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Vitamins B_1 and B_2 contents in cultivated mushrooms

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Abstract

Mushrooms have long been treated as a delicacy. Nowadays however, many researchers consider them to be nutraceutical foods, which has stimulated new and existing Brazilian producers to search for more productive techniques and to introduce other species. The objective of this study was to determine the vitamin B_1 and B_2 contents in mushrooms. The main species of mushroom cultivated in Brazil and analysed in this study are: *Agaricus bisporus* (white button mushroom and portobello), *Lentinula edodes* (shiitake) and *Pleorotus* spp. (shimeji and oyster mushroom). The methodology employed used acid hydrolysis followed by enzymatic hydrolysis and separation of the vitamins by high performance liquid chromatography using a C_{18} reverse phase column and fluorescence detector. The results obtained for thiamine (vitamin B_1) were from 0.004 to 0.08 mg/100 g and for riboflavin (vitamin B_2), from 0.04 to 0.3 mg/100 g.
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1. Introduction

Mushrooms have been used as a food for centuries. Although many cultures use mushrooms, both for their gastronomic importance and for their medicinal value, their use as a functional food is more notable in oriental cultures, in which the application of mushrooms for the maintenance of health has its roots thousands of years ago, for example, in China. The mushroom shiitake, well-known by the Japanese and other Asians, has become the second most cultivated mushroom in the world. Together with their habit of using them as food, the Asians have a strong tradition to use mushrooms medicinally, dating from more than 2000 years ago (Chang, 1996). In a revision, Sadler (2003) concluded that some species of mushrooms have been used for their beneficial proprieties for over 2000 years, including studies that suggests an important therapeutic role in cancer and other diseases.

Mushrooms have been part of human diet for thousands of years and recently their consumption has been increasing, involving a great number of species apart from the popular button mushroom (Mattila et al., 2001). More than 2000 edible species of mushroom are known, but only 25 are commercially cultivated. In Brazil the main species cultivated are *Agaricus bisporus*, *Lentinula edodes* and *Pleorotus* spp., which according to Bernaś, Jaworska, and Lisiewska (2006) currently play a significant economic hole in the global market.

The well-known button mushroom (*A. bisporus*) was the first species cultivated in Brazil and is the most cultivated species throughout the world. In Brazil, mainly in the region of the city of Mogi das Cruzes in the State of São Paulo, cultivation is still carried out in a rudimentary way, mostly by Chinese families who inherited the techniques from many generations of ancestors, but without deeper scientific knowledge (Coutinho, 2006). Recently, an increase in the consumption of the product, and hence of its production and commercialisation, has been noted in Brazil, due to greater divulgation of its nutritive and medicinal values and the fact that its price has become more accessible. The market growth can be verified by the following: in 1995 the world annual production of button mushroom was 2.0 million tons, and in the year of 2005

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the production raised to 3.3 million tons, an increase of over 60% in 10 years (Faostat).

Nutritional information on foods is becoming more and more important both for professionals in the food and health areas and for consumers, who show increasing concern about the nutritional quality of the food which makes up, or could be introduced into, their diets. However, little is known about the nutritional value of the edible mushrooms cultivated in Brazil. The vitamin content is of great value, since they have important functions in the human and animal organism, and, according to Breene (1990), mushrooms could be a good source of vitamins B_1, B_2, niacin, biotin and vitamin C. For this reason the objective of the present study was to quantify vitamins B_1 and B_2 in the mushrooms cultivated in Brazil.

2. Materials and methods

2.1. Samples

The mushroom species analysed in this study were: *A. bisporus* (white button mushrooms, portobello and button mushroom in conserve), *L. edodes* (shiitake) and *Pleorotus* spp. (white and salmon oyster mushroom and shimeji). Different samples of these mushroom species (10 brands of fresh mushrooms and five brand of conserved mushroom, five batches of each brand) were acquired from supermarkets in the city of Campinas, SP. Samples were kept under refrigeration until the moment of the analysis and were analysed before expiration date.

