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FOOD COMPOSITION AND ANALYSIS

Effect of different cooking methods on nutritional value and antioxidant activity of cultivated mushrooms

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ABSTRACT

Influence of culinary treatments (boiling, microwaving, grilling, and deep frying) on proximate composition and antioxidant capacity of cultivated mushrooms (*Agaricus bisporus*, *Lentinula edodes, Pleurotus ostreatus,* and *Pleurotus eryngii*) was studied. Proximate composition was affected by the cooking method and the mushrooms species. Frying induced more severe losses in protein, ash, and carbohydrates content but increased the fat and energy. Boiling improved the total glucans content by enhancing the β-glucans fraction. A significant decrease was detected in the antioxidant activity especially after boiling and frying, while grilled and microwaved mushrooms reached higher values of antioxidant activity. Maillard reaction products could be partially responsible, as supported by the absorbance values measured at 420 nm. Since cooking techniques clearly influence the nutritional attributes of mushrooms, the proper selection of treatments is a key factor to prevent/reduce nutritional losses. Microwaving and grilling were established as the best processes to maintain the nutritional profile of mushrooms.

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Introduction

Mushrooms have been part of the human diet for thousands of years. The consumption of edible mushrooms has risen greatly in recent times, involving a large number of species (Reis et al. 2012a). The most cultivated mushroom worldwide is *Agaricus bisporus*, followed by *Lentinula edodes* and *Pleurotus spp*. Mushrooms are considered valuable health foods, since they have a significant amount of dietary fiber and are poor in calories and fat (Reis et al. 2012a). Moreover, they have a good protein content (20–30% of dry matter) which includes most of the essential amino acids (Ghorai et al. 2009); also provide a nutritionally significant content of vitamins (B1, B2, B12, C, D, and E) and trace minerals such as zinc or selenium (Manzi et al. 2004; Mattila et al. 2001, Çağlarırmak 2007; Çağlarırmak 2009)

Mushrooms are also an important source of biologically active compounds with potential medicinal value. The medicinal use of mushrooms has a very long tradition in the Asian culture, whereas in the Western countries, the study of fungal bioactive compounds and their health effects has only recently emerged (Cheung 2010). The medicinal mushroom properties have been reported by the scientific community including antitumor, immunomodulating, antioxidant, radical scavenging, antihypercholesterolemia, antiviral, antibacterial, hepatoprotective, and antidiabetic effects (Wasser 2011). The mushrooms bioactive compounds responsible for these properties are: polysaccharides, dietary fiber, and antioxidants (vitamins C, B12 and D; folate; ergothioneine and polyphenols). Currently, authors are focused on β-glucans, a cell-wall non-starch polysaccharide with repeating units of glucose. These glucose units may branched in several ways depending on the source from which it is extracted, those synthetized by fungi and yeast have these side chains structure: β-1,3-D-glucans and β-1,6-D-glucans (Rop et al. 2009). Numerous positive effects have been associated with fungal β-glucans, for instance, in the treatment of cancer disease; in the metabolism of fats and sugars by reducing cholesterol and glucose level in blood; improving resistance against allergies by increasing the numbers of lymphocytes, among others (Wasser 2011).

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Processing of food products such as boiling, microwaving, pressure-cooking, grilling, baking, steaming, and frying induces significant changes in the texture and chemical composition. During boiling losses of vitamins, antioxidant compounds or leaching of soluble substances in the water may significantly influence the nutritional value of the final product (Faller & Fiahlo 2009). On one hand, thermal treatments can also reduce the food quality; it is well-known that most of the bioactive compounds are relatively unstable to heating (Choi et al. 2006). However, on the other hand, different chemical reactions between the food components take place and new substances can be formed, for instance, Maillard reaction products (Delgado-Andrade et al. 2007). The development of the Maillard reaction in food matrix is often responsible for the appearance of attractive aromas, colors, and flavors, and hence improved food palatability. Moreover, Maillard reaction products are associated to some positive biological actions such as an increase in antioxidant activity (Somoza 2005).

The most mushrooms are commonly cooked before being consumed, but scarce information is available about the changes in nutritional quality after culinary treatments. Lower level of nutraceuticals and antioxidant activity was reported by Jaworska et al. (2015) in blanched mushrooms (*Agaricus bisporus* and *Pleurotus ostreatus*) compared with raw ones. Accordingly, Manzi et al. (2004) also described a decrease in antioxidant activities in cooked mushrooms. However, the polyphenol concentration seems to increase by cooking treatments as described by Choi et al. (2006) for *Lentinula edodes* heated at 121 °C for 30 min. In the same line, Sun et al. (2014) also reported that the retention of total phenolic compounds after microwaving treatment was better than in others cooking methods. Thus, microwaving seems to be one of the best cooking processes to preserve the antioxidant properties of mushroom as demonstrated by Tan et al. (2015), who established that microwaved *Pleurotus eryngii* showed 17% higher Trolox equivalent antioxidant capacity (TEAC) value compared with the uncooked sample.

All these data suggest that increasing the knowledge of the nutritional consequences of culinary treatments is a good strategy to preserve the nutritional quality of mushroom. Thus, this assay aimed to evaluate the influence of different cooking methods on proximate composition, β-glucans content and antioxidant activity of four cultivated mushrooms species. Unspecific parameters as CIE Lab color and absorbance measurements at 420 nm were used as a tool to better understand emerging changes.

