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TECHNICAL NOTE

Optimization of the Protein Sequential Extraction for Quantitative Determination of Albumins, Globulins, Prolamins and Glutelins in Edible Mushrooms

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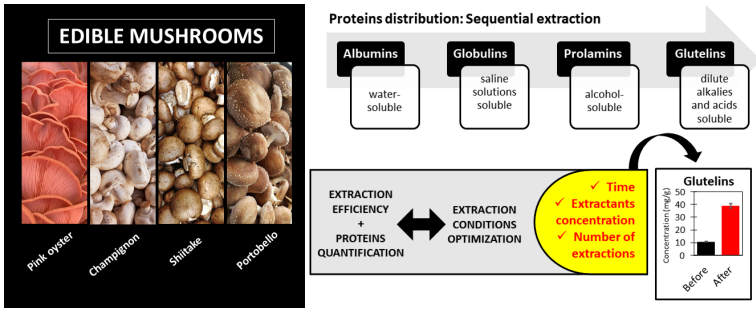

For physiological functions development and a healthy life maintenance, a balanced diet, containing carbohydrates, proteins, and lipids, should be practiced, and consumed. The proteins should be highlighted, since are essential macronutrients for cell growth and repair mechanisms in our body. There is an increase in the protein sources consumption, mainly by athletes, aiming an increase of muscle mass and to avoid muscle hypertrophy. Although animal proteins exhibit high digestibility, animal foods can not be accessible and widely consumed, due to high cost or lifestyle choice (vegetarians and vegans). In these sceneries, scientists and food industry are constantly searching for alternative proteins, such as plant and fungi proteins. In view of these information, extraction procedures are proposed to proteins fractionation. However, these procedures must be done to exhaustion to guarantee the acquisition of quantitative values. Therefore, the aims of this work were evaluated the protein distribution in edible mushrooms and optimized the sequential protein extraction procedure to obtain total concentration of albumin, globulin, prolamin and glutelin in edible mushrooms, evidencing the need to carry out extraction procedures until exhaustion to adequately attribute nutritional value to edible mushrooms (pink oyster, shiitake, portobello and champignon). The optimized extraction conditions (extractant, time, concentration, number of extractions) were as follows (H_2O, 30 min, ---, 3); (NaCl, 15 min, 0.25 mol L^{-1}, 1); (ethanol, 15 min, 50% (v v^{-1}), 1); (NaOH, 60 min, 0.25 mol L^{-1}, 8) for albumin, globulin, prolamin and glutelin

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extraction. The champignon mushrooms presented all protein group concentrations below LOD and LOQ. The portobello presented the lowest total proteins concentration. The pink oyster mushroom is the species with the highest concentration of albumin and glutelin as well as total protein concentration 4.7 times higher than shiitake mushroom, which is one of the most consumed mushroom species, showing that this exotic species can be promising mainly due to nutritional characteristics and protein source.

Keywords: edible mushrooms, protein distribution, sequential extraction, optimization

INTRODUCTION

For physiological functions development and a healthy life maintenance, a balanced diet, containing 45-55% carbohydrates, 20% proteins, and 25-30% lipids should be practiced and consumed.[1] Considering these chemical compounds, the proteins should be highlighted, since are essential macronutrients for cell growth and repair mechanisms in our body.[2,3] There is an increase in the protein sources consumption, mainly by athletes, aiming an increase of muscle mass and to avoid muscle hypertrophy.[1]

The protein effectiveness is evaluated by amino acids content and digestibility.[4,5] Of the total amino acids, there are eight essential for the body (leucine, isoleucine, methionine, valine, threonine, tryptophan, phenylalanine, and lysine).[1,5] It is important to point out that some amino acids are not synthesized by human body, being present in certain foods, such animal source. In animal proteins, there are essential amino acids, and they have high digestibility. However, the animal foods cannot be accessible and widely consumed, due to high cost or lifestyle choice (vegetarians and vegans).[1]

