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: Boletus edulis is amongst 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.

1. Introduction

Mushrooms are usually eaten fresh but due to their high water content, they become highly perishable and need to be preserved (Ezekiel 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 et al., 2103a).

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 (Shephard, 2008; Ezekiel et al., 2013).

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, 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).

2. Materials and methods

2.1. 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).

2.2. Samples and electron-beam irradiation

Boletus edulis Bull. and *Russula delica* Fr. 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 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). 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.

2.3. Chemical parameters

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 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}).

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 Heleno, Barros, Sousa, Martins, & 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 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 chromatography with flame ionization detection (GC-FID), after the extraction and derivatization procedures previously described by Heleno, Barros, Sousa, Martins, & Ferreira (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.

2.3.4. Tocopherols

Tocopherols were determined after an extraction procedure previously described by Heleno, Barros, Sousa, Martins, & 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 mg per 100 g of dry weight (dw).

2.3.5. 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 (PDA), using a Shimadzu 20A series UFLC (Shimadzu Cooperation). Detection was carried out in a PDA, 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 mg per 100 g of dry weight (dw).

2.3.6. 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 g dw.

2.4. Antioxidant parameters

2.4.1. 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.

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

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.

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.

2.5. 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 18.0 (SPSS Inc).

3. Results and discussion

3.1. Effects on chemical parameters

The nutritional parameters of *B. edulis* and *R. delica* (**Table 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, Barros, Sousa, Martins, & Ferreira, 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 et al., 2013b). Likewise, proteins revealed the highest changes in *R. delica* samples, as it became evident from the different classification 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 dissulfide 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 time, which was verified to exert, with no exception, a more relevant effect that irradiation on the chemical profiles of assayed samples.

Unlike nutritional parameters, free sugars composition (**Table 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, *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 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 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 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.

3.2. 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 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, Barros, Sousa, Martins, & Ferreira, 2010; Yaltirak, Aslim, Sahlan, & Alli, 2009) and slightly lower in *B. edulis* (Heleno et al., 2011), most likely due to seasonal variability or different geographical origin 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.

3.3. 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 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 1-7**. 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 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.

4. Conclusions

The effects of gamma-irradiation up to 2 kGy in chemical parameters of fresh wild *Boletus 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 *Macrolepiota 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 as also decontaminated samples. In this way, treated foods might be available for persons with particular food safety concerns like immunocompromised or elderly people (FAO, 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.

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References

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, 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., 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.

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.

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. (2013a). 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., & 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.

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., C. Haixia, Z. Wenchai, W, & Zhaoshuai. (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. John Wiley & Sons, USA. ISBN 0-471-35634-4.

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.

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.

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, WHO, Geneva.

Yaltirak, T., Aslim, B., Sahlan, O., & Alli, H. Antimicrobial and antioxidant activities of *Russula delica* Fr. (2009). *Food and Chemical Toxicology*, *47*, 2052-2056.

Table 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 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 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 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. Phenolic and cinnamic acids 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 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 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]$p<0.05$ 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).

Table 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 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 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]$p<0.05$ 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 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.

[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 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).

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.

