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Chitin and Chitosan—Value-Added Products from Mushroom Waste

TAO WU,[†] SVETLANA ZIVANOVIC,*[,†] F. ANN DRAUGHON,[‡] AND CARL E. SAMS[§]

Food Biopolymers Laboratories, Department of Food Science and Technology, The University of Tennessee, 2509 River Drive, Knoxville, Tennessee 37996, Food Safety Center of Excellence, The University of Tennessee, 2605 River Drive, Knoxville, Tennessee 37996, and Department of Plant Sciences, The University of Tennessee, 2431 Joe Johnson Drive, Knoxville, Tennessee 37996

Accumulation of chitinous material in *Agaricus bisporus* stalks was determined during postharvest storage at 4 and 25 °C. The chitinous material was extracted after alkali treatment and acid reflux of alkali insoluble material and analyzed for yield, purity, degree of acetylation (DA), and crystallinity. The total glucosamine content in mushroom stalks increased from 7.14% dry weight (DW) at harvest (day 0) to 11.00% DW and 19.02% DW after 15 days of storage at 4 °C and 5 days of storage at 25 °C, respectively. The yield of crude chitin isolated from stalks stored at 25 °C for 5 days was 27.00% DW and consisted of 46.08% glucosamine and 20.94% neutral polysaccharides. The DA of fungal chitin was from 75.8 to 87.6%, which is similar to commercially available crustacean chitin. The yield of crude fungal chitin of 0.65−1.15% on a fresh basis indicates the potential for the utilization of these mushroom byproducts.

KEYWORDS: ***Agaricus bisporus*; chitin; chitosan; glucosamine; waste utilization; biobased products; value-added products**

INTRODUCTION

White common mushroom, *Agaricus bisporus*, is the most consumed mushroom in the United States. Production of *Agaricus* mushrooms has been relatively constant, and sales totaled 382 million kilograms in the 2002/03 season (*1*). Waste accumulated during mushroom production and harvest consists mainly of stalks and mushrooms of irregular dimensions and shape. Depending on the size of the mushroom farm, the amount of waste ranges between 5 and 20% of production volume. This results in approximately 50000 metric tons of waste material per year with no suitable commercial use. Waste disposal creates environmental problems for producers due to the large volume and volatile degradation products. However, *A. bisporus* is rich in chitinous biopolymers that can be utilized as a biopesticide (*2*−*4*), antimicrobial agent (*5*, *6*), coating material (*7*), or for water purification (*8*).

Fungal chitinous biopolymers, or glycosaminoglycans, consist mainly of chitin and chitosan. Besides being found in fungal cell walls, chitin is the major structural biopolymer in crustacean shells, squid skeleton, and the cuticle of insects. Currently, commercial chitin and its deacetylated derivate, chitosan, are produced from shrimp and crab shells as byproducts of the seafood industry. However, the conventional industrial isolation of chitin from crustacean shells requires harsh solvents and high temperature treatments and has seasonal supplies as well as geographical limitations. Fungal biotechnology offers advantages in the production of fungal chitin and chitosan over classical processing procedures because chitinous material can be produced in a controlled environment all year round, and the extraction process is simpler and requires less harsh solvents.

Chitosan, 2-amino-2-deoxy-β(1→4)-D-glucan, is one of the structural biopolymers in fungal cell walls. However, its content depends primarily on the taxonomy of the fungi. Thus, the chemotype of the cell wall in Zygomycetes consists of a chitosan−glucan complex, while in Homobasidiomycetes, Euascomycetes, and Deuteriomycetes, it contains chitin−glucan (*9*). Chitosan is commercially produced by deacetylation of chitin from crustacean shells, but the technology requires strong alkali, high temperatures, and a long processing time (*10*). Since the late 1970s, when White et al. (*11*) proposed a laboratory-scale method for the isolation of chitosan from mycelia of *Mucor rouxii*, different protocols have been developed to utilize fungal biomass rather than crustacean shells for the production of chitosan (*12*−*16*). Mycelia of various fungi including *Absidia coerulea*, *Absidia glauca*, *Aspergillus niger*, *Colletotricum lindemuthianum*, *Gangronella butleri*, *M. rouxii*, *Phycomyces blakesleeanus*, *Pleurotus sajo-caju*, *Rhyzopus oryzae*, *Lentinus edodes*, and *Trichoderma reesei* have been suggested as alternative chitosan sources to crustaceans (*14*, *15*, *17*−*20*). However, because of variations in extraction procedures reported in the literature, it is impossible to reliably compare different fungal species as potential chitin/chitosan sources. Nevertheless, regardless of the fungal species or the method used, extraction

* To whom correspondence should be addressed. Tel: 865-974-0844. Fax: 865-974-7332. E-mail: lanaz@utk.edu.
[†] Department of Food Science and Technology.
[‡] Food Safety Center of Excellence.
[§] Department of Plant Sciences.

