1 mM; 100 µL) at 37 ºC for 1 h. The reaction was stopped by the addition of trichloroacetic acid (28% w/v, 500 µL), followed by thiobarbituric acid (TBA, 2%, w/v, 380 µL), and the mixture was then heated at 80 ºC for 20 min. After centrifugation at 3000*g* for 10 min to remove the precipitated protein, the color intensity of the malondialdehyde (MDA)-TBA complex in the supernatant was measured by its absorbance at 532 nm. The inhibition ratio (%) was calculated using the following formula: Inhibition ratio (%) = [(A - B)/A] × 100%, where A and B were the absorbance of the control and the sample solution, respectively.

Statistical analysis

All the analyses (extractions) were performed in triplicate; each replicate was quantified also three times. Data were expressed as means ± standard deviations.
The fulfillment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro-Wilk's, and the Levene's tests, respectively. For each parameter, significant differences among mean values were checked by Welch's statistics ($p<0.05$ means that the mean value of the evaluated parameter of at least one irradiation differs from the others). In the cases where statistical significance differences were identified, the dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Principal components analysis (PCA) was applied as pattern recognition unsupervised classification method. PCA transforms the original measured variables into new uncorrelated variables called principal components. The first principal component covers as much of the variation in the data as possible. The second principal component is orthogonal to the first and covers as much of the remaining variation as possible, and so on (Patras et al., 2011). The number of dimensions to keep for data analysis was evaluated by the respective eigenvalues (which should be greater than one), by the Cronbach's alpha parameter (that must be positive) and also by the total percentage of variance (that should be as higher as possible) explained by the number of components selected. The number of dimensions considered for PCA was chosen in order to allow meaningful interpretations, to ensure their reliability.

All statistical tests were performed at a 5% significance level using the SPSS software, version 22.0 (IBM, Corp., Armonk, NY: USA).

5.3.3. Results and discussion

Effects on chemical parameters

The nutritional parameters of *A. caesarea* and *A. curtipes* (**Table 5.3.1**) showed some relevant differences, despite belonging to the same genus, especially in what

concerns water and fat contents. Nevertheless, water was the major component (≈94% in *A. caesarea*; ≈84% in *A. curtipes*) in fresh weight basis, while carbohydrates (≈65% in *A. caesarea*; ≈62% in *A. curtipes*) were the major component *per* dry weight, followed by ash, proteins and fat contents. These nutritional profiles are very similar to those reported for *A. caesarea* and other species from this genus (Reis et al., 2011). In terms of the effects of EB irradiation, significant changes were detected in nearly all cases, except water content in *A. caesarea*. The highest doses seemed to have cause more notorious effects, excluding the observed for energy in both mushrooms and water and fat contents in *A. curtipes*. The highest differences among control and irradiated samples were verified for protein content, which might be related to scission of the C-N bonds in the backbone of the polypeptide chain, or physical changes like unfolding (Molins, 2001), leading to a higher availability of nitrogen atoms with consequences in the Kjeldahl reaction, used for evaluating the nutritional value after the irradiation treatment.

Table 5.3.1. Proximate composition of the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		Water (g/100 g fw)	Fat (g/100 g dw)	Protein (g/100 g dw)	Ash (g/100 g dw)	Carbohydrates (g/100 g dw)	Energy (kcal/100 g dw)
				Amanita caesarea			
EB	0 kGy	94±1	6.4±0.1^{c}	6.3±0.1^{d}	14.8±0.1^{c}	72.5±0.3^{a}	373±1^{a}
	2 kGy	93±1	7.0±0.2^{b}	12.0±0.2^{c}	17.0±0.1^{b}	64.0±0.4^{b}	367±1^{c}
	6 kGy	94±1	7.7±0.2^{a}	12.5±0.1^{b}	17.4±0.1^{a}	62.5±0.3^{c}	369±1^{b}
	10 kGy	94±2	7.9±0.3^{a}	12.9±0.3^{a}	17.4±0.2^{a}	61.9±0.5^{d}	370±2^{b}
p-values	Homoscedasticity[2]	0.002	0.023	0.004	0.058	0.788	0.008
	Normal distribution[3]	0.198	0.194	<0.001	<0.001	<0.001	0.041
	1-way ANOVA[4]	0.773	<0.001	0.004	<0.001	<0.001	<0.001
				Amanita curtipes			
EB	0 kGy	80±1^{b}	8.6±0.3^{b}	6.4±0.4^{c}	17.2±0.1^{c}	67.8±0.4^{a}	374±2^{a}
	2 kGy	85±1^{a}	9.5±0.4^{a}	10.7±0.2^{b}	19.0±0.1^{b}	60.8±0.3^{b}	371±2^{b}
	6 kGy	85±1^{a}	9.8±0.2^{a}	11.0±0.1^{b}	19.1±0.3^{b}	60.2±0.3^{c}	372±2^{ab}
	10 kGy	85±1^{a}	9.8±0.2^{a}	11.5±0.5^{a}	19.5±0.2^{a}	59.2±0.5^{d}	371±1^{b}
p-values	Homoscedasticity[2]	0.359	0.033	<0.001	0.009	0.062	0.416
	Normal distribution[3]	<0.001	0.001	<0.001	<0.001	<0.001	0.631
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001	0.001

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Continuing to the first group of individual molecules, different free sugars profiles (**Table 5.3.2**) were detected for both *Amanita*. *A. curtipes* presented a higher content, with trehalose as the most abundant sugar followed by mannitol and fructose. In the extracts of *A. caesarea*, only mannitol and trehalose were detected, and in much lower quantities. The same sugars were previously detected in *A. caesarea*, but the amounts reported herein were lower (Reis et al., 2011), which might be explained by different maturity stages. Samples submitted to EB irradiation presented significant changes in sugars contents, despite the produced effect had not been coherent in both species (sugars increase with irradiation in *A. caesarea* but not in *A. curtipes*). Irradiation is known for causing sugars degradation and, in this case, it might be hypothesized that some polysaccharide units have been hydrolysed releasing the corresponding free sugar units.

Table 5.3.2. Free sugars composition (g/100 g dw) of the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		Fructose	Mannitol	Trehalose	Sugars
			Amanita caesarea		
EB	0 kGy	nd	0.30±0.02[d]	0.58±0.03[c]	0.88±0.03[d]
	2 kGy	nd	0.52±0.02[c]	0.59±0.01[c]	1.11±0.02[c]
	6 kGy	nd	1.19±0.05[b]	1.86±0.05[b]	3.0±0.1[b]
	10 kGy	nd	1.25±0.03[a]	3.77±0.05[a]	5.0±0.1[a]
p-values	Homoscedasticity[2]	-	0.001	<0.001	0.001
	Normal distribution[3]	-	<0.001	<0.001	<0.001
	1-way ANOVA[4]	-	<0.001	<0.001	<0.001
			Amanita curtipes		
EB	0 kGy	2.3±0.1[a]	3.9±0.1[b]	8.9±0.2[b]	15.1±0.2[b]
	2 kGy	1.9±0.1[b]	3.5±0.1[c]	10.4±0.3[a]	15.8±0.3[a]
	6 kGy	1.7±0.1[c]	3.4±0.1[c]	9.2±0.2[b]	14.3±0.2[d]
	10 kGy	1.9±0.1[b]	5.2±0.2[a]	7.5±0.3[c]	14.6±0.3[c]
p-values	Homoscedasticity[2]	0.014	0.002	0.056	0.630
	Normal distribution[3]	<0.001	<0.001	0.136	0.483
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

From **Table 5.3.3**, it is possible to conclude that the composition in organic acids is quite similar for both *Amanita*, with malic acid as the main organic acid, followed by fumaric acid and oxalic acid. The only detected phenolic acid was *p*-hydroxybenzoic acid, more than ten-fold higher in *A. caesarea*, which presented also cinnamic acid, contrarily to *A. curtipes.* Some slight differences, but statistically significant, were detected for the organic acids, despite lacking an identifiable tendency. Nevertheless, cinnamic acid suffered a strong decrease in irradiated samples.

Table 5.3.4 presents the individual fatty acids (FA) quantified above 0.2% in each mushroom species: C6:0, C8:0, C10:0, C12:0, C15:0, C17:0, C17:1, C18:3, C20:1, C20:2 (not detected in *A. caesarea*), C20:3+C21:0, C20:5, C22:0, C22:1 (not detected in *A. curtipes*), C23:0 and C24:1 (not detected in *A. caesarea*) were also quantified (and included further in the principal component analysis). The most abundant FA in these *Amanita* were palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2), as it is typical in this genus (Reis et al., 2011). Saturated fatty acids seemed to be more resistant to EB irradiation, while monounsaturated species tended to increase in percentage and polyunsaturated ones showed the opposite behavior, which is in agreement with its different radiosensitivity (Stewart, 2001). Even so, fatty acid percentages did not suffer any observable severe change, which might be explained by the fact that irradiation was performed in dried mushrooms. In fact, the general mechanism of lipids radiolysis (primary ionization, followed by migration of the positive charge either toward the carboxyl carbonyl group or double bonds) is more likely to occur in fresh mushrooms (Molins, 2001).

Table 5.3.3. Organic acids, *p*-hydroxybenzoic acid and cinnamic acid composition of the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		Oxalic acid (g/100 g dw)	Malic acid (g/100 g dw)	Fumaric acid (g/100 g dw)	Organic acids (g/100 g dw)	*p*-hydroxybenzoic acid (mg/100 g dw)	Cinnamicacid (mg/100 g dw)
				Amanita caesarea			
EB	0 kGy	0.17±0.02[c]	1.1±0.1[a]	0.32±0.03[a]	1.5±0.1[a]	3.9±0.1[b]	2.48±0.02[a]
	2 kGy	0.23±0.01[b]	0.40±0.04[d]	0.14±0.01[c]	0.77±0.05[d]	4.7±0.3[a]	0.84±0.05[c]
	6 kGy	0.29±0.02[a]	0.59±0.05[b]	0.23±0.02[b]	1.11±0.05[b]	2.1±0.4[d]	0.93±0.05[b]
	10 kGy	0.23±0.02[b]	0.49±0.02[c]	0.21±0.02[b]	0.92±0.04[c]	2.7±0.1[c]	0.75±0.03[d]
p-values	Homoscedasticity[2]	0.052	0.016	0.003	0.233	<0.001	<0.001
	Normal distribution[3]	0.311	<0.001	0.026	0.003	<0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
				Amanita curtipes			
EB	0 kGy	0.27±0.03[a]	1.5±0.1[d]	0.26±0.03[c]	2.1±0.1[d]	0.32±0.01[a]	nd
	2 kGy	0.17±0.02[c]	1.8±0.1[c]	0.34±0.04[b]	2.3±0.1[c]	0.10±0.01[d]	nd
	6 kGy	0.21±0.02[b]	2.7±0.1[a]	0.54±0.04[a]	3.4±0.2[a]	0.18±0.01[c]	nd
	10 kGy	0.18±0.02[c]	2.1±0.2[b]	0.33±0.02[b]	2.6±0.2[a]	0.27±0.02[b]	nd
p-values	Homoscedasticity[2]	0.045	0.445	0.069	0.140	<0.001	-
	Normal distribution[3]	0.015	0.007	0.001	0.001	<0.001	-
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001	-

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Table 5.3.4. Fatty acids (relative percentage) profile of the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		C14:0	C16:0	C16:1	C18:0	C18:1n9	C18:2n6	C20:0	C22:0	C24:0	SFA	MUFA	PUFA
							Amanita caesarea						
EB	0 kGy	0.30±0.02^{b}	14.5±0.5^{b}	1.1±0.1^{a}	2.9±0.3^{b}	44±1^{c}	35±1^{b}	0.18±0.02^{c}	0.14±0.01^{b}	0.84±0.05^{a}	19.4±0.5^{ab}	45.6±0.5^{c}	35.0±0.2^{b}
	2 kGy	0.32±0.02^{b}	13.9±0.3^{d}	1.1±0.1^{a}	2.9±0.1^{b}	42±1^{d}	37±1^{a}	0.21±0.02^{b}	0.17±0.01^{a}	0.86±0.03^{a}	19.1±0.4^{b}	44.0±0.5^{d}	36.9±0.3^{a}
	6 kGy	0.45±0.02^{a}	15.2±0.3^{a}	1.1±0.1^{a}	2.8±0.2^{b}	48±1^{b}	31±1^{c}	0.15±0.01^{d}	0.10±0.01^{c}	0.61±0.02^{c}	19.8±0.2^{a}	49.1±0.2^{b}	31.1±0.2^{c}
	10 kGy	0.20±0.02^{c}	12.7±0.1^{d}	0.58±0.03^{b}	5.0±0.2^{a}	52±1^{a}	27±1^{d}	0.29±0.01^{a}	0.10±0.01^{c}	0.75±0.02^{b}	19.4±0.2^{ab}	53.5±0.5^{a}	27.1±0.4^{d}
p-values	Homoscedasticity[2]	0.662	<0.001	0.032	0.001	0.021	0.010	<0.001	0.182	0.001	0.001	0.030	0.013
	Normal distribution[3]	0.018	0.018	<0.001	<0.001	0.001	<0.001	<0.001	<0.001	0.008	0.060	0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
							Amanita curtipes						
EB	0 kGy	0.28±0.01^{a}	18.9±0.2^{a}	1.1±0.1^{a}	4.1±0.2^{a}	54±1^{d}	19.2±0.3^{a}	0.16±0.01^{c}	0.24±0.01^{c}	1.2±0.1^{b}	25.4±0.3^{a}	55.2±0.4^{d}	19.4±0.3^{a}
	2 kGy	0.22±0.01^{b}	18.6±0.4^{ab}	0.9±0.1^{b}	4.3±0.2^{a}	56±1^{c}	17.1±0.2^{b}	0.17±0.01^{b}	0.23±0.01^{c}	1.2±0.1^{b}	25.0±0.2^{a}	57.7±0.3^{c}	17.2±0.2^{b}
	6 kGy	0.19±0.01^{c}	17.7±0.3^{c}	1.0±0.1^{ab}	3.1±0.5^{b}	58±1^{b}	17.2±0.2^{b}	0.21±0.01^{a}	0.27±0.01^{b}	1.5±0.1^{a}	23.2±0.5^{b}	59.4±0.5^{b}	17.4±0.2^{b}
	10 kGy	0.19±0.01^{c}	18.4±0.3^{b}	0.9±0.1^{b}	1.1±0.1^{c}	61±1^{a}	15.4±0.4^{c}	0.22±0.01^{a}	0.29±0.01^{a}	1.4±0.2^{a}	21.9±0.3^{c}	62.5±0.5^{a}	15.6±0.4^{c}
p-values	Homoscedasticity[2]	0.098	0.288	<0.001	<0.001	<0.001	0.026	0.472	0.091	0.090	<0.001	<0.001	0.037
	Normal distribution[3]	0.001	0.053	0.417	<0.001	0.024	0.016	0.005	0.022	0.009	<0.001	0.018	0.015
	1-way ANOVA[4]	<0.001	<0.001	0.003	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

The tocopherols profiles (**Table 5.3.5**) revealed the presence of α-, γ- and δ-tocopherol quantified in higher quantities in *A. caesarea.* γ-Tocopherol was the dominant form in both species, with α-tocopherol as the minor isoform, in agreement with other reported results (Reis et al., 2011). In general, irradiated samples tended to present higher amounts, except for what was observed with γ-tocopherol in *A. caesarea.* This result should not, obviously, be interpreted as if irradiation causes an increase in tocopherol content but as a preservation effect of irradiation; on the other hand, the previously pointed out changes in the atmosphere of the samples containers might act by preventing the tocopherol degradation, due to a decrease in the molecular oxygen availability.

Table 5.3.5. Tocopherols composition (μg/100 g dw) of the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		α-Tocopherol	γ-Tocopherol	δ-Tocopherol	Tocopherols
		Amanita caesarea			
EB	0 kGy	6.4±0.1[b]	100±1[a]	34±1[d]	140±1[d]
	2 kGy	5.9±0.2[c]	61±1[d]	105±1[a]	172±1[b]
	6 kGy	2.5±0.1[d]	67±1[c]	79±1[c]	149±1[c]
	10 kGy	9.2±0.2[a]	72±1[b]	93±1[b]	174±1[a]
p-values	Homoscedasticity[2]	<0.001	0.017	0.001	0.019
	Normal distribution[3]	<0.001	<0.001	<0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001
		Amanita curtipes			
EB	0 kGy	4.0±0.1[b]	40±1[d]	4.1±0.1[d]	48±1[c]
	2 kGy	3.1±0.1[c]	73±1[a]	16.9±0.1[c]	93±1[b]
	6 kGy	3.9±0.2[b]	59±1[c]	29.8±0.3[a]	93±1[b]
	10 kGy	6.6±0.1[a]	70±1[b]	17.4±0.1[b]	94±2[a]
p-values	Homoscedasticity[2]	<0.001	<0.001	<0.001	0.001
	Normal distribution[3]	<0.001	<0.001	0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Effects on antioxidant parameters

The effects of using EB irradiation in the antioxidant activity of the *Amanita* extracts were evaluated by comparing the results obtained from different assays (**Table 5.3.6**). With no exception, irradiated samples (especially for the higher doses) showed to be more antioxidant, either as DPPH radicals scavengers, ferric reducers (conversion of a Fe^{3+}/ferricyanide complex to Fe^{2+}) and β-carotene bleaching or TBARS formation inhibitors. The higher antioxidant activity was coherent with the levels of phenolic compounds, which also increased with extending irradiation doses. Except for TBARS formation inhibition, *A. caesarea* showed higher antioxidant activity than *A. curtipes*, and besides this action, the assayed mushroom proved to be particularly active as reducing agents. The increased TBARS formation inhibition might be related to the high amount of tocopherols (powerful lipophilic antioxidants) detected in the irradiated samples. The EC_{50} values are in general agreement with those reported in *Amanita* genus (Reis et al., 2011).

Table 5.3.6. Antioxidant properties of extracts from the *Amanita* species submitted to electron-beam irradiation (EB).[1]

		DPPH scavenging activity	Reducing power	β-carotene bleaching inhibition	TBARS formation inhibition	Phenols
			Amanita caesarea			
EB	0 kGy	8.0±0.1[a]	1.36±0.01[a]	3.7±0.3[a]	1.5±0.1[a]	29.8±0.2[b]
	2 kGy	7.3±0.1[b]	0.85±0.01[b]	2.1±0.1[b]	1.1±0.1[b]	30.4±0.2[a]
	6 kGy	7.3±0.1[b]	0.86±0.01[b]	2.1±0.2[b]	1.1±0.1[b]	30.4±0.1[a]
	10 kGy	7.3±0.1[b]	0.70±0.01[c]	2.0±0.1[b]	1.0±0.1[c]	30.5±0.2[a]
p-values	Homoscedasticity[2]	0.001	0.001	<0.001	0.108	0.019
	Normal distribution[3]	<0.001	<0.001	<0.001	0.002	0.007
	1-way ANOVA[4]	<0.001	<0.001	<0.001	<0.001	<0.001
			Amanita curtipes			
EB	0 kGy	19.0±0.1[a]	1.53±0.02[a]	9.8±0.4[a]	0.7±0.1[a]	30.6±0.3[b]
	2 kGy	14.2±0.2[b]	1.01±0.01[c]	6.1±0.5[b]	0.6±0.1[b]	30.6±0.2[b]
	6 kGy	14.1±0.4[b]	1.21±0.01[b]	5.8±0.5[b]	0.6±0.1[b]	29.8±0.1[c]
	10 kGy	10.1±0.2[c]	0.90±0.02[d]	3.9±0.2[c]	0.5±0.1[b]	32.7±0.2[a]
p-values	Homoscedasticity[2]	<0.001	<0.001	0.001	<0.001	0.044
	Normal distribution[3]	<0.001	<0.001	0.001	<0.001	<0.001
	1-way ANOVA[4]	<0.001	<0.001	0.001	<0.001	<0.001

[1]The results are presented as the mean±SD.
[2]Homoscedasticity among EB doses was measured by the Levene test: homoscedasticity, $p>0.05$; heteroscedasticity, $p<0.05$.
[3]Normal distribution of the residuals was evaluated using Shapiro-Wilk test.
[4]$p<0.05$ indicates that the mean value of the evaluated parameter of at least one EB dose differs from the others (in this case multiple comparison tests were performed). For each species, means within a column with different letters differ significantly ($p<0.05$).

Principal component analysis (PCA)

In the former section, profiling changes resulting from EB irradiation were compared for each individual assayed parameter within each *Amanita* species. Despite the high number of detected statistically significant changes, it was not possible to present overall conclusions regarding the feasibility of this technology. Furthermore, it was intended to validate this technology independently of the mushroom species. Accordingly, in the present section the results were evaluated considering data for both species and considering all parameters simultaneously.

To conclude if EB irradiation allows maintaining the chemical and antioxidant profiles among non-irradiated and irradiated samples, principal components analysis (PCA) was applied to obtain output including the integrated effects on all parameters at once. Due to the variable magnitude between species for specific parameters (**Tables 5.3.1 - 5.3.6**), the values were normalized by subtracting the value corresponding to 0 kGy to each of the values corresponding to 2, 6 and 10 kGy. The obtained differences were further divided by the value of the respective control. In this way, the classification procedure was applied to the differences caused by irradiation and not to the absolute values measured for each parameter. Due to practical reasons, only the parameters detected in both species were included in this analysis (fructose, C20:2, C22:1, C24:1 and cinnamic acid were excluded).

The plot of object scores (**Figure 5.3.1**) for EB irradiation dose showed that the first two dimensions (first: Cronbach's α, 0.964; eigenvalue, 17.661; second: Cronbach's α, 0.946; eigenvalue, 13.478) included most of the variance of all quantified variables (37.6% and 28.7%, respectively). The inclusion of a third dimension (Cronbach's α, 0.809; eigenvalue, 4.802) would increase the percentage of explained variance with an additional 10.2%, but the produced output would not allow such a meaningful interpretation as in the case of using two dimensions. Groups corresponding to each gamma irradiation dose (0 kGy, 2 kGy, 6 kGy and 10 kGy) were not arranged individually, despite all the significant differences in **Tables 5.3.1-5.3.6**. This apparently random distribution seemed to be a direct consequence of the dissimilar effects of EB in each of the assayed species. As it can be depicted from **Figure 5.3.1**, only half of the object scores corresponding to 10 kGy were grouped individually. These scores correspond with no doubt to *A. caesarea*, since they are characterized by high increase in ash, trehalose, mannitol and total sugars,

and high decrease in carbohydrates, C16:1, C17:1, C18:3, reducing power and TBARS formation inhibition. However, the 10 kGy dose had not the same effect on *A. curtipes*, leading to the grouping of its object scores together with those corresponding to samples irradiated with 2 and 6 kGy (left side on the bottom). Accordingly, and despite the statistical significant changes identified for individual parameters, the effects of each EB dose do not seem to be distinguishable. All in all, when considering all nutritional, chemical and bioactive parameters at once, it does not seem to be relevant differences in using 2, 6 or 10 kGy.