The methodology was validated using about 2 kg of *A. bisporus* mushrooms, recently harvested from the production areas of the company Toyobo do Brasil Ltda, and freeze-dried whole for 28 h at −55 °C with a pressure of 10^{-1}–10^{-2} mbar. After freeze-drying the mushrooms were ground in a knife mill using a 20 mesh sieve, and stored at −10 °C in polyethylene flasks until analysed.

2.2. Reagents

The thiamine (B_1) and riboflavin (B_2) standards were acquired from Sigma Chemical Co., USA. The standard vitamin solutions were prepared in 0.1 N HCl. The enzyme takadiastase was acquired from Fluka®, Switzerland. The organic solvents used as the mobile phase for liquid chromatography were of chromatographic grade and the other reagents of analytical grade. Water purified in the Milli-Q (Millipore) system was used to prepare the mobile phases. All mobile phases were filtered through membranes with a pore size of 0.45 μm.

2.3. Equipment

An Edwards model Super Modullyo freeze-dryer was employed to freeze-dry the sample used in the validation of the methodology. An HP model 1100 high performance liquid chromatograph was used in the vitamin determinations, equipped with a degasser, quaternary pump, automatic injection system (0–100 μL), with a diode array and fluorescence detectors coupled in series, and a temperature control compartment for the analytical column. The detection system allowed for the simultaneous detection at various wavelengths. The overall system was controlled by the HP-Chemstation software, which also administered the data collection and treatment system.

2.4. Methodology validation

The evaluation parameters used to validate the methodology for the determination of vitamins B_1 and B_2 were linearity, the detection and quantification limits and repeatability. All the analyses were carried out using the sample of freeze-dried *A. bisporus*.

Linearity was determined by constructing calibration curves with standard solutions containing the vitamins in the mass range from 1.1 to 210 ng and from 2.3 to 202 ng for vitamins B_1 and B_2 respectively. Three injections were made at each level. The oxidation reaction of thiamine to thiochrome was the same used on the samples analysis and was carried out as described in Section 2.5.

The detection limits for vitamins B_1 and B_2 were determined from the signal/noise ratio equal to three (ACS, 1980), where the smallest amount of the standard produced a signal with amplitude three times greater than that of the noise. The quantification limits adopted for the vitamins were fixed as five times the detection limit (Thompson, Ellison, & Wood, 2002).

Recovery tests were carried out by spiking samples of freeze-dried mushrooms with standards of B_1 and B_2 at levels of 0.1 and 1.0 mg/100 g. These levels of recovery were equivalent to approximately 0.01–0.1 mg/100 g of raw mushroom. Ten determinations were carried out for the spiked samples as well for the unspiked controls. Recoveries were calculated from the differences between the spiked and unspiked samples. Repeatability of the method was evaluated through the coefficients of variation (CV) associated to measurements of the vitamins performed during recovery analysis.

2.5. Vitamin analysis

The extraction methodology used was based on that described by Esteve, Farré, Frígola, and Garcia-Cantabella (2001) and all determinations were carried out in duplicate. The mushrooms sample was ground to complete liquefaction, and homogenized using a domestic blender, followed by immediate analysis. For the acid hydrolysis, 2 g of previously disintegrated sample were used, and 30 mL 0.1 N HCl added. This solution was heated in a water bath at 95–100 °C for 30 min. The extract was then cooled and the pH adjusted to 4.0–4.5 using a 5 M sodium acetate solution. 500 mg of the enzyme takadiastase were added to this extract and incubated in a water bath at 45–50 °C for 5 h. After the enzymatic hydrolysis, 1 mL 50% trichlo-

roacetic acid was added to the extract, which was heated in a water bath for 5 min at 95–100 °C. After cooling, the volume was completed to 50 mL with 0.1 N HCl and filtered. 300 μL of potassium ferricyanide (1% in 15% NaOH) were added to 5 mL of the filtrate and left to react for 10 min in the dark. The extract was then neutralized with 200 μL of 15% orthophosphoric acid, filtered through a filter with a pore size of 0.45 μm and immediately injected into the chromatograph.