Materials and methods

Mushrooms samples and cooking methods

Fresh fruiting bodies of four mushrooms: *Agaricus bisporus* (common name: white button mushroom), *Lentinula edodes* (common name: shiitake) and two species of oyster mushrooms (*Pleurotus spp.*), *Pleurotus ostreatus,* and *Pleurotus eryngii*, commonly known as oyster mushroom and king oyster, respectively, were harvested from the cultivation rooms at CTICH facilities. Fresh mushrooms fruiting were cleaned from soil and substrate. Mushrooms were then cut along their vertical axes into slices 4–5 mm thick and submitted to one of these methods.

The experiments were carried out using 15 kg of each mushroom; this amount was randomly divided into five groups (3 kg each). One of them remained raw and the rest were cooking in four different methods (boiling, microwaving, griddling, and frying). Culinary conditions were as follows:

1. *Boiling*: Mushroom slices (300 g/batch) were boiling on a pot containing 3 L of bottled water for 10 min.
2. *Deep frying*: Mushroom slices (150 g/batch) were frying in a pan with 500 mL olive oil (160 °C) for 3 min.
3. *Microwaving*: Mushroom slices (100 g/batch) were placed in a dish and cooked in a domestic microwave at 1000 W for 1.5 min.
4. *Grilling*: Mushroom slices (180 g/batch) were cooked in an electric grill at 100 °C for 6 min (3 min on each side).

All these processes were repeated until the 3 kg of slices of each mushroom were cooked. After cooking, all samples were placed on a filter paper to drain water or oil excess. Raw and processed mushrooms were then freeze-dried, powdered, and homogenized in a commercial grinder to use in subsequent experiments.

Chemical composition

The chemical composition of the edible mushrooms, including moisture, ash, crude fat, and protein, were determined in triplicate according to AOAC methods (1995). The protein content ($N \times 4.38$) of the samples was estimated by Kjeldahl method using Kjeltec digestion apparatus (2100 Digestion Unit, Tecator, Sweden). The conversion factor nitrogen-to-protein used was 4.38, since mushrooms proteins are part of chitin which is not digestible so that digestible

proteins were calculated using the adjustment factor 4.38 (Shashirekha et al. 2002). The crude fat was determined by extracting a known weight of powdered sample with petroleum ether using a Soxhlet apparatus. The ash content was measured by incineration at 500 °C. Total carbohydrates were calculated by difference. Energy was estimated according to the following equation: energy (kcal) = 4 × (g protein + g carbohydrate) + 9 × (g fat).

The content of glucans was determined spectrophotometrically using a Mushroom and Yeast β-Glucan Assay kit (Megazyme, Bray, Ireland) according to the protocol of the manufacturer.

Antioxidant assays

Reagents

All chemicals were of analytical reagent grade or higher purity. Bidistilled deionized water was obtained from a Milli-Q purification system (Millipore, Bedford, MA). Methanol, Folin–Ciocalteau reagent, 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox), 2,2-azinobis-(3-ethylbensothiazoline)-6-sulfonic acid (ABTS), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were provided by Sigma (St. Louis, MO). 2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) for the ferric reducing power (FRAP) method was obtained from Fluka Chemicals (Fluka Chemicals, Madrid, Spain). Sodium bicarbonate, sodium carbonate and hydrochloric acid (37%) were provided by Merck (Darmstadt, Germany).

Chemical extraction

The chemical extraction of antioxidants was performed following the procedure described by Pérez-Jiménez and Saura-Calixto (2005). Briefly, 0.250 g of sample was placed in a tube and 2.5 mL of acidic methanol/water (50:50 v/v, pH 2) were added. The tube was thoroughly shaken at room temperature for 1 h and centrifuged at 2500 g for 10 min, and the supernatant was recovered. 2.5 mL of acetone/water (70:30, v/v) were added to the residue, and the shaking and centrifugation steps were repeated. The methanolic and aqueous-acetone extracts were then combined and the volume made up to 5 mL.

Antioxidant activity

Three procedures were applied to test the antioxidant activity of the samples: the ABTS and DPPH assays, to measure the free radical scavenger ability, and the FRAP method, to study the ferric reducing antioxidant power. Aqueous solutions of Trolox were used for calibration (0.01–0.1 mg/mL). Results were expressed as μmol equivalents of Trolox per 100 mg of raw or cooked mushrooms (μg TE/100 mg).

ABTS method The ABTS assay was conducted as described by Rufián-Henares and Delgado-Andrade (2009) with slight modifications. $ABTS^{+\cdot}$ was prepared 12–16 h before use by dissolving ABTS 7 mM with 2.45 mM potassium persulfate, and then diluted in ethanol:water 50:50 to an absorbance of 0.7 ± 0.02. 20 μL of the samples and 280 μL of ABTS solution were incubated for 20 min in the dark and the absorbance was read at 730 nm in a Victor X3 multilabel plate reader (Perkin-Elmer, Norwalk, CT).

DPPH method The antiradical activity was estimated following the procedure reported by Rufián-Henares and Morales (2007). Briefly, 50 μL of the sample were mixed with 250 μL of DPPH solution (74 mg/L in methanol freshly prepared). After incubation for 60 min, the absorbance was measured at 520 nm in the same plate reader, maintaining the temperature in the measurement chamber at 30 °C.

FRAP method The ferric reducing ability of the extract of each sample was estimated following the procedure described by Rufián-Henares and Delgado-Andrade (2009). About 280 μL of FRAP reagent freshly prepared and warmed at 37 °C was mixed with 20 μL of the sample. The FRAP reagent contained 2.5 mL of a 10 mM TPTZ solution in 40 mM HCl plus 2.5 mL of 20 mM FeCl3 and 25 mL of 0.3 M acetate buffer, pH 3.6. The samples were incubated at 37 °C for 30 min in the dark and the absorbance was read at 595 nm in the plate reader indicated.

