

Contents of folates in edible mushrooms commercialised in the city of Campinas, São Paulo, Brazil

Teor de folatos em cogumelos comestíveis comercializados na cidade de Campinas, São Paulo, Brasil

Regina Prado Zanes FURLANI^{1*}, Helena Teixeira GODOY²

Abstract

In this study, folates were evaluated in the main species of mushroom cultivated in Brazil. The species analysed were *Agaricus bisporus* (button mushroom), *Lentinula edodes* (shiitake) and *Pleurotus ostreatus* (shimeji). The five main forms of folate found in foods were determined: tetrahydrofolic acid (THFA), 10-methyl folic acid (10MFA), 5-methyl tetrahydrofolic acid (5MTHFA), 10-formyl folic acid (10FFA) and 5-formyl tetrahydrofolic acid (5FTHFA). The methodology employed used extraction with phosphate buffer, clean up with trichloroacetic acid and separation of the vitamins by high-performance liquid chromatography, with simultaneous ultraviolet and fluorescence detection. The results obtained for total folate were 551 to 1404 $\mu\text{g}\cdot 100\text{ g}^{-1}$ for the button mushroom, 606 to 727 $\mu\text{g}\cdot 100\text{ g}^{-1}$ for shiitake and 460 to 1325 $\mu\text{g}\cdot 100\text{ g}^{-1}$ for shimeji. The data showed that mushrooms could be considered as sources of folates and that their contribution of these vitamins to the diet was meaningful.

Keywords: mushroom; folates; edible fungi; vitamins.

Resumo

O teor de folatos nas principais espécies de cogumelos cultivados no Brasil foi avaliado neste trabalho. As espécies analisadas foram *Agaricus bisporus* (champignon de Paris), *Lentinula edodes* (shiitake) e *Pleurotus ostreatus* (shimeji). Foram determinadas as cinco principais formas de folatos presentes em alimentos: tetrahidro ácido fólico (THAF), 10-metil ácido fólico (10MAF), 5-metil tetrahidro ácido fólico (5MTHAF), 10-formil ácido fólico (10FAF) e 5-formil tetrahidro ácido fólico (5FTHAF). A metodologia empregada utilizou extração com tampão fosfato, limpeza com ácido tricloroacético e separação das vitaminas por cromatografia líquida de alta eficiência, com detecção em série por fluorescência e ultravioleta. Os resultados obtidos para o total de folatos foram 551 a 1404 $\mu\text{g}\cdot 100\text{ g}^{-1}$ para o champignon de Paris, 606 a 727 $\mu\text{g}\cdot 100\text{ g}^{-1}$ para o shiitake e 460 a 1325 $\mu\text{g}\cdot 100\text{ g}^{-1}$ para o shimeji. Os dados mostram que os cogumelos podem ser considerados fontes de folatos, e sua contribuição na dieta, para o aporte dessa vitamina, é significativa.

Palavras-chave: cogumelo; folatos; fungos comestíveis; vitaminas.

1 Introduction

Mushrooms have been used as a food for many centuries. More than 2,000 species of edible mushroom are known, but only 25 are cultivated commercially. In Brazil, the main cultivated edible species are *A. bisporus*, *L. edodes*, *P. ostreatus* and the medicinal species *A. blazei* (sun mushroom).

These edible species have been part of the oriental diet for hundreds of years and recently their consumption has increased in occidental cultures, involving a great number of species in addition to the popular button mushroom (*A. bisporus*)⁷. This well-known mushroom was the first species cultivated in Brazil and is the most cultivated species in the World. In Brazil, mostly in the region of the city of Mogi das Cruzes in the State of São Paulo, its cultivation is still carried out in a rudimentary way, generally by Chinese families who inherited the techniques from their ancestors and have no deep scientific knowledge³.

Mushrooms have been appreciated since ancient times due to a belief in their high nutritive value and medicinal potential, in addition to being classified as a noble spice in culinary dishes.

In Brazil, an increase in the consumption of the product and consequently of its production and commercialisation has been noted recently due to a greater divulgation of its nutritional and medicinal value and because its price has become a bit more accessible to the population. Nutritional information on foods has become increasingly important recently, both for professionals in the food and health sectors and for the actual consumers, who are increasingly concerned with the nutritional quality of the foods which make up their diet or could be introduced into it. However, little is known about the nutritional quality and no data can be found in the indexed literature about the folate content of edible mushrooms cultivated in Brazil. The vitamin contents of foods are of great value, since vitamins perform important functions in the human and animal organisms.