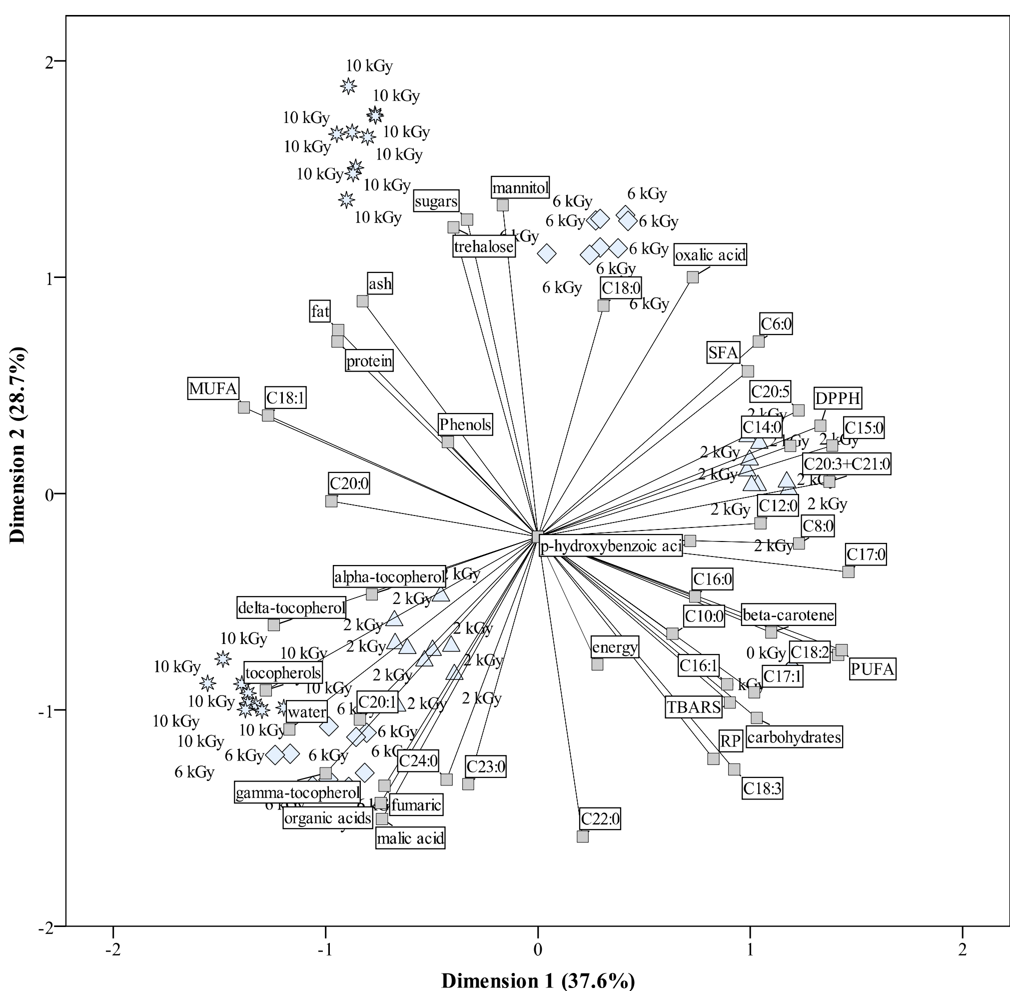

Figure 5.3.1. Biplot of object (electron-beam irradiation doses) scores and component loadings (evaluated parameters).

5.3.4. Conclusions

The effects of different EB irradiation doses were assessed by measuring the changes produced on a wide variety of nutritional, chemical and antioxidant indicators from wild dried *Amanita* mushrooms. The evaluated profiles indicated differences among non-irradiated and irradiated samples, but the produced effect was often advantageous, as in the case of oleic acid or the improvement in the antioxidant activity. Furthermore, when considering all effects at once, it could be concluded that there are no relevant differences when using different irradiation doses. This was a very interesting finding, since it indicated the possibility of using the highest assayed dose (10 kGy), which has, off course, higher effectiveness from the decontamination and disinfestation point of view, without causing more pronounced effects than the lower doses. Accordingly, it would be advised to use the 10 kGy dose, since the treated foods could also be available for persons with particular food safety concerns, and this dose did not cause stronger effects than the lower doses.

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5.3.5. References

AOAC. (1995). *Official methods of analysis* (16th Ed.). Arlington VA, USA: Association of Official Analytical Chemists.

Barros, L., Dueñas, M., Ferreira, I.C.F.R., Baptista, P., & Santos-Buelga, C. (2009). Phenolic acids determination by HPLC-DAD-ESI/MS in sixteen different

Portuguese wild mushrooms species. *Food and Chemical Toxicology*, *47*, 1076-1079

Barros, L., Pereira, C., & Ferreira, I.C.F.R. (2013). Optimized analysis of organic acids in edible mushrooms from Portugal by ultra fast liquid chromatography and photodiode array detection. *Food Analytical Methods*, *6*, 309-316.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P., & Blanco, D. (2012). Evaluation of gamma and electron-beam irradiation on the aromatic profile of black truffle (*Tuber melanosporum*) and summer truffle (*Tuber aestivum*). *Innovative Food Science and Emerging Technologies*, 13, 151-157.

Ezekiel, C.N., Sulyok, M., Frisvad, J.C, Somorin, Y.M., Warth, B., Houbraken, J., Samson, R.A., Krska, R., & Odebode, A.C. (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology*, *162*, 231-236.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Martins, A., Oliveira, M.B.P.P., & Ferreira, I.C.F.R. (2013). Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014). Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. *Innovative Food Science and Emerging Technologies*, 22, 158-166.

Heleno, S. A., Barros, L., Sousa, M. J., Martins, A., & Ferreira, I.C.F.R. (2009). Study and characterization of selected nutrients in wild mushrooms from Portugal by gas chromatography and high performance liquid chromatography. *Microchemical Journal*, *93*, 195-199.

Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., & Ferreira, I.C.F.R. (2010). Tocopherols composition of Portuguese wild mushrooms with antioxidant capacity. *Food Chemistry*, *119*, 1443-1450.

ICGFI - International Consultative Group on Food Irradiation (1999). In Facts about Food Irradiation. Buckinghamshire, United Kingdom.

ICMSF - International Commission on Microbiological Specifications for Foods (1985). Ecologia microbiana de los alimentos 2. Editorial Acribia, Zaragoza, Spain.

Lukasse, L.J.S., & Polderdijk, J.J. (2003). Predictive modelling of post-harvest quality evolution in perishables, applied to mushrooms. *Journal of Food Engineering*, *59*, 191-198.

Ma, L., Chen, H., Zhu, W., & Wang, Z. (2013) Effect of different drying methods on physicochemical properties and antioxidant activities of polysaccharides extracted from mushroom Inonotus obliquus. *Food Research International*, *50*, 633-640.

Patras, A., Brunton, N.P., Downey, G., Rawson, A., Warriner, K., & Gernigon, G. (2011). Application of principal component and hierarchical cluster analysis to classify fruits and vegetables commonly consumed in Ireland based on in vitro antioxidant activity. *Journal of Food Composition and Analysis*, *24*, 250-256.

Reis, F.S., Heleno, S.A., Barros, L., Sousa, M.J., Martins, A., Santos-Buelga, C., & Ferreira, I.C.F.R. (2011). Toward the antioxidant and chemical characterization of mycorrhizal mushrooms from northeast Portugal. *Journal of Food Science*, *76*, 824-830.

Reis, F. S., Barros, L., Martins, A., & Ferreira, I. C. F. R. (2012). Chemical composition and nutritional value of the most widely appreciated cultivated mushrooms: An inter-species comparative study. Food and Chemical Toxicology, 50, 191-197.

Rodríguez, J.A.S., Rodríguez, J.A., Prieto, O.G., Alfonso, A.T., Frade, B.L., Martín, E.A., & Jarauta, T.P. (1991). El género *Amanita* Pers. Ex Hooker en la provincia de León. *Acta Botánica Malacitana*, 16, 123-132.

Şevik, S., Aktaş, M., Doğan, H., & Koçak, S. (2013). Mushroom drying with solar assisted heat pump system. *Energy Conversion and Management*, *72*, 171-178.

Shephard, G.S. (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants*: Part A, *25*, 146-151.

Stewart, E.M. (2001). Food Irradiation Chemistry. Food irradiation: Principles and applications. R. A. Molins. New York, USA, John Wiley & Sons, 37-76.

Supriya, P., Sridhar, K.R, Nareshkumar, S., & Ganesh, S. (2012). Impact of electron beam irradiation on fatty acid profile of *Canavalia* Seeds. *Food and Bioprocess Technology*, *5*, 1049-1060.

5.4

Cogumelos silvestres como fonte de fibra alimentar: Amostras irradiadas por feixe de eletrões

Este sub-capítulo apresenta os efeitos da radiação por feixe de eletrões na composição em fibras de amostras desidratadas de Boletus edulis *Bull. (doses 2, 6 e 10 kGy) e* Macrolepiota procera *(Scop.) (doses 0,5, 1 e 6 kGy).*

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Exquisite wild mushrooms as a source of dietary fiber: Analysis in electron-beam irradiated samples

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Abstract

In the present study, electron-beam irradiation was applied to dried samples of *Boletus edulis* and *Macrolepiota procera* to evaluate the effects on their fiber composition. Both species presented an important percentage of dietary fiber, soluble and insoluble in different ratios. These high fiber levels are an interesting feature, allowing considering mushrooms as an alternative source of dietary fibers in the highly competitive market of fiber-enriched food products. In *B. edulis* samples, insoluble fiber and total fiber amounts were significantly lower in samples irradiated with 10 kGy, but soluble fiber had no significant changes for any of the assayed doses, while total available carbohydrates were significantly lower in unirradiated samples. *M. procera* samples irradiated with 6 kGy presented less total fiber, insoluble fiber and carbohydrates, but the same dose allowed the highest contents in soluble dietary fiber. In general, the irradiated samples, especially for higher doses, gave some significant changes in the total available carbohydrates and dietary fibers content. Nevertheless, the resulting differences still allow considering these species as good natural fiber sources, maintaining their potential health effects, while promoting a clean way to disinfest and decontaminate these highly perishable products.

Keywords: Wild mushrooms; Electron-beam; Soluble/Insoluble Dietary Fiber

5.4.1. Introduction

Dietary fiber and high-fiber food products have attracted attention because of their significant health benefits to consumers (Cheung, 2013; Tungland & Meyer, 2002). Nowadays, most dietary ingredients (such as cereals-based, fruits-based, and legumes-based dietary fiber) are derived from their processing byproducts (*e.g.* milling, juice extraction or de-hulling) followed by different refining steps (*e.g.*, grinding, sieving, bleaching or defatting) to come across a wide range of customers' requirements (McKee & Latner, 2000; O'Shea, Arendt, & Gallagher, 2012; Robin, Schuchmann, & Palzer, 2012). Due to the highly competitive market of fiber enriched food products, exploration of alternative sources of dietary fiber is urgent (Wong & Cheung, 2009).

Mushrooms are highly appreciated for their nutritional (Kalač, 2009) and nutraceuticals properties, being also considered as a novel source of dietary fiber, with various beneficial health effects to humans, since most of the carbohydrates in mushrooms are non-digestible (Cheung, 2013). Carbohydrates are the major components in mushrooms and the total content ranges from 35% to 70% in dry weight. Most of the carbohydrates in mushrooms are non-digestible carbohydrates (dietary fiber) including oligosaccharides (*e.g.*, trehalose) and cell wall polysaccharides (*e.g.* chitin, β-glucans and mannans) (Cheung, 2010; 2013). Mushroom dietary fiber is constituted mainly by insoluble fiber (chitin and β-glucans being the most representative), while the level of-soluble fiber is usually less than 10% dry matter (Cheung, 2013). Insoluble fibers absorb water in the colon that softens and increases the bulk of the stool. This action helps relieving constipation and preventing colon disease and hemorrhoids. While, soluble fiber form a gel and maximize the viscosity of the food matrix, slowing down digestion, which can help lower postprandrial blood glucose, insuline and cholesterol (UWH, 2013, pp. 1-6) and strengthens the immune system and antitumor activity, as well as blood glucose and lipid attenuation (Zhang, Cui, Cheung, & Wang, 2007; Cheung, 2013).

Consumption of edible mushrooms as part of our daily diet in most cases could easily provide up to 25% of the recommended dietary intake of dietary fiber (Cheung, 2010). Nonetheless, mushrooms are rapidly perishable and they start deteriorating within a day after harvest (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Therefore, fresh mushrooms have to be processed to extend their shelf life for off-season use, and drying is a widely used postharvest technology, which overcomes problems related to overproduction and short shelf-life (Walde, Velu, Jyothirmayi, & Math, 2006). However, during the drying process, microorganisms may secrete potentially toxic metabolites and contaminate mushrooms (Shephard, 2008; Ezekiel et al., 2013). In this sense, electron-beam irradiation proved its technological feasibility to be safely used in order to reduce food losses caused by deterioration (namely, by insect pest attack during storage) (Kim, Akram, Ahn, & Kwon, 2012). Moreover, the irradiation proved to be a viable technique for dried food decontamination in alternative to fumigation with microbicidal gases (Farkas, 1998). In fact, extensive research has demonstrated the nutritional safety and practical effectiveness of irradiation, which has also been approved by different international health organizations (WHO 1999; Farkas & Mohacsi-Farkas 2011). Furthermore,

dried mushrooms (grouped in the food additive class and generally used as seasonings) might be treated with doses up to 50 kGy for different technical objectives (ICGGI 1999; Akram & Kwon 2010). The emergent market of irradiated food demands effective identification methods to monitor the irradiated food in the international trade market. These methods must evaluate physical and chemical changes effecting technical and functional properties of food samples to develop a better preservation approach (Akram, Ahn, & Kwon, 2012).

There are some studies about the effects of electron-beam irradiation on nutritional and chemical parameters of wild dried (Fernandes et al., 2014a; 2014b) and cultivated (Duan, Xing, Shao, & Zhao, 2010; Rivera, Blanco, Marco, Oria, & Venturini, 2011) mushrooms, but these studies were never focused on the effects over fiber content. Therefore, in the present study, electron-beam irradiation was applied to dried samples of *Boletus edulis* Bull.: Fr. and *Macrolepiota procera* (Scop.) Singer, two highly appreciated edible mushrooms, in order to evaluate the effects on their fiber composition.

5.4.2. Materials and methods

Samples

M. procera wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011, and dried at 30 ºC in an oven. Subsequently, the samples were divided in four groups with three specimens in each group: control (unirradiated, 0 kGy); sample 1 (0.5 kGy); sample 2 (1 kGy) and sample 3 (6 kGy).

B. edulis wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012. Samples were dried at 30 ºC in an oven and subsequently divided in four groups with six specimens per group: control (unirradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy), kept in polyethylene bags.

Standards and reagents

Glucose standards and fiber enzymatic kit (TDF-100A) were purchased from Sigma (St. Louis, MO, USA). Glutamic acid (used in organic acids analysis) was

purchased from Merck (Darmstadt, Germany). Sulfuric acid, perchloric acid, hydrochloric acid, sodium hydroxide and anthrone reagent and all other general laboratory reagents were obtained from Panreac Quimica S.L.U. (Barcelona, Spain).

Electron-beam irradiation

The irradiation was performed at the INCT- Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, a graphite calorimeter, and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 µs, pulse frequency of 440 Hz and average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min and the scan frequency was 5 Hz. The absorbed dose for *M. procera* was 0.53, 0.83 and 6.10 kGy, with an uncertainty of 20% for the two first doses and 10% for the last dose. The estimated absorbed doses for *B. edulis* were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read the Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a calibrated curve (Fernandes et al., 2014a).

Total available carbohydrate (TAC) assay

The determination of TAC was carried out by the Anthrone method as described by Osborne & Voogt (1986) using 0.25 g of sample. The samples were pre-treated with 13 mL of 52% $HClO_4$ and kept for 18 h in the dark. After this period, distilled water was added, the sample was filtered and the volume of the filtrate was adjusted to 100 mL. Finally, the solution was further diluted to 10%, and 5 mL of 0.1% anthrone solution in 73% H_2SO_4 was added. Samples were kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green color, and absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW ver. 1.2. The

absorbance of the sample solution was compared to a 10-100 µg/mL concentration range standard glucose calibration curve. TAC values were expressed as g/100 g dw sample.

Soluble and insoluble dietary fiber assay

AOAC enzymatic-gravimetric methods (993.19 and 991.42) were used for soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) analysis (Latimer, 2012). In brief, freeze-dried samples were treated with alpha-amylase, protease and amyloglucosidase.

The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C, and protein content was determined in the residue. Total fiber is the sum of soluble and insoluble fiber, both were expressed as g/100 g dw sample.

Statistical analysis

For each irradiation dose and mushroom species, three independent samples were used. Each of the samples was taken after pooling the mushrooms treated in the same conditions together. Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS software, version 22.0 (IBM Corp., USA).

The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

5.4.3. Results and discussion

In previous reports describing the nutritional composition of *B. edulis* and *M. procera*, it could be observed that carbohydrates were the dominant compounds in dry mass basis, varying from 54 - 76% (Fernandes et al., 2014a), 60 - 70% (Fernandes et al., 2013a), 65 - 77% (Fernandes et al., 2013b), and 71 - 78%

(Fernandes et al., 2014b). According to the present work, it is now possible to conclude that an important percentage of those carbohydrates occur naturally as dietary fibers (**Figure 5.4.1A**). These high fiber percentages might be explained by the composition of mushrooms' cell walls, which contain a mixture of fibrillar and matrix components, including chitin (a straight-chain (1→4)-α-linked polymer of *N*-acetyl-glucosamine) and polysaccharides such as (1→3)-α-D-glucans and mannans. In fact, these components are non-digestible carbohydrates resistant to human enzymes and can be considered as dietary fiber (Cheung, 2013; Tungland & Meyer, 2002).

The distribution of total available carbohydrates (TAC; *M. procera*: 23.2 to 33.6 g/100 g DW; *B. edulis*: 19.3 to 27.3 g/100 g DW) and total dietary fibers (*M. procera*: 29.1 to 33.9 g/100 g DW; *B. edulis*: 26.7 to 30.8 g/100 g DW) was similar for both species (**Figure 5.4.1B**). Nevertheless, fiber content is often highly variable among different species: some mushrooms were found to be low in total fiber, *e.g.* for *Craterellus aureus* and *Sarcodon aspratus* values were ≈5 g/100 g DW, while for many others, *e.g.* for *Lactarius volemus* and *Lentinula edodes* up to 40 g/100 g DW contents were reported (Wang et al., 2014).

The fairly high detected levels of fiber in these mushrooms might be considered as a desirable characteristic, since fiber plays an important role in human diet (EFSA, 2010). The Recommended Dietary Allowance (RDA) establish for dietary fiber are 25 - 38 g/100g for women and men, respectively (Trumbo, Schlicker, Yates, & Poos, 2002; EFSA, 2010). In this way, the consumption of the studied edible mushrooms as part of our daily diet could provide interesting amounts of total dietary fiber, because the intake of 100 g (FW) of *M. procera* and *B. edulis* covers important percentages of the RDA. This fact might represent an additional point of interest considering the increasing public awareness of the potential health benefits of dietary fibers, which has greatly encouraged food manufacturers to develop a wide range of fiber-enriched or fiber-fortified food products (Ktenioudaki & Gallagher, 2012).

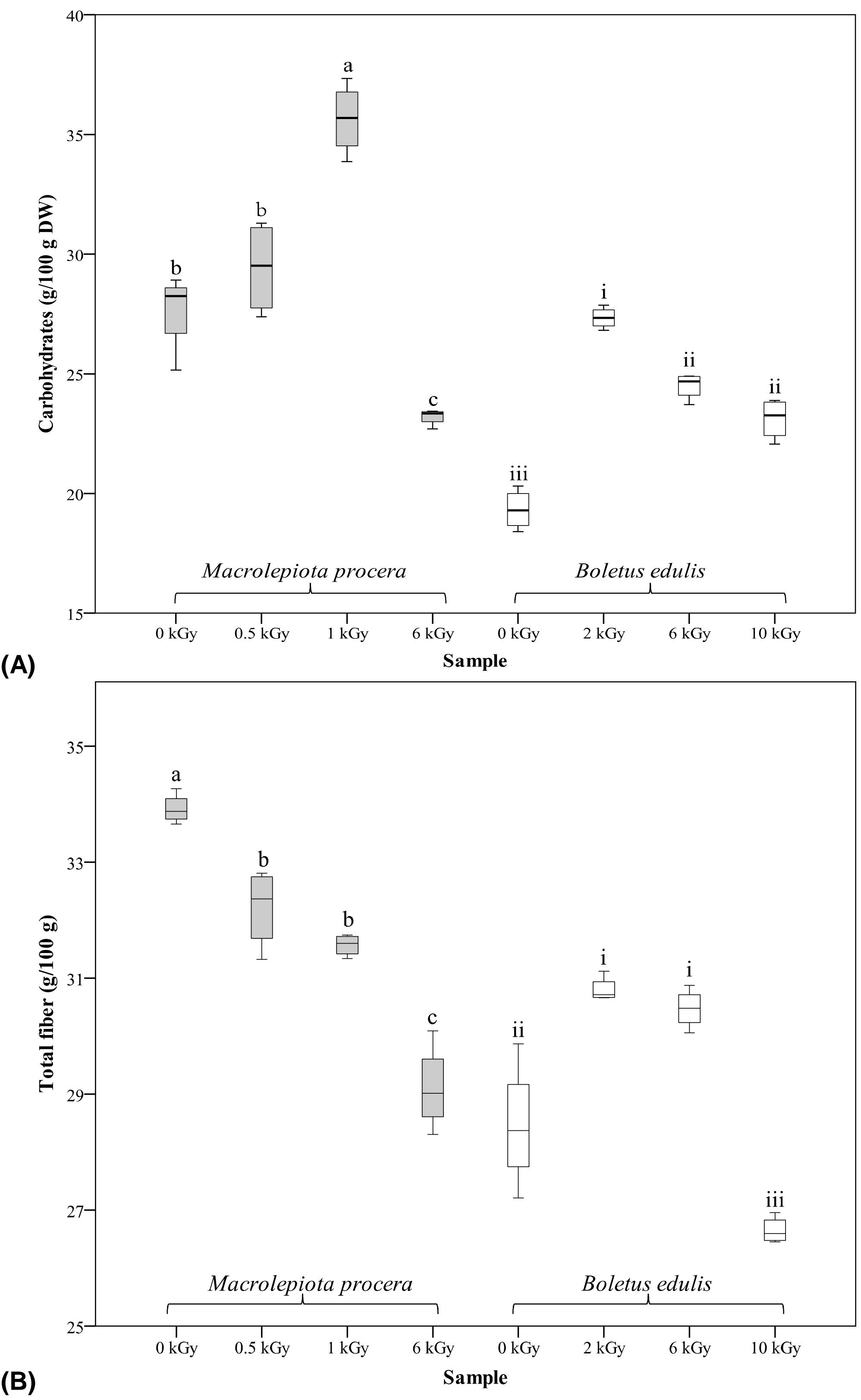

Figure 5.4.1. Boxplot scores for carbohydrates (A) and fibers (B) in *M. procera* and *B. edulis* submitted to different electron-beam irradiation doses.