In these sceneries, scientists and food industry are constantly searching for alternative proteins. The research and the industrial products have shown that plant and fungi proteins are promising sources for consumers of a healthy life.[2,6] The protein value of mushrooms varies from 19 to 37% of dry weight and, depending on mushroom variety, 100 g of this food can cover from 29 to 66% of the Recommended Dietary Allowance (RDA) for men and from 36 to 80% for women.[5] It is worth mentioning that produce a large number of proteins and peptides with interesting biological activities, such as lectins, fungal immunomodulatory proteins, ribosome inactivating proteins, antimicrobial proteins, ribonucleases, and laccases.[7]

In addition to the protein content of the edible mushrooms, they attracted great interest from the medical and scientific community, due to their other nutrients, like carbohydrates, vitamins, calcium and iron.[8,9] Beside this, they can therapeutic and medicinal properties, presenting antitumor characteristics, modulate cholesterol levels, prevent platelet aggregation in the arteries, prevent cardiovascular disease, combat hepatitis C virus, and exert antioxidant and antibacterial properties.[10-12] In addition, studies have shown that edible mushrooms have the ability to modulate intestinal microbiota as well as immune system.[13]

Approximately 2,000 edible mushroom species are estimated to exist and around 30 of them are commercially grown worldwide.[14] In many countries, mushroom consumption has been growing significantly, due to the nutritional value and market availability, which makes the product more popular and affordable. *Agaricus bisporus* mushrooms are the most consumed and marketed, followed by *Lentinus edodes* (shitake mushrooms) and different species of the *Pleurotus* (oyster mushrooms) genus.[15] However, some exotic species can also be edible such as pink oyster mushrooms (*Pleurotus djamor*) which are exotic mushrooms with salmon color and fibrous texture. It is known that this species can be used to inhibit hepatoma cell proliferation and plays a vital role in antiviral, antitumor, and immunosuppressive biological activities.[16]

Considering the importance of the mushroom proteins, extraction procedures are proposed to proteins fractionation. However, these procedures must be done to exhaustion, or rather the optimization and repetition of the experimental steps must be performed to guarantee the acquisition of quantitative values. According to Christian,[17] the quantitative extraction is most efficiently carried out by performing multiple extraction with smaller portions of the same volume of solvent. Additionally, in the sequential extraction of the different protein types, the optimization of extraction with appropriated extractants is even more

imperative, since sequentially the extractants are capable to extract the species that are not extracted by the previous extractant, highlighting the NaOH that is the last extractant used in the sequential extraction, because it is not a selective protein extractant, being capable to extract any protein group and not just glutelins.[18,19] The use of sequential extraction procedure without optimization only provides qualitative information, resulting in only albumins, globulins, prolamins and glutelins distribution. In view of the protein importance and the fact that foods are protein sources for humans, it is essential to optimize the extraction steps to obtain quantitative results mainly aiming nutritional information on foods that are widely consumed but still little chemically characterized. Therefore, the aims of this work were evaluated the protein distribution in edible mushrooms and optimized the sequential protein extraction procedure to obtain total concentration of albumin, globulin, prolamin and glutelin in different species of edible mushrooms, evidencing the need to carry out extraction procedures until exhaustion to adequately attribute nutritional value to edible mushrooms.

MATERIALS AND METHODS

Instrumentation

Mushroom species were dried in a freeze dryer (Thermo Fisher Scientific, England) before of the sequential extraction. The mixture between dried sample and extractants was performed by constant agitation on orbital shaker (model 0225M, Quimis, Brazil). A centrifuge (Spectrafuge 6C Compact model, Labnet International, USA) was used for the phase separation.

The supernatants were analyzed by a spectrophotometer (model Q898DRM5, Quimis, Brazil) equipped with tungsten lamp and wavelength range of 325-1000 nm for protein quantification.

