Exquisite wild mushrooms as a source of dietary fiber: analysis in electron-beam irradiated samples

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ABSTRACT

In the present study, electron-beam irradiation was applied to dried samples of *Boletus edulis* and *Macrolepiota procera* to evaluate the effects on their fiber composition. Both species presented an important percentage of dietary fiber, soluble and insoluble in different ratios. These high fiber levels are an interesting feature, allowing considering mushrooms as an alternative source of dietary fibers in the highly competitive market of fiber-enriched food products. In *B. edulis* samples, insoluble fiber and total fiber amounts were significantly lower in samples irradiated with 10 kGy, but soluble fiber had no significant changes for any of the assayed doses, while total available carbohydrates were significantly lower in unirradiated samples. *M. procera* samples irradiated with 6 kGy presented less total fiber, insoluble fiber and carbohydrates, but the same dose allowed the highest contents in soluble dietary fiber. In general, the irradiated samples, especially for higher doses, gave some significant changes in the total available carbohydrates and dietary fibers content. Nevertheless, the resulting differences still allow considering these species as good natural fiber sources, maintaining their potential health effects, while promoting a clean way to disinfest and decontaminate these highly perishable products.

***Keywords**:* Wild mushrooms; Electron-beam; Soluble/Insoluble Dietary Fiber

1. Introduction

Dietary fiber and high-fiber food products have attracted attention because of their significant health benefits to consumers (Tungland & Meyer, 2002; Cheung, 2013). Nowadays, most dietary ingredients (such as cereals-based, fruits-based, and legumes-based dietary fiber) are derived from their processing byproducts (*e.g.* milling, juice extraction or de-hulling) followed by different refining steps (*e.g.*, grinding, sieving, bleaching or defatting) to come across a wide range of customers' requirements (McKee & Latner, 2000; O'Shea, Arendt, & Gallagher, 2012; Robin, Schuchmann, & Palzer, 2012). Due to the highly competitive market of fiber enriched food products, exploration of alternative sources of dietary fiber is urgent (Wong & Cheung, 2009).

Mushrooms are highly appreciated for their nutritional (Kalač, 2009) and nutraceuticals properties, being also considered as a novel source of dietary fiber, with various beneficial health effects to humans, since most of the carbohydrates in mushrooms are non-digestible (Cheung, 2013). Carbohydrates are the major components in mushrooms and the total content ranges from 35% to 70% in dry weight. Most of the carbohydrates in mushrooms are non-digestible carbohydrates (dietary fiber) including oligosaccharides (*e.g.*, trehalose) and cell wall polysaccharides (*e.g.* chitin, β-glucans and mannans) (Cheung, 2010; 2013). Mushroom dietary fiber is constituted mainly by insoluble fiber (chitin and β-glucans being the most representative), while the level of-soluble fiber is usually less than 10% dry matter (Cheung, 2013). Insoluble fibers absorb water in the colon that softens and increases the bulk of the stool. This action helps relieving constipation and preventing colon disease and hemorrhoids. While, soluble fiber form a gel and maximize the viscosity of the food matrix, slowing down digestión, which can help lower postprandrial blood glucose, insuline and cholesterol (UWH, 2013) and strengthens the immune system and

antitumor activity, as well as blood glucose and lipid attenuation (Zhang, Cui, Cheung, & Wang, 2007; Cheung, 2013).

Consumption of edible mushrooms as part of our daily diet in most cases could easily provide up to 25% of the recommended dietary intake of dietary fiber (Cheung, 2010). Nonetheless, mushrooms are rapidly perishable and they start deteriorating within a day after harvest (Fernandes, Antonio, Oliveira, Martins, & Ferreira, 2012). Therefore, fresh mushrooms have to be processed to extend their shelf life for off-season use, and drying is a widely used postharvest technology, which overcomes problems related to overproduction and short shelf-life (Walde, Velu, Jyothirmayi, & Math, 2006). However, during the drying process, microorganisms may secrete potentially toxic metabolites and contaminate mushrooms (Shephard, 2008; Ezekiel et al., 2013). In this sense, electron-beam irradiation proved its technological feasibility to be safely used in order to reduce food losses caused by deterioration (namely, by insect pest attack during storage) (Kim, Akram, Ahn, & Kwon, 2012). Moreover, the irradiation proved to be a viable technique for dried food decontamination in alternative to fumigation with microbicidal gases (Farkas, 1998). In fact, extensive research has demonstrated the nutritional safety and practical effectiveness of irradiation, which has also been approved by different international health organizations (WHO 1999; Farkas & Mohacsi-Farkas 2011). Furthermore, dried mushrooms (grouped in the food additive class and generally used as seasonings) might be treated with doses up to 50 kGy for different technical objectives (ICGGI 1999; Akram & Kwon 2010). The emergent market of irradiated food demands effective identification methods to monitor the irradiated food in the international trade market. These methods must evaluate physical and chemical changes effecting technical and functional properties of food samples to develop a better preservation approach (Akram, Ahn, & Kwon, 2012).