In a more specific analysis, the results for soluble and insoluble dietary fiber (IDF) contents might be observed in **Table 5.4.1**. In either case, insoluble fibers were clearly dominant, which represents a common feature in mushrooms (Cheung, 2008). According to FEN (2013, p. 80) recommendations, the dietary ratio intake of insoluble:soluble dietary fibers (IDF/SDF) should be between 1.5 to 3. Nevertheless, the assayed species showed differences regarding the ratios (IDF:SDF), *B. edulis* presents an appropriate ratio according to the above recommendation (3:1 ratio), while *M. procera* presented a higher amount of insoluble fiber, with a 7:1 ratio. In a study performed on Italian *Boletus* spp. (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004), the SDF:IDF ratio was similar to the detected herein, but the global amounts (2% and 7% of dry weight, respectively) were quite lower, which might be related with the commercial origin of the samples, since the present study was performed in wild samples.

Considering the importance of dietary fibers, nowadays recognized as functional foods, the application of any decontamination or disinfestation technology, should maintain fiber amounts in the treated samples. Accordingly, the potential changes caused by electron-beam irradiation, were evaluated. Energetic electrons, like gamma- and X-rays, transfer their energy to the irradiated food by ejecting atomic electrons, which can then ionize other atoms in a cascade of collisions. Therefore, these energy sources might ultimately produce similar effects in any irradiated material. Accordingly, the choice for electron-beam application was defined by practical aspects such as the thickness and density of the mushrooms, dose uniformity ratio or economic factors (Cleland, 2013). In addition, the lack of significant effects, as evaluated in different chemical parameters, pointed out electron-beam irradiation as a feasible choice for mushroom disinfestation and decontamination. The selected doses were chosen according to previous results and considering that it was meant to be applied to dried samples (Fernandes et al., 2014a, 2014b).

In terms of dose-dependent changes, the Levene test showed that the assumption of equality among variances ($p > 0.05$) was verified in most cases, allowing classifying the samples according to the Tukey test. In the remaining cases (carbohydrates in *M. procera* and soluble fiber in *B. edulis*), samples were classified using the Tamhanes' T2 test as multiple comparison tool.

Regarding *B. edulis*, insoluble fiber was significantly lower in samples irradiated with 10 kGy (**Table 5.4.1**); the same result was also verified for total fiber amounts

(**Figure 5.4.1**). Irradiation is referred in literature has having a synergetic effect in reducing non-nutritive factors (Al-Kaisey, Alwan, Mohammad, & Saeed; Lima, Souza, Godoy, França, & Lima, 2011). Soluble fiber, on the other hand, had no significant changes ($p > 0.05$) for any of the assayed doses. In general terms, total available carbohydrates (TAC) were significantly lower in unirradiated samples. This could be explained by the fact that gamma and e-beam irradiation have a breaking effect on the constituents of irradiated food products.

In what concerns *M. procera*, samples irradiated with 6 kGy presented less total fiber, insoluble fiber and carbohydrates (**Figure 5.4.1; Table 5.4.1**). However, samples irradiated with the same dose gave also the highest contents in soluble dietary fiber (SDF; **Table 5.4.1**). The detected differenced might be explained because irradiation induces degradation of polysaccharides such as cellulose, starch and pectin (Fan, 2013). Cell wall polysaccharides, particularly cellulose (insoluble) and pectic (soluble) substances are partially degraded by irradiation. Degradation of both pectin and cellulose occur at approximately the dose at which tissue softening could be first demonstrated and progress with increasing dose (Fan, 2013).

In fact, irradiation was previously indicated as causing a dose-dependent degradation of fiber, which might be attributed to the breakage of glycosidic bonds, leading to the formation of carbonyl groups or double bonds (Xu, Sun, Yang, Ding, & Pang, 2007).

Table 5.4.1. Soluble and insoluble fiber content in mushrooms irradiated with different doses of electron-beam. The results are presented as the mean ± SD.

Dose (kGy)	Soluble fiber (g/100 g DW)	Insoluble fiber (g/100 g DW)
Boletus edulis		
0	7.4±0.5	21.0±0.5 b
2	7.8±0.1	23.0±0.3 a
6	7.4±0.5	23.1±0.4 a
10	7.7±0.1	19.0±0.3 c
Levene's test[a]	$p = 0.014$	$p = 0.353$
1-way ANOVA[b]	$p = 0.344$	$p < 0.001$
Macrolepiota procera		
0	3.4±0.1 b	30.5±0.2 a
0.5	3.8±0.4 b	28.4±0.4 b
1	3.6±0.2 b	28.0±0.2 b
6	5.3±0.3 a	23.8±0.5 c
Levene's test[a]	$p = 0.053$	$p = 0.417$
1-way ANOVA[b]	$p < 0.001$	$p < 0.001$

[a] Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, p value > 0.05; heteroscedasticity, p value < 0.05.
[b] $p < 0.05$ meaning that the mean value of soluble or insoluble fiber of at least one dose differs from the others (in this case multiple-comparison tests were performed).

5.4.4. Conclusion

Overall, both mushroom species proved to have potential as dietary fiber sources, raising the possibility of its inclusion in the highly competitive market of fiber-enriched food products, which seriously demands the exploration of alternative source and preparation methods of dietary fibers.

The irradiated samples, especially those treated with higher doses, gave some specific significant changes in the carbohydrates and fibers contents. Nevertheless, the resulting differences still allow considering these species as good natural fiber sources, maintaining their potential health effects, while promoting a clean way to disinfest and decontaminate this highly perishable products. Additional studies on the viscosity of irradiated mushrooms, as well as their digestibility, might be an interesting future approach.

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5.4.5. References

Akram, K., Ahn, J.J., & Kwon, J.H. (2012). Identification and characterization of gamma-irradiated dried *Lentinus edodes* using ESR, SEM, and FTIR analyses. *Journal of Food Science*, *77*, C690-C696.

Akram, K., & Kwon, J.H. (2010). Food irradiation for mushrooms: a review. *Journal of the Korean Society for Applied Biological Chemistry*, *53*, 257-265.

Al-Kaisey, M.T., Alwan, A.-K.H., Mohammad, M.H., & Saeed, A.H. (2003). Effect of gamma irradiation on antinutritional factors in broad bean. *Radiation Physics and Chemistry*, *67*, 493-496.

Cheung, P.C.K. (2013). Mini-review on edible mushrooms as source of dietary fiber: Preparation and health benefits. *Food Science and Human Wellness*, *2*, 162-166.

Cheung, P.C.K. (2010). The nutritional and health benefits of mushrooms. *Nutrition Bulletin*, *35*, 292-299.

Cheung, P.C.K. (2008) Nutritional value and health benefits of mushrooms. In P.C.K. Cheung (Ed.), Mushrooms as Functional Foods (pp 71-110). Hoboken, New Jersey: John Wiley & Sons Inc.

Cleland, M.R. (2013). Advances in electron beam and x-ray technologies for food irradiation. In X. Fan, & C. H. Sommers, (Eds.), *Food Irradiation Research and Technology* (2nd Ed.) (pp. 9-27). New Delhi: Wiley-Blackwell.

Duan, Z., Xing, Z., Shao, Y., & Zhao, X. (2010). Effect of electron-beam irradiation on postharvest quality and selected enzyme activities of the white button

mushroom, *Agaricus bisporus*. *Journal of Agricultural and Food Chemistry*, *58*, 9617-9621.

EFSA - Panel on Dietetic Products, Nutrition, and Allergies (2010). Scientific opinion on dietary reference values for carbohydrates and dietary fibre. *EFSA Journal*, *8*, 1462.

Ezekiel, C.N., Sulyok, M., Frisvad, J.C, Somorin, Y.M., Warth, B., Houbraken, J., et al. (2013). Fungal and mycotoxin assessment of dried edible mushroom in Nigeria. *International Journal of Food Microbiology*, *162*, 231-236.

Fan, X. (2013). Radiation chemistry of food components. In X. Fan, & C. H. Sommers, (Eds.) *Food Irradiation Research and Technology* (2nd Ed.) (pp. 75-97). New Delhi: Wiley-Blackwell.

Farkas, J. (1998). Irradiation as a method for decontaminating food: a review. *International Journal of Food Microbiology*, *44*, 189-204.

Farkas, J., & Mohácsi-Farkas, C. (2011). History and future of food irradiation. *Trends in Food Science and Technology*, *22*, 121-126.

FEN (2013). *Libro blanco de la nutrición en España*. Madrid: Ed. Fundación Española de la Nutrición.

Fernandes, Â., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: a review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014a). Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Technology*, 7, 1606-1617.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014b). Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. *Innovative Food Science and Emerging Technologies*, *22*, 158-166.

Fernandes, Â., Barros, L., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., et al. (2013a). Effects of different processing technologies on chemical and antioxidant parameters of *Macrolepiota procera* wild mushroom. *LWT- Food Science and Technology*, *54*, 493-499.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Santos, P.M.P., Martins, A., Oliveira, M.B.P.P., et al. (2013b). Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Research International*, *54*, 18-25.

International Consultative Group on Food Irradiation (ICGGI). (1999). Facts about food irradiation. Buckinghamshire, United Kingdom. Available from http://www.iaea.org/Publications/Booklets/foodirradiation.pdf.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: a review. *Food Chemistry*, *113*, 9-16.

Kim, G.-R., Akram, K., Ahn, J.-J., & Kwon, J.-H. (2012). Identification of gamma ray and electron-beam irradiated wheat after different processing treatments. *Journal of Cereal Science*, *56*, 347-351.

Ktenioudaki, A., & Gallagher, E. (2012). Recent advances in the development of high-fibre baked products. *Trends in Food Science and Technology*, *28*, 4-14.

Latimer, G. W. (2012). Official methods of analysis of AOAC international (18th ed.). Gaithersburg: EEUU.

Lima, K.S.C., Souza, L.B., Godoy, R.L.O., França, T.C.C., & Lima, A.L.S. (2011). Effect of gamma irradiation and cooking on cow pea bean grains (*Vigna unguiculata* L. Walp). *Radiation Physics and Chemistry*, *80*, 983-989.

Manzi, P., Marconi, S., Aguzzi, A., & Pizzoferrato, L. (2004). Commercial mushrooms: nutritional quality and effect of cooking. *Food Chemistry*, *84*, 201-206.

McKee, L.H., & Latner, T.A. (2000). Underutilized sources of dietary fiber: a review. *Plant Foods for Human Nutrition*, *55*, 285-304.

O'Shea, N., Arendt, E.K., & Gallagher, E. (2012). Dietary fibre and phytochemical characteristics of fruit and vegetable by-products and their recent applications as novel ingredients in food products. *Innovative Food Science and Emerging Technologies*, *16*, 1-10.

Osborne, D.R., & Voogt, P. (1985). *Análisis de los nutrientes de los alimentos*. (p. 258). Zaragoza: Ed. Acribia.

Rivera, C.S., Blanco, D., Marco, P., Oria, R., & Venturini, M.E. (2011). Effects of electron-beam irradiation on the shelf life, microbial populations and sensory characteristics of summer truffles (*Tuber aestivum*) packaged under modified atmospheres. *Food Microbiology*, *28*, 141-148.

Robin, F., Schuchmann, H.P., & Palzer, S. (2012). Dietary fiber in extruded cereals: Limitations and opportunities. *Trends in Food Science and Technology*, *28*, 23-32.

Shephard, G.S. (2008). Impact of mycotoxins on human health in developing countries. *Food Additives and Contaminants, Part A, Chemistry, analysis, control, exposure and risk assessment*, *25*, 146-151.

Trumbo, P., Schlicker, S., Yates, A.A., & Poos, M. (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of American Dietetics Association*, *102*, 1621-1630.

Tungland, B., & Meyer, D. (2002). Nondigestible oligo and polysaccharides (dietary fiber): Their physiology and role in human health and food. *Comprehensive Reviews in Food Science and Food Safety*, *1*, 90-109.

UWH - University of Wisconsin Hospitals and Clinics Authority. (2013). *Health Information: Health Facts for You.* Available from: http://www.uwhealth.org/healthfacts/nutrition/190.html.

Walde S.G., Velu, V., Jyothirmayi, T., & Math, R.G. (2006). Effects of pretreatments and drying methods on dehydration of mushroom. *Journal of Food Engineering*, *74*, 108-115.

Wang, X.-M., Zhang, J., Wu, L.-H., Zhao, Y.-L., Li. T., Li, J.-Q., et al. (2014). A mini-review of chemical composition and nutritional value of edible wild-grown mushroom from China. *Food Chemistry*, *151*, 279-285.

WHO. (1999). High-dose irradiation: wholesomeness of food irradiated with doses above 10 kGy. Report of a Joint FAO/IAEA/WHO Study Group on High Dose Irradiation. Geneva, Switzerland: WHO Technical Report Series, World Health Organization.

Wong, K.-H., & Cheung, P.C.-K. (2009). Enzymatic preparation of mushroom dietary fibre: A comparison between analytical and industrial enzymes. *Food Chemistry*, *115*, 795-800.

Xu, Z., Sun, Y., Yang, Y., Ding, J., Pang, J. (2007). Effect of γ-irradiation on some physiochemical properties of konjac glucomannan. *Carbohydrate Polymers*, *70*, 444-450.

Zhang, M., Cui, S.W., Cheung, P.C.K., & Wang, Q. (2007). Antitumor polysaccharides from mushrooms: a review on their isolation process, structural characteristics and antitumor activity. *Trends in Food Science and Technology*, *18*, 4-19.

CAPÍTULO 6.

Efeitos comparativos da radiação gama e feixe de eletrões em parâmetros químicos e bioativos de cogumelos

Este capítulo apresenta os efeitos comparativos da radiação gama e feixe de eletrões em parâmetros químicos e bioativos de cogumelos silvestres Boletus edulis *Bull.*, Boletus pinophilus *Pilát & Dermek*, Macrolepiota procera *(Scop.) Singer e* Russula delica *Fr.*

6.1

Perfil de triacilgliceróis como uma ferramenta química para identificar cogumelos submetidos a irradiação gama ou feixe de eletrões

Este sub-capítulo apresenta o potencial do perfil em triacilgliceróis como marcador para deteção de cogumelos silvestres irradiados: utilizaram-seamostras frescas de Boletus edulis *Bull. (doses 1 e 2 kGy) e* Boletus pinophilus *Pilát & Dermek (2 kGy) submetidas a radiação gama;* amostras frescas, desidratadas e congeladas de Macrolepiota procera *(Scop.) Singer submetidas a radiação gama (doses 0,5 e 1 kGy); amostras desidratadas de* B. edulis *Bull. e* Russula delica *Fr. (doses 2, 6 e 10 kGy) submetidas a radiação por feixe de eletrões.*

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Triacylglycerols profiling as a chemical tool to identify mushrooms submitted to gamma or electron-beam irradiation

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Abstract

In order to define irradiation treatment as a routine conservation methodology, it is imperative to develop chemometric indicators with the ability to distinguish irradiated from unirradiated foodstuffs. Electron spin resonance, photostimulated luminescence and thermoluminescence methods were employed to monitor radiation-induced markers, as well as different chemical compounds produced from the lipidic fraction of different foodstuffs. Otherwise, the specificity of triacylglycerol profiles was previously detected in mushroom species, as also the effect of irradiation

treatment in the triacylglycerol profiles of chestnut. Accordingly, its feasibility as chemometric indicator of irradiated mushrooms was evaluated. In line with the obtained results, the effects of each type of irradiation were significantly different, as it can be concluded from the correlations among discriminant functions and variables within each statistical test. Triacylglycerol profile proved to be a useful tool to detect irradiated mushrooms, independently of the species or irradiation source, especially for doses above 1 kGy.

Keywords: Triacylglycerols; wild mushrooms; gamma irradiation; electron-beam; chemometrics.

6.1.1. Introduction

Mushrooms are widely appreciated foods due to their nutritional, organoleptic (Kalač, 2009) and pharmacological properties (Lindequist, Niedermeyer & Jülich, 2005). Nevertheless, mushrooms shelf life is very short due to several postharvest changes related to the high respiration rate and lack of physical protection to avoid water loss or bacteria and moulds attack, which results in weight loss and browning (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Irradiation is a conservation/preservation technique that can minimize the mentioned losses, contributing to extend foods shelf life and reducing health hazards (Soika & Delincée, 2000).

The specific effects of radiation on mushrooms chemical composition and antioxidant activity have been progressively studied by our research group, either using gamma irradiation (Fernandes et al., 2013a) or electron-beam treatment (Fernandes et al., 2013b).

The existence of tests capable of distinguishing irradiated from unirradiated foodstuffs is imperative, in order to regulate international trade and guarantee freedom of choice to the consumer (Ndiaye, Jamet, Miesch, Hasselmann, & Marchioni, 1999).

The European Committee for Standardization validated methods to identify irradiated foods; these methods are based on the study of primary radiolytic products by Electron Paramagnetic Resonance (EPR) and thermoluminescence, or on the analysis of certain chemical compounds (*e.g.*, volatile hydrocarbons and 2-

alkylcyclobutanones) formed by the radiolysis of triglycerides (Ndiaye et al., 1999). The European Union (EU) adopted Directives 1999/2/EC and 1999/3/EC to standardize the rules of processing and marketing of irradiated foods in countries of EU for consumer protection and information (Alberti et al., 2011). At the European level, there are official protocols for the electron spin resonance (ESR) detection of irradiated foodstuffs containing bone structures (EN 1786, 1996; Alberti et al., 2011), cellulose (EN 1787, 2000; Alberti et al., 2011) or crystalline sugar (EN 13708, 2001; Alberti et al., 2011). Several ESR studies were made for the identification of irradiated seafood: fishes, crustacean, shrimps and mollusks (Alberti et al., 2011). Regarding fatty foods, the main methods are based on the chemical determination of compounds formed from the irradiation of lipid components; 2-alkylcyclobutanones (2-ACBs) are produced by the irradiation of fatty acids and glycerides (Crews, Driffield, & Thomas, 2012). 2-Dodecylcyclobutanone (2-DCB) (Blanch, Caja, Flores, & Castillo, 2009), produced from palmitic acid specifically by radiolysis (Ndiaye et al., 1999) and the alkane hydrocarbons were used as irradiation markers in sliced dry-cured ham.

These two compounds were evaluated by solid phase microextraction (SPME)-gas chromatography-mass spectrometry (GC-MS) (Blanch et al., 2009). Otherwise, gamma irradiation of papaya resulted in the appearance of a new peak in the GC-MS, which was identified as phenol, functioning as a marker of this irradiated food (Chatterjee, Variyar, & Sharma, 2012). Photostimulated luminescence (PSL) and thermoluminescence (TL) methods were also employed to monitor radiation-induced markers in gamma ray and electron-beam irradiated wheat after different processing treatments (Kim, Akram, Ahn, & Kwon, 2012).

Triacylglycerols (TAG) profile is specific of each natural matrix and it has been used for detecting adulteration of fats and oils, crystallization and recognition of oil origins, being one of the prime determinants in the study of oil oxidation (Zeb, 2012; Barreira et al. 2013). It can also act as a quality marker in roasted coffee (Toci, Neto, Torres, & Farah, 2013) and was also pointed out as a chemical taxonomical marker for mushrooms (Barreira, Ferreira, Oliveira, & 2012).

Therefore, the potential of using TAG profile as a marker for detecting irradiated foods and, in particular, mushrooms, was evaluated. In order to achieve a high broad irradiation marker, samples from mushrooms submitted to different industrial processing, irradiation type and dose were used.

6.1.2. Materials and methods

Standards and reagents

Triacylglycerols 1,2,3-tripalmitoylglycerol (PPP), 1,2,3-tristearoylglycerol (SSS), 1,2,3-trilinolenoylglycerol (LnLnLn), and 1,2,3-tripalmitoleoylglycerol (PoPoPo), of purity > 98%, and 1,2,3- trioleoylglycerol (OOO), 1,2,3-trilinoleoylglycerol (LLL), 1,2-dilinoleoyl- 3-palmitoyl-*rac*-glycerol (PLL), 1,2-dilinoleoyl-3-oleoyl-*rac*-glycerol (OLL), 1,2-dipalmitoyl-3-oleoyl-*rac*-glycerol (PPO), 1,2-dioleoyl-3-stearoyl-*rac*-glycerol (SOO), 1-palmitoyl-2-oleoyl-3-linoleoylglycerol (POL), and 1,2-dioleoyl-3-palmitoyl-*rac*-glycerol (POO), of ≈99% purity, were purchased from Sigma (St. Louis, MO, USA). Petroleum ether was analytical grade and obtained from Fisher Scientific (Leicestershire, UK). Acetonitrile and acetone were HPLC grade and obtained from Merck (Darmstadt, Germany). The code letters used for the fatty acids are: L, linoleic; Ln, linolenic; O, oleic; P, palmitic; Po, palmitoleic; S, stearic.