Folic acid is a water-soluble vitamin, also known as pteroylglutamic acid, vitamin B₉, vitamin B₁₁ and vitamin M, and it has recently come to the attention of researchers due to confirmation of its beneficial action to the human organism. The vitamin is naturally present in foods, usually in the reduced form as derivatives of polyglutamates⁵. Folate is a general term reserved for structurally similar compounds and also for those with similar activity^{1,4}. It appears that one of the effects directly connected to folate deficiency, and which has caused the greatest repercussions throughout the World, is the appearance of congenital malformations. Numerous papers have shown that

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¹ Centro de Química de Alimentos e Nutrição Aplicada, Instituto de Tecnologia de Alimentos - ITAL, Av. Brasil, 2880, CEP 13073-001, Campinas - SP, Brasil, E-mail: rfurlani@ital.sp.gov.br

² Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas - UNICAMP

*A quem a correspondência deve ser enviada

folate deficiencies can cause cardiovascular diseases, cancer and mental disorders, such as Alzheimer's disease⁶.

Thus the objective of this study was to determine five forms of folate in samples of edible mushrooms cultivated in Brazil.

2 Materials and methods

2.1 Samples

The mushroom species analysed in this study were: *A. bisporus* (button mushroom), *L. edodes* (shiitake) and *Pleurotus* spp. (shimeji). The samples were acquired from supermarkets in the city of Campinas, State of São Paulo, Brazil. Four samples from different brands were analysed for each mushroom species.

2.2 Reagents

The standards of tetrahydrofolic acid (THFA), 10-methyl folic acid (10MFA), 5-methyl tetrahydrofolic acid (5MTHFA), 10-formyl folic acid (10FFA) and 5-formyl tetrahydrofolic acid (5FTHFA) were acquired from Schircks laboratories, Switzerland. The stock standard solutions of the standards were prepared individually with a concentration of 0.03 mg.mL⁻¹. The standards were dissolved in a phosphate buffer (0.25 mol.L⁻¹ Na₂HPO₄ + 0.37 mol.L⁻¹ KH₂PO₄) together with 1% ascorbic acid. A working solution was prepared from these stock solutions, containing all the forms of folate.

The reagents used to prepare the mobile phase were of a chromatographic grade. All mobile phases were filtered through a membrane with a pore size of 0.45 µm. The water used in the preparation of the mobile phases and phosphate buffer was purified in the Milli-Q® system (MILLIPORE). All other reagents were of an analytical grade.

2.3 Equipment

The equipment used to determine the vitamins was an HP model 1100 high performance liquid chromatograph, equipped with a degasser, quaternary pump, automatic injection system (0 to 100 µL), UV-Visible with diode array and fluorescence detectors connected in series and a temperature control compartment for the analytical column. The system was controlled by the HP-Chemstation software, which also administered the data acquisition and treatment system.

2.4 Folate determination

The procedure employed used methodology developed and validated by CATHARINO².

The mushrooms were ground to complete liquefaction, homogenised in a domestic mixer and analysed immediately. For the extraction, 3 g of previously disintegrated sample were weighed and mixed with a 30 mL phosphate buffer (0.25 mol.L⁻¹ Na₂HPO₄ + 0.37 mol.L⁻¹ KH₂PO₄). This mixture was immersed in an ultrasonic bath for 15 minutes to extract the folates and

1 mL trichloroacetic acid and 10 mL acetonitrile then added to clean up the mixture and precipitate interfering substances. The volume was then completed to 50 mL with a phosphate buffer in a volumetric flask and the extract filtered first through common filter paper and then through a filter with 0.22 µm pores. The analyses were carried out in duplicate.

2.5 Chromatographic conditions

Separation of the folates was carried out by high performance liquid chromatography using a reverse phase octadecylsilyl column (Nova-pak®, Waters) with 3 µm particles, dimensions of 3.9 x 150 mm and with the temperature controlled at 25 °C. The guard column filled with the octadecylsilyl stationary phase with 5 µm particles and dimensions of 3.9 x 15 mm.

The mobile phase consisted of an aqueous acetic phase (2% acetic acid; pH adjusted to 2.8 with KOH) and acetonitrile, using gradient elution. The gradient started with 100% aqueous acetic phase, followed by a linear gradient to 24% aqueous acetic phase and 76% acetonitrile in 25 minutes. At 26 minutes the starting conditions were re-established and the run finished at 40 minutes, sufficient to re-equilibrate the system. All the forms of folate were eluted in the first 15 minutes of the run. The flow rate of the mobile phase was 0.5 mL/minute and the sample injection volume was 100 µL.

Fluorescent and ultraviolet detection were carried out simultaneously. The compounds were monitored at λ = 290 nm in the ultraviolet detector and λ_{exc.} = 290 nm and λ_{em.} = 360 and 445 nm for fluorescence. Quantification was carried out externally using the standard calibration of the various folate standards. Identification was by comparison of the absorption spectra, both in the ultraviolet region and from the fluorescence detector and also by their retention times. Standards were also added to the extracts to confirm the results.