Reagents and samples

Four species of edible mushrooms (Pink oyster mushroom (*Pleurotus djamor*), champignon mushroom (*Agaricus bisporus*), shiitake (*Lentinula edodes*), and portobello (*Agaricus bisporus))* and, for each species, four packages of 200 g were purchased at a local market in Sao Paulo.

All solutions were prepared from analytical reagent grade chemicals and using high-purity deionized water obtained from a Milli-Q water purification system (Millipore, USA).

For the sequential extraction, the following reagents (Merck, Germany) were used: acetone, chloroform, ethanol, methanol, NaCl, and NaOH. The total protein concentration in the extracts was obtained using Bradford´s reagent (BioAgency, Brazil), which was diluted five times with deionized water before analysis. The analytical curve for quantification of proteins in the extracts was prepared with stock solution with 0.2 mg mL^{-1} of ovalbumin (BioAgency, Brazil).

Preliminary sample preparation

The four packages of 200 g of the same mushroom species were mixed and cleaned with deionized water. After that, mushrooms were dried by lyophilization (*ca.* 3 days) and grounded in decontaminated pestle and mortar. All grounded samples were stored in polypropylene tubes and kept frozen at -4 °C.

Grounded samples were submitted to sequential extraction procedures, aiming to quantify albumin, globulin, prolamin, and glutelin in different species of edible mushrooms.

Sequential extraction of proteins: proteins distribution

The protein screening was obtained only for pink oyster mushrooms, using sequential extraction procedure described by Naozuka & Oliveira (2007).[20] Sample mass of approximately 200 mg was submitted to sequential extraction, using 10 mL of different extractants: methanol/chloroform mixture (1:2 v v^{-1}), acetone (75 % v v^{-1}), deionized water, 0.5 mol L^{-1} NaCl, 70 % (v v^{-1}) ethanol, and 0.5 mol L^{-1} NaOH. The mixture methanol/chloroform and acetone were used to remove lipids and polyphenols, respectively. Subsequently, the extractants water, NaCl, ethanol, and NaOH were used, producing four supernatants containing albumins, globulins, prolamins, and glutelins, respectively. In Figure 1 is shown a schematic diagram of the sequential extraction experimental setup.

The mixture between samples and extractants were carried out using an orbital shaker at 1520 x g for 30 minutes. The supernatant separation was executed by centrifugation at 4000 rpm for 10 minutes. Protein determination was performed by the Bradford (1976) method.[21] Spectrophotometer calibration was performed using analytical reference solutions of 4, 6, 8, 10, 12, 16, and 20 µg of ovalbumin in 1.0 mL of Bradford reagent. Before analysis, water and NaOH supernatants were diluted with deionized water 2-20 times, while ethanol and NaCl supernatants were not diluted.

