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THE COMPOSITION OF FRESH AND STORED OYSTER MUSHROOMS (*PLEUROTUS OSTREATUS*)

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Key Word Index—*Pleurotus ostreatus*; oyster mushroom; composition; storage; respiration; carbohydrate; polysaccharide; cell wall; protein.

Abstract—Soluble carbohydrate, protein, polysaccharide and cell wall composition were assayed in freshly harvested *Pleurotus ostreatus* sporophores and those stored for 4 days at 2° or 18°. Mannitol and trehalose were present at 1.8 and 6.5% dry wt respectively in fresh sporophores, and at reduced levels in those stored at 18°. In sporophores stored at 2°, trehalose levels increased by up to 122%. Soluble polysaccharide appeared to be composed of glycogen-like material, which was susceptible to post-harvest breakdown, and components containing mannose and other sugars. The total protein content was 42% dry wt; no protein degradation was seen in sporophores stored at 2°, but about 25% of the protein disappeared during storage at 18°. Cell wall polysaccharide was utilised during storage. Respiration rate was about 8–10 ml CO_2/g dry wt/hr at harvest and declined to about 5 ml/g dry wt/hr after 40 hr storage at 18°.

INTRODUCTION

In recent years the development of commercial culture methods for 'mushrooms' of *Pleurotus* spp. [1] has led to an increasing interest in their proximate composition. The protein content of the sporophore is high [2, 3] and is of good nutritional value [4, 5], making the mushroom a potentially useful addition to the human diet. However little appears to be known of the rest of the sporophore constituents. Harvested mushrooms are stored prior to consumption and, therefore, data on the changes which occur in composition during such storage are desirable in order to determine the optimum conditions for preservation of nutritional value.

Pleurotus undergoes rapid post-harvest deterioration at room temperature, but the storage period is extended at 2° [6]. This paper reports on the composition of the mushroom and its changes during short-term storage at 2° and 18°.

RESULTS

Respiration

Production of CO_2 from freshly harvested sporophores was 8–10 ml/g dry wt/hr. After 5 hr storage at 18°, this dropped by about 30%; then a slow decrease was seen to about 50% of the original value at 40 hr.

Composition of sporophore

Changes in fr. and dry wts of the sporophore after harvest are given in Table 1. Two soluble carbohydrates were detected by GLC in 80% ethanol extracts of the sporophores; mannitol and trehalose. The mannitol level in fresh sporophores varied between 1.4 and 2.6% dry wt, this level was reduced during storage at both 2 and 18° (Table 1). Trehalose content was between 4 and 9.6% dry wt of fresh sporophores. During storage at 2° the trehalose level increased by between 40 and 122% in 4 days, but after storage at 18° the content was reduced to values from 1.2 to 5.7% sporophore dry wt (Table 1).

Digestion of the freeze-dried tissue with amyloglucosidase released a considerable quantity of reducing sugars. This was about 11% sporophore dry wt in freshly harvested sporophores and 5.5 and 4.9% sporophore dry wt in those stored at 2° and 18° respectively (Table 1). Non-structural polysaccharides were extracted in hot water and partially purified by ethanol precipitation. About 50% of the isolated material was carbohydrate as determined by the anthrone method. Digestion of the isolated material with amyloglucosidase released reducing power equivalent to 56.5 and 35.6% of the total carbohydrate (determined by anthrone) in the material from fresh and 18° stored sporophores respectively (Table 2). When the polysaccharide was incubated with *Cytophaga* lytic enzyme a small amount of reducing power was released (Table 2). After partial hydrolysis of the polysaccharide with HCl, the major monosaccharide was glucose in material from both fresh (86%) and 18° stored (66%) sporophores. There was also a significant amount of mannose in the hydrolysate and the proportion of this and other minor monosaccharides increased during storage (11% in fresh to 22% in 18° stored for mannose); sorbose, ribose and xylose increased from 1% each to 8, 6 and 4% respectively after 4 days at 18°. The major disaccharide detected was maltose (α and β). There were also small quantities of isomaltose and unidentified disaccharides. The absorbance spectrum of the I_2 complex of an aqueous solution of the polysaccharide was determined. A broad peak with λ_{max} at 400 nm was observed with an E_{max}, for 0.02% polysaccharide solution, of 0.07. No significant difference was seen between the material isolated from fresh or stored sporophores. In the presence of half saturated $(NH_4)_2SO_4$ the λ_{max} was 408 nm and the E_{max} 0.27 and 0.19 for polysaccharide from fresh and stored sporophores respectively.

