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Measurement of β-glucan and other nutritional characteristics in distinct strains of *Agaricus subrufescens* mushrooms

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β-Glucan is a polysaccharide with anticancer properties, and it is present in the mushroom *Agaricus subrufescens*. The aim of this study was to compare two analytical methods to quantify β-glucan in mushrooms and analyze the nutritional characteristics, the concentration of phenolic compounds and the mineral composition, for *A. subrufescens* strains. An enzymatic extraction was performed, and β-glucan was quantified by spectrophotometry and high performance liquid chromatography (HPLC). No significant differences were found between the two methods of quantification of β-glucan. The CS7 and CS10 strains were found to have greater amounts of β-glucan, similar to the levels found in *Pleurotus eryngii* and *Pleurotus-sajor-caju*. *Pleurotus ostreatus* and *Lentinula edodes* samples contained even higher amounts of β-glucan. Significant differences were found in the nutritional characteristics for all parameters assessed, except for fat content. There was no difference between the strains with regard to phenolic compounds or certain mineral components. Fresh *A. subrufescens* mushrooms are not considered to be a high-protein food. However, they are a good source of fiber and minerals, in addition to being low in fat. Spectrophotometry presents the advantages of being quicker and lower in cost; this technique may be suggested as the standard for measurement of the β-glucan.

Key words: β-Glucan, medicinal mushroom, royal sun mushroom, almond portobello.

INTRODUCTION

The nutritional quality of edible and/or medicinal mushrooms may vary according to species, cultivation substrate, environmental conditions and strain (Bonatti et al., 2004; Fan et al., 2007; Guardia et al., 2005; Liu et al., 2005; Ragunathan and Swaminathan, 2003; Toro et al., 2006). The mushroom *Agaricus subrufescens* has drawn the attention of the scientific community, due mainly to its medicinal properties; these properties include anticancer and antioxidant effects, which are characteristics that are associated with the cell wall components (1→6)-(1→3)-β-D-glucan and (1→4)-α-glucan (Bellini et al., 2006; Dong et al., 2002; Firenzuoli et al., 2008; Huang and Mau, 2006; Machado et al., 2005). These polysaccharides have also been associated with the medicinal characteristics of other mushroom species, such as *Pleurotus sp.*, *Lentinula edodes* and *A. bisporus* (Adams et al., 2008; Carbonero et al., 2006; Pramanik et al., 2007).

Studies of genetic diversity (Colauto et al., 2002; Tomizawa et al., 2007) have demonstrated that there are few differences between the various isolates of *A. subrufescens* cultivated in different regions of Brazil; however, some isolates are genetically distinct and are

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Table 1. *Agaricus subrufescens* strains used in the study and its origin.

Strain	Origin (City/State)
CS1	Vitória/ES*
CS5	Araçatuba/SP*
CS7	Porto Alegre/RS*
CS9	Eloi Mendes/MG*
CS10	Belo Horizonte/MG*

*ES, Espírito Santo State; SP, São Paulo State; MG, Minas Gerais State; RS, Rio Grande do Sul State.

considered to be different strains of *A. subrufescens*. These facts imply that distinct strains may have differences in their nutritional characteristics and in their β-glucan content. Investigating the amount of β-glucan and the nutritional and chemical constitution of these strains is of great importance for selecting strains for mushroom production and breeding and for obtaining new strains with desirable characteristics.

Several methods have been used for the extraction and quantification of β-glucans, and the results may vary according to the methods used (Dallies et al., 1998; Manzi and Pizzoferrato, 2001; Park et al., 2003; Rhee et al., 2008). Previously, there was no standard method that fulfilled the requirements of being both quick and highly reproducible for use in ordinal analysis in laboratories. Therefore, in addition to nutritional analysis of the chemical composition, we have tested two methods of β-glucan quantification in the *A. subrufescens* strains cultivated in Brazil.

MATERIALS AND METHODS

Materials

The phosphoric and sulfuric acids, sodium hydroxide, ethanol (95%) and ether were obtained from Sigma-Aldrich (St. Louis, MO). The enzymes α-amylase (Termamyl120L, New Nordisk), protease (New Nordisk)andamyloglicosidase(AMG300,NewNordisk) were obtained from Novozymes Latin America (Araucaria, Parana, Brazil). The kit used for glucose quantification, containing 4-(dimethylamino)-1,5dimethyl-2-phenylpyrazol-3-one (0.025 M), phenol (0.055 M), glycosidase (1U/ ml), peroxidase (0.15 U / ml) and the standard glucose, was obtained from Laborlab Ltd. (Guarulhos, São Paulo, Brazil).