There are some studies about the effects of electron-beam irradiation on nutritional and chemical parameters of wild dried (Fernandes et al., 2014a; Fernandes et al., 2014b) and cultivated (Duan, Xing, Shao, & Zhao, 2010; Rivera, Blanco, Marco, Oria, & Venturini, 2011) mushrooms, but these studies were never focused on the effects over fiber content. Therefore, in the present study, electron-beam irradiation was applied to dried samples of *Boletus edulis* Bull.: Fr. and *Macrolepiota procera* (Scop.) Singer, two highly appreciated edible mushrooms, in order to evaluate the effects on their fiber composition.

2. Materials and methods

2.1. Samples

Macrolepiota procera (Scop.) Singer wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2011, and dried at 30 ºC in an oven. Subsequently, the samples were divided in four groups with three specimens in each group: control (unirradiated, 0 kGy); sample 1 (0.5 kGy); sample 2 (1 kGy) and sample 3 (6 kGy).

Boletus edulis Bull.: Fr. wild samples were obtained in Trás-os-Montes, in the Northeast of Portugal, in November 2012. Samples were dried at 30 ºC in an oven and subsequently divided in four groups with six specimens per group: control (unirradiated, 0 kGy); sample 1 (2 kGy); sample 2 (6 kGy) and sample 3 (10 kGy), kept in polyethylene bags.

2.2. Standards and reagents

Glucose standards and fiber enzymatic kit (TDF-100A) were purchased from Sigma (St. Louis, MO, USA). Glutamic acid (used in organic acids analysis) was purchased from Merck (Darmstadt, Germany). Sulfuric acid, perchloric acid, hydrochloric acid, sodium

hydroxide and anthrone reagent and all other general laboratory reagents were obtained from Panreac Quimica S.L.U. (Barcelona, Spain).

2.3 Electron-beam irradiation

The irradiation was performed at the INCT- Institute of Nuclear Chemistry and Technology, in Warsaw, Poland. To estimate the dose during the irradiation process three types of dosimeters were used: a standard dosimeter, a graphite calorimeter, and two routine Gammachrome YR and Amber Perspex dosimeters, from Harwell Company (UK). The irradiation took place in an e-beam irradiator of 10 MeV of energy with pulse duration of 5.5 μs, pulse frequency of 440 Hz and average beam current of 1.1 mA; the scan width was 68 cm, the conveyer speed was settled to the range 20-100 cm/min and the scan frequency was 5 Hz. The absorbed dose for *M. procera* was 0.53, 0.83 and 6.10 kGy, with an uncertainty of 20% for the two first doses and 10% for the last dose. The estimated absorbed doses for *B. edulis* were 2.5, 6.2 and 10.9 kGy, with an uncertainty of 20%. To read the Amber and Gammachrome YR dosimeters, spectrophotometric methods were used at 603 nm and at 530 nm, respectively, to estimate the dose from the value of absorbance according to a previous calibration curve. For the graphite calorimeter dosimeter the electrical resistance was read and converted in dose according to a calibrated curve (Fernandes et al., 2014a).

2.4. Total available carbohydrate (TAC) assay

The determination of TAC was carried out by the Anthrone method as described by Osborne & Voogt (1986) using 0.25 g of sample. The samples were pre-treated with 13 mL of 52% $HClO_4$ and kept for 18 h in the dark. After this period, distilled water was added, the sample was filtered and the volume of the filtrate was adjusted to 100 mL.