Total polyphenols

Total polyphenol content was determined following the Folin–Ciocalteau colorimetric method as described by Saura-Calixto and Goñi (2006) with modifications. About 10 μL of sample and 10 μL of Folin–Ciocalteau reagent were mixed in 96-well multi-well plates and let stand for 3 min. Sodium carbonate solution (75 g/L) of 200 μL were added, the volume was made up to 250 μL with Milli-Q water, mixed and allowed to stand in the dark for 60 min. The absorbance was measured at 750 nm using a Victor X3 multilabel plate reader (Waltham, MA) against a standard curve of gallic acid (0–200 mg/L). The total polyphenols content was expressed as μg gallic acid equivalent

per 100 mg of raw or cooked mushroom (μg GAE/100 mg).

Measurement of color

The color of different samples was determined using a Chroma Meter CR-400 optical sensor (Konica Minolta Sensing, Inc., Osaka, Japan) according to the CIE Lab scale (CIE Colorimetric Committee 1974). The system provides the values of three color components: L^* (black–white component, luminosity) and the chromaticity coordinates, a^* (+red to − green component) and b^* (+yellow to − blue component). The samples were placed in a 34 mm optical glass cell and illuminated with D65-artificial daylight (10° standard angle) in accordance with the instructions of the manufacturer. The E index is calculated from the equation: $E = (L^{*2} + a^{*2} + b^{*2})^{1/2}$ and the yellowing index (YI) was estimated from the equation $YI = 142.86 \times b^*/L^*$. Each color value reported was the mean of three determinations at 22–24 °C.

Measurement of absorbance at 420 nm

The browning associated with Maillard reaction development was determined at 420 nm. The progress of the reaction involves the production of final and high molecular weight compounds, termed melanoidins, with chromophore groups with a characteristic absorbance maximum at 420 nm (Morales & Jiménez-Pérez 2004). Briefly, the measurement of browning was performed using the extracts prepared for antioxidant assays, they were measured at 420 nm in an UV/Vis spectrophotometer (UV-1700 Pharmaspec, Shimazu Corporation, Japan). Analyses were performed in triplicate.

Statistical analysis

Statistical significance of the data was tested by one-way analysis of the variance (ANOVA), followed by the Duncan test to compare the means that showed significant variation ($p < .05$). Analyses were performed using Statgraphics Centurion XVI software (StatPoint Technologies, Inc., Warrenton, VA). Relationship between the different variables was carried out by computing the relevant correlation coefficient (Pearson's linear correlation) at the $p < .05$ confidence level.

Results and discussion

Proximate composition

Data from the proximate composition of raw and cooked mushrooms are presented in Table 1. On an average, 60 g of *A. bisporus* was obtained from 100 g of fresh mushrooms, regardless the culinary treatment. However, in *Pleurotus* mushrooms, after boiling and microwaving methods, the culinary yield was greater (80%) comparing with the treatments that involved higher temperatures, grilling, and deep frying. Regarding *L. edodes,* in general, the culinary yields

Table 1. Cooking yield (%) and proximate composition (g/100 g dry weight) of raw and cooked mushrooms[a].

