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

Ângela Fernandes[a,b], Amilcar L. Antonio[a,c,d], João C.M. Barreira[a,b], M. Luisa Botelho[c], M. Beatriz P.P. Oliveira[b], Anabela Martins[a], Isabel C.F.R. Ferreira[a,*]

[a]CIMO-ESA, Instituto Politécnico de Bragança Campus de Santa Apolónia, Apartado 1172, 5301-855 Bragança, Portugal.

[b]REQUIMTE/ Depto. de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua Jorge Viterbo Ferreira n.º 228, 4050-313 Porto, Portugal.

[c]IST/ITN, Instituto Tecnológico e Nuclear, Estrada Nacional 10, 2686-953 Sacavém, Portugal.

[d]Departamento de Física Fundamental, Universidade de Salamanca, Plaza de la Merced, 37008 Salamanca, Spain.

*Author to whom correspondence should be addressed (I.C.F.R. Ferreira: iferreira@ipb.pt, tel. +351-273303219, fax +351-273325405).

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 a Co-60 experimental equipment. Nutritional value was accessed by macronutrients analysis and determination of energetic value; fatty acid, sugar and tocopherol profiles were determined by GC-FID, HPLC-RI 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

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 (US-FDA, 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, *Lactarius 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.

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 sulfuric 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 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 eighteen 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 267 TBq (6.35 kCi) in November 2011 (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), the 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 the irradiation, 4 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, U.K.). The samples were rotated upside down (180^{o}) 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 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 analysed, 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 AOAC procedures (AOAC, 1995). The crude protein content (N × 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 High Performance Liquid Chromatography (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 g 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 by using calibration curves obtained from commercial standards of each compound. The results were expressed in mg per 100 g of dry weight (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 g of extract.

DPPH radical scavenging activity. This methodology was performed by 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 though 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 2h of assay/initial absorbance) × 100.

TBARS assay. Lipid peroxidation inhibition in porcine (*Sus scrofa*) brain homogenates was evaluated by the decreasing in thiobarbituric acid reactive substances (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] × 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 GLM (General Linear Model) procedure of the SPSS software, version 18.0 (SPSS, Inc.). All dependent variables were analyzed using a 2-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.

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 1**), phenolics and antioxidant activity EC_{50} values (**Table 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.

Chemical composition

Regarding nutritional composition, the results are in agreement with former studies in non-irradiated samples of *L. deliciosus* (Barros et al. 2007b and 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 1**; **Figure 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 1B**), 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 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 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 1C**) 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 1**).

The results obtained for tocopherols content (**Table 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 1D**). 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 2-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 2-

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 acids results were included in the model. The classification for sugars (88.9%, for original grouped cases; 85.2%, for cross-validated grouped cases), antioxidant activity EC_{50} values (85.2%, for original grouped cases; 84.0%, for cross-validated grouped cases), nutritional parameters (71.6%, for original grouped cases; 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 2A**). 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 standardized coefficients might be seen in **Table 3**.

Regarding ID, the model defined also two significant functions, which comprised 100.0% of the observed variance (**Figure 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**.

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. 2011). 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. Sugars 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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Table 1. Chemical composition, main fatty acids, sugars and tocopherols profile of non-irradiated and irradiated *Lactarius deliciosus* samples, after different times of storage. The results are presented as mean±SD[a] (n=27, for each storage time, ST, and each irradiation dose, ID).

	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

[a]Results 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 less than 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 2. Phenolic content and antioxidant activity of non-irradiated and irradiated *Lactarius deliciosus* samples, after different times of storage. The results are presented as mean±SD[a] (n=27, for each storage time, ST, and each irradiation dose, ID).

	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

[a]Results 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 less than 0.001.