2.6. Chromatographic conditions

The mobile phase consisted of an aqueous phase of 0.3% triethylamine (TEA) adjusted to pH 7.4 with sulphuric acid, and an organic phase of methanol. The initial gradient was composed of 80% aqueous phase and 20% organic phase, held for 1.5 min. At 1.51 min, the gradient was changed to 84% aqueous phase and 16% methanol and a linear gradient then followed, arriving at 30% aqueous phase and 70% organic phase in 15 min. The system was then returned to the initial conditions but the column did not require equilibrating before the next injection. The flow rate was 1.0 mL/min^{-1}. The analytical column used was a 3.9 × 150 mm Nova-pak® C_{18} column (Waters) with 4 μm particles, protected by a 3.9 × 15 mm guard column containing the stationary phase octadecylsilyl (Varian), with particles of 5 μm and temperature controlled at 25 °C. The sample injection volume was 100 μL. The vitamins eluted were monitored using a programmed fluorescence detector. Detection started at $\lambda_{exc} = 365$ nm and $\lambda_{em} = 436$ nm for vitamin B_1 and after 7.5 min was altered to $\lambda_{exc} = 422$ nm and $\lambda_{em} = 515$ nm to detect B_2. Quantification was by external standardisation using the vitamin B_1 and B_2 standards. Identification was by comparison of the fluorescence spectra and retention times. In addition, standards were added to the extracts for confirmation.

2.7. Statistical analysis

The variance analysis and Tukey's test were applied using the Statistica (2000) software. The significant differences ($p < 0.05$) were evaluated for the vitamin B_1 and B_2 contents of each sample analysed ($n = 10$), independent of batch or collection date, and also for each sample individually, comparing the values found between batches.

3. Results and discussion

3.1. Method validation

The linearity was determined by constructing calibration curves and three injections were made at each level, resulting in mean variation coefficients for the injections of 4.2% and 1.2% for B_1 and B_2 respectively. The values for the correlation coefficients (r) were >0.99.

The detection limits for the vitamin B_1 and B_2 standards were 0.026 and 0.029 ng for B_1 and B_2, respectively.

The recoveries obtained for vitamin B_1 and B_2 ranged from 86% to 97% and the CV ranged from 3.0% to 4.3%. For the unspiked controls, the values found were 0.23 mg/100 g (CV = 11.2%) and 2.21 mg/100 g (CV = 5.6%) for vitamin B_1 and B_2, respectively. The recovery values obtained are similar to the ones presented by Esteve et al. (2001), who obtained for mushroom samples values of 91.6% and 96.7% for vitamins B_1 and B_2, respectively.

3.2. Contents in mushrooms

Table 1 shows the vitamins contents in the 11 brands of mushrooms analysed. Values ranged from 0.004 to 0.08 mg/100 g for thiamine and 0.037 to 0.298 mg/100 g for riboflavin.

Vitamin B_2 contents in the analysed mushrooms, with exception of mushroom in conserve, are higher than the levels present in many vegetables. The *A. bisporus* mushroom in particularly contains levels higher than the ones present in milk and dairy products (USDA, 2006).

According to Table 1, *L. edodes* and *Pleurotus* spp. present riboflavin levels lower than the *A. bisporus* species.

The vitamin B_1 levels in the analysed species is not very high when compared with other foods usually considered as thiamine sources such as grains, but otherwise these levels are higher than the ones present in eggs and are similar to the ones contained in vegetables.

Some differences were found when comparing the values determined for vitamins B_1 and B_2 in the present study with those reported in the few papers available involving the analysis of vitamins in mushrooms.