Mushroom strains and cultivation

To measure the β-glucan content and the nutritional characteristics of *A. subrufescens,* five strains were used (CS1, CS5, CS7, CS9 and CS10) (Table 1). The amount of β-glucan obtained from *A. subrufescens* strains was compared with that of the following mushrooms: *Pleurotus ostreatus* (PO), *Pleurotus eryngii* (PE), *Pleurotus-sajor-caju* (PC), *Lentinula edodes* (LE) and *Agaricus bisporus* (AB). All species were obtained from the fungi collection in the Laboratory of Edible Mushrooms in the Department of Biology at the Federal University of Lavras (UFLA).

All strains, including *A. subrufescens*, were maintained on Potato dextrose agar (PDA) medium. Spawn was prepared on paddy rice grains supplemented with 10% wheat bran, 2% gypsum and 2% limestone, using the methods of Siqueira et al. (2009). All mushrooms were produced from strains belonging to the fungi collection of the Laboratory of Edible Mushrooms, except for *L. edodes*, which was obtained from the local market. *Pleurotus* mushrooms were produced in sterilized substrate, using chopped bean straw that was autoclaved twice at 121°C, according to the methods described by Dias et al. (2003). *A. bisporus* and *A. subrufescens* were cultivated according to the standard procedures for this mushroom, as described by Chang and Miles (2004) and Siqueira et al. (2011), using coastcross hay and sugar cane bagasse-based compost, supplemented with superphosphate (1%), limestone (2%), gypsum (2%) and urea (2%).

Mushrooms were cultivated in pots (*Agaricus*) or polypropylene bags (*Pleurotus*), in four replicates. Fruiting bodies were picked and dried for 24 h at approximately 60°C and were subsequently ground in a Wiley mill and homogenized.

β-Glucan extraction

Insoluble fiber, which is mainly composed of β-glucan (Manzi and Pizzoferrato, 2000) was extracted according to the method originally described by Prosky et al. (1988) with the modifications developed by the Japanese Food Analysis Center, as described by Park et al. (2003). 1 g of ground mushroom was added to a 500 mL Erlenmeyer flask containing 50 mL phosphate buffer (80 mmol L^{-1}, pH 6.0). The sample was then subjected to three enzymatic treatment stages: A. 0.1 mL of thermostable α-amylase (Termamyl 120L, New Nordisk) was added, and the flask was incubated in a boiling water bath for 30 min; B, 0.1 mL of neutral bacterial protease (New Nordisk) was added, and the pH was adjusted to 7.5 with 25 mmol L^{-1} NaOH, followed by incubation for 30 min at 60°C; C, 0.3 mL of amyloglucosidase (AMG 300, New Nordisk) was added, and the pH was adjusted to 4.5, followed by incubation for 30 min at 60°C.

After the three enzymatic stages, 200 mL of 95% ethyl alcohol was added to the solution and incubated at 60°C for 01h00. The insoluble fiber that was precipitated from the ethanol was filtered with Whatman number 5B filter paper (samples were weighed before filtering and after fiber removal) and washed three times into a solution of 80% ethyl alcohol and 20% acetone. The fiber precipitate was subsequently dried at room temperature and carefully removed from the filter paper.

For the measurement of β-glucan concentrations in insoluble fiber, each sample was transferred to a 300 mL Erlenmeyer flask and hydrolyzed by adding 10 mL of 72% H_2SO_4 and incubating at room temperature overnight. Distilled water (140 mL) was added, and the solution was incubated for 02h00 in a boiling water bath. The pH of the solution was adjusted to 7.0 using 5 mol L^{-1} NaOH, and the final volume was adjusted to 250 mL. The solution was then filtered with Whatman number 5B filter paper, followed by filtration with Durapore membrane with 0.22 µm pores (Millipore).