Table 3. Standardized 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

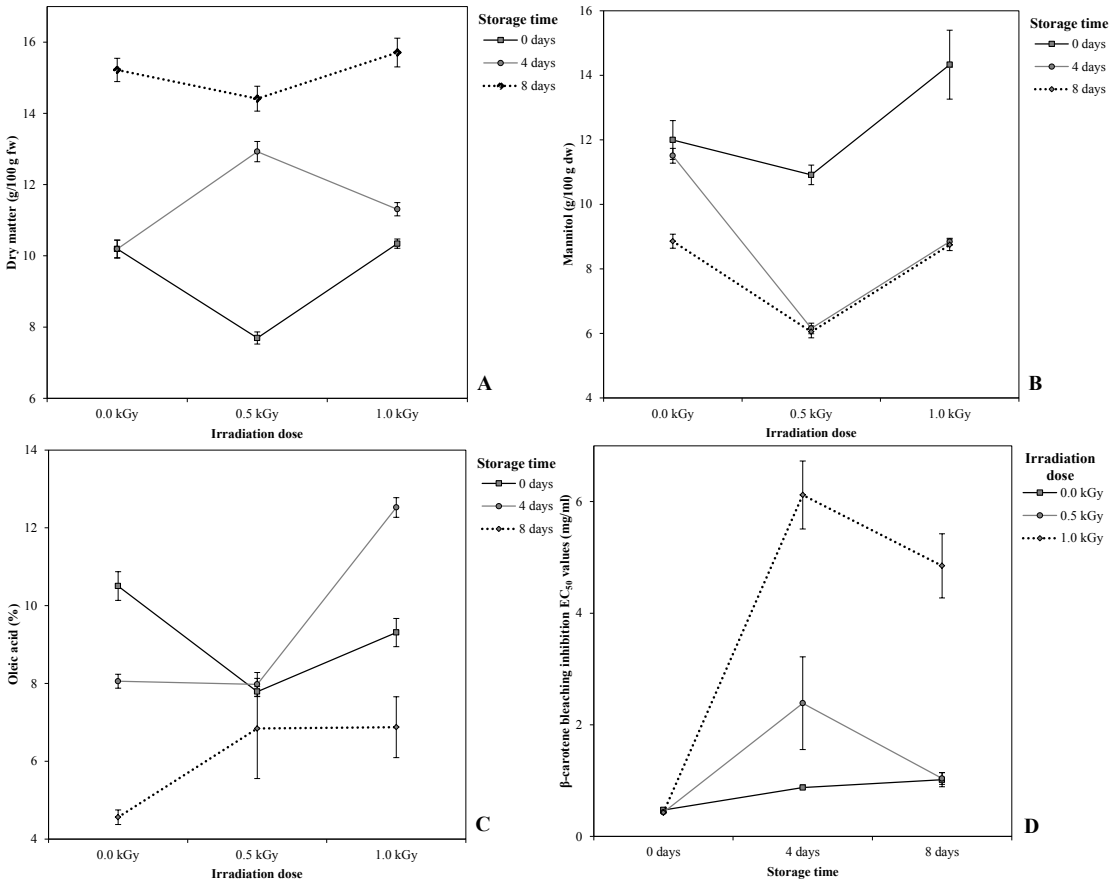

Figure 1. Interactions between ST and ID effects on *Lactarius deliciosus* extracts. Influence on: A- dry matter; B- mannitol; C- oleic acid; D- β-carotene bleaching inhibition.

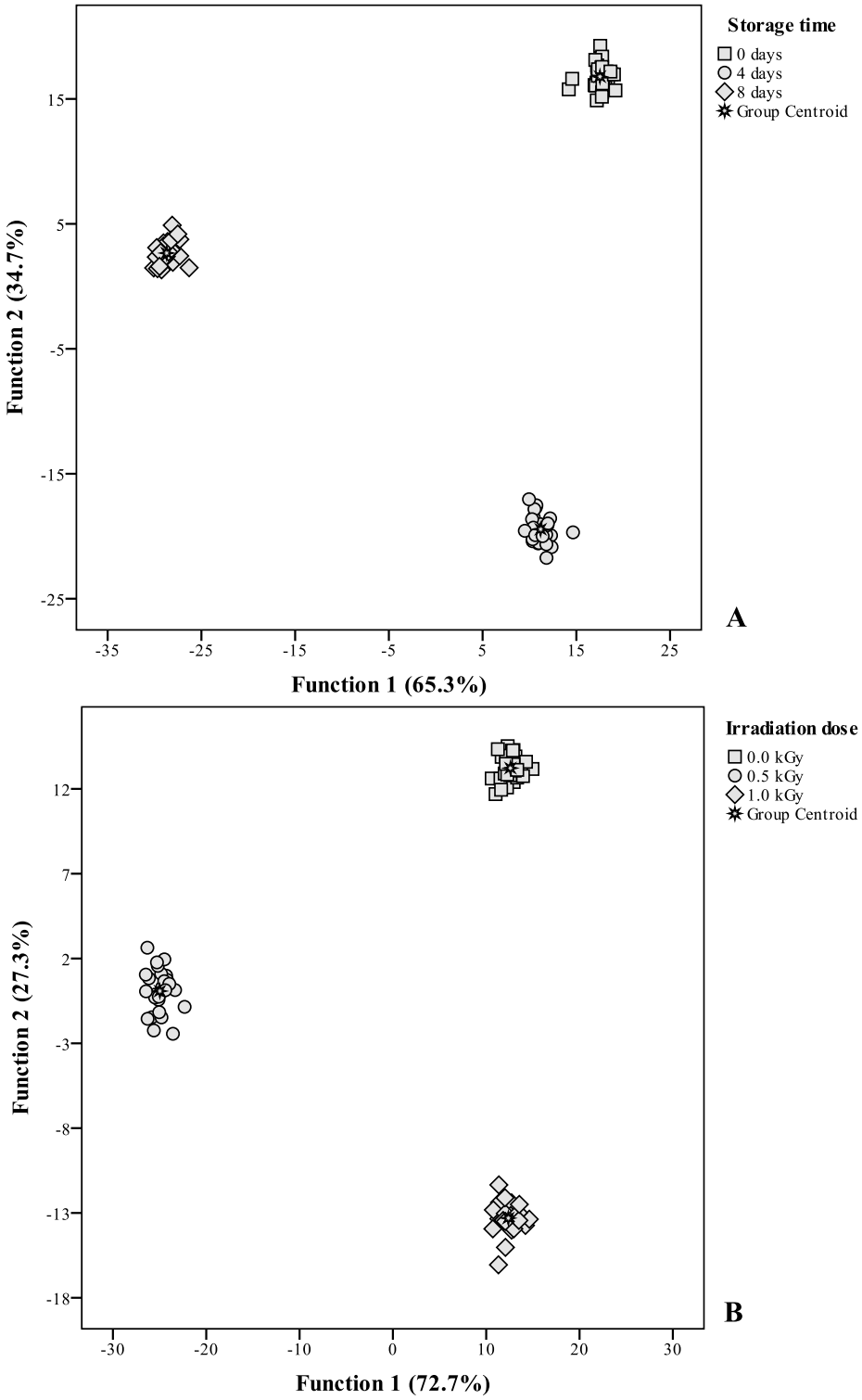

Figure 2. Score plots defined for the assayed parameters using ST (A) or ID (B) as grouping variables.