Finally, the solution was further diluted to 10%, and 5 mL of 0.1% anthrone solution in 73% H_2SO_4 was added. Samples were kept in a boiling water bath for 12 min where the anthrone reaction with sugars yielded a green colour, and absorbance was measured at 630 nm on a UV/Vis Spectrometer EZ210 (Perkin Elmer, Waltham, MA, USA) equipped with Lambda software PESSW ver. 1.2. The absorbance of the sample solution was compared to a 10-100 µg/mL concentration range standard glucose calibration curve. TAC values were expressed as g/100 g dw sample.

2.5. Soluble and insoluble dietary fiber assay

AOAC enzymatic-gravimetric methods (993.19 and 991.42) were used for soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) analysis (Latimer, 2012). In brief, freeze-dried samples were treated with alpha-amylase, protease and amyloglucosidase.

The soluble and insoluble fractions were separated by vacuum filtration. Waste from the digests was dried at 100 °C, and protein content was determined in the residue. Total fiber is the sum of soluble and insoluble fiber, both were expressed as g/100 g dw sample.

2.6. Statistical analysis

For each irradiation dose and mushroom species, three independent samples were used. Each of the samples was taken after pooling the mushrooms treated in the same conditions together. Data were expressed as mean ± standard deviation. All statistical tests were performed at a 5% significance level using SPSS software, version 22.0 (IBM Corp., USA).

The fulfilment of the one-way ANOVA requirements, specifically the normal distribution of the residuals and the homogeneity of variance, was tested by means of the Shapiro Wilk's and the Levene's tests, respectively. All dependent variables were compared using

Tukey's honestly significant difference (HSD) or Tamhane's T2 multiple comparison tests, when homoscedasticity was verified or not, respectively.

3. Results and discussion

In previous reports describing the nutritional composition of *B. edulis* and *M. procera*, it could be observed that carbohydrates were the dominant compounds in dry mass basis, varying from 54 to 76% (Fernandes et al., 2014a), 60 to 70% (Fernandes et al., 2013a), 65 to 77% (Fernandes et al., 2013b), and 71 to 78% (Fernandes et al., 2014b). According to the present work, it is now possible to conclude that an important percentage of those carbohydrates occur naturally as dietary fibers (**Figure 1**). These high fiber percentages might be explained by the composition of mushrooms' cell walls, which contain a mixture of fibrillar and matrix components, including chitin (a straight-chain (1→4)-α-linked polymer of *N*-acetyl-glucosamine) and polysaccharides such as (1→3)-α-D-glucans and mannans. In fact, these components are non-digestible carbohydrates resistant to human enzymes and can be considered as dietary fiber (Cheung, 2013; Tungland & Meyer, 2002).

The distribution of total available carbohydrates (TAC; *M. procera*: 23.2 to 33.6 g/100 g DW; *B. edulis*: 19.3 to 27.3 g/100 g DW) and total dietary fibers (*M. procera*: 29.1 to 33.9 g/100 g DW; *B. edulis*: 26.7 to 30.8 g/100 g DW) was similar for both species (Fig. 1b). Nevertheless, fiber content is often highly variable among different species: some mushrooms were found to be low in total fiber, *e.g.* for *Craterellus aureus* and *Sarcodon aspratus* values were ≈5 g/100 g DW, while for many others, *e.g.* for *Lactarius volemus* and *Lentinula edodes* up to 40 g/100 g DW contents were reported (Wang et al., 2014).

The fairly high detected levels of fiber in these mushrooms might be considered as a desirable characteristic, since fiber plays an important role in human diet (EFSA, 2010). The Recommended Dietary Allowance (RDA) establish for dietary fiber are 25 to 38

g/100g for women and men, respectively (Trumbo, Schlicker, Yates, & Poos, 2002; EFSA, 2010). In this way, the consumption of the studied edible mushrooms as part of our daily diet could provide interesting amounts of total dietary fiber, because the intake of 100 g (FW) of *M. procera* and *B. edulis* covers important percentages of the RDA. This fact might represent an additional point of interest considering the increasing public awareness of the potential health benefits of dietary fibers, which has greatly encouraged food manufacturers to develop a wide range of fiber-enriched or fiber-fortified food products (Ktenioudaki & Gallagher, 2012).