			%					
Samples	Cooking methods	Cooking yield %	Moisture	Ash	Protein[b]	Fat	Carbohydrates[c]	Energy[c]
Agaricus bisporus	Raw		89.71 ± 0.25[a]	9.40 ± 0.06[a]	24.64 ± 0.17[a]	2.34 ± 0.03[a]	63.66 ± 0.24[a]	374.2 ± 0.5[a]
	Boiling	57.3	85.87 ± 0.24[b]	5.46 ± 0.09[b]	26.56 ± 0.37[b]	1.19 ± 0.02[b]	66.55 ± 0.37[b]	384.4 ± 0.2[b]
	Microwaving	60.9	82.14 ± 0.72[c]	8.21 ± 0.04[c]	23.87 ± 0.14[c]	1.42 ± 0.01[b]	66.62 ± 0.09[b]	374.4 ± 0.2[a]
	Grilling	60.6	79.62 ± 1.09[d]	8.81 ± 0.10[d]	23.67 ± 0.18[c]	3.27 ± 0.08[c]	64.06 ± 0.04[a]	381.0 ± 1.0[c]
	Deep frying	61.5	56.24 ± 1.01[e]	5.01 ± 0.05[e]	15.27 ± 0.14[d]	45.08 ± 0.19[d]	34.81 ± 0.10[c]	605.3 ± 0.6[d]
Lentinula edodes	Raw		87.83 ± 1.08[a]	7.36 ± 0.01[a]	16.82 ± 0.36[a]	2.06 ± 0.01[ab]	73.43 ± 0.21[a]	380.9 ± 0.1[a]
	Boiling	109.1	89.14 ± 0.24[a]	4.49 ± 0.10[b]	16.88 ± 0.08[a]	1.57 ± 0.03[a]	77.02 ± 0.14[b]	389.5 ± 0.3[c]
	Microwaving	84.8	81.11 ± 0.75[b]	6.73 ± 0.07[c]	16.75 ± 0.10[a]	1.98 ± 0.03[ab]	74.40 ± 0.14[a]	382.9 ± 0.2[ab]
	Grilling	73.9	78.31 ± 0.54[c]	6.89 ± 0.07[c]	16.50 ± 0.04[a]	3.00 ± 0.04[b]	73.62 ± 0.01[a]	387.5 ± 0.7[bc]
	Deep frying	86.3	39.44 ± 0.26[d]	2.66 ± 0.02[d]	5.90 ± 0.08[b]	62.27 ± 0.75[c]	29.20 ± 0.88[c]	700.6 ± 3.7[d]
Pleurotus ostreatus	Raw		89.41 ± 0.27[a]	6.73 ± 0.05[a]	12.55 ± 0.24[a]	2.46 ± 0.01[a]	78.35 ± 0.36[a]	382.5 ± 0.1[a]
	Boiling	88.0	88.05 ± 0.47[ab]	3.50 ± 0.09[b]	12.85 ± 0.11[a]	2.14 ± 0.08[ab]	81.33 ± 0.01[b]	396.3 ± 0.3[b]
	Microwaving	80.8	83.87 ± 1.12[bc]	6.02 ± 0.15[c]	12.82 ± 0.08[a]	1.52 ± 0.01[b]	79.70 ± 0.24[c]	383.4 ± 1.1[a]
	Grilling	71.2	81.81 ± 1.05[c]	6.05 ± 0.09[c]	12.69 ± 0.06[a]	2.03 ± 0.02[ab]	79.19 ± 0.03[ac]	385.6 ± 0.1[a]
	Deep frying	61.7	54.01 ± 2.21[d]	3.14 ± 0.01[d]	5.96 ± 0.10[b]	50.38 ± 0.48[c]	40.47 ± 0.33[d]	639.4 ± 2.4[c]
Pleurotus eryngii	Raw		88.16 ± 0.17[a]	5.39 ± 0.04[a]	12.30 ± 0.04[a]	1.60 ± 0.02[ab]	80.74 ± 0.02[a]	386.5 ± 0.2[a]
	Boiling	84.8	88.09 ± 0.18[a]	3.22 ± 0.04[b]	13.01 ± 0.05[b]	1.67 ± 0.01[a]	82.15 ± 0.06[b]	395.4 ± 0.3[b]
	Microwaving	81.0	80.58 ± 0.54[b]	5.08 ± 0.05[c]	12.05 ± 0.31[a]	1.57 ± 0.01[b]	81.03 ± 0.06[a]	387.6 ± 0.2[c]
	Grilling	67.7	77.80 ± 0.56[c]	5.24 ± 0.02[d]	13.97 ± 0.04[c]	2.02 ± 0.05[c]	78.79 ± 0.12[c]	389.2 ± 0.2[d]
	Deep frying	59.6	45.95 ± 1.40[d]	2.93 ± 0.04[e]	7.38 ± 0.21[d]	51.03 ± 0.02[d]	38.50 ± 0.16[d]	643.5 ± 0.4[e]

[a]Values are means ± SE, $n = 3$. Different letters within a column indicate significant differences between raw and cooked samples of each mushroom ($p < .05$).
[b]$N \times 4.38$.
[c]Calculated by difference.

were higher than in the others mushrooms assayed, especially after boiling where the value exceeded the 100%. Probably, because of its own structure, *L. edodes* could absorb more water during the boiling process than the others; in fact, this mushroom presented the highest values of moisture and less ash content. Similar values to the average yield data of this assay were found in the study of Manzi et al. (2004) after cooking mushrooms for 10 min in the grill.

The moisture content of cooked samples was significantly lower than the uncooked ones in all the mushrooms species (Table 1). The values of moisture in frying samples were found to be the lowest ones, with a reduction of up to 50% in *L. edodes* and *P. eryngii*. A decrease in moisture content after cooking of mushroom has been previously reported (Jaworska et al. 2015). Similar results were observed in the Ramírez-Anaya et al. (2015) assay comparing raw and cooked vegetables: a strong reduction in moisture was detected in fried vegetables, while in samples cooked by other method as boiling or sautéed, only a slight decrease was noted. In the same line, a reduction in ash content was shown in all cooked samples compared with the raw ones in the present assay. In this case, boiled and fried mushrooms exhibited the lowest values, probably due to the leaching of soluble substances in the water or in the oil. Comparing the type of mushroom, *A. bisporus* had the major ash content values follows by *L. edodes*, *P. ostreatus*, and *P. eryngii*. The ash values of the four uncooked mushroom are in the same range that those reported by Crisan and Sands (1978).

Regarding the protein content, on one hand, fried mushrooms shown a significant reduction compared with the raw and cooked samples. This decrease could be explained by the high temperatures during deep frying since the oil can reach up to 175 °C. It is well known that heat treatment can reduce the amount of protein and destroy some amino acids, changing the quality of protein composition in food (Henry 1998). On the other hand, the frying process implies the penetration of fat in the food matrix and exerts a "dilution effect" in the rest of the nutrients present in the food. Mushrooms processed by other cooking methods had similar protein content to uncooked ones. Once again, *A. bisporus* was the mushroom with major values compared with others species, even two-fold the protein content of *Pleurotus spp*.

The fat content significantly increased in fried mushrooms in the four species compared with raw, boiled, microwaved, and grilled samples which had values of 1–3%. Fried mushrooms contained between 45 and 60% of fat depending on the specie, this increase during the frying process is due to the oil penetration into the mushroom after water is partially lost by evaporation (Saguy & Dana 2003), greatly contributing to the composition of the final product. As a result of this fat penetration during frying, the caloric value of fried mushrooms was two-fold higher than that of raw and cooked mushrooms. The data are in agreement with the study published by Pogoń et al. (2013) who observed a pronounced increase of fat and energy values when a mushroom called *Lactarius delicioius* was fried.