Samples

Macrolepiota procera, *Boletus edulis, Russula delica* and *Boletus pinophilus* were collected in Trás-os-Montes, in the Northeast of Portugal; the first mushroom specie were collected in November 2011 and the other three species were collected in November 2012.

M. procera fruiting bodies were divided in three groups with nine mushrooms per group, and further submitted to different processing technologies: freezing (at -20º C in a freezer), drying (at 30 ºC in an oven) and the third group was kept fresh (stored at 4 ºC in a refrigerator). Each group was further subdivided in three subgroups: control (non-irradiated, 0 kGy); sample 1 (irradiated with 0.5 kGy) and sample 2 (irradiated with 1 kGy).

B. edulis fruiting bodies were divided in two groups, and further submitted to drying (at 30 ºC in an oven) or kept fresh (stored at 4 ºC in a refrigerator). Drying samples group was then subdivided in four subgroups submitted to electron-beam irradiation: control (non-irradiated, 0 kGy), sample 1 (irradiated with 2 kGy), sample 2 (irradiated with 6 kGy) and sample 3 (irradiated with 10 kGy) with six mushrooms per subgroup; fresh samples group was subdivided in three subgroups treated with

gamma irradiation: control (non-irradiated, 0 kGy), sample 1 (irradiated with 1 kGy) and sample 2 (irradiated with 2 kGy), with 3 mushrooms per subgroup

Besides the former mushrooms, which stand among the species producing in highest quantity, two additional species were studied. A second Boletus species (*B. pinophilus*) was studied following the same sampling used for *B. edulis* fresh samples, except the intermediate dose, which was not tested.

A brittle gill mushroom (*R. delica*) was also studied as an example of a less appreciated, despite edible, species. The same sampling as that used for *B. edulis* dried samples was followed, except for the higher number (6) of samples per group.

All the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples for subsequent analysis.

Samples irradiation

Gamma irradiation

The irradiation of the samples was performed in a Co-60 experimental chamber with four sources, total activity 198 TBq (5.33 kCi), (Precisa 22, Graviner Manufacturing Company Ltd, U.K.). After irradiation geometry dose rate estimation, using the Fricke dosimeter and the procedure described in the standards (ASTM, 1992), all groups were placed in Poly(methyl methacrylate) (PMMA) box, or acrylic glass, and irradiated at ambient atmosphere and temperature (15 ºC). To monitor the process during the irradiation, 4 routine dosimeters were used for each group for the higher dose (Amber Perspex dosimeters, batch V, from Harwell company, U.K.). The samples were rotated upside down (180º) at half of the time, to increase the dose uniformity. The Amber Perspex dosimeters were read in a UV-VIS Spectrophotometer (Shimadzu mini UV 1240 spectrophotometer) at 603 nm, two readings for each, to estimate the dose according to a previous calibration curve.

The estimated doses after irradiation for *M. procera* were 0.6±0.1 kGy and 1.1±0.1 kGy for samples 1 and 2, respectively, at a dose rate of 2.3 kGy h^{-1}. The estimated doses and dose rates were: 1.14±0.23 kGy, 1.71 kGy h^{-1} and 1.99±0.32 kGy, 1.49 kGy h^{-1} for *B. edulis* sample 1 and 2 respectively; for *B. pinophilus,* the estimated doses and dose rates were: 2.09±0.16 kGy and 1.57 kGy h^{-1}.

Electron-beam irradiation

For *B. edulis* and *R. delica* the irradiation was performed at the INCT - Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used a standard dosimeter, graphite calorimeter, and two routine dosimeters, Gammachrome YR and Amber Perspex, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 µs, a pulse frequency of 440 Hz, and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min and the scan frequency was 5 Hz. The estimated absorbed doses were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the Graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a previous calibration curve (Carocho et al., 2012).

Triacylglycerols analysis

Each sample (~3 g) was then submitted to an extraction with petroleum ether (40-60 ºC) performed in Soxhlet apparatus for 1.5 h. The chromatographic analyses were carried out according to the procedure previously described (Barreira, Casal, Ferreira, Oliveira, & Pereira, 2009), with a Jasco (Tokyo, Japan) HPLC system, equipped with a PU-1580 quaternary pump and a Jasco AS-950 automatic sampler with a 10 µL loop. Detection was performed with an evaporative light-scattering detector (ELSD) (model 75-Sedere, Alfortville, France). The chromatographic separation of the compounds was achieved with a Kromasil 100 C_{18} (5 µm; 250 mm × 4.6 mm) column (Teknokroma, Barcelona, Spain) operating at room temperature (≈20 ºC). The mobile phase was a mixture of acetone and acetonitrile (70:30), in an isocratic mode, at an elution rate of 1 mL/min. Detection was performed with an evaporative light-scattering detector (ELSD) (model 75-Sedere, Alfortville, France) with the following settings: evaporator temperature 40 ºC, air pressure 3.5 bar and photomultiplier sensitivity 6. Taking into account the selectivities (R, relative retention times to LLL), peaks were identified according to the logarithms of R in relation to

homogeneous TAG standards. Quantification of the peaks was made by internal normalization of chromatographic peak area, and the results were expressed in relative percentage, assuming that the detector response was the same for all the compounds within each analysis. Data were analyzed using the Borwin-PDA Controller Software (JMBS, France).

Statistical analysis

For each combination of processing technology, irradiation type and dose, three samples were analysed, with all the assays being also carried out in triplicate. Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS software, version 18.0.

Analysis of variance

The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively. Results obtained for *B. pinophilus* were classified using a simple *t*-test for equality of means (after checking the equality of variances through a Levene's test), since there were fewer than three groups.

Stepwise Linear Discriminant Analysis (LDA)

LDA was used to check for significant differences in TAG profiles in result of being submitted to different processing technologies, irradiation types and doses. A stepwise technique, using the Wilks' λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove), was applied to select variables. This procedure follows a combination of forward selection and backward elimination steps; *i.e.*, before a new variable is selected to be included, it is verified whether all previously selected variables remain significant (Cunha & Oliveira, 2006; Hill & Lewicki, 2006). The combination of varieties is defined in a way that the first function furnishes the most

general discrimination between groups, the second provides the second most, and so on (López, García, & Garrido, 2008). To verify which canonical discriminant functions were significant, the Wilks' λ test was applied. To keep a more realistic data modulation, a leave-one-out cross-validation procedure was carried out to assess the model performance. Moreover, the sensibility and specificity of the discriminant model were computed from the number of individuals correctly predicted as belonging to an assigned group (Benitez, Nogales, Campos, & Ruano, 2006). Sensibility was calculated by dividing the number of samples of a specific group correctly classified by the total number of samples belonging to that specific group. Specificity was calculated by dividing the number of samples of a specific group classified as belonging to that group by the total number of samples of any group classified as belonging to that specific group.

6.1.3. Results and discussion

In a previous work conducted to evaluate the usefulness of mushrooms' triacylglycerol (TAG) profile as a chemical fingerprint for different taxonomic ranks, the highest intrinsic differences were found among mushroom species, indicating a high degree of specificity, possibly derived from the genetic control of the stereospecific distribution of fatty acids on the glycerol molecule (Barreira, Ferreira, & Oliveira, 2012). In addition, the effects of gamma and electron-beam irradiation on TAG profiles were also reported as being significant, especially with the highest doses (1 and 3 kGy in both cases), in a study performed on chestnut samples.

Accordingly, TAG profiles in mushrooms submitted to different irradiation types and doses were characterized. Four mushroom species were selected, using samples processed according to the most common industrial availability (fresh, dried and frozen) for wild edible mushrooms.

Table 6.1.1 shows the mean values obtained for TAG profiles of each mushroom species, according to the processing type and irradiation treatment. The values are presented in relative percentage due to limitations in the availability of high-purity standards. Nevertheless, the peak areas might be readily converted into relative TAG concentration, assuming linearity and uniformity of the detector signal, regardless of the TAG molecule and absolute concentration (Rombaut, De Clercq, Foubert, & Dewettinck, 2009). The usual chromatographic elution order is ttt, stt, mtt,

stt, ddt, mdt, sdt, ddd, mmt, smt, mdd, sst, sdd, mmd, smd, mmm, ssd, smm, ssm and sss (s = saturated, m = monoenoic, d = dienoic and t = trienoic acids (Fuchs et al., 2011). TAGs quantified in **Table 6.1.1** followed the expected order: LLnLn (dtt), LLL (ddd), OLL (mdd), PLL (sdd), OOL (mmd), POL (smd), OOO (mmm), POO (smm), PPO (ssm), SPO (ssm) and SPP (sss), except for SOO (smm).

Table 6.1.1. Triacylglycerol profiles according to processing type, irradiation source and dose. The results are presented as the mean ± SD[1].

Processing	Irradiation	Dose (kGy)	LLnLn	LLL	OLL	PLL	OOL	POL	OOO	POO	PPO	SOO	SPO	SPP
Boletus edulis														
		0.0	nd	10.0±0.5	11.0±0.5 b	2.0±0.2	23.6±0.3 b	21.5±0.5 b	15.5±0.4 b	nd	15.6±0.1 a	0.7±0.1	nd	nd
Fresh	Gamma	1.0	nd	9.7±0.5	10.5±0.1 b	1.7±0.1	25.2±0.1 a	19.8±0.2 c	17.5±0.3 a	nd	14.8±0.5 ab	0.8±0.1	nd	nd
		2.0	nd	10.6±0.3	12.3±0.2 a	1.8±0.4	22.3±0.3 c	24.6±0.5 a	13.5±0.3 c	nd	14.2±0.5 b	0.6±0.2	nd	nd
Levene test[1]			-	$p = 0.631$	$p = 0.034$	$p = 0.127$	$p = 0.347$	$p = 0.020$	$p = 0.430$	-	$p = 0.043$	$p = 0.225$	-	-
1-way ANOVA[2]			-	$p = 0.051$	$p = 0.001$	$p = 0.213$	$p < 0.001$	$p < 0.001$	$p < 0.001$	-	$p = 0.007$	$p = 0.457$	-	-
		0.0	nd	10.4±0.5 c	11±1 bc	1.9±0.2	24.7±0.5 a	19.5±0.2 ab	14.8±0.3 a	nd	17.1±0.3 ab	nd	nd	nd
Dried	E-beam	2.0	nd	11.7±0.5 b	10.4±0.4 c	1.9±0.3	23.5±0.4 b	19.8±0.1 a	15.0±0.2 a	nd	17.7±0.5 a	nd	nd	nd
		6.0	nd	13.1±0.2 a	12.6±0.3 ab	1.8±0.1	22.3±0.4 c	19.8±0.1 a	13.6±0.1 b	nd	16.9±0.3 b	nd	nd	nd
		10.0	nd	12.5±0.4 ab	13±1 a	1.6±0.4	22.8±0.4 bc	19.2±0.2 b	14.2±0.4 b	nd	16.8±0.4 b	nd	nd	nd
Levene test[1]			-	$p = 0.441$	$p = 0.274$	$p = 0.040$	$p = 0.849$	$p = 0.122$	$p = 0.159$	-	$p = 0.210$	-	-	-
1-way ANOVA[2]			-	$p < 0.001$	$p = 0.001$	$p = 0.236$	$p < 0.001$	$p = 0.001$	$p < 0.001$	-	$p = 0.023$	-	-	-
Boletus pinophilus														
Fresh	Gamma	0.0	nd	0.25±0.01	7.8±0.3	nd	22.2±0.1	1.6±0.3	35.2±0.5	4.4±0.5	0.040±0.001	21.0±0.5	6.7±0.5	0.68±0.04
		2.0	nd	0.28±0.05	8.7±0.4	nd	23.0±0.5	1.57±0.04	34.1±0.1	5.5±0.2	0.031±0.003	20.0±0.4	5.9±0.2	0.99±0.05
Independent samples *t*-test			-	$p = 0.044$	$p = 0.556$	-	$p = 0.005$	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.079$	$p = 0.950$	$p = 0.337$	$p = 0.564$
1-way ANOVA[2]			-	$p = 0.297$	$p = 0.015$	-	$p = 0.036$	$p = 0.722$	$p = 0.019$	$p = 0.016$	$p = 0.002$	$p = 0.019$	$p = 0.019$	$p < 0.001$
Macrolepiota procera														
		0.0	0.76±0.05	5±1 ab	13±1 a	4.6±0.5	21±1	9.7±0.4 b	4.9±0.5 ab	24.6±0.4 b	17.1±0.3 b	nd	nd	nd
Fresh	Gamma	0.5	0.80±0.05	3.5±0.2 b	11.5±0.5 b	4.0±0.4	21.3±0.5	10.2±0.3 ab	5.6±0.1 a	25.5±0.3 a	17.6±0.2 a	nd	nd	nd
		1.0	0.87±0.04	6.0±0.5 a	10.8±0.3 b	3.6±0.2	20.4±0.1	10.8±0.1 a	4.1±0.1 b	25.7±0.3 a	17.8±0.2 a	nd	nd	nd
Levene test[1]			$p = 0.346$	$p = 0.112$	$p = 0.117$	$p = 0.148$	$p = 0.298$	$p = 0.136$	$p = 0.029$	$p = 0.794$	$p = 0.794$	-	-	-
One-way ANOVA			$p = 0.093$	$p = 0.002$	$p = 0.001$	$p = 0.068$	$p = 0.064$	$p = 0.003$	$p = 0.001$	$p = 0.004$	$p = 0.004$	-	-	-
		0.0	0.94±0.05 ab	7±1 ab	10.2±0.2 b	3.8±0.3 a	20.0±0.5 a	11.1±0.3 a	3.9±0.1 b	25.5±0.3 c	17.6±0.2 c	nd	nd	nd
Dried	Gamma	0.5	0.99±0.04 a	5±1 b	11.0±0.5 a	3.5±0.5 ab	20±1 a	10.1±0.5 b	4.7±0.5 ab	26.3±0.4 b	18.2±0.3 b	nd	nd	nd
		1.0	0.87±0.04 b	7±1 a	10.1±0.4 b	2.7±0.3 b	18.8±0.2 b	9.3±0.4 c	5.2±0.3 a	26.9±0.1 a	18.6±0.1 a	nd	nd	nd
Levene test[1]			$p = 0.223$	$p = 0.428$	$p = 0.369$	$p = 0.065$	$p = 0.003$	$p = 0.638$	$p = 0.033$	$p = 0.044$	$p = 0.034$	-	-	-
1-way ANOVA[2]			$p = 0.030$	$p = 0.041$	$p = 0.016$	$p = 0.045$	$p = 0.018$	$p = 0.001$	$p = 0.006$	$p < 0.001$	$p < 0.001$	-	-	-
		0.0	0.82±0.05	8±1	8.8±0.5 b	3.6±0.5	19.5±0.5	10±1 b	5.5±0.3 a	26.1±0.4 a	18.1±0.3 a	nd	nd	nd
Frozen	Gamma	0.5	0.95±0.01	6±1	11±1 a	4.5±0.3	19.7±0.5	10.5±0.5 ab	4.6±0.4 b	25.2±0.4 b	17.5±0.2 b	nd	nd	nd
		1.0	0.88±0.05	8±2	9.4±0.5 ab	3±1	21±1	12±1 a	4.2±0.2 b	24.9±0.1 b	17.2±0.1 b	nd	nd	nd
Levene test[1]			$p = 0.164$	$p = 0.058$	$p = 0.171$	$p = 0.221$	$p = 0.798$	$p = 0.851$	$p = 0.215$	$p = 0.135$	$p = 0.129$	-	-	-
1-way ANOVA[2]			$p = 0.073$	$p = 0.196$	$p = 0.017$	$p = 0.051$	$p = 0.080$	$p = 0.023$	$p = 0.001$	$p = 0.002$	$p = 0.001$	-	-	-
Russula delica														
		0.0	nd	0.28±0.02 b	26±2 a	nd	32.5±0.4	12.5±0.2 a	14±1 c	12±1 b	0.28±0.02 a	0.8±0.1 c	0.55±0.03 d	nd
Dried	E-beam	2.0	nd	0.13±0.01 d	25±1 ab	nd	32±1	10.2±0.1 c	18.0±0.4 a	11.8±0.2 b	0.27±0.01 ab	2.15±0.03 b	0.77±0.02 c	nd
		6.0	nd	0.21±0.01 c	24.4±0.4 b	nd	31.2±0.5	10.6±0.2 c	15.2±0.2 bc	12.4±0.2 b	0.24±0.01 b	2.6±0.2 a	3.2±0.1 b	nd
		10.0	nd	0.35±0.03 a	22.2±0.3 c	nd	32±1	11.7±0.2 b	15.7±0.4 b	13.5±0.5 a	0.30±0.03 a	1.01±0.03 c	3.13±0.02 a	nd
Levene test[1]			-	$p = 0.054$	$p = 0.037$	-	$p = 0.033$	$p = 0.534$	$p = 0.005$	$p = 0.001$	$p = 0.055$	$p = 0.040$	$p < 0.001$	-
1-way ANOVA[2]			-	$p < 0.001$	$p < 0.001$	-	$p = 0.108$	$p < 0.001$	$p < 0.001$	$p = 0.002$	$p = 0.009$	$p < 0.001$	$p < 0.001$	-

[1]Homoscedasticity among cultivars was tested by means of the Levene test: homoscedasticity, p value > 0.05; heteroscedasticity, p value < 0.05. [2]$p < 0.05$ meaning that the mean value of the evaluated TAG of at least one dose differs from the others (in this case multiple-comparison tests were performed).

In this first set of data, mean values were calculated for each irradiation dose, irradiation source and processing type. As a preliminary overview, it is possible to conclude that the effect of electron-beam irradiation is more pronounced than that obtained for gamma irradiation (except for dried *M. procera* samples), as it can be observed by the *p*-values for 1-way ANOVA test. However, this result might be explained by the higher doses used in electron-beam irradiation, instead of the irradiation source. Following the same reasoning, changes in dried samples were more evident than those verified in fresh or frozen samples.

Regarding some particular changes, the Levene test showed that the assumption of equality among variances could be made in most cases, allowing applying Tukey test as a multiple comparison test. In the remaining cases samples were classified by means of the Tamhanes' T2 test.

Fresh *B. edulis* samples submitted to gamma irradiation presented higher percentages of OLL and POL and less OOL, OOO and PPO, especially for 1 kGy dose. The effects of electron-beam in dried samples of the same mushroom were particularly observable for 6 and 10 kGy doses, which produced similar changes: higher percentages in LLL and OLL and lower percentages in OOL, OOO and PPO.

Results obtained for *B. pinophilus* were classified using a simple *t*-test for equality of means (after checking the equality of variances through a Levene's test), since less than three groups were available. OLL, OOL and POO showed to be significantly higher in irradiated samples, while OOO, PPO, SOO and SPO presented higher values in unirradiated samples.

Concerning *M. procera*, the effects of gamma irradiation were more marked in dried samples, since the mean value of at least one dose differs from the others for all TAG. Among fresh samples, irradiation tended to increase LLL, POL, POO and PPO percentages; the same effect was observable for LLL, OOO, POO and PPO in dried samples and OLL and POL in frozen samples. On the other hand, fresh unirradiated samples presented higher contents in OLL, PLL, dried unirradiated samples in OOL and POL, and unirradiated frozen samples in OOO, POO and PPO.

In line with the observed for *B. edulis*, the effects of electron-beam irradiation were significant for most TAG molecules quantified in *R. delica* samples: LLL, POO and SPO had maximum percentages in samples irradiated with a 10 kGy dose, while OLL and POL were highest in unirradiated samples, which simultaneously presented the least values in OOO, SOO and SPO.

The significant differences found among the mean values for each TAG were a good preliminary indicator of TAG profiles' ability to act as an irradiation treatment indicator. This assumption was checked by applying different linear discriminant analyses (LDA), chosen as a supervised classification technique. Primarily, it was intended to verify if the significant differences among irradiated and unirradiated samples could be enough to recommend TAG analysis as a reliable indicator of irradiation treatment independently of general state of mushrooms (fresh, dried or frozen), irradiation type (gamma or electron-beam) and irradiation doses (0, 0.5, 1.0, 2.0, 6.0 or 10.0, depending on each case).

Since the driving force was finding differences among irradiated and unirradiated samples in a general way, and the assayed mushrooms presented qualitative and quantitative differences in their TAG profiles, the set of data was normalized to overcome unrealistic results derived from biased results such as differences in TAG profiles of each control. The applied normalization consisted of calculating relative differences among each quantified value (irradiated samples) and its respective control (unirradiated samples). Hence, LDA was applied to the relative differences found for all measurements and not to the absolute values presented in **Table 6.1.1**.

The significant independent variables (TAG) were selected using the stepwise procedure of the LDA, according to the Wilks' ë test. Only variables with a statistically significant classification performance ($p < 0.05$) were kept in the analysis.

In the first approach, all doses (for both irradiation types) were used as grouping variables. In this case, 5 significant functions were defined, from which the first three were plotted (**Figure 6.1.1**). The three plotted functions integrated 95.1% of the observed variance (first, 68.1%; second, 16.5%; third, 10.5%). As can be observed, the naturally occurring groups (each assayed dose) were not individually clustered. Nevertheless, the classification performance was satisfactory, resulting in 69% of correctly classified samples (sensitivity) within the leave-one-out cross-validation procedure (**Table 6.1.2**). All unirradiated samples were correctly classified and none of samples irradiated with 6 or 10 kGy resulted in a false negative (unirradiated). Hence, the obtained model seems to be effective to detect irradiation treatment above 2 kGy, independently of irradiation source. The satisfactory performance of the proposed classification procedure is also confirmed by the overall specificity (72%) achieved for the cross-validation procedure. The analysis kept SPO, LLL, PLL, SPP,

OOL and SOO in the final discriminant model, being verified that OOL and SPO were the variables with highest correlation with function 1, SPO and SPP with function 2 and PLL and LLL with function 3.