Quantification of β-glucan content by the enzymatic method

Quantification of β-glucan based on the enzymatic release of glucose was performed according to McClear and Glennie-Holmes (1985). The kit has the following composition: Standard reactive (glucose solution 100 mg dL^{-1}), enzyme reactive (glucose oxidase (EC 1.1.3.4) ≥ 0.001 L K U^{-1}, peroxidase (EC 1.11.1.7) ≥ 15.10^{-5} L K U^{-1}), color reagent 1 (4-aminophenazone 25 mol m^{-3}, 920 mol m^{-3} Tris) and color reactive 2 (55 mol m^{-3} phenol). The working reagent was prepared by adding in a 250 mL volumetric flask containing

225 mL of distilled water, 12.5 mL of color reagent 1, 12.5 mL of color reagent 2 and 0.75 mL enzyme reactive. The reaction was made of 2.0 mL of working reagent with 20 µL of each sample after extraction for 15 min at 37°C. Absorbance (505 nm) was measured with a UV-VISIBLE spectrophotometer, model UV-1601PC (Shimadzu, Corp., Japan). For standardization, the reaction was made of 2.0 mL of working reagent and 20 µL of standard reactive in the same conditions described above. The results were analyzed using the following Equation 1:

$$\beta - glucan\ \left(\frac{g}{kg}\right) = A\ x f\ x\ 0.9\ x\ 2.5 \quad (1)$$

Where, A is the Reaction absorbance of each sample after acid treatment; $f = 100\ mg\ dL^{-1}/P$, where P is the standard absorbance [20 µL standard reactive (glucose solution 100 mg dL^{-1}) + 2.0 mL reactive work (glycosidase ≥ 3000U; peroxidase ≥ 400U; 4-aminofenazone 0.0015 mol; TRIS buffer 0.0015 mol; phenol 0.00275 mol; pH 7.4 ± 0.1)]. Factor 0.9: 162/180, the factor of free glucose conversion that was determined for anhydrous glucose, which occurs in β-glucan (McClear and Glennie-Holmes (1985)); Factor 2.5: The conversion factor to convert from mg dL^{-1} to g Kg^{-1}, once the final volume of the extraction is 250 mL.

Quantification of β-glucan content by HPLC

For chromatographic analysis, samples were obtained using the previously described procedures. The samples were transferred from storage at -20 to 10°C and were kept at 10°C for approximately 12h00. The samples were then left at room temperature for approximately 04h00 before being analyzed. After temperature stabilization, 25 µL of each sample was diluted by a factor of 20 with ultrapure water, which had been filtered through an ultrafilter Durapore membrane with a 0.20 µm pore size (Millipore). A 20 µL volume of the diluted sample was injected into the chromatograph for analysis.

Values were determined using the AOAC (1992) technique, as modified by Schwan et al. (2001) and Shimadzu (1998). A high performance liquid chromatograph (HPLC), model LC-10Ai (Shimadzu Corp., Japan) was used. It was equipped with refraction index detectors (model RID-10A). For measurement of glucose, a cationic exchange column was used (Shim-Pack SCR-101H) (7.9 mm in diameter x 30 cm in length). For carbohydrate measurement, the column was operated at room temperature, with a mobile phase of ultrapure water adjusted to pH 2.1 and a flux of 0.6 mL min^{-1}. Quantification was done by comparison with a glucose standard curve, which was made using certified standards (Supelco-Sigma-Aldrich, St. Louis, MO, USA). The results read in the HPLC were analyzed using the following Equation 2:

$$\beta - glucan\ \left(\frac{g}{100\,g}\right) = G\ x\ 0.9\ x\ 250 \quad (2)$$

Where, G is the Glucose concentration in mg/mL, determined using a standard curve; Factor 0.9: factor that accounts for the proportion of glucose coming from β-glucan; Factor 250: e conversion factor to convert from mg mL^{-1} to g Kg^{-1}, once the final extraction volume is 250 mL.

Chemical analysis

Samples from different strains of *A. subrufescens* mushrooms were dried, ground in a Wiley Mill and homogenized. They were then conditioned in glass flasks (closed and kept under refrigeration at approximately 5°C). Chemical analyses were performed with four replicates for each treatment.