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Table 1. Changes in *Pleurotus ostreatus* sporophores during storage at 2° and 18°

Parameter	Harvest	Stored (4 days) 2°	18°	
Fr. wt (g)*	12.0	10.7	5.6	
Dry wt (g)*	1.1	1.1	0.8	
	% dry wt†	% dry wt†	% dry wt†	% of dry wt at harvest
Mannitol	1.8 ± 0.2	0.9 ± 0.1	0.7 ± 0.1	0.5
Trehalose	6.5 ± 0.9	10.8 ± 1.3	3.7 ± 0.7	2.8
Amyloglucosidase digestible polysaccharide	11.0 ± 2.0	5.5 ± 0.4	4.9 ± 0.9	3.7
Cell wall	32.3 ± 2.2	33.7 ± 1.4	35.9 ± 2.1	27.3
Carbohydrate	17.2	16.5	15.0	11.4
Protein	5.4	6.0	8.7	6.6
Chitin	4.9	5.2	7.2	5.5
Total protein	42.1 ± 1.1	43.9 ± 1.3	40.4 ± 1.6	30.7

* Per sporophore

† Values are means of 8 samples in 4 experiments ± standard error. Results are given as % of sporophore dry wt on day measured, and for those stored at 18° % of original dry wt at harvest.

The cell wall content of the sporophores remained relatively constant during storage (Table 1). The three cell wall constituents measured were chitin, anthrone-positive carbohydrate and protein (Table 1). There was little change in the composition of the cell wall during storage at 2°, although a small decrease was seen in the anthrone-positive carbohydrate in all experiments. After storage at 18° the carbohydrate content of the cell wall was reduced by 22%, with a concomitant increase in chitin and protein levels (Table 1). Digestion of isolated cell wall material with amyloglucosidase led to a release of reducing sugar equivalent to approximately 4% sporophore dry wt (22% of cell wall anthrone-positive material) in fresh sporophores and 2% sporophore dry wt (13% cell wall anthrone-positive material) in those stored at 18° (Table 2). The cell wall carbohydrate was not significantly digested by lytic enzyme (Table 2). After partial hydrolysis of the cell wall with HCl the major monosaccharide detected was glucose with only traces of other sugars. Small quantities of maltose were tentatively identified.

The total protein content of the sporophore was approximately 40% dry wt. There was little change in protein content as a percentage of dry wt during storage, but when protein levels were calculated to g dry wt of sporophore at harvest a real loss of 25% of the protein during 4 days storage at 18° was apparent (Table 1). The amino acid content of the protein was similar to that reported previously for *Pleurotus* spp. [5, 7, 8]. There were some changes in the composition of the protein during storage at 18°, the largest of these being an increase in the glutamic acid content. In the fresh sporophore the glutamate content was approximately 7% of the protein

Table 2. Extent of digestion of cell wall and soluble polysaccharide by amyloglucosidase and *Cytophaga* enzyme

	% anthrone positive material digested	% sporophore dry wt digested
Amyloglucosidase		
Cell wall (harvest)	22	4
Cell wall; day 4, 18°	13	2
Water-soluble polysaccharide (harvest)	57	*
Water-soluble polysaccharide: day 4, 18°	36	*
Cytophaga lytic enzyme		
Cell wall (harvest)	1	*
Water-soluble polysaccharide (harvest)	2	*

* Not determined.

wt: after storage at 2° this increased to 9%, and at 18° to 19%. The tyrosine and lysine content of the protein increased by about 80% during storage at 18°.

DISCUSSION

Much of the interest in *Pleurotus* spp. as a food source has centred on its high protein content, and the high nutritional value of the protein [2–5, 7, 8]. The total protein values reported here are at the higher end of the range of previous determinations. These differences may be due to strain and species or to methodological differences. About 13% of the total protein was found to be in the cell wall in this investigation. The changes in protein composition, and the loss in protein during storage at 18° show that care is necessary in storing the sporophores if their nutritional value is not to be altered.