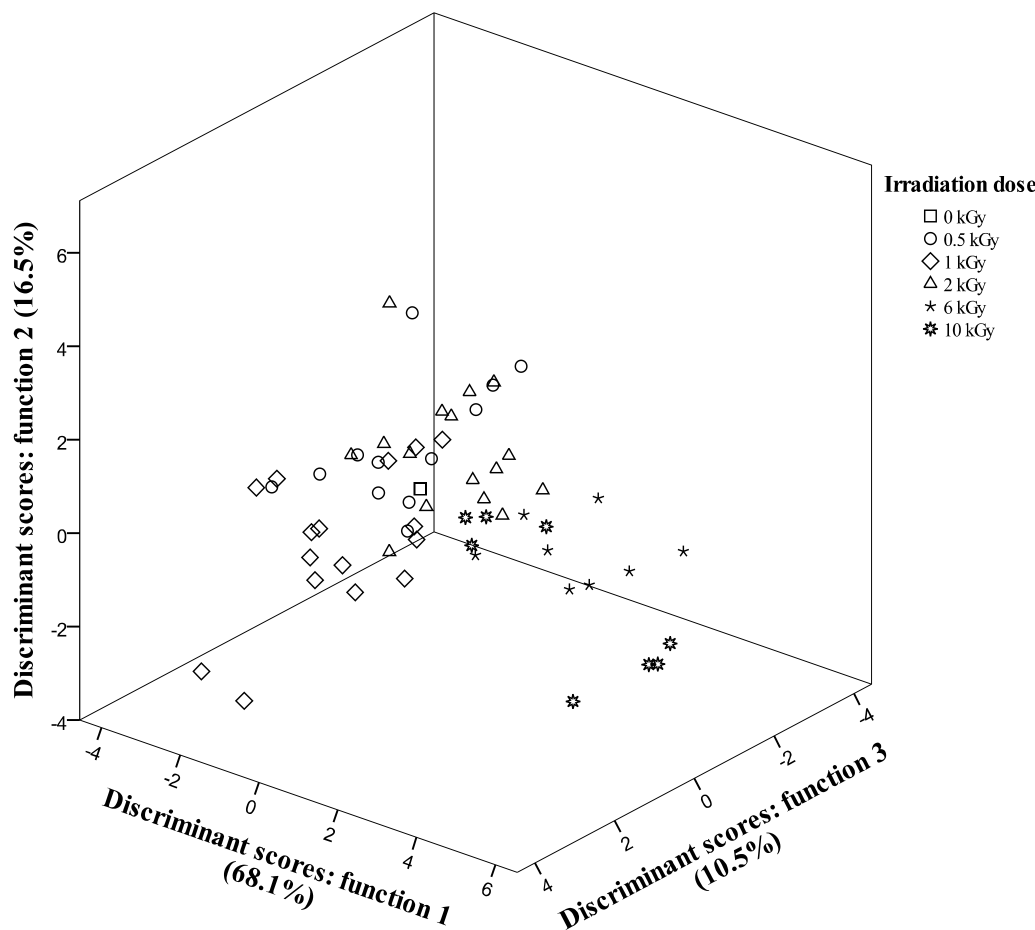

Figure 6.1.1. Mean scores of different gamma and electron-beam irradiation doses (all assembled) projected for the three rotated discriminant functions defined from TAG profiles.

Table 6.1.2. Contingency matrix obtained using LDA based on TAG profiles of mushroom species.

Irradiation dose	Predicted Group Membership							
	0 kGy	0.5 kGy	1 kGy	2 kGy	6 kGy	10 kGy		
	Gamma and electron-beam irradiation						Total	Sensitivity (%)
0 kGy	28	0	0	0	0	0	28	100
0.5 kGy	4	6	2	0	0	0	12	50
1 kGy	2	4	8	2	0	0	16	50
2 kGy	3	1	2	9	1	0	16	56
6 kGy	0	0	0	2	6	0	8	75
10 kGy	0	0	0	3	1	4	8	50
total	37	11	12	16	8	4	88	69
Specificity (%)	76	55	67	56	75	100	72	

	Gamma irradiation					
	0 kGy	0.5 kGy	1 kGy	2 kGy	Total	Sensitivity (%)
0 kGy	20	0	0	0	20	100
0.5 kGy	5	6	1	0	12	50
1 kGy	3	4	9	0	16	56
2 kGy	0	0	0	8	8	100
total	28	10	10	8	56	77
Specificity (%)	71	60	90	100	80	

	Electron-beam irradiation					
	0 kGy	2 kGy	6 kGy	10 kGy	Total	Sensitivity (%)
0 kGy	8	0	0	0	8	100
2 kGy	1	6	0	1	8	75
6 kGy	0	0	6	2	8	75
10 kGy	0	1	2	5	8	63
total	9	7	8	8	32	78
Specificity (%)	89	86	75	63	78	

In order to abolish the effect of irradiation type, two additional LDA were applied fixing the results obtained for gamma and electron-beam irradiation treatment. In the first case, the discriminant model was defined with 3 significant functions (**Figure 6.1.2A**), which included 100.0% of the observed variance (function 1: 92.7%, function 2: 5.0%, function 3: 2.3%). The model showed a better classification performance (sensitivity = 77%). In fact, as can be observed from **Table 6.1.2**, all unirradiated samples were correctly classified and none of samples irradiated with 2 kGy resulted in a false negative (unirradiated). Hence, the obtained model seems to be effective to detect gamma irradiation treatment above 1 kGy. The satisfactory performance of the proposed classification procedure is also confirmed by the overall specificity (80%)

achieved for the cross-validation procedure. SPP, POL (more correlated with the first function), LLL, PPO (more correlated with the second function), LLnLn, OOL and POO (more correlated with the third function) were the variables kept in the final discriminant model.

Regarding e-beam irradiation, the discriminant model selected also 3 significant functions (**Figure 6.1.2B**), which included 100.0% of the observed variance (function 1: 76.2%, function 2: 16.2%, function 3: 7.6%). The model showed a similar classification performance (sensitivity = 78%). Once again, the unirradiated samples were correctly classified in all cases; furthermore, none of samples irradiated with 6 or 10 kGy resulted in a false negative, allowing considering the feasibility of the model to detect electron-beam irradiation treatment above 2 kGy. The performance of the proposed classification procedure is also confirmed by the overall specificity (78%) achieved for the cross-validation procedure. SPO, LLL (more correlated with the first function), OOO, SOO and PPO (more correlated with the second function), were the variables kept in the final discriminant model.

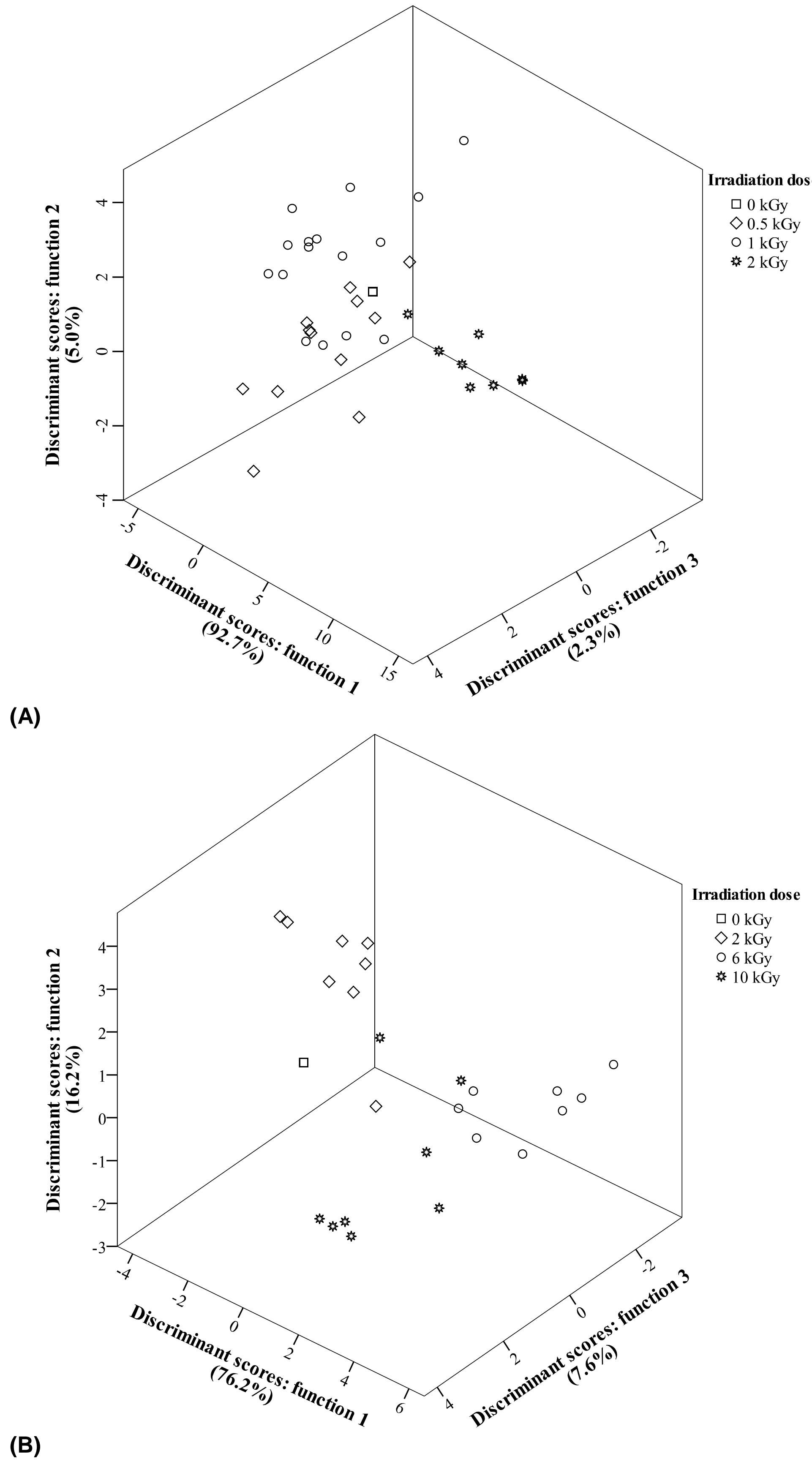

Figure 6.1.2. Mean scores of different gamma (a) or electron-beam (b) irradiation doses projected for the three rotated discriminant functions defined from TAG profiles.

Overall, TAG profile might be a practical tool to detect irradiated mushrooms, independently of mushroom species or irradiation source. The effects of each type of irradiation were significantly different, as it can be concluded from the correlations among discriminant functions and variables within each statistical test. The suggested chemometric parameter was more reliable for the higher assayed doses, indicating that the lowest doses had only a slight effect on TAG profiles. Other mushrooms species might be scrutinized in order to increase the broadness of application of this particular chemometric indicator.

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6.1.4. References

Alberti, A., Chiaravalle, E., Fuochi, P., Macciantelli, D., Mangiacotti, M., Marchesani, G., & Plescia, E. (2011). Irradiated bivalve mollusks: Use of EPR spectroscopy for identification and dosimetry. *Radiation Physics and Chemistry*, *80*, 1363-1370.

ASTM, American Society for Testing and Materials. (1992). Practice for Using the Fricke Reference Standard Dosimetry System, ASTM E1026. Annual Book of ASTM Standards, 12.02, Philadelphia, PA.

Barreira, J.C.M., Casal, S., Ferreira, I.C.F.R., Oliveira, M.B.P.P., & Pereira, J.A. (2009). Nutritional, fatty acid and triacylglycerol profiles of *Castanea sativa* Mill. cultivars: a compositional and chemometric approach. *Journal of Agricultural and Food Chemistry, 57*, 2836-2842.

Barreira, J.C.M, Ferreira, I.C.F.R., & Oliveira, M.B.P.P. (2012). Triacylglycerol profile as a chemical fingerprint of mushroom species: evaluation by principal component and linear discriminant analyses. *Journal of Agricultural and Food Chemistry*, *60,* 10592-10599.

Barreira, J.C.M., Carocho, M., Ferreira, I.C.F.R., Antonio, A.L., Kaluska, I., Botelho, M. L., Bento, A., & Oliveira, M.B.P.P. (2013). Effects of gamma and electron beam irradiations on the triacylglycerol profile of fresh and stored *Castanea sativa* Miller samples. *Postharvest Biology and Technology*, *81*, 1-6.

Benitez, E., Nogales, R., Campos, M., & Ruano, F. (2006). Biochemical variability of olive-orchard soils under different management systems. *Applied Soil Ecology*, *32*, 221-231.

Blanch, G.P., Caja, M.M., Flores, G., & Castillo, M.L.R. (2009). Identification of 2-dodecylcyclobutanone and linear-alkanes as irradiation markers in sliced dry-cured ham. *Food Chemistry*, *113*, 616-620.

Carocho, M., Barreira, J.C.M., Antonio, A.L., Bento, A., Kaluska, I., & Ferreira, I.C.F.R. (2012). Effects of Electron Beam Radiation on Nutritional parameters of Portuguese Chestnuts (*Castanea sativa* Mill). *Journal of Agricultural and Food Chemistry*, *60*, 7754-7760.

Chatterjee, S., Variyar, P.S., & Sharma, A. (2012). Post-irradiation identification of papaya (*Carica papaya* L.) fruit. *Radiation Physics and Chemistry*, *81*, 352-353.

Crews, C., Driffield, M., & Thomas, C. (2012). Analysis of 2-alkylcyclobutanones for detection of food irradiation: Current status, needs and prospects. *Journal of Food Composition and Analysis*, *26*, 1-11.

Cunha, S.C., & Oliveira, M.B.P.P. (2006). Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD. *Food Chemistry*, *95*, 518-524.

EN 1786. (1996). Foodstuffs: detection of irradiated food containing bone - method by ESR spectroscopy. European Committee of Standardization (CEN), Brussels.

EN 1787. (2000). Foodstuffs: detection of irradiated food containing cellulose by ESR spectroscopy. European Committee of Standardization (CEN), Brussels.

EN 13708 (2001). Foodstuffs: detection of irradiated food containing crystalline sugar by ESR spectroscopy. European Committee of Standardization (CEN), Brussels.

Fernandes, Â., Antonio, A.L., Oliveira, M.P.P., Martins, A., & Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Antonio, A.L., Barreira, J.C.M., Botelho, L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2013a). Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2013b). Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food Bioprocess Technology*. DOI 10.1007/s11947-013-1179-4.

Fuchs, B., Rosmarie, Suss R., Teuber, K., Eibisch, M., & Schiller, J. (2011). Lipid analysis by thin-layer chromatography- A review of the current state. *Journal of Chromatography A*, *1218*, 2754-2774.

Hill, T., & Lewicki, P. (2006). Statistics: Methods and Applications. A Comprehensive Reference for Science, Industry, and Data Mining; StatSoft: Tulsa, OK, USA.

Kalač, P. (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. *Food Chemistry*, *113*, 9-16.

Kim, G.-R., Akram, K., Ahn, J.-J., & Kwon, J.-H. (2012). Identification of gamma ray and electron-beam irradiated wheat after different processing treatments. *Journal of Cereal Science*, *56*, 347-351.

Lindequist, U., Niedermeyer, T.H.J., & Jülich, W.-D. (2005). The Pharmacological Potential of Mushrooms - review. *eCAM*, *3*, 285-299.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, *106*, 369-378.

Ndiaye, B., Jamet, G., Miesch, M., Hasselmann, C., & Marchioni, E. (1999). 2-Alkylcyclobutanones as markers for irradiated foodstuffs II. The CEN (European Committee for Standardization) method: field of application and limit of utilization. *Radiation Physics and Chemistry*, *55*, 437-445.

Rombaut, R., De Clercq, N., Foubert, I., & Dewettinck, K. (2009). Triacylglycerol analysis of fats and oils by evaporative light scattering detection. *Journal of the American Oil Chemists' Society*, *86*, 19-25.

Soika, C., & Delincée, H. (2000). Thermoluminescence analysis for detection of irradiated food - luminescence characteristics of minerals for different types of radiation and radiation doses. *Lebensmittel-Wissenschaft & Technologie*, *33*, 431-439.

Toci, A.T., Neto, V.J.M.F., Torres, A.G., & Farah, A. (2013). Changes in triacylglycerols and free fatty acids composition during storage of roasted coffee. *LWT - Food Science and Technology*, *50*, 581-590.

Zeb, A. (2012). Triacylglycerols composition, oxidation and oxidation compounds in camellia oil using liquid chromatography-mass spectrometry. *Chemistry and Physics of Lipids*, *165*, 608-614.

6.2

Viabilidade da utilização da irradiação gama e por feixe de eletrões como tecnologias de conservação de cogumelos silvestres: efeito nos macro e micro elementos

Este sub-capítulo apresenta o estudo dos efeitos da radiação nos macro e micro elementos de amostras frescas de Boletus edulis *Bull. e* Macrolepiota procera *(Scop.) (doses 1 e 2 kGy de radiação gama), e de amostras desidratados de* Boletus edulis *Bull. (doses 2, 6 e 10 kGy de feixe de eletrões) e* Hydnum repandum *L. Fr. (doses 0,5, 1 e 6 kGy de feixe de eletrões).*

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Gamma and electron-beam irradiation as viable technologies for wild mushrooms conservation: effects on macro- and micro-elements

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Abstract

The consumption of mushrooms is increasing all over the world as a result of their sensorial and nutritional qualities. Among their nutrients, mushrooms present high levels of macro- and micro-elements. The qualitative composition in these elements is, however, often unknown. Mushrooms are known also as being very perishable products. In this sense, gamma rays or electron-beam irradiation have been applied to improve their shelf life and decrease health hazards caused by microorganisms. In addition, the effects of irradiation on the physicochemical and nutritional parameters of wild mushrooms have been studied by our research group. Nevertheless, the effects on essential macro- and micro-elements of these natural matrices are still unknown. Therefore, the effects of gamma and electron-beam irradiation on the macro- and micro-elements profiles were evaluated in *Boletus edulis*, *Hydnum repandum* and *Macrolepiota procera*. The same elements were detected in the three species with some quantitative differences. The profiles obtained allowed the definition of proper dietary intakes, thus preventing undesirable effects derived from consuming mushrooms in quantities that exceed threshold levels of these minerals. The applied irradiation doses did not show a systematic effect on the macro- and micro-elements profiles, except for the 10 kGy. Accordingly, irradiation treatment, using gamma rays or electron-beam up to 6 kGy, is a suitable technique to disinfest and/or decontaminate wild mushrooms, independently of their species or physical state.

Keywords: Wild mushrooms; Irradiation; Micro-elements; Macro-elements; Linear Discriminant Analysis.

6.2.1. Introduction

The consumption of wild edible mushrooms is increasing due to their aroma and taste, as well as their nutritional properties, especially their low fat content and high levels of carbohydrates, proteins and vitamins (Agrahar-Murugkar & Subbulakshmi, 2005). Furthermore, mushrooms are considered excellent sources of minerals (Kalač & Svoboda, 2000; Demirbaş, 2001; Gençcelep, Uzun, Tunçtürk, & Demirel, 2009; Falandysz & Borovička, 2013), depending on mushroom species, age of mycelium,

interval between the fructifications and on their ecosystems (Seeger, 1982; Kalač & Svoboda, 2000).

The essential minerals are well known regarding their physiological functions and requirements. From a nutritional point of view, mineral elements have been classified into the following groups: i) macro-elements, such as K, Na, Ca, Mg and P, which are required in amounts of about 100 mg/day or higher; ii) micro-elements, also known as oligo-elements, such as Fe, Zn or Mn, which are required in lower amounts and are mostly essential to maintain the body functions (Koyyalamudi, Jeong, Manavalan, Vysetti, & Pang, 2013; Mahan, Escott-Stump, & Raymond, 2013).

The functions of macro-elements include maintaining acid-base balance, osmotic regulation and oxygen transport (McDowell, 2003). On the other hand, the micro-elements are important in catalytic processes (especially as enzymatic cofactors) associated with metabolic, endocrine and immune systems (Tomkins, 2002; Koyyalamudi, Jeong, Manavalan, Vysetti, & Pang, 2013).

The daily intake of these nutrients is likely to pose no risk of adverse effects. However, some essential (*e.g.* Zn, Se, Fe, Ca, Mg, P, Mn) and non-essential (*e.g.* Cd, Hg, Ba, As, Al) trace elements might be unhealthy when ingested above the threshold levels (FNB, 2001; Falade, Adepoju, Owoyomi, & Adewusi, 2008; Gençcelep, Uzun, Tunçtürk, & Demirel, 2009). Accordingly, it is important to know the levels of essential elements in food products (Işiloğlu, Yilmaz, & Merdivan, 2009).

Mushrooms are very perishable products, and treatments like using gamma rays or electron-beam irradiation have been applied to improve their shelf life and reduce health hazards caused by pathogenic microorganisms (Akram & Kwon, 2010; Rivera, Blanco, Marco, Oria, & Venturini, 2011; Culleré, Ferreira, Venturini, Marco, & Blanco, 2012; Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). The effects of irradiation on physicochemical and nutritional parameters of wild mushrooms, including composition in free sugars, tocopherols, fatty acids (Fernandes et al., 2013a; 2013b; 2014a; 2014b), organic acids (Fernandes et al., 2014c) and triacylglycerols (Fernandes et al., 2014d) have been extensively studied by our research group, but, studies on the effects on essential macro- and micro-elements in wild mushrooms are scarce. Therefore, in the present study gamma and electron-beam irradiation were applied to three highly appreciated edible mushrooms: *Boletus edulis* Bull.: Fr., *Hydnum repandum* L.: Fr. and *Macrolepiota procera* (Scop.) Singer.

Afterwards, the effects of irradiation source and dose on the macro- and micro-elements profiles were assessed using a pairwise approach.

6.2.2. Materials and methods

Standards and reagents

Standard solutions of the minerals to be analyzed - Na, K, Ca, Mg, Fe, Cu, Mn and Zn - were made from > 99% purity commer cial reagents and La_2O_3 (99% purity) were supplied by Merck (Darmstadt, Germany). Other general use laboratory reagents (ethyl ether, HCl, H_2SO_4, HNO_3, $HClO_4$, NaOH, KSO_4 and $CuSO_4$) were analytical grade and purchased from Sigma-Aldrich (St. Louis, MO, USA).

Samples

Macrolepiota procera wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011, and dried at 30 ºC in an oven until constant weight was reached. Subsequently, the samples were divided into four groups with three specimens in each group: control (unirradiated, 0 kGy); sample 1 (0.5 kGy); sample 2 (1 kGy) and sample 3 (6 kGy).

Boletus edulis and *Hydnum repandum* wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012. Fresh samples of each species were divided into three groups with three specimens of each: control (unirradiated, 0 kGy); sample 1 (1 kGy) and sample 2 (2 kGy). Different samples belonging to the same initial pool of *B. edulis* were dried at 30 ºC in an oven and subsequently divided into four groups with six specimens per group: control (unirradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy).