The following procedures were carried out for chemical analysis, according to the methods adopted by AOAC (2000): Measurement of moisture by drying at 105°C for 06h00, determination of fat content by ether extraction and gravimetry in a Soxhlet extractor, ash measurement by sample incineration and crude fiber measurement by acid digestion. The protein content was determined using the Kjeldahl method, with the amount of crude protein in the mushroom determined based on nitrogen content using a conversion factor (N) of 4.38 (Silva et al., 2007; Tsai et al., 2008). Glucose was measured using the method developed by Somogyi (1945) and Nelson (1944), and total sugar was calculated using the antrona method.

For the measurement of minerals, analyses were performed in triplicate with 0.5 g of dry mushroom for each strain or species. Calcium, magnesium, copper, zinc and iron were measured in Atomic Absorption Spectrometer AA-50 model GTA-110 (Agilent Technologies, Santa Clara, CA, United States). Phosphorus and sulfur were measured by spectrophotometry at 420 nm and boron at 540 nm in Spectrophotometer LAMBDA 25 (PerkinElmer, Waltham, MA, United States) while potassium was measured using a Flame Spectrophotometer model B-262 (Micronal, São Paulo, SP, Brazil) (Malavolta et al., 1997).

Statistical analysis

The means of the results were evaluated using analysis of variance (ANOVA), and the Scott-Knott test was used to compare differences ($p < 0.05$) among the β-glucan content, methods of quantitation of β-glucan and chemical composition. The SISVAR software (Ferreira, 2011) was used for the statistical analysis.

RESULTS AND DISCUSSION

β-Glucan content

β-Glucan extraction from the fungal cell wall can be done using several methods. The method described by Prosky et al. (1988) has been used, with some variations, to extract this polysaccharide for later quantification from soluble and insoluble fiber contained in the fungal cell wall (Dallies et al., 1998; Manzi and Pizzoferrato, 2001; Park et al., 2003; Brauer et al., 2008; Manzi et al., 2004).

In this work, a modified version of the method described by Prosky et al. (1988) was used to extract β-glucan, which was later quantified by spectrophotometry and HPLC. In addition to the *A. subrufescens* strains, other mushroom species were analyzed for comparison. The results of the β-glucan measurements are shown in Table 2. No significant differences were found when results obtained using the different quantification methods (spectrophotometry and HPLC) were compared. The samples quantified by the two tested methods were prepared using the same methods of extract preparation and filtration through filter paper as well as a 0.20 µm ultrafilter membrane. Filtration through an ultrafilter membrane is of extreme importance for avoiding overestimation of the amount of β-glucan and is also necessary prior to HPLC analysis to avoid column block-

Table 2. β-Glucan concentration (g Kg^{-1}) of edible and medicinal mushroom samples determined by enzimatic method and by HPLC.

Sample*	HPLC	Enzimatic method
LE	81.2± 1.29A	90.0± 1.43A
PO	89.2± 0.68A	96.3± 2.04A
PE	60.0± 0.29B	67.2± 1.95B
CS10	57.5± 0.81B	60.3± 0.43B
PC	48.7± 0.85C	70.3± 0.83B
CS7	42.0± 0.65C	50.0± 1.00C
CS9	40.0± 0.29C	42.7± 0.71C
CS5	38.4± 0.38C	41.0± 1.10C
CS2	30.5± 0.50D	32.6± 1.22C
AB	29.9± 0.84D	33.6± 1.02C
CS1	27.0± 1.25D	21.1± 0.67C

Values followed by same letters in the column do not differ among themselves, by Scott-Knott test ($p < 0.05$). * CS1 a CS10: *A. subrufescens* strains; AB, *A. bisporus*; LE: *L. edodes*; PE, *P. eryngii*; PC, *P. sajor-caju*; PO, *P. ostreatus*.

age. For spectrophotometric analysis, ultrafiltration or ultracentrifugation of the sample is important because at the end of the extraction process, small particles present in the sample may affect the absorbance reading, resulting in an overestimation of the β-glucan content. Park et al. (2003) extracted β-glucan from *A. subrufescens* mushrooms using the method of Prosky et al. (1988); however, these authors did not report a second filtration of the samples (through an ultrafilter membrane) before submitting them to spectrophotometric analysis.

The authors (Park et al., 2003) found that the concentration of β-glucan varied from 76.0 to 101.0 g Kg^{-1} of dehydrated mushroom. These values are higher than the ones obtained in the present study, in which the highest β-glucan concentrations were 50.0 and 60.3 g Kg^{-1} for strains CS7 and CS10, respectively. Different strains may have different amounts of β-glucan, but because ultrafiltration was not used by Park et al. (2003), it is possible that their results were an overestimation.