In a more specific analysis, the results for soluble and insoluble dietary fiber (IDF) contents might be observed in **Table 1**. In either case, insoluble fibers were clearly dominant, which represents a common feature in mushrooms (Cheung, 2008). According to FEN (2013) recommendations, the dietary ratio intake of insoluble:soluble dietary fibers (IDF/SDF) should be between 1.5 to 3. Nevertheless, the assayed species showed differences regarding the ratios (IDF:SDF), *B. edulis* presents an appropriate ratio according to the above recommendation (3:1 ratio), while *M. procera* presented a higher amount of insoluble fiber, with a 7:1 ratio. In a study performed on Italian *Boletus* spp. (Manzi, Marconi, Aguzzi, & Pizzoferrato, 2004), the SDF:IDF ratio was similar to the detected herein, but the global amounts (2% and 7% of dry weight, respectively) were quite lower, which might be related with the commercial origin of the samples, since the present study was performed in wild samples.

Considering the importance of dietary fibers, nowadays recognized as functional foods, the application of any decontamination or disinfestation technology, should maintain fiber amounts in the treated samples. Accordingly, the potential changes caused by electron-beam irradiation, were evaluated. Energetic electrons, like gamma- and X-rays, transfer their energy to the irradiated food by ejecting atomic electrons, which can then ionize other

atoms in a cascade of collisions. Therefore, these energy sources might ultimately produce similar effects in any irradiated material. Accordingly, the choice for electron-beam application was defined by practical aspects such as the thickness and density of the mushrooms, dose uniformity ratio or economic factors (Cleland, 2013). In addition, the lack of significant effects, as evaluated in different chemical parameters, pointed out electron-beam irradiation as a feasible choice for mushroom disinfestation and decontamination. The selected doses were chosen according to previous results and considering that it was meant to be applied to dried samples (Fernandes et al., 2014a,b).

In terms of dose-dependent changes, the Levene test showed that the assumption of equality among variances ($p > 0.05$) was verified in most cases, allowing classifying the samples according to the Tukey test. In the remaining cases (carbohydrates in *M. procera* and soluble fiber in *B. edulis*), samples were classified using the Tamhanes' T2 test as multiple comparison tool.

Regarding *B. edulis*, insoluble fiber was significantly lower in samples irradiated with 10 kGy (**Table 1**); the same result was also verified for total fiber amounts (**Figure 1**). Irradiation is referred in literature has having a synergetic effect in reducing non-nutritive factors (Al-Kaisey, Alwan, Mohammad, & Saeed; Lima, Souza, Godoy, França, & Lima, 2011). Soluble fiber, on the other hand, had no significant changes ($p > 0.05$) for any of the assayed doses. In general terms, total available carbohydrates (TAC) were significantly lower in unirradiated samples. This could be explained by the fact that gamma and e-beam irradiation have a breaking effect on the constituents of irradiated food products.

In what concerns *M. procera*, samples irradiated with 6 kGy presented less total fiber, insoluble fiber and carbohydrates (**Figure 1; Table 1**). However, samples irradiated with the same dose gave also the highest contents in soluble dietary fiber (SDF; **Table 1**). The detected differenced might be explained because irradiation induces degradation of

polysaccharides such as cellulose, starch and pectin (Fan, 2013). Cell wall polysaccharides, particularly cellulose (insoluble) and pectic (soluble) substances are partially degraded by irradiation. Degradation of both pectin and cellulose occur at approximately the dose at which tissue softening could be first demonstrated and progress with increasing dose (Fan, 2013).

In fact, irradiation was previously indicated as causing a dose-dependent degradation of fiber, which might be attributed to the breakage of glycosidic bonds, leading to the formation of carbonyl groups or double bonds (Xu, Sun, Yang, Ding, & Pang, 2007).

4. Conclusion

Overall, both mushroom species proved to have potential as dietary fiber sources, raising the possibility of its inclusion in the highly competitive market of fiber-enriched food products, which seriously demands the exploration of alternative source and preparation methods of dietary fibers.