Carbohydrates, calculated by difference, followed the same trend as protein content; similar values were found between raw and cooked samples, except in fried ones which presented an important reduction of carbohydrates content. As it mentioned above, the high amount of oil absorbed by these samples led to changes in their nutritional composition. In the rest of cooking methods, some significant increases have been detected in carbohydrates, protein, and energy values with respect to raw ones, this fact could be due to a loss of moisture during the mushrooms processing and a subsequent concentration of nutrients (Manzi et al. 2001, 2004). Dikeman et al. (2005) also reported cooking losses and, therefore, a concentration of dry matter constituents in *Agaricus bisporus* and *Lentinula edodes*, especially for carbohydrates (starch and total dietary fiber).

The composition of the cooked mushrooms was comparable with the data available in the literature (Barros et al. 2007; Pogoń et al. 2013) and the values of uncooked samples are in agreement with those previously described for each species of mushroom studied (Diez & Alvarez 2001; Manzi et al. 2004).

Glucans content

Results of total glucans, α-glucans, and β-glucans are depicted in Table 2. Glucan concentration varied depending on the cooking method to which mushrooms have been submitted. In *A. bisporus* and *L. edodes*, boiling treatment significantly increased the total glucans content, followed by microwaving and grilling method. Mushrooms processed by these three methods presented similar or even more total glucans content than the raw ones. The same trend can be observed in α-glucans content; however, in this case, boiled samples presented lower amount of these compounds than microwaved and grilled ones. Therefore, the percentage of β-glucans, calculated by difference, only increased significantly after boiling process. Results from *L. edodes* showed that despite some significant differences in α-glucans between raw and

Table 2 Contents of total glucans, α-glucans and β-glucans (g/100 g dry weight) in raw and cooked mushrooms[a].

		%		
Samples	Cooking methods	Total glucans	α-Glucans	β-Glucans
Agaricus bisporus	Raw	15.97 ± 0.69^{a}	3.03 ± 0.11^{a}	12.94 ± 0.81^{a}
	Boiling	20.71 ± 0.44^{b}	3.71 ± 0.16^{b}	17.00 ± 0.33^{b}
	Microwaving	18.51 ± 0.31^{c}	4.17 ± 0.13^{c}	13.44 ± 0.42^{a}
	Grilling	17.58 ± 0.39^{c}	4.14 ± 0.03^{c}	14.35 ± 0.39^{a}
	Deep frying	12.91 ± 0.38^{d}	2.35 ± 0.11^{d}	10.56 ± 0.44^{c}
Lentinula edodes	Raw	34.81 ± 1.23^{a}	2.26 ± 0.01^{a}	32.55 ± 1.13^{a}
	Boiling	43.48 ± 0.61^{b}	3.39 ± 0.04^{b}	40.09 ± 0.63^{b}
	Microwaving	36.94 ± 0.30^{c}	3.09 ± 0.06^{c}	33.85 ± 0.29^{ac}
	Grilling	36.67 ± 0.24^{ac}	2.13 ± 0.03^{d}	34.55 ± 0.23^{c}
	Deep frying	14.66 ± 0.11^{d}	1.04 ± 0.04^{e}	13.62 ± 0.08^{d}
Pleurotus ostreatus	Raw	49.15 ± 0.52^{ab}	8.29 ± 0.06^{a}	40.86 ± 0.57^{a}
	Boiling	51.23 ± 0.29^{a}	7.90 ± 0.09^{b}	43.33 ± 0.32^{a}
	Microwaving	49.48 ± 0.78^{ab}	7.88 ± 0.10^{b}	41.60 ± 0.86^{a}
	Grilling	48.36 ± 0.91^{b}	5.18 ± 0.05^{c}	43.18 ± 0.95^{a}
	Deep frying	26.89 ± 1.29^{c}	2.80 ± 0.12^{d}	24.09 ± 1.37^{b}
Pleurotus eryngii	Raw	54.25 ± 0.43^{a}	9.79 ± 0.14^{a}	44.47 ± 0.30^{a}
	Boiling	56.06 ± 0.88^{a}	12.60 ± 0.55^{b}	43.46 ± 0.77^{ab}
	Microwaving	55.32 ± 0.30^{a}	12.40 ± 0.18^{b}	42.92 ± 0.22^{ab}
	Grilling	48.60 ± 0.65^{b}	6.44 ± 0.04^{c}	42.16 ± 0.62^{b}
	Deep frying	25.04 ± 0.30^{c}	2.01 ± 0.07^{d}	23.03 ± 0.27^{c}

[a]Values are means ± SE, $n = 3$. Different letters within a column indicate significant differences between raw and cooked samples of each mushroom ($p < .05$).

cooked samples were observed, the boiled mushrooms had the highest values of β-glucans compared with raw ones but, in this case, a slight increase was also detected in grilled mushrooms. β-Glucans are considered as bioactive compounds with medicinal properties, so it is important to know which culinary method better preserves or even increases the β-glucans content. The increase of these compounds in boiled mushrooms could be due to the leaching of soluble substances during boiling, which could result in a concentration effect of the fraction of insoluble carbohydrates (Pogoń et al. 2013).

In *Pleurotus ostreatus*, despite some differences between cooked and raw samples observed in total and α-glucans, the β-glucan content were unchanged in all the samples except in the fried one. The same occurred in *P. eryngii* although, in this case, the amount of β-glucan in grilled mushroom also decreased respect to the uncooked one.