Among *A. subrufescens* strains, the highest β-glucan content was seen in the CS10 strain (60.3 g Kg^{-1}), followed by the CS7 strain (50.0 g Kg^{-1}); the lowest β -glucan content was seen in the CS1 strain (21.1 g Kg^{-1}). Therefore, the CS7 and CS10 strains of *A. subrufescens*, which contained higher concentrations of β-glucan than the others, may have a greater potential for commercial exploitation.

In the comparison between species, the highest β-glucan contents were seen in *L. edodes* (90.0 g Kg^{-1}) and *P. ostreatus* (96.3 g Kg^{-1}), followed by *P. sajor-caju* (70.3 g Kg^{-1}) and *P. eryngii* (67.2 g Kg^{-1}). Several studies have highlighted the medicinal potential of *A. subrufescens* mushrooms (Bellini et al., 2006; Machado et al., 2005; Menoli et al., 2001; Oliveira et al., 2002), which is usually correlated with β-glucan content (Firenzuoli et al., 2008). However, in this work, *P. ostreatus* and *L. edodes* mushrooms were the species that contained the highest amounts of this polysaccharide. It is known that the medicinal properties of mushrooms are related to the type of linkages in their polysaccharide branches as well as the total quantity of polysaccharides (Siqueira et al., 2011; Kawagishi et al., 1989).

A comparison of the two β-glucan quantification methods (spectrophotometry and HPLC) used in this study shows that the spectrophotometric method may be the better choice for this type of analysis because it is a quicker and easier method to execute. It is important to observe that, except for *P. sajor-caju*, all other species and strains kept the same relative positions with regard to β-glucan content when measured by either method. In other words, the species with higher β-glucan concentrations when measured by HPLC also had higher concentrations under the spectrophotometric analysis (Table 2).

Shimizu et al. (2003) reported using the same extraction method described in this work for the β-glucan analysis of two strains of *Pholiota adiposa* mushrooms and observed meaningful differences between the strains. These results corroborate the idea that different strains of the same species may contain different amounts of β-glucan, as was observed in the present study.

Rhee et al. (2008) reported β-glucan extraction from *Inonotus obliquus* mushrooms using two methods. The first method was by enzymatic extraction, using Termamyl and amyloglucosidase enzymes, a method similar to the one used in the present study. The second method consisted of alkaline extraction in sodium carbonate–bicarbonate buffer (pH 10), followed by treatment with HCl. According to the authors, no significant differences were observed between the two extraction methods; this finding demonstrates that different methods may be used to extract β-glucan without compromising the validity of the results.

However, Manzi and Pizzoferrato (2000) reported β-glucan measurements in different *Pleurotus* and *L. edodes* species that were much lower than the values reported in other studies. According to those authors, β-glucan concentrations of 5.3 g Kg^{-1} for *Pleurotus pulmonarius*, 3.8 g Kg^{-1} for *P. ostreatus* and 2.2 g Kg^{-1} for *L. edodes* were observed. However, in that study, the samples were subjected to lichenase hydrolysis and further degradation by β-glucosidase, instead of acid hydrolysis, prior to β-glucan analysis.

Enzymatic hydro-lysis ensures that glucose is released only from β-glucan; however, the hydrolysis may be incomplete. Acid hydroly-sis is normally complete, although it is nonspecific. Given these facts, acid hydrolysis may be a good method for β-glucan analysis in mushrooms, considering that the insoluble fraction of mushrooms contains mainly β-glucan, in addition to some

Table 3. Chemical and nutritional composition (g Kg^{-1}) of dry mushrooms from distinct *Agaricus subrufescens* strains.

Strain	Protein	Lipid	Nifest	Fiber	Ash	Moisture
CS1	245.0± 2.01B	18.6± 0.08A	383.7± 1.6C	165.0± 0.17C	63.6± 0.25C	124.1± 0.28A
CS5	270.7± 0.50C	17.0±0.10A	349.1± 1.5D	175.0± 0.17B	71.7± 0.11A	116.5± 0.92B
CS7	220.8± 0.46A	17.0± 0.10A	431.5± 0.49A	170.0± 0.15C	63.7± 0.19C	97.0± 0.43C
CS9	233.7± 0.46A	18.4± 0.09A	404.8± 0.96B	183.0± 0.66A	67.2± 0.07B	92.9± 0.09C
CS10	248.4± 1.11B	17.9±0.09A	416.1± 1.25B	163.0± 0.19C	67.5± 0.06B	87.1± 0.15D

Values followed by same letters, in columns, do not differ among themselves by Scott-Knott test ($p < 0.05$). Values overwritten represent average pattern deviation.