The irradiated samples, especially those treated with higher doses, gave some specific significant changes in the carbohydrates and fibers contents. Nevertheless, the resulting differences still allow considering these species as good natural fiber sources, maintaining their potential health effects, while promoting a clean way to disinfest and decontaminate this highly perishable products. Additional studies on the viscosity of irradiated mushrooms, as well as their digestibility, might be an interesting future approach.

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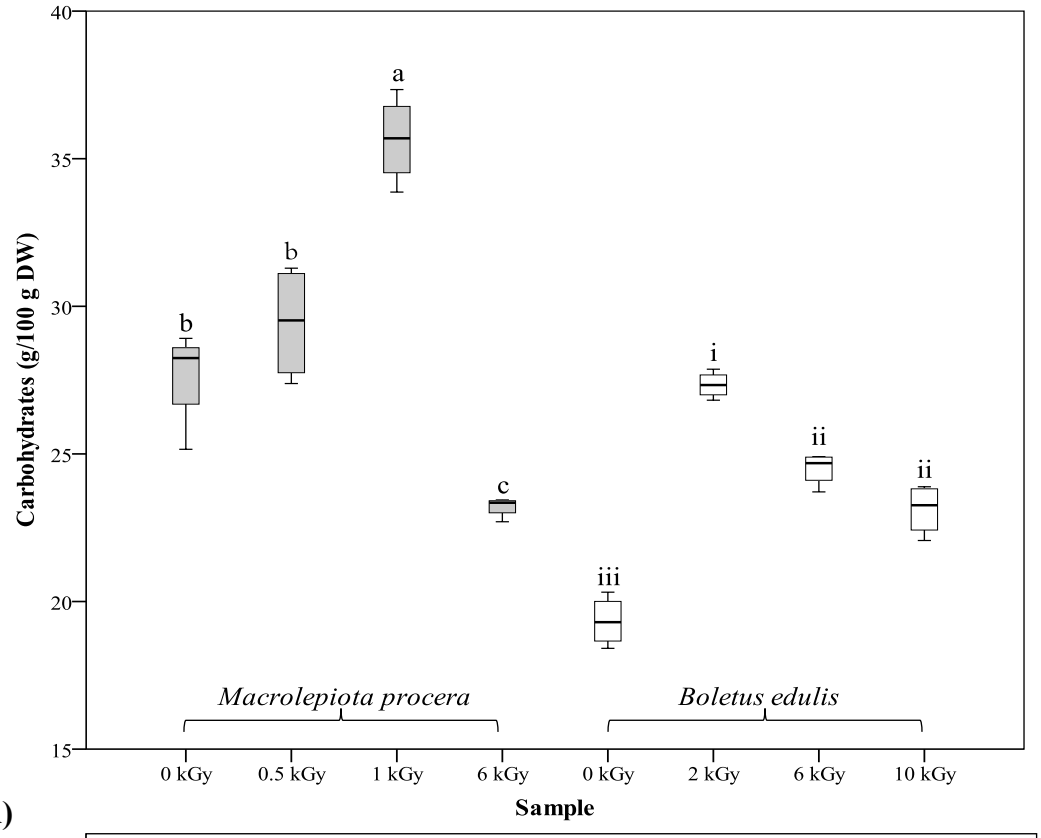
Figure 1. Boxplot scores for carbohydrates (**A**) and fibers (**B**) in *M. procera* and *B. edulis* submitted to different electron beam irradiation doses.

Table 1. Soluble and insoluble fiber content in mushrooms irradiated with different doses of electron-beam. The results are presented as the mean±SD.

