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Beta-glucans in edible mushrooms

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Abstract

Edible mushrooms contain interesting functional components. In particular beta glucans, homo- and hetero-glucans with β(1→3), β(1→4) and β(1→6) glucosidic linkages, are supposed to be responsible for some healthy properties of mushrooms. In this research the amount of beta glucans in different edible mushroom species has been evaluated and their distribution within the soluble and insoluble fractions of dietary fibre has also been assessed. Beta glucans have been analysed by a modification of an enzymatic method originally developed for cereals and based on lichenase and β-glucosidase hydrolyses followed by a spectrophotometric determination of the released free glucose. A large variability can be observed in the studied mushroom species; the beta glucan concentration ranges from 0.21 to 0.53 g/100 g on a dry basis. Furthermore, the beta glucan in the dietary fibre fractions varies according to species of mushrooms.

1. Introduction

For centuries edible mushrooms have been used to maintain health and increase longevity in ancient civilisations or have been consumed as hallucinogens by American tribes. Nowadays, mushrooms are undoubtedly consumed much more for their texture and flavour than for their nutritional and medicinal properties. Mushrooms are considered to be healthy because they are poor in calories and in fat but rich in proteins, minerals and dietary fibre (Manzi, Aguzzi, Vivanti, Paci & Pizzoferrato, 1999).

Dietary fibre, a mixture of polysaccharides, lignin and other plant cell wall constituents resistant to hydrolysis by human enzymes, has some interesting properties. It speeds up the transit of bowel contents, increases faecal bulk and frequency and protects the body from colon cancer, diverticular diseases and irritable bowel syndrome. Finally, it lowers the level of cholesterol in blood, thus protecting against coronary disease (Southgate, Waldron, Johnson & Fenwich, 1990). These biochemical properties are mainly due to the soluble fraction of dietary fibre, characterised by gel-forming properties, while the insoluble fraction is more efficient as a "mechanical" agent.

Edible mushrooms are a potential source of dietary fibre: fungal cell walls contain chitin, other hemicelluloses, mannans and, among the most interesting functional components, beta glucans. These compounds, homo- and hetero-glucans with β(1→3), β(1→4) and β(1→6) glucosidic linkages, are supposed to play a key role in some healthy properties of mushrooms, such as enhancement of macrophage function and host resistance to many bacterial, viral, fungal and parasitic infections, activation of a non-specific immune stimulation, reduction of blood cholesterol and blood glucose levels (Cheung, 1998; Rajarathnam, Shashirekha & Bano, 1998).

The aim of this research is the evaluation of the amount of beta glucans in different edible mushrooms species and the identification of their presence, as soluble components, in the dietary fibre fraction.

2. Materials and methods

2.1. Samples

Pleurotus ostreatus (SMR 125, SMR 127, SMR 138), *Pleurotus pulmunarius* (SMR 126), *Pleurotus eryngii* (SMR 172, SMR 173, SMR 133) and *Lentinula edodes* (SMR 90), belonging to the mushroom collection of the National Research Council (Montelibretti, Roma-I),

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were cultivated in an Italian farm (Castelluccio, Senise Potenza-I) and analysed immediately after harvesting.

2.2. Chemicals

Lichenase [EC 3.2.1.73] 1000 U/ml, β-glucosidase [EC 3.2.1.21] 40 U/ml, and glucose standards were obtained from Megazyme Int. (Ireland Ltd). The enzyme kit, containing α-amylase, amyloglucosidase and protease according to the official method for dietary fibre (Association of Official Analytical Chemists, 1990), was from Sigma (Milano, I).

2.3. Methods

Beta-glucan content was determined according to a modification of the method of McCleary and Holmes (1985). Mushroom samples are submitted with or without a pre-treatment with aqueous ethanol (50% v/v) solution, to a lichenase hydrolysis and further degraded by β-glucosidase. The released free glucose is measured spectrophotometrically at 510 nm against a blank necessary to subtract the free glucose eventually present in the sample. Lichenase acts at 40°C on mixed-links β(1→3) (1→4) and the hydrolysis of the other links β(1→4), β(1→3), β(1→6) is catalysed by β-glucosidase. The addition of β-glucosidase is carried out after separating the solid residue of lichenase action by filtration in order to avoid any interference due to other beta-linked saccharides (e.g. cellulose).

Dietary fibre fractions were prepared following the analytical procedure of Prosky, Asp, Schweizer, De Vreis and Furda (1988). Briefly, samples are gelatinised at 100°C with termamyl (heat stable α-amylase) to hydrolyse digestible starch and then enzymatically digested with protease and amyloglucosidase to remove any digestible proteins. The mixture is filtered and the residue washed with water, collecting the filtrate and the washing solution. The residue, washed with ethanol and acetone, contains insoluble salts, undigested proteins and the insoluble fraction of dietary fibre.