Doses were selected according to international recommendations, since the advised dose for extending the shelf-life of fresh mushroom in different countries (such as Argentina, China, Croatia, Hungary, Israel, Korea, Mexico, Poland and United Kingdom) is 1-3 kGy, while the advised doses for decontamination of dried mushrooms may vary from 10 to 50 kGy (ICGFI, 1999; Akram & Kwon, 2010).

Sample irradiation

Gamma irradiation. The estimated dose rate for the irradiation position was obtained using a Fricke dosimeter, and the irradiation of the fresh samples was performed in a Co-60 experimental chamber with four sources, total activity 226 TBq (6.08 kCi) in November 2011 (Precisa 22, Graviner Manufacturing Company Ltd., U.K.), following the procedure previously described by the authors (Fernandes et al., 2013a).

The estimated doses, dose rates and dose uniformity ratios (Dmax/Dmin) were: 1.14 ± 0.23 kGy, 1.71 kGy/h, 1.72 and 1.99 ± 0.32 kGy, 1.49 kGy/h, 1.44, for *B. edulis*; and 1.02 ± 0.04 kGy, 1.53 kGy/h, 1.08 and 1.66 ± 0.46 kGy, 1.24 kGy/h, 1.98, *H. repandum*.

Electron-beam irradiation. The irradiation of dried samples was performed at the INCT - Institute of Nuclear Chemistry and Technology, Warsaw, Poland. To estimate the dose during the irradiation process, three types of dosimeters were used: a standard dosimeter, a graphite calorimeter and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV energy with pulse duration of 5.5 μs, a pulse frequency of 440 Hz and an average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was set in the range 20-100 cm/min and the scan frequency was 5 Hz. The absorbed dose for *M. procera* was 0.53, 0.83 and 6.10 kGy, with an uncertainty of 20% for the two first doses and 10% for the last dose. The estimated absorbed doses for *B. edulis* were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read the Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the Graphite calorimeter dosimeter the electrical resistance was read and converted to dose according to a calibrated curve (Fernandes et al., 2014a).

Macro- and Micro-elements

After the irradiation procedures, all the samples were lyophilized (FreeZone 4.5 model 7750031, Labconco, KS, USA), reduced to a fine dried powder (20 mesh) and mixed to obtain homogenized samples, for macro- and micro-elements analyses.

The total mineral content (ashes) and mineral elements analyses were performed on lyophilized samples. Method 930.05 of AOAC procedures was used (Horwitz & Latimer, 2005). 500 mg of each sample were subjected to dry-ash mineralization at 450 °C. The residue after incineration was extracted with HCl (50% v/v) and HNO_3 (50% v/v) and made up to an appropriate volume with distilled water, where Fe, Cu, Mn and Zn were directly measured. An additional 1/10 (v/v) dilution of the sample extracts and standards was performed to avoid interference between different elements in the atomic absorption spectroscopy (AAS) procedure: for Ca and Mg analysis in 1.16% La_2O_3/HCl (leading to $LaCl_2$); for Na and K analysis in 0.2% CsCl (Ruiz-Rodríguez et al., 2011). All measurements were performed using atomic absorption spectroscopy with air/acetylene flame in Analyst 200 Perkin Elmer equipment (Perkin Elmer, Waltham, MA, USA), comparing absorbance responses with > 99.9% purity analytical standard solutions for AAS made with Fe $(NO_3)_3$, Cu $(NO_3)_2$, Mn $(NO_3)_2$, Zn $(NO_3)_2$, NaCl, KCl, $CaCO_3$ and Mg bands, supplied by Merck (Darmstadt, Germany) and Panreac Química (Barcelona, Spain). The mineralization was performed in triplicate and further extracted.

Statistical analysis

For each combination irradiation type, irradiation dose, fresh and dried mushrooms, three independent samples were used. Each of the samples was taken after pooling the mushrooms treated under the same conditions. Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS software, version 22.0 (IBM Corp., USA).

Analysis of variance. The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilks's and Levene's tests, respectively. All dependent variables were compared using Tukey's honestly significant difference

(HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

Stepwise Linear Discriminant Analysis (LDA). LDA was used to check for significant changes in the macro- and micro-elements profiles as a result of irradiation treatment. A stepwise technique, using the Wilks's λ method with the usual probabilities of *F* (3.84 to enter and 2.71 to remove) was applied to select variables. This procedure followed a combination of forward selection and backward elimination steps; *i.e.*, before a new variable is selected to be included, it is verified whether all previously selected variables remain significant (Hill & Lewicki, 2006). The combination of variables is defined in a way that the first function furnishes the most general discrimination between groups, the second provides the second most, and so on (López, García, & Garrido 2008). To verify which canonical discriminant functions were significant, the Wilks's λ test was applied. To keep a more realistic data modulation, a leave-one-out cross-validation procedure was carried out to assess the model performance.

6.2.3. Results and discussion

The work described in the present study represents a step further in the characterization of the chemometric changes that might occur as the result of applying irradiation to disinfest and/or decontaminate different mushroom species. Despite the wide range of studies previously performed to evaluate the effects of irradiation on the profiles of several components (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012; 2013a; 2013b; 2014a; 2014b; 2014c; 2014d), the potential changes in the macro- and micro-elements in mushroom species have not yet been studied. In fact, the minerals content, which is generally considered to be high in mushrooms, (Kalač & Svoboda, 2000; Demirbaş, 2001; Gençcelep, Uzun, Tunçtürk, & Demirel, 2009; Falandysz & Borovička, 2013), depends on factors intrinsic to mushrooms, such as the species, age of mycelium, interval between the fructifications and ecosystem conditions (Seeger, 1982; Kalač & Svoboda, 2000). Accordingly, we selected mushroom species that could be analyzed in their fresh form or submitted to a drying process, which is the most common industrial procedure to commercialize wild mushrooms. A pairwise study was conducted using gamma or electron-beam irradiation, which are the types of irradiation used more

frequently in the food industry. Besides the international recommendations, the doses were also selected according to previous results, to whether samples were fresh or dried and to the effects of each type of irradiation on the physical properties of mushrooms.

Tables 6.2.1 and **6.2.2** shows the mean values obtained for the micro- and macro-element profiles of each mushroom species, according to the processing type and irradiation treatment. The mean values were calculated for each irradiation dose, irradiation source and for fresh and dried samples separately. Despite the quantitative differences, all mushrooms presented the same profile in macro-elements: K>Na>Mg>Ca; Fe and Zn were the most abundant micro-elements, whilst Mn was detected in the lowest amounts. Due mostly to its K content, *H. repandum* was the mushroom with the highest mineral content; on the other hand, *B. edulis* samples gave lower mineral contents.

The concentrations in K are higher than those reported in similar works, which represents a great nutritional benefit to the consumer, since potassium from fruit and vegetables can reduce blood pressure. Levels in Na were also slightly higher, but it still might be considered as being relatively low. On the other hand, the amounts of Ca and Mg were lower than those commonly reported in mushroom species. Fe (very important in order to decrease the incidence of anemia), Zn (Mushrooms are known as zinc accumulator), Cu (present in concentrations quite below the toxicity levels) and Mn values in in the present study are in agreement with previous reported (Gençcelep, Uzun, Tunçtürk, & Demirel, 2009).

Considering a species-to-species comparison, Turkish wild *B. edulis* studied by Ayaz et al. (2011), showed similar values of Ca, Cu and Zn and higher K, Mg and Mn contents. Also, *H. repandum* samples used in this work exhibited higher contents of Na, K, Ca, Mg and Zn, similar contents of Fe and Cu and less Mn.

According to Regulation (EU) No 1169/2011 (2011), FNB (2001) and Trumbo Schlicker, Yates, and Poos (2002), 100 g (fw) of these wild mushrooms might represent important percentages of the daily requirements in K, Fe and Zn (respectively 20%, 12% and 10% of the RDA). These contributions are significant as these trace elements perform important functions for human health acting as cofactors of antioxidant enzymes and playing an important role in the prevention of chronic diseases (McDermott, 2000).

Regarding the effects of irradiation treatment, the resulting changes were not significant ($p > 0.05$, in one-way ANOVA), as expected due to the chemical nature of micro- and macro-elements, in most cases. In addition, the elements with significant variation did not show a marked tendency (except Cu and Mg in gamma-irradiated fresh samples of *B. edulis* and Na and K in electron-beam irradiated samples of the same mushroom), which might indicated that the observed are more probably related with natural variability in mineral accumulation as a result of growing in different ecosystems. In fact, the variability of water uptake and the dilution processes of dissolved minerals during rainfall are contributing factors which could explain the difference in the concentrations of mineral elements (Koyyalamudi, Jeong, Manavalan, Vysetti, & Pang, 2013).

Despite the relatively high doses used in electron-beam irradiation, it seems that neither of the irradiation types had a more significant effect on the micro- or macro-elements profiles. Likewise, no particular differences were observed in terms of fresh or dried samples. In line with previous results, the significant effects detected were not specifically associated with any of the mushroom species investigated.

Regarding individual changes, Levene's test showed that the assumption of equality among variances could be made in most cases, allowing the application of the Tukey test as a multiple comparison test. In the remaining cases, samples were classified by means of the Tamhane's T2 test.

In general, significant differences found between the mean values for each macro- and micro-element did not appear to be linked to any of the factors tested: irradiation type, irradiation dose, mushroom species or mushroom processing. These results might indicate that irradiation had no recognizable effect over macro- and micro-elements profiles. Nevertheless, this assumption was checked by applying a supervised classification statistical technique, which has the advantage of offering a global perspective regarding the effects of irradiation doses on the variations of each quantified macro- and micro-element, irrespective of the mushroom species or state (fresh or dried).

Since one of the primary objectives was verifying the existence of differences between irradiated and unirradiated samples, and considering that the assayed mushrooms presented qualitative and quantitative differences in their macro- and micro-elements profiles, the set of data was normalized to prevent biased results. For each group of results, the relative differences between each quantified value

(irradiated samples) and its respective control (unirradiated samples) were calculated and divided by the control value. Afterwards, LDA was applied to the normalized relative differences found for all measurements and not to the absolute values as shown in **Tables 6.2.1** and **6.2.2**.

The significant independent variables (macro- and micro-elements) were selected using the stepwise procedure of the LDA, according to the Wilks's λ test. Only variables with a statistically significant classification performance (p < 0.05) were kept in the analysis.

The discriminant model selected 5 significant functions, from which the first 3 (function 1: 60.7%, function 2: 22.8%, function 3: 13.3%) were plotted (**Figure 6.2.1**). Among the 8 quantified elements, Mn, Mg and Fe were not selected in the definition of the discriminant functions. On the other hand, K (more correlated with the first function), Cu (more correlated with the second function), Ca (more correlated with the third function), Zn and Na were the variables kept in the final discriminant model. As it was expected from the results presented in **Tables 6.2.1** and **6.2.2**, the defined model did not show ability to discriminate the groups corresponding to each irradiation dose (as it can be concluded from the unsystematic distribution of the markers corresponding to different irradiation doses), with 88.1% of samples correctly classified for the original grouped cases and 64.3% for the cross-validation procedure. Group corresponding to 10 kGy, however, was placed far from the remaining groups, indicating that it might not be advised to apply this dose to disinfest/decontaminate wild mushrooms.

Table 6.2.1. Micro-elements content (mg/100 g dw) according to processing type, irradiation source and dose. The results are presented as the mean ± SD.

			Micro-elements (mg/100 g)			
Processing	**Irradiation**	**Dose (kGy)**	**Fe**	**Cu**	**Mn**	**Zn**
			Boletus edulis			
Fresh	Gamma	0	7±1	1.6±0.2 a	0.3±0.1	7.5±0.4
		1	7.9±0.3	1.3±0.1 ab	0.4±0.1	7.4±0.1
		2	7.4±0.3	1.2±0.1 b	0.2±0.1	7.4±0.2
Levene's test[1]			$p = 0.509$	$p = 0.064$	$p = 0.231$	$p = 0.097$
One-way ANOVA[2]			$p = 0.068$	$p = 0.025$	$p = 0.133$	$p = 0.877$
Dried	E-beam	0	3±1	3±1 ab	2±1	13±1 a
		2	3.6±0.2	2.9±0.1 ab	2.5±0.1	12±1 ab
		6	4±1	2.8±0.2 b	1.3±0.1	10±1 b
		10	3.8±0.3	4±1 a	1.9±0.1	13±1 a
Levene's test[1]			$p = 0.017$	$p = 0.065$	$p = 0.007$	$p = 0.725$
One-way ANOVA[2]			$p = 0.739$	$p = 0.035$	$p = 0.073$	$p = 0.001$
			Macrolepiota procera			
Dried	E-beam	0	6±1	6.2±0.1	1.5±0.2 a	8.7±0.2 a
		0.5	6.0±0.2	5.8±0.4	1.6±0.1 a	7.6±0.4 b
		1	6±1	6±1	0.9±0.1 b	9.2±0.3 a
		6	6.5±0.4	6±1	1.4±0.3 ab	8.5±0.5 ab
Levene's test[1]			$p = 0.195$	$p = 0.088$	$p = 0.185$	$p = 0.232$
One-way ANOVA[2]			$p = 0.519$	$p = 0.925$	$p = 0.006$	$p = 0.008$
			Hydnum repandum			
Fresh	Gamma	0	18±1 a	0.9±0.1	0.5±0.2	7±1 a
		1	12.2±0.2 c	0.9±0.2	0.5±0.1	5.6±0.4 b
		2	15±1 b	0.7±0.1	0.5±0.1	6.0±0.3 b
Levene's test[1]			$p = 0.069$	$p = 0.284$	$p = 0.526$	$p = 0.250$
One-way ANOVA[2]			$p < 0.001$	$p = 0.264$	$p = 0.855$	$p = 0.006$

[1]Homoscedasticity among mushroom species was tested by means of Levene's test: homoscedasticity, p value > 0.05; heteroscedasticity, p value < 0.05.
[2]$p < 0.05$ meaning that the mean value of the evaluated micro-element of at least one dose differs from the others (in this case multiple-comparison tests were performed).

Table 6.2.2. Macro-elements content (mg/100 g dw) according to processing type, irradiation source and dose. The results are presented as the mean±SD[1].

			Macro-elements (mg/100 g)			
Processing	**Irradiation**	**Dose (kGy)**	**Ca**	**Mg**	**Na**	**K**
			Boletus edulis			
Fresh	Gamma	0	22±2 b	130±12 a	696±2 a	3042±165
		1	39±5 a	126±3 ab	593±12 c	3038±144
		2	27±2 b	110±3 b	640±14 b	2955±145
Levene's test[1]			$p = 0.129$	$p = 0.081$	$p = 0.037$	$p = 0.972$
One-way ANOVA[2]			$p = 0.003$	$p = 0.028$	$p < 0.001$	$p = 0.743$
Dried	E-beam	0	23±1	151±25	288±20 a	3545±219 a
		2	18.6±0.5	128±6	285±17 ab	3158±142 ab
		6	15±3	115±2	243±2 bc	2961±136 b
		10	19±5	124±5	226±19 c	2870±120 b
Levene's test[1]			$p = 0.165$	$p = 0.008$	$p = 0.167$	$p = 0.816$
One-way ANOVA[2]			$p = 0.058$	$p = 0.052$	$p = 0.004$	$p = 0.004$
			Macrolepiota procera			
Dried	E-beam	0	23±1 a	155±6	650±6	4120±192
		0.5	27±3 a	160±2	632±43	4038±84
		1	22±1 a	163±4	504±94	3967±119
		6	12±1 b	165±6	503±88	3836±54
Levene's test[1]			$p = 0.221$	$p = 0.328$	$p = 0.028$	$p = 0.341$
One-way ANOVA[2]			$p < 0.001$	$p = 0.128$	$p = 0.058$	$p = 0.104$
			Hydnum repandum			
Fresh	Gamma	0	53±5 a	147±7	624±12 a	5276±250
		1	41±4 b	138±6	580±13 b	5121±162
		2	54±2 a	141±3	605±15 ab	5107±139
Levene's test[1]			$p = 0.263$	$p = 0.334$	$p = 0.922$	$p = 0.352$
One-way ANOVA[2]			$p = 0.010$	$p = 0.214$	$p = 0.020$	$p = 0.519$

[1]Homoscedasticity among mushroom species was tested by means of Levene's test: homoscedasticity, p value > 0.05; heteroscedasticity, p value < 0.05.
[2]$p < 0.05$ meaning that the mean value of the evaluated macro-element of at least one dose differs from the others (in this case multiple-comparison tests were performed).

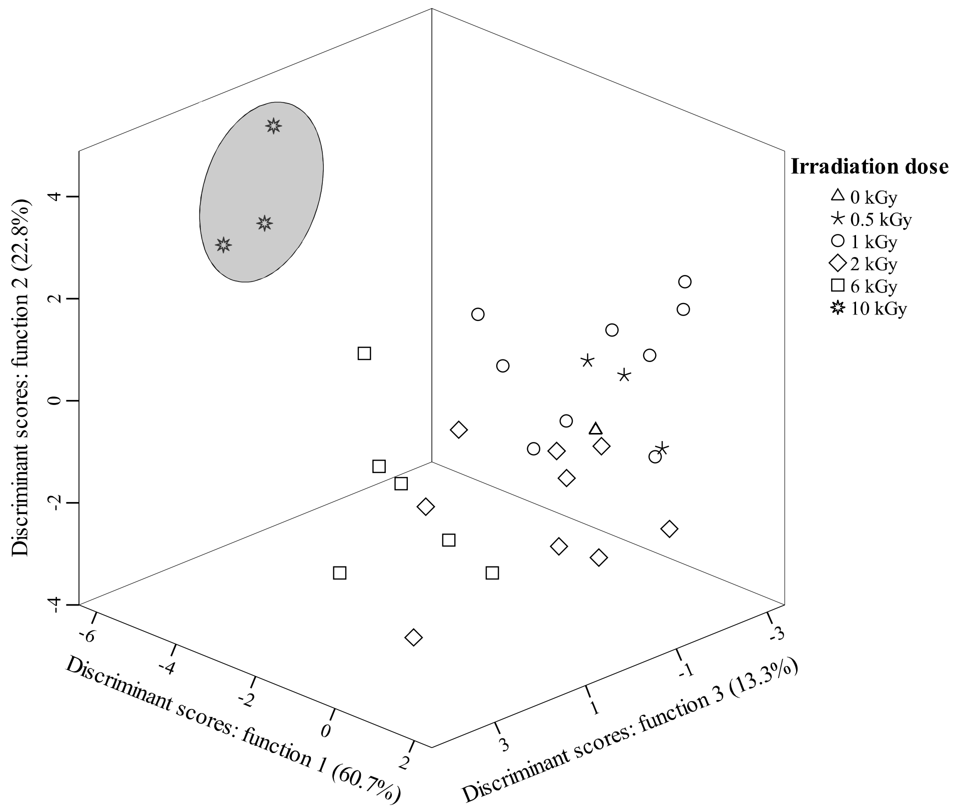

Figure 6.2.1. Mean scores for different irradiation doses projected for the discriminant functions defined from macro- and micro-elements profiles.

6.2.4. Conclusions

Overall, macro- and micro-elements profiles of these mushroom species were completely characterized, allowing the definition of healthy dietary intakes and preventing undesirable effects resulting from mushroom consumption above the threshold concentration levels.

The effects of each type of irradiation showed some dissimilarity, but Na, K and Ca were the elements with the most significant changes in both types of irradiation, as it can be concluded from the correlations among discriminant functions and variables.

The results obtained suggest that irradiation might be applied as potential disinfestation and decontamination technique without affecting the macro- and micro-elements profiles, especially up to a 6 kGy dose. Other mushrooms species might be evaluated to validate these results.

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6.2.5. References

Ayaz, F.A., Torun, H., Colak, A., Sesli, E., Millson, M., & Glew, R.H. (2011). Macro- and microelement contents of fruiting bodies of wild-edible mushrooms growing in the East Black Sea region of Turkey. *Food and Nutrition Sciences*, *2*, 53-59.

Akram, K., & Kwon, J.-H. (2010). Food Irradiation for Mushrooms: A Review. *Journal of the Korean Society for Applied Biological* Chemistry, 53, 257-265.

Agrahar-Murugkar, D., & Subbulakshmi, G. (2005). Nutritional value of edible wild mushrooms collected from the Khasi hills of Meghalaya. *Food Chemistry*, *89*, 599-603.

Culleré, L., Ferreira, V., Venturini, M.E., Marco, P., & Blanco, D. (2012). Evaluation of gamma and electron-beam irradiation on the aromatic profile of black truffle (*Tuber melanosporum*) and summer truffle (*Tuber aestivum*). *Innovative Food Science & Emerging Technologies*, *13*, 151-157.

Demirbaş, A. (2001). Heavy metal bioaccumulation by mushrooms from artificially fortified soils. *Food Chemistry*, *74*, 293-301.

Falade, O.S., Adepoju, O.O., Owoyomi, O., & Adewusi, S.R. (2008). Chemical composition and toxic trace element composition of some Nigerian edible wild mushroom. *International Journal of Food Science & Technology*, *43*, 24-29.

Falandysz, J., & Borovička, J. (2013). Macro and trace mineral constituents and radionuclides in mushrooms: health benefits and risks. *Applied Microbiology and Biotechnology*, *97*, 477-501.

Fernandes, Â., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2012). Effect of gamma and electron beam irradiation on the physico-chemical

and nutritional properties of mushrooms: A review. *Food Chemistry*, *135*, 641-650.

Fernandes, Â., Antonio, A.L., Barreira, J.C.M., Botelho, L., Oliveira, M.B.P.P., Martins, A., et al. (2013a). Effects of gamma irradiation on the chemical composition and antioxidant activity of Lactarius deliciosus L. wild edible mushroom. *Food and Bioprocess Technology*, *6*, 2895-2903.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Santos, P.M.P., Martins, A., Oliveira, M.B.P.P., et al. (2013b). Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Research International*, *54*, 18-25.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014a). Combined effects of electron-beam irradiation and storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food and Bioprocess Technology*, *7*, 1606-1617.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., & Ferreira, I.C.F.R. (2014b). Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. *Innovative Food Science & Emerging Technologies*, *22*, 158-166.

Fernandes, Â., Barros, L., Antonio, A.L., Barreira, J.C.M., Oliveira, M.B.P.P., Martins, A., et al., (2014c). Using gamma irradiation to attenuate the effects caused by drying or freezing in *Macrolepiota procera* organic acids and phenolic compounds. *Food and Bioprocess Technology*, DOI 10.1007/s11947-013-1248-8.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A., Ferreira, I.C.F.R. (2014d). Triacylglycerols profiling as a chemical tool to identify mushrooms submitted to gamma or electron beam irradiation. *Food Chemistry*, *159*, 399-406.