Table 4. Average values of phenolic compounds and sugars (g Kg^{-1}) in *A. subrufescens* mushrooms in dehydrated matter.

Strain	Reducing sugar	Non reducing sugar	Polyphenols
CS1	0.54± 0.01A	6.1± 0.05B	18.2± 0.42A
CS5	0.45± 0.01C	4.5± 0.01C	22.0± 0.11A
CS7	0.48± 0.02B	7.6± 0.10A	19.6± 0.26A
CS9	0.44± 0.02C	6.3± 0.02B	17.8± 0.08A
CS10	0.39± 0.01D	5.2± 0.01C	16.5± 0.12A

Values followed by same letters, in columns, do not differ among themselves by Scott-Knott test ($p < 0.05$). Values overwritten represent average pattern deviation.

chitin and hemicellulose. The sugars produced by the hydrolysis of chitin and hemicellulose are different from the glucose released from glucan hydrolysis; thus, acid hydrolysis, followed by spectrophotometric measurement of the free glucose released, allows for an efficient quantification of β-glucan. According to Park et al. (2003), the method described by Prosky et al. (1988) and modified by the Japanese Food Analysis Center is the official method in Japan for the analysis of β-glucan.

Rhee et al. (2008) attempted unsuccessfully to standardize a method of β-glucan measurement in *I. obliquus* mushrooms by testing two quantification methods (a gravimetric method and HPLC analysis). Their results were not statistically distinct (101.0 to 107.0 g Kg^{-1} dehydrated matter by the gravimetric method and 81.0 to 83.0 g Kg^{-1} dehydrated matter by HPLC). The authors suggested that HPLC measurement would be more appropriate for quantifying β-glucan when it is present at high levels.

The method of extracting β-glucan used in this work has proven to be efficient for *A. subrufescens* mushrooms and for other mushroom species. Both of the quantification methods (HPLC and spectrophotometry) were found to be equally efficient for measuring the amount of β-glucan in the mushroom samples. The amount of β-glucan in the mushroom samples was the same for both methods. Methods of β-glucan by HPLC are more laborious and more expensive than spectrophotometric methods.

Chemical analysis

With the aim of better understanding the nutritional properties of *A. subrufescens*, nutritional characteristics, concentration of phenolic compounds and mineral composition of five strains were evaluated. Significant differences ($p < 0.05$) were observed for all of the nutritional variables assessed, except for fat content (Table 3). Among the five strains, the CS5 strain had the highest concentrations of protein (270.7 g Kg^{-1}) and ash (71.7 g Kg^{-1}). However, the CS9 strain had the highest crude fiber concentration (183.0 g Kg^{-1}), followed by the CS5 strain (175.0 g Kg^{-1}). These data are very similar to those reported by Tsai et al. (2008) for samples of an *A. subrufescens* strain purchased in a market in Taiwan. Crude fiber is composed of multiple polysaccharides, including β-glucan, which is a major target for studies of compounds with antitumor activity (Bellini et al., 2006; Firenzuoli et al., 2008; Machado et al., 2005; Park et al., 2003). Therefore, fiber content may be related to the concentration of β-glucan in mushrooms. There were no significant differences with regard to the percentage of fat, and all of the strains contained less than 20 g Kg^{-1} fat. A low concentration of lipids is generally considered a common feature of mushrooms (Liu et al., 2005). The various *A. subrufescens* strains showed statistically significant differences in the concentrations of reducing and non-reducing sugars (Table 4). The CS7 strain had the highest concentration of non-reducing sugars (7.6 g

Table 5. Average mineral composition (g Kg^{-1}) of mushrooms from different *A. subrufescens* strains in dried matter.