Ethanol is added to the collected filtrates to precipitate the soluble fraction of dietary fibre. The precipitate, filtered and washed with ethanol and acetone, contains minerals, undigested proteins and the soluble fraction of dietary fibre.

3. Results and discussion

The analytical method for beta glucans available in the literature was developed for cereal analysis (McCleary & Holmes, 1985) and is absolutely specific for mixed-linkage beta glucans. In fact, cereals contain essentially beta glucans with β(1→3) (1→4) mixed links, while mushrooms present larger amounts of β(1→4) (1→6) than β(1→3) (1→4) mixed links and different single linkages such as β(1→3), β(1→4) and β(1→6) (Mullins, 1990). To adapt the method for the measurement of beta glucans in mushrooms, some preliminary tests have been performed, changing (separately) the enzyme concentration and the reaction time.

An optimisation of the beta glucosidase amount was achieved by increasing the enzyme concentration from 0.2 to 1 U. The relevant results of the beta glucan determination follow an increasing trend with enzyme concentration and differences are statistically significant comparing 0.8–1 U with 0.2–0.4 U enzyme concentrations ($p < 0.01$). On the other hand, within the range 0.8–1 U and 0.2–0.4 U, the differences are not significant ($p > 0.05$). The results obtained (Table 1) show that the amount of β-glucosidase necessary to complete the beta glucan hydrolysis in mushrooms is higher than that suggested for cereals (0.8 vs 0.2 U). Mushroom glucans characterised by different β links probably require a more incisive action of this enzyme, which is effective in catalysing the hydrolysis of beta links without a particularly high specificity.

After selecting the β glucosidase concentration (0.8 U), a further test was performed to optimise the reaction time. As shown in Table 1, the incubation time was prolonged from 15 to 60 min to give increasing results. However, 30 min seemed to be the more suitable time to significantly improve the beta glucan recovery ($p < 0.01$).

A test has also been performed to verify the effectiveness of the lichenase action and an opportunity to increase the enzyme concentration. Table 1 shows the beta glucan levels measured after a 60 min hydrolysis catalysed by an amount of lichenase ranging from 0 to 20 U. Levels increase significantly with enzyme concentration in the range 0–10 U; a further increase from 10 to 20 U is ineffective. These results confirm that the

Table 1
Comparison of beta glucan contents in *Pleurotus ostreatus* (SMR 138), using different test conditions

Lichenase (U)	Reaction time (min)	Beta glucosidase (U)	Reaction time (min)	Beta glucans (g/100 g dry basis)[a,b]
10	60	0.2	15	0.14 ± 0.01a
10	60	0.4	15	0.15 ± 0.01a
10	60	0.8	15	0.20 ± 0.02b
10	60	1.0	15	0.19 ± 0.01b
10	60	0.8	15	0.21 ± 0.02c
10	60	0.8	30	0.29 ± 0.01d
10	60	0.8	60	0.30 ± 0.03d
0	60	0.8	30	0.18 ± 0.01e
5	60	0.8	30	0.23 ± 0.01f
10	60	0.8	30	0.29 ± 0.01g
20	60	0.8	30	0.27 ± 0.04g

[a] Means with different letters are statistically different ($p < 0.01$).
[b] Means with the same letters are not significantly different ($p < 0.05$).

lichenase enzyme, highly specific towards the β(1→3)(1→4) mixed link, is not greatly involved in mushroom beta glucan hydrolysis where the key step is probably the hydrolysis catalysed by beta glucosidase. Nevertheless, if the test is performed without the pre-treatment with lichenase (0 U), the beta glucan level is underestimated and, in this case, the recovery is nearly 60% (0,18 vs 0.29 g/100 g). With other mushroom species, a different recovery might be expected, depending on the presence, genetically regulated, of glycosidic mixed-links.