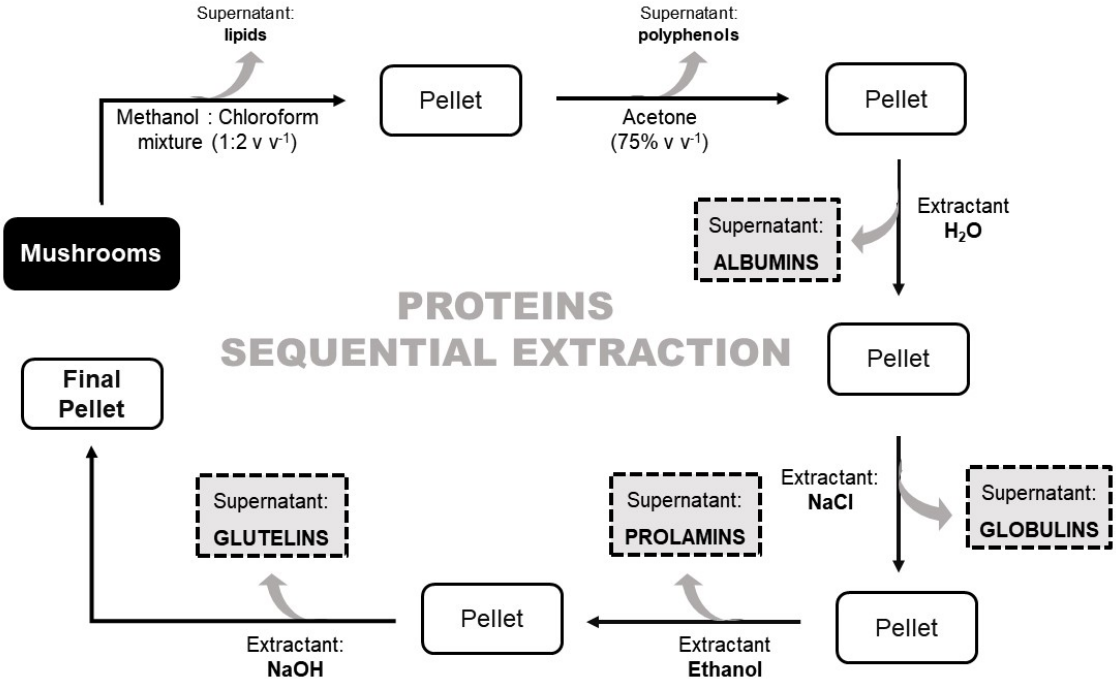

Figure 1. Schematic diagram of the protein sequential extraction experimental setup.

Sequential extraction of proteins: optimization

The sequential extraction optimization is important to obtain total albumins, globulins, prolamins and glutelins concentrations. Time, extractant concentrations and number of consecutives extraction were evaluated only for pink oyster mushrooms using the univariate analysis. Before sequential extraction, a sample mass of 200 mg was submitted to extraction with 10 mL of methanol/chloroform mixture (1:2 v v^{-1}) and acetone (75% v v^{-1}) to remove lipid and polyphenols, respectively.

The time (15, 30 and 60 min) of mixture between sample and extractants was firstly evaluated. The extractants used were deionized water, NaCl (0.5 mol L^{-1}), ethanol (70% v v^{-1}), and NaOH (0.5 mol L^{-1}). The separation of the solid phase was carried out by centrifugation at 1520 x g for 10 minutes. The best time was chosen based on the greatest extracted proteins concentration.

In the best time, the extractants concentration (0.25, 0.5 and 1.0 mol L^{-1} (NaCl and NaOH) and 50, 60, and 70% v v^{-1} (ethanol) was studied. Again, appropriated extractant concentration was chosen considering the greatest extracted proteins concentration.

Finally, with the optimal conditions of time and concentration kept fixed, the number of consecutive extractions was evaluated for each extractant. In each supernatant, proteins were quantified, and the number of consecutive extractions was obtained when the protein concentration was smaller than limit of detection (LOD).

The optimized conditions were applied to different species of edible mushrooms: pink oyster (*Pleurotus djamor*), champignon (*Agaricus bisporus*), shiitake (*Lentinula edodes*), and portobello (*Agaricus bisporus),* aiming the albumins, globulins, prolamins and glutelins determination.

Protein determination was performed by the Bradford (1976) method.[21] Spectrophotometer calibration was performed using analytical reference solutions of 4, 6, 8, 10, 12, 16, and 20 µg of ovalbumin in 1.0 mL of Bradford reagent. Before analysis, water and NaOH supernatants were diluted with deionized water 2-20 times, while ethanol and NaCl supernatants were not diluted.