Strain	P	K	Ca	Mg	S	B	Cu	Mn	Zn	Fe
CS1	11.2± 0.03C	19.4± 0.05A	3.8± 0.02A	1.3± 0.01A	3.7± 0.01B	32.0± 2.11A	0.0286±0.38C	0.0085±0.55B	0.0620±4.70B	0.0703± 7.77B
CS5	12.3± 0.07A	19.5± 0.02A	3.4± 0.02A	1.3± 0.01A	4.1± 0.01A	40.0± 1.50A	0.0539±0.65A	0.0082± 0.29B	0.0515± 0.57D	0.0912± 6.53A
CS7	10.8± 0.01D	19.4± 0.03A	1.8± 0.01B	1.3± 0.01A	3.5± 0.01C	34.0± 1.76A	0.0306± 0.15B	0.0072± 0.10C	0.0463± 0.87E	0.0678± 0.49B
CS9	10.8± 0.02D	19.5± 0.02A	3.6± 0.03A	1.4± 0.01A	3.4± 0.01C	21.7± 0.06A	0.0237± 0.25D	0.0094± 0.40A	0.0560± 0.68C	0.0670± 0.83B
CS10	11.6± 0.02B	19.4± 0.01A	3.7± 0.01A	1.5± 0.01A	3.7± 0.01B	20.0± 0.60A	0.0274± 0.93C	0.0080± 0.12B	0.0679± 0.84A	0.0690± 0.60B

Values followed by same letters, in columns, do not differ among themselves by Scott-Knott test ($p < 0.05$). Values overwritten represent average pattern deviation.

Kg^{-1}) among the five strains; however, the values obtained were very low overall, and the concentration of reducing sugars was negligible. *A. subrufescens* mushrooms are known to possess antioxidant properties, and this characteristic in mushrooms is associated with the presence of phenolic compounds (Barros et al., 2008; Elmastas et al., 2007). The measurement of phenolic compounds from different strains of *A. subrufescens* mushrooms reported in the present study did not show any significant differences between strains (Table 4). The values are similar to those found for *Agaricus arvensis* (28.3 g Kg^{-1}) and are lower than those reported for species from other genera, such as *L. edodes* (47.9 g Kg^{-1}), *Volvariella volvacea* (150.0 g Kg^{-1}), *Leucopaxillus giganteus* (62.9 g Kg^{-1}) and *Sarcodon imbricatus* (37.6 g Kg^{-1}) (Barros et al., 2007; Cheung et al., 2003).

The mineral composition of *A. subrufescens* mushrooms is represented in Table 5, with significant differences ($p < 0.05$) between strains treatments. No significant differences were found between strains for potassium, magnesium, boron and iron. The CS5 strain contained the highest concentrations of phosphorus, sulfur and copper of all of the strains. The highest concentration of manganese was found in the CS9 strain, and the highest concentration of zinc in the CS1 strain. The CS7 strain contained significantly the lowest concentrations of calcium and manganese. Besides Besides, CS7 was among the strains with lowest phosphorus, sulfur and iron concentrations.

It is worth noting that the results presented here refer to dry, rather than fresh, mushrooms. Considering that the amount of moisture in the fresh mushrooms varied from 832.5 to 917.5 g Kg^{-1} depending on the strain, the dry components account for only 82.5 to 167.5 g Kg^{-1} of the total composition of the mushrooms. However, fresh mushrooms should be considered an important source of minerals and fiber and a better source of protein than other vegetables. Furthermore, it is important to emphasize that the results presented in this work demonstrate that the genetic differences observed by Tomizawa et al. (2007) result in different chemical and nutritional properties for different strains. Experiments with different strains of *Pleurotus* spp., *Flammulina velutipes, Lentinula edodes* and *Agaricus bisporus* also demonstrated differences in chemical composition among strains of the same species (Toro et al., 2006; Calonje et al., 1995; Yang et al., 2001).

Conclusions

Fresh *A. subrufescens* mushrooms are not considered to be a high-protein food. However, they are a good source of fiber and minerals, in addition to being low in fat. Therefore, these mushrooms can be considered a beneficial source of nutrients in the diet. Two different methods of quantification (HPLC and spectrophotometry) may be used to efficiently measure the amount of β-glucan in *A. subrufescens*. Spectrophotometry presents the advantages of being quicker and lower in cost, allowing for routine use in the laboratory. Based on these characteristics, this technique may be suggested as the standard for measurement of the β-glucan concentration in edible and medicinal mushrooms.

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