FNB (Food and Nutrition Board), 2001. Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Washington, DC: Institute of Medicine, *Food and Nutrition Board*. National Academy of Sciences.

Gençcelep, H., Uzun, Y., Tunçtürk, Y., & Demirel, K. (2009). Determination of mineral contents of wild-grown edible mushrooms. *Food Chemistry*, *113*, 1033-1036.

Hill, T., & Lewicki, P. (2006). Statistics: Methods and Applications. A Comprehensive Reference for Science, Industry, and Data Mining; StatSoft: Tulsa, OK, USA.

Horwitz, W., & Latimer, G.W. (2005). Official methods of analysis of AOAC International, 18th Edition. Gaithersburg, Md.: AOAC International.

ICGFI - International Consultative Group on Food Irradiation, 1999. Facts about Food Irradiation. Buckinghamshire, United Kingdom.

Işiloğlu, M., Yilmaz, F., & Merdivan, M. (2009). Concentrations of trace elements in wild edible mushrooms. *Food Chemistry*, *73*, 169-175.

Kalač, P., & Svoboda, L. (2000). A review of trace element concentrations in edible mushrooms. *Food Chemistry*, *69*, 273-281.

Koyyalamudi, S.R., Jeong, S.-C., Manavalan, S., Vysetti, B., & Pang, G. (2013). Micronutrient mineral content of the fruiting bodies of Australian cultivated *Agaricus bisporus* white button mushrooms. *Journal of Food Composition and Analysis*, *31*, 109-114.

López, A., García, P., & Garrido, A. (2008). Multivariate characterization of table olives according to their mineral nutrient composition. *Food Chemistry*, *106*, 369-378.

Mahan, L.K., Escott-Stump, S., & Raymond, J.L. (2012). Krause Dietoterapia (13th ed.) Elsevier, Mexico.

McDermott, J.H. (2000). Antioxidant nutrients: current dietary recommendations and research update. *Journal of American Pharmacists Association*, *40*, 785-99.

McDowell, L.R. (2003). Minerals in Animal and Human Nutrition, 2nd ed. Amsterdam. The Netherlands, Elsevier.

Regulation (EC) No 1169/2011 of the European Parliament and of the Council, of 25 October 2011, on the provision of food information to consumers. *Official Journal of the European Union*. 22.11.2011. L 304/18- 63.

Rivera, C.S., Blanco, D., Marco, P., Oria, R., & Venturini, M.E. (2011). Effects of electron-beam irradiation on the shelf life, microbial populations and sensory characteristics of summer truffles (*Tuber aestivum*) packaged under modified atmospheres. *Food Microbiology*, *28*, 141-148.

Ruiz-Rodríguez, B., Morales, P., Fernández-Ruiz, V., Sánchez-Mata, M.C.; Cámara, M., Díez-Marqués, C., et al. (2011). Valorization of wild strawberry tree fruits (*Arbutus unedo* L.) through nutritional assessment and natural production data. *Food Research International*, *44*, 1244-1253.

Seeger, R. (1982). Toxische schwermetalle in Pilzen. *Dtsch Apoth Ztg*, *122*, 1835-1844.

Tomkins, A. (2002). Nutrition, infection and immunity: public health implications. In: Calder, P.C., Field, C.J., Gill, H.S. (Eds.), Nutrition and Immune Function. CABI Publishing, Wallingford, UK, 375-412.

Trumbo, P., Schlicker, S., Yates, A.A., & Poos, M. (2002). Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. *Journal of the American Dietetic Association*, *102*, 1621-1630.

6.3.

Como é que a radiação gama e por feixe de eletrões afeta a atividade antimicrobiana de diferente extractos de cogumelos silvestres?

Este sub-capítulo apresenta as propriedades antibacterianas de extratos preparados a partir de amostras frescas de Boletus edulis *Bull. e* Hydnum repandum *L. Fr. (doses 1 e 2 kGy de radiação gama), amostras desidratadas e congeladas de* Macrolepiota procera *(Scop.) Singer (doses 0,5 e 1 kGy de radiação gama) e amostras desidratadas de* Russula delica *Fr. (doses 2, 6 e 10 kGy de feixe de eletrões), contra isolados clínicos com diferentes perfis de resistência da Unidade Local de Saúde de Mirandela, Nordeste de Portugal.*

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ORIGINAL ARTICLE

How gamma-rays and electron-beam irradiation would affect the antimicrobial activity of differently processed wild mushroom extracts?

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Abstract

Aims: The effects of irradiation (gamma-rays and electron-beams), up to 10 kGy, in the antimicrobial activity of mushroom species (*Boletus edulis*, *Hydnum repandum*, *Macrolepiota procera* and *Russula delica*) differently processed (fresh, dried, freeze) were evaluated.

Methods and Results: Clinical isolates with different resistance profiles from hospitalized patients in Local Health Unit of Mirandela, Northeast of Portugal, were used as target micro-organisms. The mushrooms antimicrobial activity did not suffer significant changes that might compromise applying irradiation as a possible mushroom conservation technology.
Conclusion: Two kGy dose (independently of using gamma-rays or electron-beams) seemed to be the most suitable choice to irradiate mushrooms.
Significance and Impact of the Study: This study provides important results in antimicrobial activity of extracts prepared from irradiated mushroom species.

Keywords: Electron-beam irradiation; Gamma-irradiation, irradiation technology, Multi-resistant bacteria, Wild mushroom extracts.

6.3.1. Introduction

The interest of the scientific community for mushrooms (extracts and derived compounds) has been increasing due to their potential in prevention or treatment of different diseases of the modern world. Different mushroom species were previously reported for their anticancer, anti-inflammatory, immunosuppressive and antibacterial properties (Asfors and Ley 1993; Wieczorek *et al*. 1993; Ferreira *et al*. 2010; Alves *et al*. 2014).

In particular, several authors reported the antimicrobial activity of extracts prepared from different mushroom species including *Boletus edulis* Bull. (Kosanić *et al*. 2011), *Hydnum repandum* L. Fr., (Ozen *et al*. 2011), *Macrolepiota procera* (Scop.) Singer, and *Russula delica* Fr. (Türkoğlu *et al*. 2007; Yaltirak *et al*. 2009; Alves *et al*. 2012b). In this sense, mushrooms have been recognized as functional foods and as a source for the development of drugs and nutraceuticals (Lindequist *et al*. 2005; Poucheret *et al*. 2006).

Nevertheless, and in spite of these undeniable qualities, mushrooms are one of the most perishable products and tend to loose quality immediately after harvest. A short shelf life (1-3 days at room temperature) is one of the disadvantages for their distribution and marketing as a fresh product. Their shelf life is limited due to

postharvest changes, such as browning, cap opening, stipe elongation, cap diameter increase, weight loss and texture changes; but also due to a high respiration rate and lack of physical protection to avoid water loss or microbial attack (Akram and Kwon 2010; Fernandes *et al*. 2012).

Irradiation is recognized as a safe and effective preservation method, being used worldwide to increase the shelf life of foods (*e.g*, fruits and legumes, spices, grains, meat or seafood) (Andrews *et al*. 1998; Fernandes *et al*. 2012). Gamma-irradiation (Beaulieu *et al*. 2002) and electron-beam (Koorapati *et al*. 2004) are considered potential tools in extending the shelf life of fresh mushrooms. Furthermore, different regulatory agencies ensure that food irradiation is a safe process in relation to the processing of food for humans (USFDA 1991; WHO 1994).

Our research group has been investigating the effects of mushrooms (including the above mentioned species *B. edulis*, *H. repandum*, *M. procera* and *R. delica*) irradiation by evaluating their nutritional, physical and chemical parameters, concluding that these parameters are not affected in high extension with this preservation technology (Fernandes *et al*. 2013a-c, 2014a-c).

Nevertheless, to the authors' knowledge, there are no available reports about the effects of irradiation on mushrooms antimicrobial activity. Therefore, in the present study, the antibacterial properties of extracts prepared from irradiated (gamma-radiation and electron-beam) wild mushrooms were assessed against clinical isolates with different resistance profiles from hospitalized patients in Local Health Unit of Mirandela, Northeast of Portugal.

6.3.2. Materials and methods

Standards and Reagents

To estimate the dose and dose rate of gamma-irradiation it was used a chemical solution sensitive to ionizing radiation, Fricke dosimeter, prepared in the lab following the standards (ASTM 1992) and Amber Perspex dosimeters (batch V, from Harwell Co., UK). To prepare the acid aqueous Fricke dosimeter solution, the following reagents were used as follows: ferrous ammonium sulphate(II)

hexahydrate, sodium chloride and sulphuric acid, all purchased from Panreac S.A. (Barcelona, Spain) with purity PA (proanalysis), and water treated in a Milli-Q water purification system (Millipore, model A10, USA).

Methanol was of analytical grade purity from Lab-Scan (Lisbon, Portugal). The culture media Muller Hinton broth (MHB), Wilkins-Chalgren Broth (WCB) and Columbia agar (CA) with 5% horse blood were obtained from Biomerieux (Marcy l´Etoile, France), respectively. The dye *p*-iodonitrotetrazolium chloride (INT) was purchased from Sigma–Aldrich (Spruce Street; St. Louis, USA) to be used as microbial growth indicator.

Samples and samples irradiation

Macrolepiota procera, Boletus edulis, Hydnum repandum and *Russula delica* (**Figure 6.3.1)** were collected in Trás-os-Montes, in the Northeast of Portugal; the first mushroom species were collected in November 2011 and the three other species were collected in November 2012.

B. edulis and *H. repandum* fresh fruiting bodies were divided in three groups (each species) with three mushrooms per group and submitted to gamma-irradiation according to the procedure described by Fernandes *et al*. (2013a): control (non-irradiated, 0 kGy), sample 1 (irradiated with 1 kGy) and sample 2 (irradiated with 2 kGy). *M. procera* fruiting bodies were divided in two groups with nine mushrooms per group, and further submitted to different processing technologies according to the procedure described by Fernandes *et al*. (2014a): freezing (at -20º C in a freezer) and drying (at 30 ºC in an oven). Each group was further subdivided in three subgroups and submitted to gamma-irradiation: control (non-irradiated, 0 kGy); sample 1 (irradiated with 0.5 kGy) and sample 2 (irradiated with 1 kGy). *R. delica* fruiting bodies were divided in four groups with six mushrooms per group, dried at 30 ºC in an oven and then submitted to electron-beam irradiation according to the procedure described by Fernandes *et al*. (2014b): control (non-irradiated, 0 kGy), sample 1 (irradiated with 2 kGy), sample 2 (irradiated with 6 kGy), and sample 3 (irradiated with 10 kGy).

Figure 6.3.1 Representative samples of the control population of *M. procera*, *B. edulis*, *H. repandum* and *R. delica* (from the left to the right). Mushrooms of the other groups (irradiated) are similar to the control.

Extracts preparation

Each mushroom lyophilized sample (approx. 1.5 g) was extracted using a methanol : water (80 : 20; 30 mL) mixture at -20 °C for 1.5 h. After 15 min in an ultrasonic bath, filtered through Whatman nº 4 paper. The residue was then re-extracted with additional 30 mL of methanol : water mixture at -20 °C for 1.5 h and the previous steps were repeated. The combined extracts were evaporated at 40 ºC under reduced pressure to remove methanol (rotary evaporator Büchi R-210, Flawil, Switzerland), lyophilized, redissolved in water, at a concentration of 200 mg/mL, and stored at -20 ºC for further use.

Micro-organisms and culture media

The micro-organisms used were clinical isolates from patients hospitalized in various departments of the Local Health Unit of Mirandela, Northeast of Portugal.

Two Gram-negative bacteria (*Escherichia coli* and *Proteus mirabilis*, isolated from urine) and two Gram-positive bacteria (MSSA- Methicillin-sensitive *Staphylococcus aureus*, isolated from Wound exudate and MRSA- methicillin-

resistant *Staphylococcus aureus*, isolated from expectoration) were used to screen the antimicrobial activity of the mushroom extracts.

Characterization of antibiotic susceptibility of target strains

The characterization of antibiotic susceptibility and the identification of the target strains were performed using VITEK® 2 Compact card (Biomérieux, Lyon, France). These cards allow the simultaneous determination of susceptibility to antimicrobial agents and the strain identification, including aerobic and facultative anaerobic Gram-negative bacilli (VITEK® 2 Compact AST-N192), and Gram-positive cocci such as some fastidious aerobic Gram positive (VITEK® 2 Compact AST-P619). The sensibility of the micro-organisms to each antibiotic is identified by the MIC.

Determination of the antimicrobial activity of the extracts

MIC determinations were performed by the microdilution method and the rapid *p*-iodonitrotetrazolium chloride (INT) colorimetric assay following the methodology suggested by Kuete *et al*. (2011a, b) with some modifications.

Initially, 50 µL of each mushroom extract (200 mg/mL) was diluted in 450 µL of MHB (final concentration of 20 mg/L) and then, 200 µL of this extract solution was added in each well (96-well microplate). Dilutions were carried out over the wells containing 100 µL of MHB and afterwards, 10 µL of inoculum (1 $\times 10^8$ CFU/mL) were added to all the wells. Two negative (one with MHB and the other with the mushroom extract) and one positive (with MHB and the inoculum) controls were prepared. The plates were incubated at 37 °C, for 24 h, in an oven (Jouan, Berlin, Germany).

The MIC of the samples was detected after adding INT (0.2 mg/mL, 40 µL) and incubating at 37 °C for 30 min. Viable micro-organisms reduced the yellow dye to a pink color. MIC was defined as the lowest mushroom extract concentration that prevented this change and exhibited complete inhibition of bacterial growth. All the assays were carried out in duplicate.

6.3.3. Results

Before analysing the potential antimicrobial activity of the studied mushrooms, the resistance profiles of the bacterial isolates are described in **Table 6.3.1**. From its interpretation, it is possible to conclude that *E. coli* and MSSA constitute wild strains with small resistance to the tested antibiotic groups, excluding the result obtained with MSSA against benzylpenicillin. On the other hand, *P. mirabilis* and MRSA showed resistance against the quinolone group (ciprofloxacin and levofloxacin) and β-lactamic antibiotics (amoxicillin/clavulanic acid). In addition, *P. mirabilis* was also resistant to trimethoprim/sulfasoxazole (antimethabolic) and nitrofurantoin. Overall, the obtained MIC values are in the same range as those obtained in a previous study (Alves *et al*. 2012b).

The values depicted in each table should not be directly compared, as results in **Table 6.3.1** were achieved using pure compounds, while those in **Table 6.3.2** derive from using crude extracts with several possible interferences.

Centering the discussion in the antimicrobial activity of mushroom extracts, a primary observation allows concluding that the Gram-positive bacteria presented more susceptibility, in agreement with other reports (Alves *et al*. 2012b; Barros *et al*. 2007; Barros *et al*. 2008a, b; Venturini *et al.* 2008), despite the results obtained by Ozen *et al*. (2011), which indicated that *H. repandum* extracts had a maximal antibacterial activity against a Gram-negative bacteria (*Pseudomonas aeruginosa*).

Among all tested extracts, those obtained from fresh *B. edulis* presented the highest activity against all tested bacterial strains, followed by dried samples of *M. procera*. Similar resistance levels were previously reported for *B. edulis* samples assayed against *E. coli* and MSSA (Kosanić *et al*. 2012). The results reported herein for dried *R. delica* extracts indicate slightly higher antibacterial activity, namely against *P. mirabilis* and MRSA, than those obtained in our laboratory with the same species (Alves *et al*. 2012b). Nevertheless, the results from both studies might be considered as belonging to the same range. As the studied species have no phenolic compounds (Vaz *et al*. 2011), the antimicrobial activity could be due to the presence of steroids, sesquiterpenes, organic acids, or peptides, which represent antimicrobial compounds commonly found in mushrooms (Alves *et al*. 2012a).

The mushroom species studied herein, among several others, might benefit from an increased shelf life when submitted to irradiation, either using gamma-rays (Beaulieu *et al*. 2002; Fernandes *et al*. 2013a-c, 2014a) or electron-beams (Koorapati *et al*. 2004; Fernandes *et al*. 2014b, c) as energy sources. In all studied cases, the chemical composition and antioxidant activity of each mushroom species were not negatively affected by irradiation treatment. Considering the ability to maintain the antioxidant activity, we hypothesized that irradiated mushrooms might likewise present the same antimicrobial activity. The results obtained for differentially processed mushrooms (fresh, freeze or dried) treated with gamma irradiation or electron-beam irradiations with varying doses are presented in **Table 6.3.2**. As a first remark, it should be clarified that the used heterogeneous samples were defined to prove the feasibility of irradiation treatment in the maintenance of antimicrobial activity, independently of mushroom species, mushroom processing, irradiation source and irradiation dose. As it can be concluded from **Table 6.3.2**, the antimicrobial activity was not adversely affected in most of the cases, except gamma-irradiated *B. edulis* against *P. mirabilis*. In fact, this activity was sometimes potentiated, as it was verified for the frozen samples of *M. procera* against *E. coli* and MSSA, or the dried sample of *R. delica* against *E. coli*.

Some matrix effects must indeed be considered. For instance, the 1 kGy dose does not seem to be the preferable choice to treat *B. edulis* and *H. repandum* (which have a better response to the 2 kGy dose), but the same dose had advantageous effects when used to irradiate the *M. procera* samples. This indicates that the chemical composition of a determined mushroom might impart different outcomes as a result of the same irradiation dose. Nevertheless, the results obtained for irradiated samples and controls are very similar, indicating that there are no significant changes in the antimicrobial activity. As a general conclusion, the results indicate that the 2 kGy should be considered as the best choice in terms of antimicrobial activity maintenance.

Table 6.3.1 MIC values (µg/mL) of different antibiotics against Gram-negative bacteria and Gram-positive bacteria.

	Escherichia coli		*Proteus mirabilis*		*MRSA*		*MSSA*	
Benzylpenicillin					≥ 0.5	R	≥ 0.5	R
Ampicillin	≤ 2	S	≤ 2	S				
Oxacillin					≥ 4	R	≤ 0.25	S
Claritromycin					na	S	na	R
Clindamycin					≤ 0.25	S	≤ 0.25	S
Daptomycin					0.25	S	0.25	S
Erythromycin					≤ 0.25	S	≥8	R
Fosfomycin					≤ 8	S	≤ 8	S
Moxifloxacin					1	I	≤ 0.25	S
Gentamicin	≤ 1	S	≤ 1	S	≤ 0.5	S	≤ 0.5	S
Ciprofloxacin	≤ 0.25	S	≥ 4	R	na	R	na	S
Levofloxacin	≤ 0.12	S	≥ 8	R	4	R	0.25	S
Linezolid					2	S	2	S
Teicoplanin					≤ 0.5	S	≤ 0.5	S
Tigecycline					≤ 0.12	S	≤ 0.12	S
Tetracycline					≤ 1	S	≤ 1	S
Trimethoprim/Sulfasoxazole	≤ 20	S	≥ 320	R	≤ 10	S	≤ 10	S
Vancomycin					≤ 0.5	S	1	S
Amoxicillin/Clavulanic acid	≤ 2	S	16	R	na	R	na	S
Cephalothin	4	S	8	S				
Cefuroxime	4	S	≤ 1	S				
Cefuroxime Axetil	4	S	≤ 1	S				
Cefotaxime	≤ 1	S	≤ 1	S	na	R	na	S
Ceftazidime	≤ 1	S	≤ 1	S				
Ceftriaxona	na	S			na	R	na	S
Ertapenem	≤ 0.5	S	1	I				
Meropenem	≤ 0.25	S	0.8	S				
Amikacin	≤ 2	S	≤ 2	S				
Tobramycin	≤ 1	S	≤ 1	S				
Fusidic acid					≤ 0.5	S	≤ 0.5	S
Mupirocin					≤ 2	na	≤ 2	na
Rifampicin					≤ 0.5	na	≤ 0.5	na
Nitrofurantoin	≤ 16	S	≥ 512	R	≤ 16	S	≤ 16	S
Piperacillin/Tazobactam	≤ 4	S	≤ 4	S				

S- susceptible; I- intermediate; R- resistant (this classification was made according to the interpretative breakpoints suggested by Clinical and Laboratory Standards Institute - CLSI); na- not applicable.

Table 6.3.2 MIC values (mg/mL) of irradiated wild mushrooms against clinical isolates of Gram-negative and Gram-positive bacteria.

		Escherichia coli	*Proteus mirabilis*	*MRSA*	*MSSA*
		gamma-irradiated *Boletus edulis*			
0 kGy	fresh	10	5	2.5	5
1 kGy	fresh	10	20	5	5
2 kGy	fresh	10	10	2.5	5
		gamma-irradiated *Hydnum repandum*			
0 kGy	fresh	10	>20	10	5
1 kGy	fresh	20	>20	20	10
2 kGy	fresh	10	>20	10	5
		gamma-irradiated *Macrolepiota procera*			
Control	dried	20	20	5	10
	frozen	20	>20	5	10
0.5 kGy	dried	20	20	5	10
	frozen	10	>20	5	5
1 kGy	dried	20	20	5	10
	frozen	10	>20	5	5
		electron-beam irradiated *Russula delica*			
Control	dried	>20	10	5	10
2 kGy	dried	>20	10	5	5
6 kGy	dried	20	10	5	10
10 kGy	dried	20	10	5	5

MIC- minimum inhibitory concentration; MRSA- methicillin-resistant *Staphylococcus aureus*; MSSA- Methicillin-sensitive *Staphylococcus aureus*.

6.3.4. Discussion

The development of antibacterial agents' resistance and the decrease of effective antifungal agents are leading the researchers towards investigating novel chemical structures and considering new natural sources of compounds. Usually, compounds found in natural matrices exert their antimicrobial activity by interacting with the microorganism's cell membrane or cell wall, altering its permeability and causing cell destruction. The antimicrobial activity might also be explained by infiltration into bacterial cells, followed by promoting the coagulation of the cell content (Taguri *et al*. 2006; Tian *et al*. 2009).