The other major components of *Pleurotus ostreatus* sporophores were carbohydrates. Mannitol and trehalose were present as in the cultivated mushroom, *Agaricus bisporus* [9], but mannitol levels were much lower and trehalose levels slightly higher. Amyloglucosidase-digestible polysaccharide was present in both the water-extractable fraction of the cell and the cell wall in the approximate ratio (calculated from cell wall digestion) of 2:1 in fresh sporophores and 1:1 in those stored at 18°. The water extractable polysaccharide appeared to be heterogeneous; its I_2 staining properties including the increase in λ_{max} and E_{max} in the presence of $(NH_4)_2SO_4$, and the identification of maltose and isomaltose in the hydrolysate were consistent with the presence of a glycogen-like component [10]. However, the incomplete digestion by amyloglucosidase and the detection of mannose and other monosaccharides in the hydrolysate indicate that other structures are present. The low activity of *Cytophaga* enzyme against the soluble polysaccharide suggests that β1,3 and α1,6 glucans are not present in significant quantities. Three fractions have been isolated from the water-soluble polysaccharide in a study of its antitumour activity; one of the fractions contained galactose, which was not detected in the present investigation, as a major component [11]. Results from the digestion and HCl hydrolysis of water-soluble polysaccharide from 18° stored sporophores suggest that there is a change in its composition during storage. The increase in mannose and other sugars relative to glucose, and the decrease in digestibility by amyloglucosidase are consistent with a decrease in the 'glycogen' content of the polysaccharide during storage. The smaller increase in E_{max} on addition of $(NH_4)_2SO_4$ to the I_2 stained polysaccharide from stored sporophores also supports this. The cell wall polysaccharide appeared to be mainly glucan, about 20% of which was susceptible to amyloglucosidase digestion but more detailed structural studies of both water-soluble and cell wall polysaccharides are necessary before their identity can be established.

The cell wall makes up about 30% of the total dry weight of the sporophore. This is nearly twice the amount of cell wall material found in *A. bisporus* sporophores [12], and may account for the 'chewy' [6] texture of *Pleurotus*. However, although the proportion of cell wall in *Pleurotus* was higher than in *A. bisporus*, the chitin content of the cell wall was appreciably lower, leading to a slightly lower content on a whole tissue basis. Both glucan and protein contents of the cell wall were higher than in *A. bisporus* [12].

Pleurotus sporophores are different from some other species of edible mushroom such as *A. bisporus*, *Volvariella volvaceae* and *Lentinus edodes* in that they do not undergo obvious development involving expansion of gill tissue and perhaps stipe extension after harvest. There was no increase in cap diameter or stipe length even during 4 days storage at 18°. Thus the large changes seen in the structural components and respiratory metabolism of *A. bisporus* after harvest [9, 12] would not be expected in *Pleurotus*. In general this seemed to be the case; respiration declined after harvest without the secondary peak seen in *A. bisporus* [9] and there was no net synthesis of cell wall material. However, the respiration rate at 18° was about double that seen in *A. bisporus* [9] which must lead to more rapid consumption of respiratory substrates. Mannitol, trehalose, and soluble polysaccharide appear to be used as the main respiratory substrates at 18°. In addition, some degradation of protein, apparently from the non-cell wall fraction, occurs. Thus most of the major cell components contribute to post-harvest respiration.

Storage of the sporophores at 2° retarded the changes in composition, presumably through a reduction in metabolic rate. There was still a considerable decrease in amyloglucosidase-digestible glucan, mostly from the soluble fraction. This was reflected by an increase in the trehalose content of the sporophore. The reason for this transfer of hexose into trehalose is obscure, but it is presumably due to a change in the balance between hexose production from polysaccharide breakdown and hexose consumption in respiration. There was no detectable rise in free hexoses during 2° storage, but trehalose production could still be used to 'mop up' excess hexoses.

The results of this investigation are in agreement with those of Gormley and O'Riordain [6] who concluded from physical parameters that 1–2 days was the maximum storage period at 17°; changes in the composition of the sporophores over the 4 day storage period used here were too large to be acceptable. The high respiration rate must contribute to this and suppression of respiration, as in *A. bisporus* [12], would be expected to improve the post-harvest life. At 2° the changes were retarded, with the exception of the transfer of glucose from polysaccharide to trehalose.