Mushrooms from frying treatment had the lowest values of total and α-glucans, and subsequently of β-glucans in the four mushrooms species. Although few studies have described the effect of processing on mushrooms-derived biologically active polysaccharides, it is logical to think that the high temperatures could affect the content and concentration of biological compounds. Radzki et al. (2016) investigated the impact of some processing methods in *P. ostreatus,* confirming that the content and the activity of polysaccharides decreased due to the culinary processing, and that temperature and heating time are key factors.

Taking into account the values of β-glucans within the different species of mushrooms, *P. eryngi* had higher content of β-glucans than the other three species. It is well known that Pleuran, a specific β-glucan isolated from *P. ostreatus*, and Lentinan, from *L. edodes*, are currently the most frequently glucans used for their pharmacological properties. However, there are scant studies about the bioactive properties of *P. eryngii* and the most of the researchers have published lower values of β-glucans for this mushroom (Manzi et al. 2001, 2004) than those reported in the present study. Only the paper published by Synytsya et al. (2008) is in agreement with our data for *P. eryngii* and *P. ostreatus*. In view of these results, *P. eryngii* seems to be a promising candidate as medicinal mushroom and further studies are needed to explore its potential.

Antioxidant activity and polyphenols content

Total polyphenols content and antioxidant activity of raw and cooked mushrooms are shown in Table 3. Total polyphenol content was expressed as μg GAE/100 mg in dry weight and the antioxidant activity as μmol TE/100 mg in dry weight.

The amount of polyphenols present in the mushrooms is not as important as those found in fruits or vegetables (Kettawan et al. 2011). Therefore, our purpose was not to identify the phenolic compounds found in mushrooms by HPLC but we wanted to know the total polyphenol quantity to correlate them

Table 3. Antioxidant activities (μmol TE/100 mg) and total polyphenols content (μg GAE/100 mg) in raw and cooked mushrooms[a].

Samples	Cooking methods	Total polyphenols μg GAE/100 mg	ABTS μmol TE/100 mg	DPPH μmol TE/100 mg	FRAP μmol TE/100 mg
Agaricus bisporus	Raw	181.99 ± 2.29[a]	2.333 ± 0.018[a]	0.752 ± 0.005[a]	2.358 ± 0.030[a]
	Boiling	96.31 ± 1.21[b]	1.120 ± 0.025[b]	1.268 ± 0.015[b]	1.243 ± 0.008[b]
	Microwaving	170.79 ± 1.97[c]	1.388 ± 0.033[c]	1.880 ± 0.020[c]	1.927 ± 0.017[c]
	Grilling	166.87 ± 2.68[c]	1.581 ± 0.021[d]	1.808 ± 0.039[d]	2.198 ± 0.032[d]
	Deep frying	130.87 ± 2.35[d]	1.372 ± 0.012[c]	1.426 ± 0.018[e]	1.811 ± 0.013[e]
Lentinula edodes	Raw	104.35 ± 1.13[a]	0.413 ± 0.007[a]	1.012 ± 0.024[a]	1.376 ± 0.017[a]
	Boiling	54.64 ± 0.66[b]	0.383 ± 0.004[b]	0.350 ± 0.006[b]	0.439 ± 0.006[b]
	Microwaving	147.03 ± 1.91[c]	0.405 ± 0.003[a]	2.310 ± 0.015[c]	2.100 ± 0.014[c]
	Grilling	151.67 ± 1.62[d]	0.480 ± 0.004[c]	2.221 ± 0.027[d]	1.985 ± 0.014[d]
	Deep frying	44.01 ± 0.49[e]	0.329 ± 0.004[d]	0.334 ± 0.009[b]	0.393 ± 0.006[e]
Pleurotus ostreatus	Raw	82.30 ± 1.23[a]	0.770 ± 0.013[a]	0.467 ± 0.010[a]	0.480 ± 0.005[a]
	Boiling	30.08 ± 0.31[b]	0.100 ± 0.006[b]	0.150 ± 0.014[b]	0.163 ± 0.002[b]
	Microwaving	91.21 ± 1.23[c]	0.456 ± 0.004[c]	0.517 ± 0.007[c]	0.519 ± 0.004[c]
	Grilling	86.75 ± 1.62[d]	0.432 ± 0.007[d]	0.463 ± 0.006[a]	0.588 ± 0.007[d]
	Deep frying	47.86 ± 0.80[e]	0.431 ± 0.005[d]	0.308 ± 0.005[d]	0.524 ± 0.007[c]
Pleurotus eryngii	Raw	76.55 ± 0.89[a]	0.396 ± 0.007[a]	0.412 ± 0.003[a]	0.539 ± 0.006[a]
	Boiling	40.67 ± 0.43[b]	0.172 ± 0.005[b]	0.259 ± 0.004[b]	0.318 ± 0.005[b]
	Microwaving	82.79 ± 1.00[c]	0.196 ± 0.009[c]	0.427 ± 0.004[a]	0.524 ± 0.005[a]
	Grilling	96.58 ± 1.54[d]	0.462 ± 0.009[d]	0.589 ± 0.005[c]	0.604 ± 0.007[c]
	Deep frying	70.68 ± 0.79[e]	0.455 ± 0.005[d]	0.382 ± 0.007[d]	0.500 ± 0.004[d]

[a]Different letters within a column indicate significant differences between raw and cooked samples of each mushroom ($p < .05$).

with the antioxidant activity measured as DPPH, ABTS, and FRAP.