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of fungal chitinous material always starts with a dilute alkali treatment to remove proteins, glycoproteins, and branched polysaccharides. In a subsequent step, chitosan is isolated from alkali insoluble material (AIM) by acid extraction, and chitin and β-glucan remain as alkali/acid insoluble residues.

Fungal chitin and chitosan potentially differ from those isolated from crustaceans in molecular weight, degree of acetylation (DA), and distribution of charged groups (*21*−*23*). These differences can alter their functional properties and bioactivity. However, research is needed to evaluate the most economical way of obtaining chitinous material from fungal sources. The objectives of this study were to determine the content and properties of chitinous material in *A. bisporus* stalks and to design a process for extraction of these bioactive polymers from mushroom waste.

MATERIALS AND METHODS

Mushroom Samples. White button mushrooms, *A. bisporus*, were donated from Monterey Mushrooms, Inc. (Loudon, TN). Whole fruit bodies were harvested in the "closed cap" stage with a cap diameter of 30 ± 5 mm and transported to the laboratory within 1 h after harvest. Immediately upon arrival at the laboratory, stalks were separated from the caps, packed in paper bags (100 g of stalks per bag, one bag representing one replication), and stored at 4 and 25 °C. Triplicate samples were taken for analysis after 0, 1, 2, 3, 4, and 5 days of storage at 25 °C and after 0, 3, 6, 9, 12, and 15 days at 4 °C. At each sampling time, fresh weight losses and dry weight (DW) content were determined and the rest of the stalks were freeze-dried, ground to a powder with a Thomas Wiley mill (Thomas Co., Philadelphia, PA), sieved through a #40 mesh, and stored in a desiccator at room temperature for further analyses and extraction. Commercial chitins and chitosans obtained from crustacean shells were provided by Primex, Co. (Iceland) and purchased from ICN Biomedicals, Inc. (Aurora, OH), Fluka (Lausanne, Switzerland), and Aldrich (Milwaukee, WI).

Glucosamine Determination. The procedure for glucosamine determination was based on the 3-methyl-2-benzothiazolone hydrazone hydrochloride (MBTH) colorimetric assay of Tsuji et al. (*24*) with minor modifications. Chitin and chitosan standards and samples at various stages of extraction were hydrolyzed with 6 M HCl at 110 °C. Hydrolysis by strong acid causes depolymerization of the polysaccharides and deacetylation of acetylglucosamine units, resulting in free glucosamine residues (*25*). However, the time necessary for complete hydrolysis of each type of the chitinous material depends on the level of crystallinity between the macromolecules and was determined in preliminary experiments. Hydrolyzate was neutralized with sodium acetate (*26*), and glucosamine was deaminated with nitrous acid to yield the anhydromannose. After the reaction, the excess nitrous acid was neutralized with ammonium sulfamate, and MBTH and $FeCl_2$ were added to produce a blue color complex with anhydromannose. The absorbance was measured at 650 nm. A calibration curve was prepared from glucosamine chloride standards (10−150 μg/mL).

Extraction of Chitinous Material. Chitinous material was obtained after treatments with alkali and acid by a procedure adapted from Rane and Hoover (*16*) and Synowiecki and Al-Khateeb (*27*) (**Figure 1**). The freeze-dried stalk powder was stirred with 1 M NaOH (w:v/1:40) and refluxed at 95 °C for 30 min to extract proteins, alkali soluble polysaccharides, and small molecules (e.g., monosaccharides, phenolics, amino acids, and salts). The slurry was centrifuged (12000*g*, 20 min, 22 °C), and AIM was washed two times with deionized water and 95% ethanol. After the final centrifugation, the alkali insoluble residue was freeze-dried and ground to a fine powder. To obtain insoluble crude fungal chitin, AIM was refluxed in 2% acetic acid (w:v/1:100) for 6 h at 95 °C. The acid-treated slurry was centrifuged (12000*g*, 20 min, 22 °C) and washed with d.i. water and ethanol as described for AIM. Chitin, if present, remained as an insoluble residue (crude chitin), and chitosan, if present, was extracted with aqueous acetic acid and precipitated from the supernatant after adjustment of the pH to 10.