RESULTS AND DISCUSSION

Proteins distribution in pink oyster mushroom

Protein quantification was carried out by Bradford method at 595 nm wavelength.[21] The Bradford method is commonly used for protein quantification in solutions, due to its simplicity, easy application, and high sensitivity providing satisfactory analytical response. This method consists of the non-covalent bond between the anionic form of Coomassie Blue Brilhant Blue (G-250) dye with proteins.[17] The dye reacts with the positively charged portion of the protein chain, usually arginine residues. Poor interactions are observed with basic (histidine and lysine) and aromatic (tyrosine, tryptophan and phenylalanine) residues. Thus, there is an equilibrium displacement of the dye to its ionic form, which corresponds to the species absorbing at 595 nm wavelength.[18,22] Furthermore, according to Zaia et al.,[23] there are few interferents substances in the Bradford method, which they can react with proteins or with the dye, increasing the absorbance. In food, possible interferents are lipids and polyphenols, [23] but in the proposed method, the initial extractions with methanol:chloroform and acetone (Figure 1) were capable to minimize the lipids and polyphenols presence. Considering the extractants (water, NaCl solution, ethanol, and NaOH solution), only NaCl solution above 1 mol L^{-1} can provide negative results in the Bradford method.[23] In view of this information, the interferences in the proposed method is strongly minimized, adding the fact that extracts are diluted, mainly globulins supernatant, before analysis.

The characteristic parameters of the analytical calibration curves (linear range, correlation coefficient (R^2) and sensibility), LOD and limits of quantification (LOQ) are shown in Table I. The LOD was calculated using the standard deviation of 10 measurements of the analytical blank sample ($3 \times \sigma_{blank}$, where σ is the standard deviation) and the LOQ was calculated as 3 × LOD. For the sequential extraction, the values were obtained in mg g^{-1}, considering a sample mass of 200 mg and a final volume of 10 mL.

Table I. Characteristic parameters of the analytical method

	Linear range (µg mL^{-1})	R^2	Sensibility	Analytical blank	LOD (mg g^{-1})	LOQ (mg g^{-1})
Sequential extraction	20–120	0.9791	0.0015	Water	0.6	1.7
				NaCl	0.3	0.9
				Ethanol	0.4	1.2
				NaOH	0.6	1.8

The separation of lipids and polyphenols was performed using a mixture of methanol/chloroform and acetone, respectively. In the absence of lipids and polyphenols, it was possible to separate different protein groups, applying the sequential extraction procedure.

Proteins are amino acids polymers, which are linked by peptide bonding. The amino acids can carboxyl and amino groups, besides non-protein parts. The amino acids composition with polar or non-polar groups influences the protein solubility. The charge arrangements in the proteins depends on acidic (*eg.* aspartyl and glutamyl) and basic (*eg*. histidyl, arginyl, and lysyl) amino acids. The non-protein parts (lipids, carbohydrates, and phosphates) can also alter the proteins solubility.[18] So, it is possible to separate different proteins groups, using appropriated solvents. According to the Osborne (1924) method, a sequential extraction with water, saline (*eg* NaCl) solutions, alcoholic (*eg* 70-80% v v^{-1} ethanol) solutions and acidic or alkaline (*eg* NaOH) solutions is capable to separate albumin, globulin, prolamin and glutelin, respectively.[20,24] The protein distribution in pink oyster mushrooms are shown in Table II.

According to Table II, it was verified that the highest protein concentration was found in the NaOH extract, corresponding to the glutelin group. The NaOH is the last extractant used in the sequential extraction, because it is not a selective protein extractant, being capable to extract any protein group.[18,19] In this way, high glutelin concentrations can occur when extractions with water, NaCl and ethanol are inefficient due to inadequate times, volumes and/or concentrations. Therefore, the sequential extraction procedure optimization should ensure complete proteins extraction from each protein group, allowing quantitative determination of albumin, globulin, prolamin, and glutelin in edible mushrooms.

Table II. Proteins distribution in pink oyster mushroom

Mushroom	Concentration (mg g^{-1}) ± standard deviation (n = 3)				
	Albumin	Globulin	Prolamin	Glutelin	Total*
Pink oyster	4.8 ± 0.2	< LOQ	< LOQ	10.7 ± 0.2	15.5 ± 0.3

*Concentration sum of each protein type.

Sequential protein extraction optimization

Protein distribution studies are important for a first investigation of different proteins present in the samples. Although protein distribution cannot provide the total concentrations of albumins, globulins, prolamins and glutelins, it contributes with important information about nutritional characteristics and potentialities. The value of the protein content in edible mushrooms requires the quantitative determination of albumin, globulin, prolamin, and glutelin, obtained after the sequential extraction optimization. For this, it was done an univariably optimization evaluating time, extractants concentrations, and number of consecutive extractions parameters.