After submitting *L. edodes* to different cooking treatments, an appreciable decrease in polyphenol content and antioxidant activity measured by ABTS, DPPH, and FRAP methods was observed in boiled and fried samples with respect to the raw ones and the other cooking procedures. In contrast, in grilled and microwaved *L. edodes,* the polyphenol content and the antioxidant activity increased significantly compared with the uncooked ones. Previous studies also reported an increase of the antioxidant activity autoclaved *L. edodes* at 121 °C during 30 min (Choi et al. 2006). The authors explained this fact with two reasons: (I) the heat treatment might disrupt the cell wall and liberate antioxidant compounds from insoluble portion of mushroom increasing the pool of bioaccessible antioxidant compounds, it has been described that many antioxidant compounds in plant materials are mainly present as a covalently bound form with insoluble polymers (Peleg et al. 1991); and (II) the formation of novel compounds having antioxidant activity during heat treatment or thermal processing, such as Maillard reaction products.

Results from *P. ostreatus* and *P. eryngii* shown a similar trend in polyphenols content and antioxidant activity as *L. edodes*. A decrease in antioxidant capacity in boiled and fried mushrooms was also detected, although in this case, the decline in fried samples was not as pronounced as the boiled ones. Previously, several authors have demonstrated that the boiling process significantly decreased antioxidant activity and polyphenol content in different mushrooms varieties, *P. eryngii, L. edodes*, and *P. ostreatus* among them (Kettawan et al. 2011; Lam & Okello 2015)

When *P. ostreatus* and *P. eryngii* were cooked by microwave or grill, the content of polyphenol and antioxidant activity increased significantly (Table 3). As it stated above, the increase could be explained by the release of antioxidant compounds which were previously linked to other molecules increasing the polyphenol content and then the antioxidant activity and/or for the development of the Maillard reaction during thermal treatment, leading to the formation of high molecular weight compounds with a strong antioxidant capacity. Grilling is the best treatment to cook *P. eryngii,* since this treatment induced the major values for antioxidant activity and polyphenols content, which is consistent with data published by Manzi et al. (2004) who reported an increase in total phenols when *P. eryngii* was grilled for 10 min. In the case of *P. ostreatus*, according with the literature (Sun et al. 2014) and with our own data, the microwave treatment was better in the retention of total phenols. Regarding the antioxidant capacity, values from DPPH and FRAP methods also increased in the microwave samples but not in ABTS method where the antioxidant activity decreased respect to the raw one. It should be taken into account that each antioxidant test is based on different principles and mechanisms (Barros et al. 2007), so it is possible that a food sample shows high antioxidant activity with a single measuring method but not with another antioxidant test (Kettawan et al. 2011). This is the reason for what different antioxidant assays, at least three methods, should be used to measure antioxidant activity.

Interestingly, results from *Agaricus bisporus* shown a different tendency than the rest of the species studied. A decrease in total polyphenols and in ABTS and FRAP procedures was detected in all cooked samples with respect to the uncooked one, more pronounced in boiled and fried mushroom. However, the DPPH radical scavenging ability increased in all the culinary methods compared with the raw one. The lack of concordance between the results from different antioxidant techniques again underlines the importance of applying several antioxidant tests for a better consideration of the antioxidant response of a food matrix.

As mentioned, when *A. bisporus* is cooked by different methods, the antioxidant activity decreased, contrary to that occurred in the other assayed mushrooms. Probably, this fact could be due to the particular structure and shape of each mushroom variety, which could affect the cooking process as well as the yielding extraction of antioxidants during the chemical extraction, thus leading to different release of final antioxidant material from the mushroom matrix (Kettawan et al. 2011). Thermal treatment influences the tissue of the mushrooms, the membranes denaturize, and their permeability increases, which leads to a loss of water and softening of the cells and causes a change in their structure (Pogoń et al. 2013). It can be hypothesized that *A. bisporus* could be more affected by high temperatures than others species.

Comparing the data of polyphenols and antioxidant activity between the different species, *A. bisporus* showed the highest values of both parameters. Several authors have reported higher antioxidant activities in *Agaricus bisporus* compared with others mushrooms. Reis et al. (2012b) analyzed the antioxidant properties of the most cultivated worldwide species and concluded that *A. bisporus* had the highest antioxidant activity compared with the others mushrooms studied. Dubost et al. (2007) also demonstrated that *A. bisporus* had significantly higher antioxidant potential with respect to *Lentinula edodes, Pleurotus ostreatus,* and *Pleurotus eryngii.*

Strong positive correlations were observed between polyphenol content and antioxidant activity measured by the ABTS ($r = 0.7423$, $p = .0000$), DPPH ($r = 0.7622$, $p = .0000$), and FRAP ($r = 0.9346$, $p = .0000$) methods, indicating the important contribution of polyphenols to the antioxidant profile of these mushrooms.

Unspecific indicators of Maillard reaction development in cooked mushrooms

Color analysis

The color of foods is the result of colored natural products associated with the raw material and/or the colored compounds generated as a result of processing (Giangiaconmo & Messina 1988). The L^* parameter measures the luminosity, reflecting the black–white component, so that a decrease on L^* values indicates darkening. As can be observed in Table 4, the L^* data in the present assay decreased in all cooked samples

Table 4. Unspecific indicators of Maillard reaction development in cooked mushrooms. Colorimetric parameters[a].