The antimicrobial activity of different mushroom species was previously reported, particularly in what regards their effectiveness against Gram positive and Gram negative bacteria (Türkoğlu *et al.* 2007; Venturini *et al.* 2008; Yaltirak *et al.* 2009; Ozen *et al*. 2008; Alves *et al*. 2012a,b; Kosanić *et al*. 2012; Alves *et al.* 2014; Smolskaitė *et al*. 2014). In this study, instead of using commercial bacterial cultures, the antibacterial activity was evaluated using clinical isolates from different body fluids. The obtained bacteria had the common feature of showing some resistance against the typically used antibiotics.

After several studies have been conducted proving that neither the chemical composition nor the antioxidant activity of different mushroom extracts were adversely changed by irradiation treatment, the feasibility of this technology was tested herein by verifying its effects in the antimicrobial activity of different mushroom species. Besides using different mushrooms, samples were processed differently (fresh, dried, freeze) according to the most common practices applied to mushrooms. Likewise, different doses (0.0, 0.5, 1, 2, 6 and 10 kGy) and two irradiation sources (gamma-rays and electron-beams) were evaluated. Under all the assayed conditions, the antimicrobial activity did not suffer significant changes that might compromise applying irradiation as a possible mushroom conservation technology. As a final remark, the 2 kGy dose (independently of using gamma-rays or electron-beams) seemed to be the most suitable choice. Nevertheless, future assays with additional mushrooms and/or bacterial cultures will certainly be needed before guaranteeing the complete adequacy of this treatment.

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Conflict of Interest

The authors have no conflicts of interest.

6.3.5. References

Akram, K. and Kwon, J.-H. (2010) Food Irradiation for Mushrooms: A Review. *J Korean Soc Appl Biol Chem* **53**, 257-265.

Alves, M.J., Ferreira, I.C.F.R., Dias, J., Teixeira, V., Martins, A. and Pintado, M. (2012a) A review on antimicrobial activity of mushroom (Basidiomycetes) extracts and isolated compounds. *Planta Medica* **78**, 1707-1718.

Alves, M.J., Ferreira, I.C.F.R., Martins, A. and Pintado, M. (2012b) Antimicrobial activity of wild mushroom extracts against clinical isolates resistant to different antibiotics. *J Appl Microbiol* **113**, 466-475.

Alves, M.J., Ferreira, I.C.F.R., Lourenço, I., Castro, A., Pereira, L., Martins, A. and Pintado, M. (2014) Wild mushrooms extracts potentiate the action of standard antibiotics against multi-resistant bacteria. *J Appl Microbiol* **116**, 32-38.

Andrews, L.S., Ahmedna, M., Grodner, R. M., Liuzzo, J.A., Murano, P.S. and Murano, E.A. (1998) Food preservation using ionizing radiation. *Review Environm Contam Toxicol* **154**, 1-53.

Asfors, K.E. and Ley, K. (1993) Sulfated polysaccharides in inflammation. *J Labor Clinical Med* **121**, 201-202.

ASTM, American Society for Testing and Materials (1992) Practice for Using the Fricke Reference Standard Dosimetry System, ASTM E1026. Annual Book of ASTM Standards, 12.02, Philadelphia, PA.

Barros, L., Cruz, T., Baptista, P., Estevinho, L.M. and Ferreira, I.C.F.R. (2008a) Wild and commercial mushrooms as source of nutrients and nutraceuticals. *Food Chem Toxicol* **46**, 2742-2747.

Barros, L., Venturini, B.A., Baptista, P., Estevinho, L.M. and Ferreira, I.C.F.R. (2008b) Chemical composition and biological properties of Portuguese wild mushrooms: a comprehensive study. *J Agric Food Chem* **56**, 3856-3862.

Barros, L., Calhelha, R.C., Vaz, J.A., Ferreira, I.C.F.R., Baptista, P. and Estevinho, L.M. (2007) Antimicrobial activity and bioactive compounds of Portuguese wild edible mushrooms. *Eur Food Res Technol* **225**, 151-156.

Beaulieu, M., D'Aprano, G. and Lacroix, M. (2002) Effect of dose rate of gamma irradiation on biochemical quality and browning of mushrooms *Agaricus bisporus*. *Radiation Physics and Chemistry* **63**, 311-315.

BIOMERIEUX . Bacteriana VITEK teste de sensibilidade aos antibióticos identificação 2 [acesso em 27 de janeiro de 2014]. Disponível em: http://www.biomerieux.com/en/bacterial-identification-antibiotic-susceptibility-testing-vitek2.

Fernandes, Â., Antonio, A.L., Oliveira, M.P.P., Martins, A. and Ferreira, I.C.F.R. (2012) Effect of gamma and electron beam irradiation on the physico-chemical and nutritional properties of mushrooms: A review. *Food Chem* **135**, 641-650.

Fernandes, Â., Antonio, A.L., Santos, P.M.P., Oliveira, M.P.P., Martins, A. and Ferreira, I.C.F.R. (2013a) Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Res Intern* **54**, 18-25.

Fernandes, Â., Antonio, A.L., Barreira, J.C.M., Botelho, L., Oliveira, M.P.P., Martins, A. and Ferreira, I.C.F.R. (2013b) Effects of gamma irradiation on the chemical composition and antioxidant activity of *Lactarius deliciosus* L. wild edible mushroom. *Food Bioprocess Technol* **6**, 2895-2903.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Santos, P.M.P., Martins, A., Oliveira, M.B.P.P. and Ferreira, I.C.F.R. (2013c) Study of chemical changes and antioxidant activity variation induced by gamma-irradiation on wild mushrooms: Comparative study through principal component analysis. *Food Res Intern* **54**, 18-25.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A. and Ferreira, I.C.F.R. (2014a) Effects of gamma irradiation on chemical composition and antioxidant potential of processed samples of the wild mushroom *Macrolepiota procera*. *Food Chem* **149**, 91-98.

Fernandes, Â., Barreira, J.C.M., Antonio, A. L., Oliveira, M.B.P.P., Martins, A. and Ferreira, I.C.F.R. (2014b) Feasibility of electron-beam irradiation to preserve wild dried mushrooms: Effects on chemical composition and antioxidant activity. *Innov Food Sci Emerg Technol* **22**, 158-166.

Fernandes, Â., Barreira, J.C.M., Antonio, A.L., Oliveira, M.B.P.P., Martins, A. and Ferreira, I.C.F.R. (2014c) Combined effects of electron-beam irradiation and

storage time on the chemical and antioxidant parameters of wild *Macrolepiota procera* dried samples. *Food Bioprocess Technol* **7**, 1606-1617.

Ferreira, I.C.F.R., Vaz, J.A., Vasconcelos, M.H. and Martins, A. (2010) Compounds from wild mushrooms with antitumor potential. *Anti-cancer Agents Med Chem* **10**, 424-436.

Koorapati, A., Foley, D., Pilling, R. and Prakash, A. (2004) Electron-beam irradiation preserves the quality of white button mushrooms (*Agaricus bisporus*) slices. *J Food Sci Technol* **6**, 25–29.

Kosanić, M., Ranković, B. and Dašić, M. (2012) Mushrooms as Possible Antioxidant and Antimicrobial Agents. *Ira J Pharm Res* **11**, 1095-1102.

Kuete, V., Ango, P.Y., Fotso, G.W., Kapche, G.D., Dzoyem, J.P., Wouking, A.G., Ngadjui, B.T. and Abegaz, B.M. (2011a) Antimicrobial activities of the methanol extract and compounds from Artocarpus communis (Moraceae). *BMC Complement Alt Med* **25**, 11-42.

Kuete, V., Kamga, J., Sandjo, L.P., Ngameni, B., Poumale, H.M., Ambassa, P. and Ngadjui, B.T. (2011b) Antimicrobial activities of the methanol extract, fractions and compounds from Ficus polita Vahl (Moraceae). *BMC Complement Alt Med* **26**, 11-16.

Lindequist, U., Niedermeyer, T.H.J. and Julich, W.D. (2005) The pharmacological potential of mushrooms. *eCAM* **2**, 285-299.

Ozen, T., Darcan, C., Aktop, O. and Turkekull, I. (2011) Screening of antioxidant, antimicrobial activities and chemical contents of edible mushrooms wildly grown in the Black Sea region of Turkey. *Comb Chem Hight Throughput Screen* **14**, 72-84.

Poucheret, P., Fons, F. and Rapior, S. (2006) Biological and pharmacological activity of higher fungi: 20-year retrospective analysis. *Mycolog* **27**, 311-333.

Smolskaitė, L., Venskutonis, P.R. and Talou, T. (2014) Comprehensive evaluation of antioxidant and antimicrobial properties of different mushroom species. *LWT - Food Sci Technol*, *in press.*

Taguri, T., Tanaka, T. and Kouno, I. (2006) Antibacterial spectrum of plant polyphenols and extracts depending upon hydroxyphenyl structure. *Biol Pharm Bull* **29**, 2226-2235.

Tian, F., Li, B., Ji, B., Zhang, G. and Luo, Y. (2009) Identification and structure-activity relationship of gallotannins separated from *Galla chinensis*. *LWT - Food Sci Technol* **42**, 1289-1295.

Türkoğlu, A., Duru, M.E. and Mercan, N. (2007) Antioxidant and Antimicrobial Activity of *Russula delica* Fr: An Edidle Wild Mushroom. *Eur J Anal Chem* **2**, No 1.

USFDA. (1991) Irradiation in the production, processing and handling of food. *Code Federal Register*, Title 21, part 179.

Vaz, J.A., Barros, L., Martins, A., Morais, J.S., Vasconcelos, M.H. and Ferreira, I.C.F.R. (2011) Phenolic profile of seventeen Portuguese wild mushrooms. *LWT - Food Sci Technol* **44**, 343-346.

WHO (World Health Organisation) (1994) Safety and nutritional adequacy of irradiated food. WHO, Geneva.

Wieczorek, Z., Siemion, I.Z., Zimecki, M., Bolewska-Pedyczak, E. and Wieland, T. (1993) Immunosuppressive activity in the series of cycloamanide peptides from mushrooms. *Peptides* **14**, 1-5.

Yaltirak, T., Aslim, B., Ozturk, S. and Alli, H. (2009) Antimicrobial and antioxidant activities of *Russula delica* Fr. *Food Chem Toxicol* **47**, 2052-2056.

Venturini, M.E., Rivera, C.S., González, C. and Blanco, D. (2008) Antimicrobial activity of extracts of edible wild and cultivated mushrooms against foodborne bacterial strains. *J Food Prot* **71**, 1701-1706.

CAPÍTULO 7

Considerações finais e perspetivas futuras

Este capítulo apresenta as principais conclusões da tese e as perspetivas futuras

7.1. Considerações finais

A investigação planeada e executada durante o desenvolvimento desta tese de doutoramento teve sempre em mente obter informações que pudessem fundamentar a aplicação da irradiação em cogumelos silvestres. As diferentes tarefas desenvolvidas permitiram concretizar os vários objetivos previamente estabelecidos.

Dada a preferência dos consumidores por cogumelos frescos, foram avaliados os efeitos da radiação gama em diferentes espécies de cogumelos silvestres comestíveis: *Lactarius deliciosus*, *Boletus edulis*, *Hydnum repandum*, *Boletus pinophilus* e *Clitocybe subconnexa*.

- Nas amostras de *L. deliciosus* demonstrou-se que até à dose de 1 kGy, a combinação da radiação gama e o armazenamento a frio, não afetou as propriedades físicas, o perfil em açúcares nem a atividade antioxidante. Os efeitos nos macronutrientes/valor energético e na composição em tocoferóis, foram menos significativos do que os efeitos provocados pelo tempo de armazenamento (até 8 dias).
- Nas amostras de *Boletus edulis* e *Hydnum repandum*, apesar das diferenças observadas em alguns compostos individualmente, os macronutrientes/valor energético (muito relevantes em termos da aceitabilidade pelos consumidores de cogumelos) foram menos afetados.
- O efeito observado na atividade antioxidante de *Boletus pinophilus* e *Clitocybe subconnexa* foi bastante interessante, uma vez que se verificou um aumento da bioatividade e as alterações químicas detetadas foram admissíveis.

Tendo em conta que os cogumelos não são consumidos apenas em fresco, processaram-se amostras de *M. procera* de acordo com as práticas mais comuns (congelamento e desidratação). Comparou-se esse efeito com a aplicação de radiação gama em amostras frescas. Verificou-se que a congelação e a desidratação provocaram diferenças significativas nos parâmetros químicos avaliados, tendo a irradiação gama revelado maior capacidade de retenção do perfil químico das amostras frescas.

Aplicando, posteriormente, radiação gama a amostras processadas (desidratadas e congeladas) verificou-se que a irradiação atenuou os efeitos causados pela desidratação e/ou congelação; combinando a congelação com a dose de 0,5 kGy conseguiu preservar-se o teor em tocoferóis totais. No que respeita à composição em ácidos orgânicos e em compostos fenólicos individuais, as diferenças causadas pelo tipo de processamento (congelação e desidratação) foram maiores do que as verificadas com diferentes doses de irradiação. Assim, em vez de se aplicar apenas uma metodologia, a radiação gama pode atuar como um complemento de outras técnicas de conservação, nomeadamente a congelação e a desidratação.

Atendendo à presença significativa de cogumelos desidratados no mercado, aplicou-se a este tipo de amostras uma outra tecnologia de irradiação, feixe de eletrões, que consegue atravessar alimentos de menor espessura e, portanto, adequado para este tipo de amostras. Tal como nos estudos de aplicação de radiação gama, estudaram-se diferentes espécies de cogumelos silvestres: *Macrolepiota procera*, *Boletus edulis*, *Russula delica*, *Amanita caesarea* e *Amanita curtipes*.

- Nas amostras de *M. procera* verificou-se que o tempo de armazenamento (até 12 meses) teve um efeito significativo em todos os parâmetros analisados, comparativamente com as doses de irradiação, exceto para os ácidos gordos, que sofreram alterações significativas.
- A irradiação aplicada às amostras de *B. edulis* e *R. delica*, especialmente em doses mais elevadas (10 kGy), teve efeitos significativos nos perfis químicos (o teor em proteínas, açúcares e ácidos orgânicos diminuiu ligeiramente, enquanto que os ácidos gordos insaturados, tocoferóis e compostos fenólicos individuais apresentaram níveis mais elevados nas amostras irradiadas) e na atividade antioxidante que aumentou nas amostras irradiadas.
- Os perfis avaliados em *A. caesarea* e *A. curtipes* indicaram diferenças entre as amostras irradiadas e as amostras controlo (não irradiadas), mas com elevada similaridade entre as diferentes doses aplicadas (2, 6 e 10 kGy), verificando-se uma melhoria da atividade antioxidante.

- Observaram-se também algumas alterações significativas no teor em hidratos de carbono e fibras, em amostras de *M. procera* e *B. edulis*, especialmente as tratadas com as doses mais altas (6 e 10 kGy, respetivamente).

O perfil de triacilgliceróis monstrou ser uma ferramenta com potencial de utilização para deteção de cogumelos irradiados, independentemente da espécie, fonte de irradiação ou tipo de processamento. Efetivamente, verificou-se que os efeitos de cada tipo de radiação foram significativamente diferentes e que, até mesmo as doses mais baixas, tiveram um ligeiro efeito sobre os triacilgliceróis.

Os efeitos da radiação gama e por feixe de eletrões nos perfis em macro e microelementos também foram avaliados, em amostras de *B. edulis*, *H. repandum* e *M. procera*. As doses de irradiação aplicadas não mostraram um efeito sistemático sobre os perfis referidos, com exceção da dose de 10 kGy. Na, K e Ca foram os elementos com alterações mais significativas com os dois tipos de irradiação.

Em todas as condições ensaiadas, a atividade antimicrobiana não sofreu alterações significativas que possam comprometer a aplicação da irradiação como uma possível tecnologia de conservação de cogumelos.

Têm sido realizados vários estudos de irradiação, sobretudo em cogumelos cultivados, que demonstram que nem a composição química nem a atividade antioxidante são alteradas negativamente. O presente estudo, pioneiro em cogumelos silvestres, demonstrou o mesmo. No entanto, tal como qualquer outro método de processamento de alimentos, a irradiação pode preservar alguns componentes e degradar outros. O equilíbrio entre as suas vantagens e desvantagens, em comparação com outros métodos de preservação, deve ser considerado na seleção deste tipo de tecnologia, proporcionando ao consumidor um produto que cumpra os melhores critérios de qualidade e segurança.

Uma das principais consequências da irradiação em glúcidos é a quebra das ligações C-H e o rompimento das ligações éter (WHO, 1999). Os ácidos gordos insaturados são mais propensos à oxidação uma vez que são mais instáveis do que

os ácidos gordos saturados. O mecanismo geral da radiólise dos lípidos envolve a ionização primária, seguida da migração da carga positiva, quer para o grupo carboxílico, quer para as ligações duplas (Stewart, 2001), aumentando assim a degradação de ácidos gordos insaturados.

As proteínas estão entre os indicadores de irradiação mais confiáveis, especialmente devido às reações de degradação, como a cisão das ligações C-N ou a divisão das ligações dissulfito, e às mudanças físicas, como desenrolamento, desdobramento e agregação (Stewart, 2001). No entanto, o facto de a irradiação alterar as proteínas não cria um problema significativo do ponto de vista nutricional, uma vez que os aminoácidos, estando no interior da estrutura proteica, sobrevivem ao processo (Stewart, 2001).

Quando da aplicação da irradiação, tal como em tratamentos térmicos, ocorrem algumas perdas de vitaminas que, geralmente, aumentam com a dose. A irradiação com doses elevadas requer, frequentemente condições de processamento que minimizem os efeitos sensoriais indesejáveis, condições que também contribuem para uma redução das perdas de vitaminas. A vitamina E é considerada a vitamina mais sensível (WHO, 1999), podendo ser um bom indicador dos efeitos da irradiação (Stewart, 2001).

7.1.1. Bibliografia

WHO (1999). High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy: Report of a Joint FAO/IAEA/WHO Study Group, Geneva, ISBN: 92 4 120890 2.

Stewart, E. M. (2001). Food Irradiation Chemistry. In R. A. Mollins (Ed.), Food Irradiation Chemistry: Food irradiation: Principles and applications (pp. 37-76). New York, NY: John Wiley & Sons, Inc.

7.2. Perspetivas futuras

Os cogumelos apresentam excelentes propriedades medicinais e nutricionais mas, sobretudo, propriedades organoléticas de elevada especificidade e muito apreciadas.

Para além da composição química, a análise sensorial de cogumelos (aroma, sabor e textura) submetidos à irradiação ionizante é um aspeto importante para a aprovação deste tipo de processamento, uma vez que determina o grau de aceitação por parte dos consumidores. Assim, constitui uma perspetiva futura no seguimento deste trabalho.

Uma outra perspetiva futura deste trabalho será a avaliação dos efeitos da irradiação em aspectos biológicos (insetos) e microbiológicos (bactérias e/ou vírus), validando a técnica do ponto de vista da descontaminação em cogumelos frescos, desidratados e congelados. Neste sentido, é importante validar também o efeito das diferentes doses e tecnologias de irradiação sobre pragas e na carga microbiana, aferindo efetivamente se diminui e/ou inativa os insetos e/ou microrganismos que causam a deterioração do alimento e podem pôr em risco a saúde do consumidor.

No entanto, a maior tarefa parece ser o esclarecimento dos consumidores relativamente a alimentos irradiados. Interpretações erradas e a falta de informação sobre a irradiação de alimentos têm limitado o uso desta tecnologia. Neste contexto, deverão ser implementadas atitudes começando pela consciencialização, educação e divulgação da segurança e dos benefícios da irradiação.

E a este respeito...

Apesar desta tecnologia de processamento de alimentos estar exaustivasmente estudada pela comunidade científica, a irradiação de alimentos ainda permanece com baixa aceitabilidade pelo consumidor devido a razões não-científicas, por existir uma associação errada entre alimentos irradiados e contaminação radioativa (presença de radioisótopos no alimento). No entanto, deve realçar-se que na radiação por feixe de eletrões a energia utilizada é limitada a 10 MeV e para os raios-X é 5 MeV; na radiação gama, a energia emitida pelo ^{60}Co é de cerca de 1 MeV e que estes valores não são suficientes para interagir com o núcleo das moléculas, não induzindo radioatividade nos alimentos.

A ideia de que a irradiação é um “aditivo”, mantido no interior do alimento, foi inicialmente referida em certos documentos legislativos, induzindo a equívocos

científicos no que se refere aos processos físicos que utilizam a radiação eletromagnética no processamento de alimentos (Nordion, 2013).

Outra preocupação do consumidor é a formação de produtos radiolíticos ou produtos secundários que possam ter efeitos sobre a saúde. No entanto, esta questão foi clarificada pela *Organização Mundial de Saúde* no relatório sobre a "*Salubridade dos alimentos irradiados*", através do comité de especialistas da FAO/IAEA/WHO e do relatório da "*Segurança e Adequabilidade Nutricional de Alimentos Irradiados*", que reviram mais de quatrocentos estudos científicos (WHO, 1981, 1994).

Os radicais formados pela irradiação são de vida curta, cerca de 10^{-11} segundos, reagindo rapidamente com outros componentes e formando produtos estáveis (EFSA, 2011). O processamento por radiação ionizante gera menor quantidade de sub-produtos do que outros tratamentos térmicos, como o ato de cozinhar, congelar ou pasteurizar (WHO, 1999).

Apesar de haver consenso dentro da comunidade científica sobre a segurança dos alimentos irradiados, a não aceitação por parte dos consumidores tende a persistir por parte dos consumidores e só a formação e educação podem mudar esta situação...

7.2.1. Bibliografia

Codex (2003). General standard for Irradiated Foods. CODEX STAN 106-1983, REV.1-2003, Codex Alimentarius Commission.

EFSA (2011). Scientific Opinion on the Chemical Safety of Irradiation of Food. *European Food Safety Authority Journal*, *4*, 1-57.

Nordion (2013). *The History of Food Irradiation.* http://www.nordion.com/documents/the-history-of-food-irradiation.pdf (accessed August 20, 2014).

WHO (1981). Wholesomeness of irradiated food. Report of a Joint FAO/IAEA/WHO Expert Committee. World Health Organization, Geneva.

WHO (1991). Food Irradiation - A Technique for Preserving and Improving the Safety of Food. World Health Organization, Geneva.

WHO (1999). High-dose irradiation: Wholesomeness of food irradiated with doses above 10 kGy: Report of a Joint FAO/IAEA/WHO Study Group, Geneva, ISBN: 92 4 120890 2.