Utilising the conditions selected in the described analytical trials (lichenase 10 U, beta glucosidase 0.8 U for 30 min), two different procedures, as indicated by the McCleary and Holmes (1985) method, have been compared. In the first procedure (A), mushroom samples were directly incubated with lichenase and, after a β-glucosidase degradation, analysed for glucose. In the second procedure (B), a pre-treatment with an aqueous-ethanolic solution (50% v/v, incubated in a boiling water bath for 5 min) aims to clean up the sample from sugars and highly soluble carbohydrates, allowing application without any possible interference. The relevant data are reported in Table 2. The concentrations of beta glucans, determined according to methods (A) and (B) are different; the former is higher than the latter. Actually, if the supernatant separated after the ethanolic extraction is submitted to the analytical procedure, the missing amount of beta glucans can be determined, thus giving comparable data from methods (A) and (B). These results strongly suggest that beta glucans present in mushrooms show different water affinities. This particular behaviour is probably due to different molecular structures or, more simply, to the simultaneous presence of beta glucan molecules of different polymerisation degrees and, consequently, different molecular weights.

On this basis, a contemporary presence of beta glucans in the soluble and in the insoluble fractions of mushroom dietary fibre can be hypothesised. To verify this hypothesis, the soluble and insoluble fractions of dietary fibre have been isolated from mushrooms, utilising the Prosky et al. (1988) analytical procedure as a preparative method. The two isolated residues have been analysed for beta glucans without any washing procedures and the recovery percentages of beta glucans have been calculated in the soluble and insoluble dietary fibre fractions.

As reported in Table 3, the distribution of beta glucan in the dietary fibre fractions is variable in the different species or interspecies of mushrooms. In particular, *Lentinula edodes* contains high levels of beta glucans in the soluble fraction and, assuming that "the more soluble, the more effective", interesting physiological activities of this mushroom could be hypothesised. Actually, *Letinula edodes* is reported to inhibit the growth of mouse sarcoma 180 in mice, probably due to the presence of an unspecified water-soluble polysaccharide (Rai, 1995).

Further work is now in progress to understand the reason for the poor recovery of total beta glucan in dietary fibre. The low amount of sample, and the presence of inert material in the fibre residue, might hinder the diffusion of the enzymes during the beta glucans determination; it is also possible that a natural fungal beta glucanase can be activated during the dietary fibre determination. Moreover, the presence of beta glucans of very low molecular weight, escaping the ethanol precipitation of the soluble dietary fibre fraction, is also possible. Accordingly, some beta glucan present in the mushroom could be lost during the assay and this could contribute to the discrepancy between the important physiological activities and the moderately high levels of soluble dietary fibre in mushrooms (Manzi, Aguzzi et al., 1999a; Manzi, Gambelli, Marconi, Vivanti & Pizzoferrato, 1999b).

Finally, beta glucan contents of 8 edible mushrooms are reported in Table 4. A large variability can be observed in the mushroom species; the beta glucan concentration ranges from 0.22 to 0.53 g/100 g on a dry basis. According to these preliminary data, *Pleurotus pulmunarius* seems to be the richest source of fungal beta glucans, but more analyses are necessary to confirm these results.

In conclusion, this research shows that beta glucans in mushrooms are distributed both in the soluble and in the insoluble dietary fraction and, probably, the physiological effects of mushrooms are due to a cumulative action of different fibre components; soluble beta glucans play a key role, but also chitin/chitosan presents interesting functions. Our research is now oriented in this direction.

Table 2
Beta glucans (g/100 g on a dry basis) in mushrooms. A comparison between two different procedure: (A) without any pretreatments and (B) with a previous aqueous-ethanolic washing (50% v/v)

Mushroom samples		(A)	(B)		
			Residue	Solution	Residue + solution
Pleurotus ostreatus	(SMR 138)	0.29	0.15	0.11	0.26
Lentinula edodes	(SMR 90)	0.22	0.18	0.06	0.24

Table 3
Distribution (%) of beta glucan in the soluble and insoluble fraction of dietary fibre

Mushroom samples		Soluble	Insoluble
Pleurotus ostreatus	(SMR 127)	37.8	62.2
Pleurotus ostreatus	(SMR 138)	27.1	72.9
Pleurotus eryngii	(SMR 172)	16.8	83.2
Lentinula edodes	(SMR 90)	46.0	53.9

Table 4
Beta glucan contents in edible mushroom. Data are as g/100 g on a dry basis

Mushroom samples		Beta glucans means ± SD
Pleurotus ostreatus	(SMR 125)	0.38 ± 0.02
Pleurotus ostreatus	(SMR 127)	0.24 ± 0.03
Pleurotus ostreatus	(SMR 138)	0.29 ± 0.01
Pleurotus eryngii	(SMR 133)	0.38 ± 0.01
Pleurotus eryngii	(SMR 173)	0.29 ± 0.03
Pleurotus enyngii	(SMR 172)	0.22 ± 0.01
Pleurotus pulmunarius	(SMR 126)	0.53 ± 0.04
Lentinula edodes	(SMR 90)	0.22 ± 0.05

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