Initially, the extraction time was varied and the obtained results for pink oyster mushroom are presented in Table III. Comparing the concentrations for each proteins group, the optimum time for the extraction were chosen considering the highest concentration obtained as well as standard deviation values. Therefore, 30 and 60 min for albumin and glutelin, respectively. For globulins and prolamins, it was maintained 15 min, since concentrations were below the LOQ for any extraction time.

At the fixed optimal time, the extractants concentrations were optimized, except for deionized water. The results are shown in the Table IV. The NaCl, ethanol and NaOH concentrations were, respectively, divided in three groups: minimum (0.25 mol L^{-1}, 50% (v v^{-1}) and 0.25 mol L^{-1}), intermediate (0.5 mol L^{-1}, 60% (v v^{-1}) and 0.5 mol L^{-1}) and maximum (1.0 mol L^{-1}, 70% (v v^{-1}) and 1.0 mol L^{-1}). For all protein types, the minimum extractant concentration (NaCl 0.25 mol L^{-1}, ethanol 50% v v^{-1}, and NaOH 0.25 mol L^{-1}) was more appropriate.

Table III. Concentration of albumin, globulin, prolamin and glutelin in pink oyster mushrooms at different extraction times

Extraction time	Protein concentration (mg g^{-1}) ± standard deviation (n = 3)			
	Albumin	Globulin	Prolamin	Glutelin
15 min	2.5 ± 0.5	< LOQ	< LOQ	8.8 ± 0.7
30 min	4.8 ± 0.2	< LOQ	< LOQ	11.1 ± 0.1
60 min	4.6 ± 0.2	< LOQ	< LOQ	15 ± 1

*Concentration sum of each protein type.

Table IV. Concentration of albumin, globulin, prolamin and glutelin in pink oyster mushrooms at different reagent concentrations

Extractant concentration	Protein concentration (mg g^{-1}) ± standard deviation (n = 3)				
	Albumin	Globulin	Prolamin	Glutelin	Total*
Minimum	4.6 ± 0.2	< LOQ	< LOQ	17 ± 4	22 ± 4
Intermediate	4.6 ± 0.2	< LOQ	< LOQ	15 ± 1	20 ± 1
Maximum	4.6 ± 0.2	< LOQ	< LOQ	15 ± 1	20 ± 1

*Concentration sum of each protein type.

After optimization of time and extractants concentrations, the extraction quantity with the same extractant was studied, being each sequential extraction step consecutively repeated until that protein determined concentrations were below the LOD and LOQ. This study was performed only for albumins and glutelins, because the globulin and prolamin concentrations were already below the LOD and LOQ. In the Figure 2 is shown the optimization results for the number of extractions. It is possible to observe that 3 (Figure 1A) and 8 (Figure 1B) consecutives extractions with water and NaOH solution completely extracted albumin and glutelin, respectively, considering the LOQ values presented previously in Table I. Considering 3 and 8 consecutives extractions with water and NaOH solution, respectively, the albumin and glutelin concentrations had an increase, when compared with results shown in the Table II (proteins distribution).