		CIE Lab color				
Samples	Cooking methods	L^*	a^*	b^*	*YI* index[b]	*E* index[c]
Agaricus bisporus	Raw	80.01 ± 0.61^{a}	0.31 ± 0.09^{a}	13.11 ± 0.11^{a}	23.40 ± 0.37^{a}	81.07 ± 0.58^{a}
	Boiling	76.86 ± 0.02^{b}	-0.33 ± 0.04^{b}	11.87 ± 0.06^{b}	22.06 ± 0.11^{b}	77.77 ± 0.02^{b}
	Microwaving	73.47 ± 0.17^{c}	1.03 ± 0.06^{c}	11.23 ± 0.07^{c}	21.84 ± 0.19^{b}	74.33 ± 0.15^{c}
	Grilling	74.51 ± 0.37^{c}	0.69 ± 0.01^{d}	11.93 ± 0.13^{b}	22.86 ± 0.13^{a}	75.46 ± 0.39^{c}
	Deep frying	61.38 ± 0.37^{d}	0.86 ± 0.04^{cd}	17.65 ± 0.14^{d}	41.08 ± 0.08^{c}	63.87 ± 0.39^{d}
Lentinula edodes	Raw	73.92 ± 0.37^{a}	1.80 ± 0.02^{a}	10.14 ± 0.01^{a}	19.59 ± 0.11^{a}	74.63 ± 0.36^{a}
	Boiling	65.07 ± 0.01^{b}	1.99 ± 0.01^{b}	8.26 ± 0.12^{b}	18.14 ± 0.26^{b}	65.62 ± 0.02^{b}
	Microwaving	67.70 ± 0.06^{c}	1.93 ± 0.03^{b}	9.32 ± 0.10^{c}	19.67 ± 0.23^{a}	68.37 ± 0.05^{c}
	Grilling	67.01 ± 0.10^{d}	1.99 ± 0.01^{b}	10.12 ± 0.07^{a}	21.58 ± 0.12^{c}	67.80 ± 0.11^{c}
	Deep frying	35.24 ± 0.09^{e}	2.54 ± 0.08^{c}	7.23 ± 0.10^{d}	29.32 ± 0.48^{d}	36.06 ± 0.06^{d}
Pleurotus ostreatus	Raw	81.66 ± 0.66^{a}	-1.47 ± 0.24^{a}	12.17 ± 0.34^{a}	21.29 ± 0.76^{a}	82.57 ± 0.59^{a}
	Boiling	71.68 ± 0.19^{b}	0.84 ± 0.01^{b}	8.16 ± 0.13^{b}	16.27 ± 0.31^{b}	72.15 ± 0.17^{b}
	Microwaving	72.84 ± 0.25^{b}	-1.34 ± 0.02^{a}	13.31 ± 0.01^{c}	26.11 ± 0.07^{c}	74.05 ± 0.24^{c}
	Grilling	71.43 ± 0.30^{b}	-0.94 ± 0.03^{c}	14.03 ± 0.14^{d}	28.05 ± 0.15^{d}	72.80 ± 0.32^{bc}
	Deep frying	43.42 ± 0.35^{c}	2.23 ± 0.01^{d}	11.09 ± 0.15^{e}	36.49 ± 0.20^{e}	44.87 ± 0.37^{d}
Pleurotus eryngii	Raw	82.26 ± 0.09^{a}	-1.00 ± 0.06^{a}	11.67 ± 0.06^{a}	19.56 ± 0.12^{a}	86.06 ± 0.08^{a}
	Boiling	80.77 ± 0.01^{b}	0.58 ± 0.01^{b}	7.95 ± 0.08^{b}	14.05 ± 0.14^{b}	81.16 ± 0.01^{b}
	Microwaving	80.68 ± 0.07^{b}	-0.14 ± 0.05^{c}	10.41 ± 0.25^{c}	18.43 ± 0.43^{c}	81.35 ± 0.10^{b}
	Grilling	79.59 ± 0.19^{c}	0.05 ± 0.02^{d}	11.60 ± 0.01^{a}	20.82 ± 0.05^{d}	70.43 ± 0.18^{c}
	Deep frying	51.76 ± 0.31^{d}	2.91 ± 0.04^{e}	13.94 ± 0.16^{d}	38.47 ± 0.21^{e}	56.83 ± 0.34^{d}

[a]Values are means ± SE, $n = 3$. Different letters within a column indicate significant differences between raw and cooked samples of each mushroom ($p < .05$).
[b]$YI = 142.86 \times b^*/L^*$.
[c]$E = (L^*2 + a^*2 + b^*2)^{1/2}$.

A noteworthy and significant relationship was depicted between absorbance values at 420 nm and the antioxidant activity with any of the method used (DPPH, $r = 0.7734$; FRAP, $r = 0.8430$; and ABTS, $r = 0.9049$, all p values $= .0000$), a fact pointing to certain implication of MRP formed during culinary treatments in the antioxidant capacity detected.

Conclusion

This study evaluates the effects of four cooking methods (boiling, microwaving, grilling, and deep frying) on the proximate composition, β-glucans content, and antioxidant activity in four of most consumed mushroom worldwide. The results show a decrease in protein and ash content in cooked mushrooms with respect to raw ones. Frying treatment produced more severe losses in protein, ash, and carbohydrates content but increased the fat and energy. Boiling improved the total glucans content by enhancing the amount of β-glucans; this increase could be due to the leaching of soluble substances during boiling, which could result in a concentration effect of the fraction of insoluble carbohydrates. Regarding the antioxidant activity, a significant decrease was detected specially after boiling but also after frying, while grilled and microwaved mushrooms had higher values of antioxidant activity. This increase could be due to Maillard reaction development as supported by the highest absorbance values measured at 420 nm. Microwaving and grilling were the more adequate culinary treatments to preserve the nutritional profile of mushrooms. Therefore, the cooking technique clearly influences the nutritional value and the antioxidant activity of mushrooms so that the adequate selection of the culinary method is a key factor to preserve the nutritional profile of this highly consumed food.

Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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