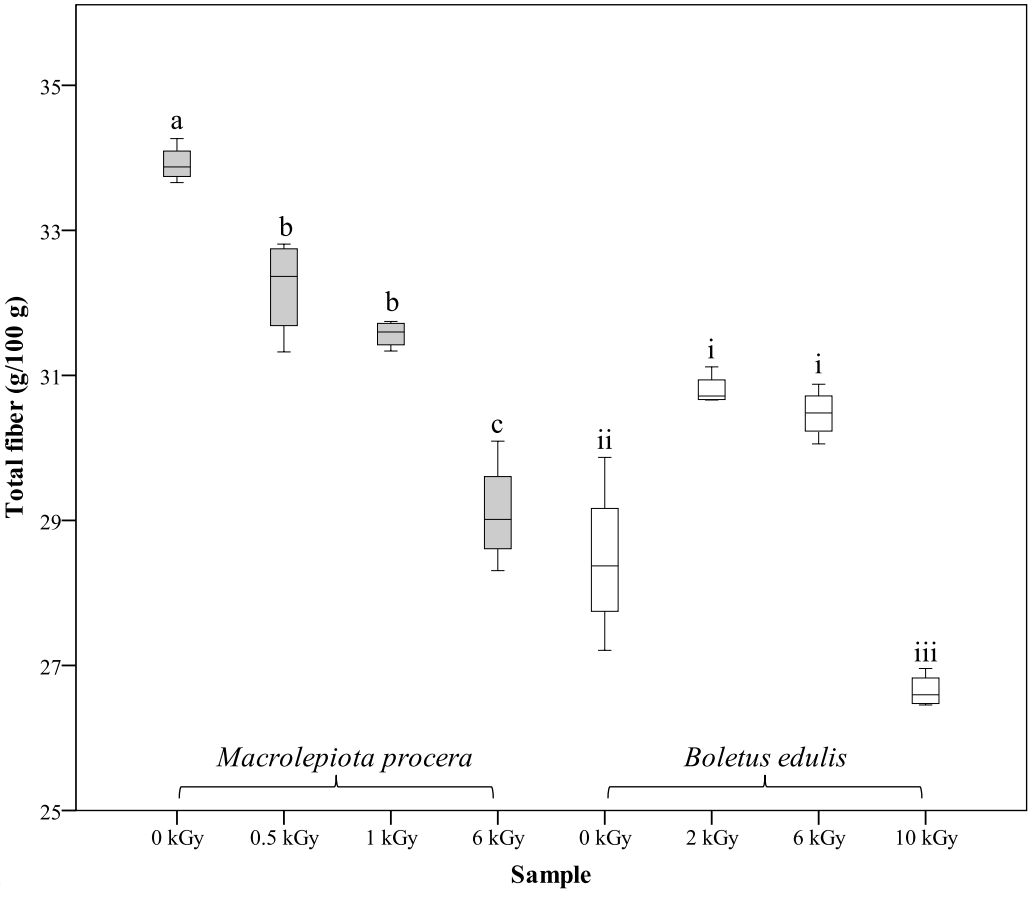
	Soluble fiber (g/100 g DW)	**Insoluble fiber (g/100 g DW)**
Dose (kGy)	*Boletus edulis*	
0	7.4±0.5	21.0±0.5 b
2	7.8±0.1	23.0±0.3 a
6	7.4±0.5	23.1±0.4 a
10	7.7±0.1	19.0±0.3 c
Levene's test[1]	$p = 0.014$	$p = 0.353$
1-way ANOVA[2]	$p = 0.344$	$p < 0.001$
	Macrolepiota procera	
0	3.4±0.1 b	30.5±0.2 a
0.5	3.8±0.4 b	28.4±0.4 b
1	3.6±0.2 b	28.0±0.2 b
6	5.3±0.3 a	23.8±0.5 c
Levene's test[1]	$p = 0.053$	$p = 0.417$
1-way ANOVA[2]	$p < 0.001$	$p < 0.001$

[1]Homoscedasticity among irradiation doses was tested by means of the Levene test: homoscedasticity, p value > 0.05; heteroscedasticity, p value < 0.05. [2]$p < 0.05$ meaning that the mean value of soluble or insoluble fiber of at least one dose differs from the others (in this case multiple-comparison tests were performed).

Figure 1.


Carbohydrates (g/100 g DW)
40
35
30
25
20
15
a
b
b
c
iii
i
ii
ii
Macrolepiota procera
Boletus edulis
0 kGy
0.5 kGy
1 kGy
6 kGy
0 kGy
2 kGy
6 kGy
10 kGy
Sample

(A)


Total fiber (g/100 g)
35
33
31
29
27
25
a
b
b
c
ii
i
i
iii
Macrolepiota procera
Boletus edulis
0 kGy
0.5 kGy
1 kGy
6 kGy
0 kGy
2 kGy
6 kGy
10 kGy
Sample

(B)