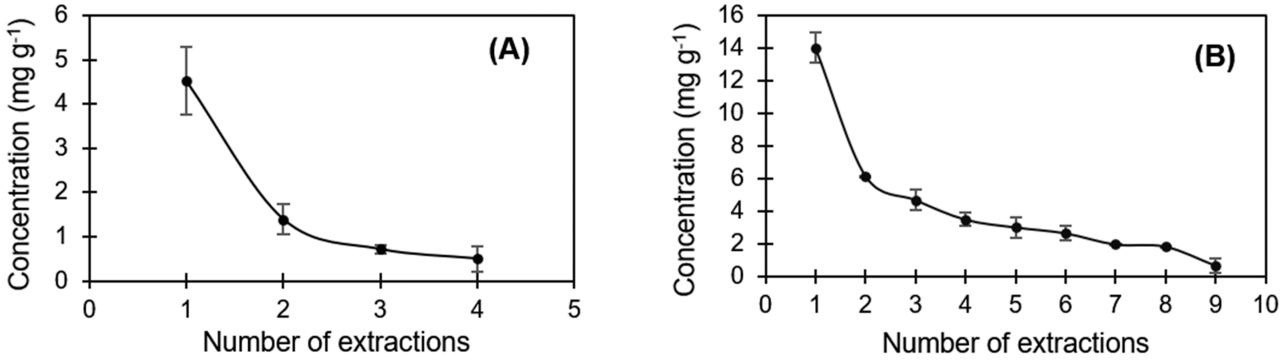

Figure 2. Concentration of albumin (A) and glutelin (B) in pink oyster mushrooms applying consecutive extractions

So, the quantitative extraction of albumins, globulins, prolamins and glutelins in mushrooms was accomplished using the optimized conditions summarized in the Table V.

Table V. Optimized parameters for sequential extraction of proteins

Parameters	Extractant			
	H_2O	NaCl	Ethanol	NaOH
Extraction time	30 min	15 min	15 min	60 min
Extractant concentration	--	0.25	50 % (v v^{-1})	0.25
Number of extractions	3	1	1	8

Protein quantitative determination in edible mushrooms

The albumin, globulin, prolamin, and glutelin concentrations in pink oyster, champignon, shiitake, and portobello mushrooms after sequential extraction using the optimized conditions are presented in Table VI.

Previous investigations determined the total protein concentrations in edible mushrooms[25,26], corresponding to 252, 234, 217, and 294 mg g^{-1} for pink oyster, champignon, shiitake, and portobelo, respectively. In this work, the total protein concentrations, Table VI, were obtained by the masses sum of all extracts, and comparing the literatures[25,26] and obtained results, it was possible to extract 18, 5 and 2% w/w for pink oyster, shiitake, and portobelo, respectively. For champignon, albumins, globulins, prolamins and glutelins were not found using the proposed method. Therefore, there are other protein groups in these edible mushrooms, and albumins, globulins, prolamins and glutelins are a small part of the whole proteins.

Table VI. Concentration of albumin, globulin, prolamin and glutelin in different mushroom species after optimized sequential extraction

Mushroom	Origin	Protein concentration (mg g^{-1}) ± standard deviation (n = 3)					DRV (%)
		Albumin	Globulin	Prolamin	Glutelin	Total[a]	
Pink oyster	Indoor growing	1.5 ± 0.1	0.9 ± 0.2	< LOQ	28 ± 2	30 ±2	0.40
Pink oyster	Commercial	7 ± 1	< LOQ	< LOQ	39 ± 2	46 ± 2	0.61
Champignon	Commercial	< LOQ	< LOQ	< LOQ	< LOQ	**n.d.	**n.d.
Shiitake	Commercial	0.7 ± 0.1	< LOQ	< LOQ	9.1 ± 0.2	9.8 ± 0.2	0.13
Portobello	Commercial	< LOQ	< LOQ	< LOQ	5.2 ± 0.1	5.2 ± 0.1	0.07

*%DRV refers to the percentage of the Daily Reference Value of each component supplied per 100 g portion of fresh mushroom in a 2000 kcal or 8,400 kJ diet, according to ANVISA RDC Resolution No. 360 December 23, 2003.[19]

**not determined (n.d.). [a]Total protein from sum of each protein type.

Comparing the proteins distribution (Table II) in pink oyster mushrooms with proteins concentrations obtained after sequential extraction conditions optimization, it is possible to observe that the albumins and glutelins concentrations suffered alteration (Table VI). Additionally, only pink oyster and shiitake mushrooms showed concentrations above the LOD and LOQ for albumin group while pink oyster mushroom was the species with the highest concentration of albumin and glutelin as well as total protein concentration 4.7 times higher than shiitake mushroom, which is one of the most consumed mushroom species, showing that this exotic species can be promising mainly due to nutritional characteristics and protein source.

