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 retain chemical profile of fresh *Macrolepiota procera* wild mushroom, when compared to freeze 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. A linear discriminant analysis revealed that chemical profiles obtained for each processing technology showed higher accuracy in defining the individual clusters corresponding to the naturally occurring groups, indicating that the tested dose caused weaker changes in the samples. *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.

1. Introduction

Mushrooms are rapidly perishable and they start deteriorating within a day after 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, the cryogenic method has been gaining 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). The freezing allows a better retention of nutritional values as well as sensory characteristics such as color, aroma, flavor 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 ionizing 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 some time 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 aging; destroying 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 (ICGFI, 1999; Akram & Kwon, 2010).

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 et al., 2013a). The obtained data shown that, until 1 kGy, gamma irradiation might provide a useful alternative to ensure the quality and extend their 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 the fresh samples (Fernandes et al., 2013b). *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 effects, 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.

2. Materials and methods

2.1. Samples and samples irradiation

Macrolepiota procera fruiting bodies were obtained from the region of 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 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 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 (7.216 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 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 lyophilized (FreeZone 4.5 model 7750031, Labconco, Kansas, USA), reduced to a fine dried powder (20 mesh), mixed to obtain homogenate samples and promptly analyzed.

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

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 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, Greenville, SC, USA).

2.3. Chemical composition

2.3.1. 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 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).

2.3.2. 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 Reis, Barros, Martins, & Ferreira (2012a), 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), auto-sampler (AS-2057 Jasco, Easton, MD, USA) 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).

2.3.3. Fatty acids. Fatty acids were determined by gas-liquid chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures described previously (Reis et al., 2012a). The analysis was carried out with a DANI

model GC 1000 instrument (Milan, Italy) 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.

2.3.4. Tocopherols. Tocopherols were determined after an extraction procedure previously described, using tocol as IS (Reis et al., 2012a). The analysis was carried out in the HPLC system described above connected to a fluorescence detector (FP-2020; Jasco, Easton, MD, USA) 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).

2.4. Antioxidant parameters

2.4.1. Extraction preparation. 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 (Reis, Martins, Barros, & Ferreira, 2012b) 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.

2.4.2. 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.

2.4.3. 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, 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.

2.4.4. 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.

2.4.5. 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.

2.4.6. 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 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.

2.5. 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 analyzed 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 effect 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 (Maroco, 2003; López, García, & Garrido, 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 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).

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 exception, PT×GID interaction was a significant ($p < 0.001$) source of variation for the results obtained in all the performed analytical assays. Accordingly, 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 generally not shown) some overall conclusions could be drawn.

3.1. Chemical composition

The values obtained for proximate composition (**Table 1**) of *M. procera* were similar to those reported in previous works (Fernandes et al., 2013b; 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 matter (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 practices (Barreira, Pereira, Oliveira, & Ferreira, 2010). Mannitol and trehalose were the major quantified sugars (**Table 1**). Dried samples presented the highest (**Figure 1A**) total free sugars content (19±1 g/100 g of dw), 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 conducted to 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 et al., 2013b).

The fatty acids profile of *M. procera* included 24 compounds (the most abundant were presented in **Table 2**) with the prevalence of linoleic acid (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 accepted. 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 can 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 it can be seen in **Figure 1B**, the 0.5 kGy dose minimized the amount of C16:1 when combined with freeze treatment, but the same dose maximized 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; Kavishree, Hemavathy, Lokesh, Shashirekha, & Rajarathnam, 2008; Fernandes et al., 2013b).

In a previous work from our research group (Fernandes et al., 2013b), it could be concluded that the application of a determined PT caused a decreased in total tocopherols content, when compared with fresh samples. However, as it can be reasoned from **Table 3**, the combination of two different PT tended to preserve the level of total tocopherols. If we focused in this vitamin, combining the freeze treatment with a 0.5

kGy dose would optimize the amount of total tocopherols in *M. procera* samples (**Figure 1C**). Furthermore, frozen and dried samples presented the 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.

3.2. Antioxidant parameters

The EC_{50} values calculated in each antioxidant activity evaluation assay remained nearly constant, independently of the applied GID (**Table 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 dissimilarly in each assay. In what concerns 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 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).

3.3. Statistical analysis

In general, *M. procera* samples are characterized 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 signed out 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 1-4** were evaluated separately regarding their discriminant power, according with 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 notorious that PT seemed to exert a higher influence. This assumption ended to be confirmed in the performed LDA assays. The plotted outputs are presented only for nutritional value, since it would be unpractical presenting them in all cases.

In what concerns 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 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 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, either for the original groups as well as for the cross-validation procedure for all the LDA analyses, except the 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).

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, conducting merely to 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 2**, since all samples were correctly classified cases 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 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 at al., 2013b), indicating that rather than a conservation methodology *per si*, gamma irradiation might act as a useful adjuvant to other conservation techniques.

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Table 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.

Table 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

Table 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.

Table 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