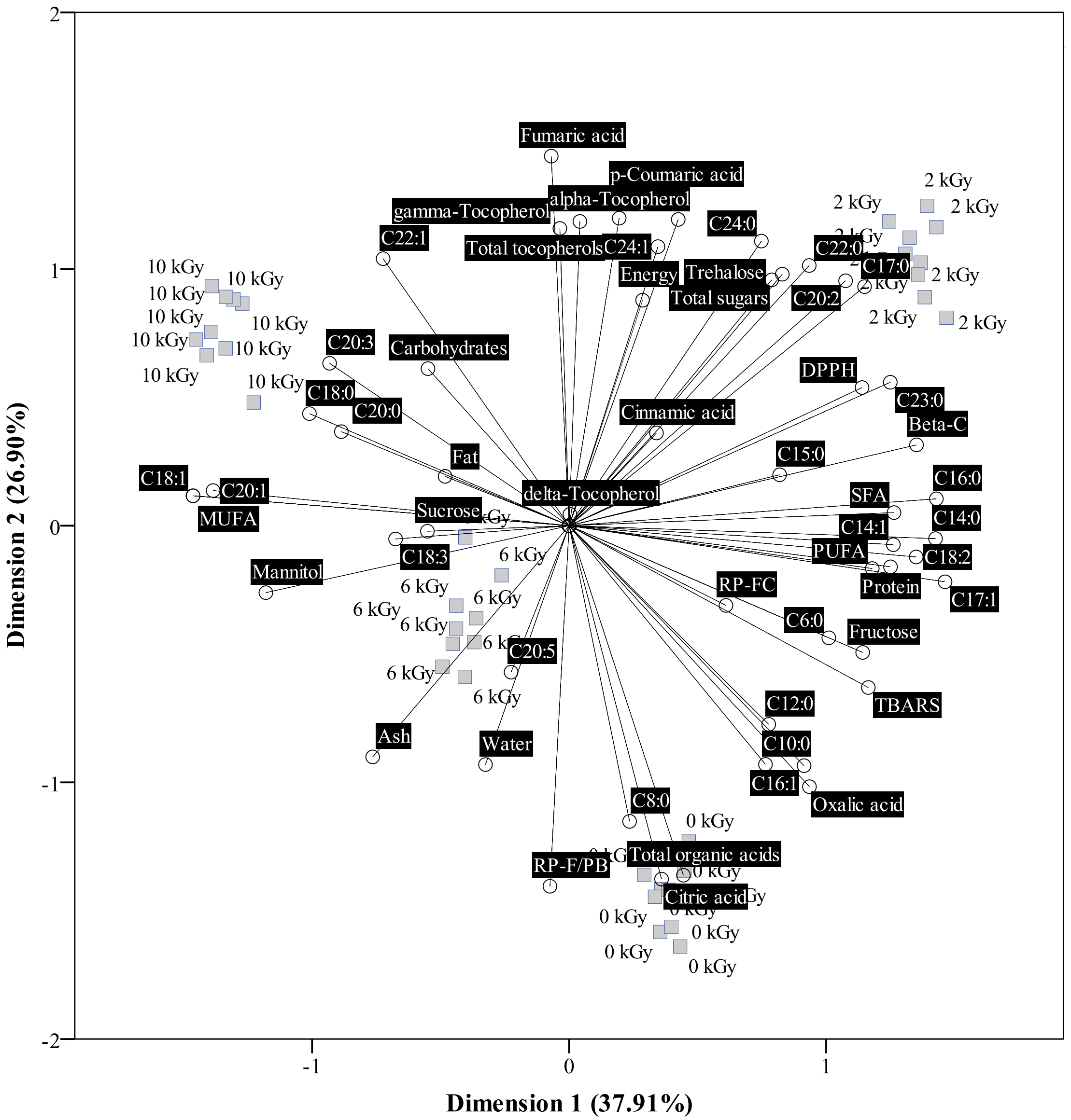

Figure 1. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *B. edulis*.

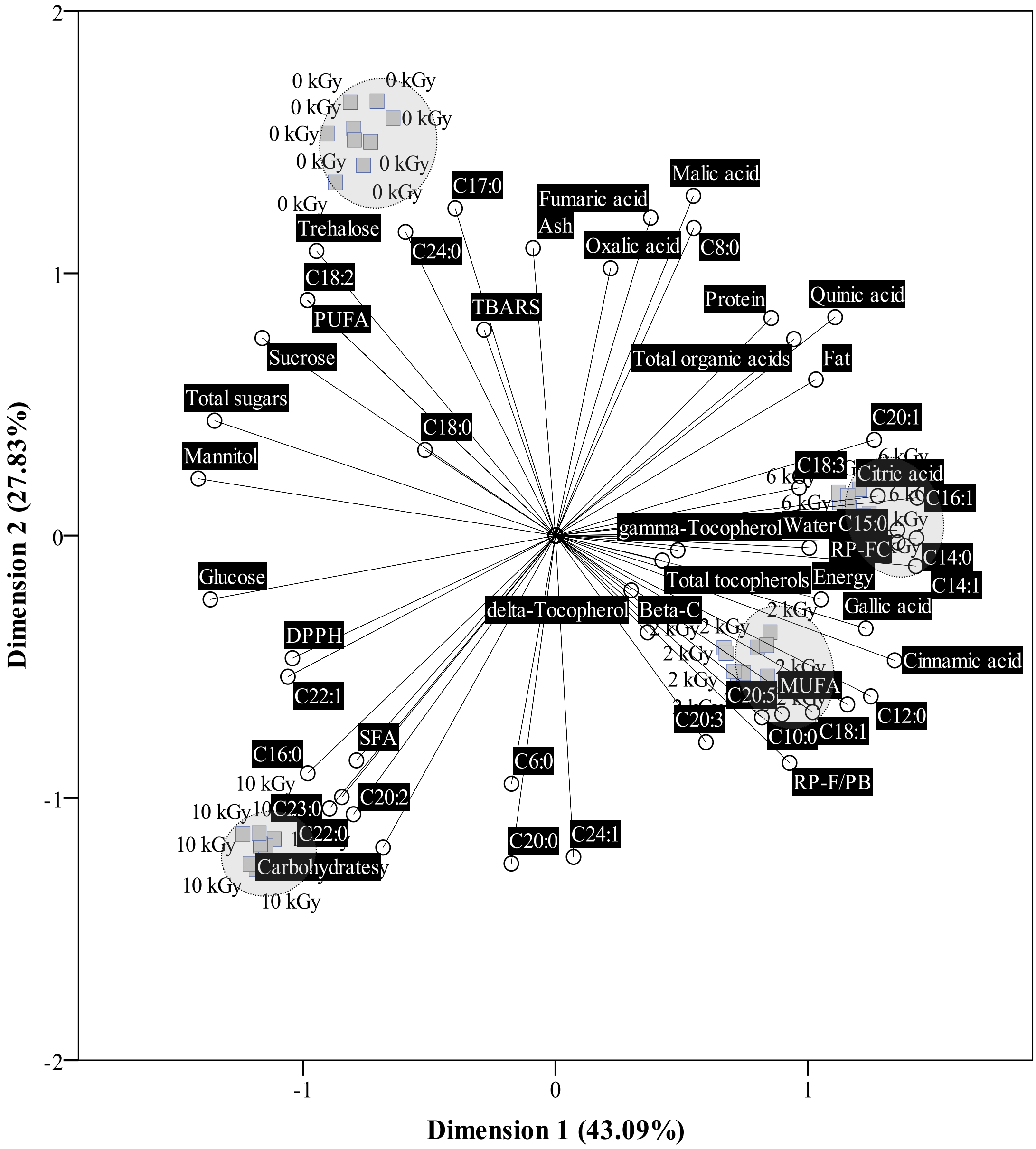

Figure 2. Biplot of objects (irradiation doses) and component loadings (evaluated parameters) for *R. delica*.