The champignon mushrooms presented all protein group concentrations below LOD and LOQ. So, this proposed method was not capable to determine proteins in this mushroom and comparatively this mushroom species is not rich in proteins like pink oyster, portobello, and shiitake. Possibly, increasing the sample mass or decreasing the extractant volume, it would be possible to determine the albumin, globulin, prolamin and glutelin concentrations in champignon mushroom. The portobello can be considered the second mushroom species with the lowest total concentration of proteins, just showing glutelins group.

It is known that mushrooms can colonize different types of agricultural and agro-industrial waste such as sawdust, sugarcane bagasse, corn husk, and coffee pulp.[27,28] Therefore, it is important to point out that comparisons between published results for other authors are difficult, since cultivation conditions (climate and irrigation), substrate composition and maturation phase of the fruiting bodies mushrooms can promote variations in proteins distribution.[29-32] Besides that, extraction procedures differ notably from each other.

Although protein distribution is based on solubility differences and plays a fundamental biological role in uptake, digestion and absorption of proteins, there is still limited data provided on protein distribution for mushrooms.[33] A study published by Petrovska (2001) evaluated the albumin, globulins, prolamins, and glutelin distribution in mushrooms.[34] However, the extraction procedure showed difference when compared with proposed method, being the prolamins extraction performed with 55% v v^{-1} isopropyl alcohol, while for glutelin extraction, borate buffer (pH 10) with 0.6% v v^{-1} 2-mercaptoethanol and 0.5% w v^{-1} sodium dodecyl sulfate were used.

The results presented in Table VI are in agreement with those published by Helm et al. (2009) in which the *Pleurotus* mushrooms also presented the highest concentration of total proteins (37.51% (w w^{-1})) when compared to other mushrooms species.[35] On the other hand, in edible Macedonian and *Pleurotus eryngii* mushrooms, different results were obtained, since extraction procedures without optimization and different extractants for prolamins and glutelins were applied.[34,36] In Macedonian mushrooms, the abundance of protein fraction was as follow: albumins > glutelins > globulins > prolamins.[34] The total protein concentration presented in Table VI for champignon mushroom (*Agaricus bisporus*) was lower than 36.3% dry wt. of protein content in *A. bisporus* mushrooms reported earlier by Akyüz et al. (2010).[37]

Lastly, it was verified that pink oyster mushroom supplies 0.47% of the recommended daily protein intake (Daily Reference Values, DRV = 75.0 g) followed by shiitake mushroom (0.13%),[38] considering the daily intake of 100 g of fresh mushrooms with an average fresh edible mushrooms moisture of 90% and the total protein concentration the sum of albumin, prolamin, globulin and glutelin concentration.

CONCLUSIONS

For quantitative determination of different protein groups (albumin, globulin, prolamin and glutelin) in edible mushrooms, it is essential to optimization of sequential extraction procedure. It was observed that the optimization significantly altered the glutelin concentration in pink oyster mushroom.

The optimized sequential extraction was applied for pink oyster, shiitake, portobello and champignon mushrooms. The champignon mushrooms presented all protein group concentrations below LOD and LOQ. The portobello can be considered the second mushroom species with the lowest total concentration of proteins, just showing glutelins group. The pink oyster mushroom is the species with the highest concentration of albumin and glutelin as well as total protein concentration 4.7 times higher than shiitake mushroom, which is one of the most consumed mushroom species, showing that this exotic species can be promising mainly due to nutritional characteristics and protein source.

In this way, the evaluation of the quantitative protein distribution in different edible mushroom species can contribute with data capable of improving the nutritional information about the conscious use of these foods, since proteins play an essential role in the human organism and in the composition of the studied mushrooms.

Conflicts of interest

The authors declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects.

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