EXPERIMENTAL

Pleurotus ostreatus (Hauser International, strain P11) was grown under commercial conditions on a straw based substrate. The stalks were trimmed from the sporophores before storage in styrene punnets at 2° (95% RH) or 18° (65% RH). At higher temperature the punnets were loosely covered with a perforated plastic film to prevent excessive water loss. At the end of the experimental period the sporophores (5–6 per sample) were sliced into liquid N_2 and freeze-dried. The freeze-dried tissue was ground to fine powder before analysis.

Carbohydrate analysis. Freeze-dried tissue (20 mg) was mixed with 1 ml dry pyridine containing 1 mg xylose internal standard. TMS; ethers were formed and analysed by GLC as described in ref. [9]. Amyloglucosidase digestible polysaccharide was assayed as described in ref. [12] except that the reducing sugar content of the digest was measured using the Nelson–Somogyi procedure [13]. Glucose standards were used. Cell wall carbohydrate and carbohydrate content of the crude soluble polysaccharide preparation were assayed by the anthrone method [13], using glucose standards.

Soluble polysaccharide isolation. After extraction of 2 g freeze-dried tissue in 80% EtOH at room temp. for 30 min, the suspension was filtered. The residue was suspended in water, frozen and thawed × 3 then sonicated for 3 min. The suspension was heated in a boiling water bath for 30 min and centrifuged at 6000 *g* for 5 min. The supernatant was reduced in a rotary evaporator and EtOH added to 70%. The sample was stood for 2 hr at 4° before centrifuging for 15 min at 6000 *g*. The pellet was redissolved in hot H_2O and reprecipitated by addition of EtOH to 70%. After centrifugation the pellet was dried in an oven at 60°. Partial hydrolysis of the polysaccharide and cell wall was carried out using 11 M HCl for 1 hr at room temp. The hydrolysate was reduced to dryness in a rotary evaporator, the TMSi ethers formed and analysed by GLC [9]. Digestion of the isolated polysaccharide and cell wall with amyloglucosidase was carried out as previously [12]. *Cytophaga* enzyme (lytic enzyme L1, BDH) (0.03 g) was dissolved in 25 ml 20 mM Tris–maleate buffer (pH 7.0) and incubated with 2 mg polysaccharide or 5 mg cell wall overnight at 35°. Reducing sugars released were assayed as above. Enzyme and polysaccharide blanks were used.

Crude cell wall isolation. This was carried out by aqueous extractions of the freeze-dried powder as in ref. [12]. The freeze-dried cell wall preparations were weighed before assaying for chitin, protein and carbohydrate. Chitin was assayed by hydrolysis in HCl [12] and determination of glucosamine in the hydrolysate [14]. Glucosamine and chitin standards were used.

Protein and amino acid determinations. Total and cell wall protein contents were assayed by the Biuret method [13] using freeze-dried material. Bovine serum albumin standards were used. Protein amino acids were assayed as follows: 10 mg freeze-dried powder was added to 10 ml 6 M HCl in a test-tube, and the tube purged with N_2 and sealed. The samples were heated overnight at 110°. The hydrolysate was evapd to dryness, redissolved in H_2O and shaken with Zerolit 225 beads (H^+) (14–52 mesh). The solution was discarded and the beads shaken with NH_4OH (2 M in 75% EtOH). The beads were filtered off and the filtrate evapd to dryness in a stream of N_2 at 50°. TMSi ethers of the amino acids were formed and assayed by GLC as in ref. [15]. Acetonitrile (0.25 ml containing 0.05 mg phenanthrene internal standard) and BSTFA (0.25 ml) were added to the dry hydrolysate, the tubes were sealed and heated at 150° for 2.5 hr. After cooling the sample was analysed by GLC using a 1.5 m column packed with celite coated with 10% OV11. The free amino acids in the sample were measured separately and were not sufficiently high to interfere significantly with the protein amino acid determinations.

Respiration measurement. Three sporophores were placed in a sealed glass flask at 18° through which humidified, CO_2-free air was flowing at a metered rate (9.0 l/hr). The CO_2 level in the effluent was measured using a Grubb–Parsons SB2 infrared gas analyser after removal of the water vapour in a cold finger. The CO_2 level was monitored continuously by chart recorder.

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