The mean level of vitamin B_1 for fresh *A. bisporus* was 0.03 mg/100 g. This value is relatively lower than the ones reported on the literature which range from 0.05 to 0.19 mg/100 g (Bautista-Justo, Alanis-Guzman, Gonzalez-De-Mejia, & Garcia-Diaz, 1998; Esteve et al., 2001; Llanos et al., 1993; Mattila et al., 2001; Olleta, Llanos, Barcos, Ancin, & Martin, 1993). The *L. edodes* and *Pleurotus* species presented mean vitamin B_1 levels of 0.009 and 0.042 mg/100 g, respectively. These values are also lower than the ones reported in the literature by Mattila et al. (2001) of 0.05 mg/100 g for *L. edodes* and 0.07 mg/100 g for *Pleurotus*.

The mean level of vitamin B_2 for the *A. bisporus* mushroom was 0.25 mg/100 g. Olleta et al. (1993), Llanos et al. (1993), Mattila et al. (2001) and Bautista-Justo et al. (1998) reported values similar to those found in the present study: 0.29 mg/100 g and 0.3 mg/100 g. In another study, Esteve et al. (2001) found higher vitamin B_2 level: 0.62 mg/100 g. The *L. edodes* and *Pleurotus* species presented lower levels (0.057 and 0.083 mg/100 g, respectively) than the ones reported by Mattila et al. (2001): 0.15 mg/100 g for *L. edodes* and 0.20 mg/100g for *Pleurotus.*

The sample presenting the lowest vitamins content was the mushroom in conserve, which differed significantly from the other samples analysed. The mean levels for mushroom in conserve in the present study were very low when comparing to data reported by other authors (Llanos

Table 1
Vitamin B_1 and B_2 contents in mushrooms commercialised in the city of Campinas, SP

Species	Common name	B_1	B_2
		mg/100 g[a]	
A. bisporus	Portobello	0.040 ± 0.005	0.279 ± 0.090
	White button mushroom	0.031 ± 0.013	0.254 ± 0.052
		0.027 ± 0.009	0.298 ± 0.052
		0.016 ± 0.007	0.165 ± 0.032
		0.024 ± 0.013	0.237 ± 0.053
	Mushroom in conserve	0.004 ± 0.002	0.037 ± 0.010
Pleurotus spp.	Oyster salmon mushroom	0.025 ± 0.027	0.107 ± 0.050
	Oyster white mushroom	0.013 ± 0.010	0.075 ± 0.049
	Shimeji	0.050 ± 0.013	0.091 ± 0.079
		0.080 ± 0.042	0.059 ± 0.033
L. edodes	Shiitake	0.009 ± 0.003	0.057 ± 0.013
MSD		0.025	0.077

[a] Mean and estimate of the standard deviation ($n = 10$); MSD – minimum significant difference.

et al., 1993; Martin Belloso & Llanos-Barriobero, 2001; Olleta et al., 1993). The values found were 10 and 5 times lower for vitamin B_1 and vitamin B_2, respectively.

As vitamins are very unstable, a lower value in the sample in conserve was expected, since heat processing was used as well as contact with chemical and whitening agents such as sulphites and sulphur dioxide, which could contribute to the losses.

Since the samples analysed were from different brands, the soil and climatic conditions, as also the strains and families, could have contributed to these differences. It must be added that in some cases the companies only commercialised the mushrooms, buying them from various producers and only packaging and distributing them. The different substrates used for cultivation could also have altered the composition of the mushrooms (Rios-Hurtado, Torres-Torres, & Medina-Rivas, 2003; Sturion & Oetterer, 1995).

The contents of vitamins B_1 and B_2 found in the analyzed mushrooms indicate that mushrooms could not be considered as sources of vitamins B_1 and B_2, since their contribution in terms of these vitamins to the diet is not significant although they may contribute to the sums of these nutrients in the diet.

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