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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. Apart from these methods, the specificity of triacylglycerol profiles has previously been detected in mushroom species, as has the effect of irradiation treatment in the triacylglycerol profiles of chestnut. Accordingly, the feasibility of using this as a chemometric indicator of irradiated mushrooms was evaluated. In line with the obtained results in literature, the effects of each type of irradiation were significantly different, as can be concluded from the correlations among discriminant functions and variables within each statistical test. Triacylglycerol profiling proved to be a useful tool to detect irradiated mushrooms, independently of the species or irradiation source, especially for doses above 1 kGy.

1. Introduction

Mushrooms are widely appreciated foods due to their nutritional, organoleptic (Kalač, 2009) and pharmacological properties (Lindequist, Niedermeyer, & Jülich, 2005). Nevertheless, the shelf life of mushrooms is very short due to several postharvest changes related to the high respiration rate and lack of physical protection, allowing water loss or bacteria and mould 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 extended food shelf life and reducing health hazards (Soika & Delincée, 2000).

The specific effects of radiation on the chemical composition of mushrooms 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 to 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 all countries of the 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 (Alberti et al., 2011; EN 1786, 1996), cellulose (Alberti et al., 2011; EN 1787, 2000) or crystalline sugar (Alberti et al., 2011; EN 13708, 2001). Several ESR studies were made for the identification of irradiated seafood: fish, crustaceans, 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

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(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 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).

The triacylglycerols (TAG) profile is specific to 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 (Barreira et al., 2013; Zeb, 2012). 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 for 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.

2. Materials and methods

2.1. 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-oleoylrac- 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-racglycerol (POO), of ≈99% purity, were purchased from Sigma (St. Louis, MO, USA). Petroleum ether of analytical grade was obtained from Fisher Scientific (Leicestershire, UK). The 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.

2.2. Samples

Macrolepiota procera, Boletus edulis, Russula delica and *Boletus pinophilus* were collected in Trás-os-Montes, in the Northeast of Portugal; the first two mushroom species were collected in November 2011 and the other species were collected in November 2012.

B. edulis fruiting bodies were divided into two groups with twelve mushrooms per group, and further submitted to drying (at 30 °C in an oven) or kept fresh (stored at 4 °C in a refrigerator). Each dried sample group was then subdivided in three subgroups submitted to gamma irradiation: control (non-irradiated, 0 kGy), sample 1 (irradiated with 1 kGy) and sample 2 (irradiated with 2 kGy), with 4 mushrooms per subgroup; each fresh sample group was subdivided in four subgroups treated with 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 3 mushrooms per subgroup.

M. procera fruiting bodies were divided into three groups with nine mushrooms per group, and further submitted to different processing technologies: freezing (at −20 °C in a freezer), drying (at 30 °C in an oven) and the third group was kept fresh (stored at 4 °C 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).

Besides the former mushrooms, which are among the species found in highest abundance, two additional species were studied. A second Boletus species (*B. pinophilus*) was studied following the same sampling used for the *B. edulis* fresh samples, except for the intermediate irradiation dose, which was not tested.

A brittlegill mushroom (*R. delica*) was also studied as an example of a less appreciated, although still 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.

2.3. Samples irradiation

2.3.1. 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), in November 2012 (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 °C). 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 (180°) for 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 previously calculated 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}.

2.3.2. 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 a 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 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 the dose Amber and Gammachrome YR dosimeters were used, with spectrophotometric methods at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previously