2.6 Statistical analyses

The analysis of variance and Tukey's test were applied, using the Statistica software⁹ to identify possible significant differences (p < 0.05) between the different batches of each species for each form of folate and for the total folate. The analysis of variance and Tukey's test were also applied to evaluate possible differences in total folate contents between the species.

3 Results and discussion

Table 1 shows the results obtained for the samples analysed.

The major forms found in the samples of button mushroom were tetrahydrofolic acid and 10-methyl folic acid. For shiitake they were 10-methyl folic acid and 5-formyl tetrahydrofolic acid and for shimeji, tetrahydrofolic acid and 5-methyl tetrahydrofolic acid.

On analysing separately the species studied, significant differences (p < 0.05) were noted between the forms of folate found in the different brands. For the button mushroom, of the

Table 1. Folate contents in mushrooms commercialised in the city of Campinas.

Species	Brand	$\mu\text{g} \cdot 100 \text{ g}^{-1} \text{ }^a$					Total
		10 MFA	5 MTHFA	5 FTHFA	10 FFA	THFA	
Button mushroom	H	86 ^b	8 ^a	nd	nd	570 ^b	664 ^b
	A-1	508 ^a	nd	nd	nd	42 ^c	551 ^b
	A-2	599 ^a	2 ^b	98	17 ^a	692 ^b	1409 ^a
	B	238 ^b	2.7 ^b	nd	24 ^a	1166 ^a	1431 ^a
	Mean ^b	358 ± 225	4 ± 2	98 ± 10	20 ± 9	618 ± 432	1014 ± 449 ^a
Shiitake	B	596 ^a	5 ^a	126 ^b	nd	nd	727 ^a
	A	602 ^a	4.0 ^a	ND	nd	nd	606 ^a
	E-1	404 ^a	29 ^a	181 ^a	nd	nd	614 ^a
	E-2	579 ^a	15.5 ^a	89.9 ^c	nd	nd	684 ^a
	Mean ^b	545 ± 96	13 ± 12	132 ± 41	nd	nd	658 ± 65 ^a
Shimeji	H	nd	6.0 ^d	70 ± 2	nd	591 ^a	666 ^b
	B	nd	964 ^a	nd	nd	361 ^b	1325 ^a
	A	nd	459 ^b	nd	nd	266 ^{bc}	725 ^b
	F	59	251 ^c	nd	nd	150 ^c	459 ^c
	Mean ^b	59 ± 21	420 ± 377	70 ± 2	nd	342 ± 175	794 ± 345 ^a

nd – not detected; ^amean of duplicate; Values in the same column with the same letter showed no significant difference between the means for each species or for the final means ($p < 0.05$) according to Tukey's test. ^bMean and standard deviation estimate ($n = 4$) for the respective species. THFA: tetrahydrofolic acid; 10MFA: 10-methyl folic acid; 5MTHFA: 5-methyl tetrahydrofolic acid; 10FFA, 10-formyl folic acid and 5FTHFA: 5-formyl tetrahydrofolic acid.

four samples analysed, two being of the same brand, differences existed for the five forms of folate. The two samples from the same brand did not show significant differences ($p > 0.05$) except for 10MFA. For shitake, the results were only significantly different ($p < 0.05$) for 5FTHFA. 10FFA and THFA were not detected in this species. For shimeji, 10MFA and 5FTHFA were not detected in three of the four samples analysed and 10FFA was not found in any of the samples analysed.

These differences could have been caused by differences in the soil and climate (season and cultivation region) apart from the stage of maturation and post-harvest storage conditions. The differences could also be due to the substrate used, since according to STURION and OETTERER¹⁰ and RIOS-HURTADO, TORRES-TORRES and MEDINA-RIVAS⁸, different substrates lead to different chemical compositions, and in this study on folates, samples from different brands were analysed, which were probably grown on distinct substrates.

The total folate contents varied between 459 and 1431 $\mu\text{g} \cdot 100 \text{ g}^{-1}$ for all the species analysed. For the button mushroom, the total folates mean for all the brands analysed was 1014 $\mu\text{g} \cdot 100 \text{ g}^{-1}$ and there were no significant differences ($p > 0.05$) between brands A-2 and B and between brands H and A-1. The brands A-2 and B showed higher values than the other two brands. For shitake, the total mean for all the batches analysed was 658 $\mu\text{g} \cdot 100 \text{ g}^{-1}$ and there was no significant difference ($p > 0.05$) between brands. Finally, for shimeji, the batches analysed were significantly different ($p < 0.05$) for the brands B and F, and brands H and A did not differ from each other ($p > 0.05$), but did differ from the others. The statistical analysis of the total folate contents for all species revealed no significant differences ($p > 0.05$) between them.

4 Conclusions

The results showed that mushrooms can be considered as a source of folates, and their contribution of this vitamin to the diet is significant.

Acknowledgements

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