Characterization of Extracted Chitinous Material. Glucosamine and glucan contents in AIM, insoluble residue, and supernatants were determined by the MBTH (*24*) and the anthrone method (*28*), respectively. Crystallinity of chitinous material was estimated by a Nexus-670 FT-IR spectrometer using attenuated total reflection sampling accessory with germanium crystal (ThermoNicolet, Mountain View, CA). The FT-IR analysis was performed between 700 and 4000 cm^{-1} with 64 scans and resolution of 4 cm^{-1}. Calculations were performed using OMNIC 6.1 software (ThermoNicolet Co.) based on the ratio of peak area at 1379 and 2900 cm^{-1} in spectra obtained in the absorption mode (*29*). The DA was determined after hydrolysis of chitin/chitosan samples following the method of Niola et al. (*30*). Hydrolysis was performed in 12 M H_2SO_4, with an oxalic and propionic acid standard mixture, at 155 °C for 1 h by using vacuum hydrolysis tubes and heating blocks (Pierce Biotechnology, Inc., Rockford, IL). Acetic acid liberated during hydrolysis was analyzed on a HPX 87H column (BioRad, Hercules, CA) using a high-performance liquid chromatography system with a photodiode array detector (Dionex, Sunnyvale, CA). The mobile phase was 10 mM H_2SO_4, the flow rate was 0.60 mL/min, the injection volume was 10 μL, and the absorbency was monitored at 210 nm.

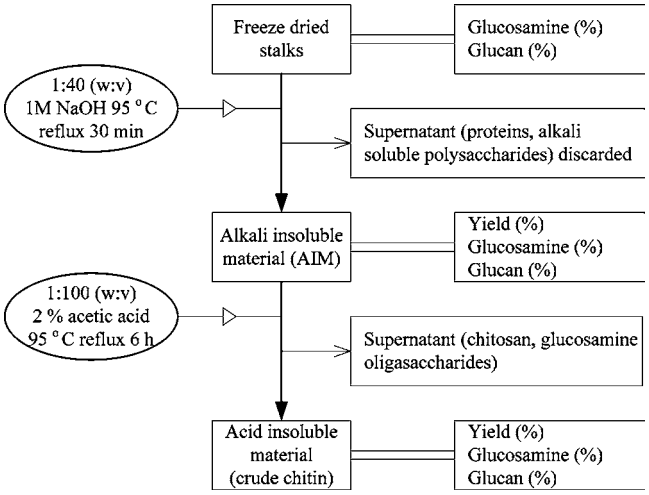

Figure 1. Extraction of chitinous material from *A. bisporus* stalks.

Data are shown as the means ± standard deviation (SD) of triplicate determinations. Statistical analysis included analysis of variance and comparisons of least squares means in the model using the Student's test ($p < 0.05$) (JMP, SAS Institute Inc., 2003).

RESULTS AND DISCUSSION

Fresh Weight Losses and DW Content. The fresh weight of mushroom stalks declined during storage, more rapidly at 25 than at 4 °C (**Figure 2A**). The average fresh weight loss was 35.65% after 5 days at 25 °C and 26.50% after 15 days at 4 °C. The weight reduction of fresh mushrooms due to extensive moisture loss has previously been reported (*31*). Moisture loss presents a significant problem in marketing of fresh mushrooms and is commonly reduced by modified atmosphere packaging. However, the reduction of fresh weight in mushroom waste has no adverse effects on waste handling and is advantageous due to the reduction of waste to be processed. However, it appears that losses during storage were caused not only by water evaporation but also due to DW reduction (**Figure 2B**). DW also declined more rapidly at the higher storage temperature. At harvest, fresh stalks contained 7.79% DW. However, DW declined to 6.79% after 15 days at 4 °C and to 4.27% after 5 days at 25 °C. The DW decrease can be explained by biochemical processes, such as respiration, that continue within the cells even after harvest (*32*). Although this fact can have negative implications on the potential utilization of the mushroom waste for extraction of structural biopolymers, an increase in chitin content in cell walls may compensate for the decrease in total dry matter (*33*).

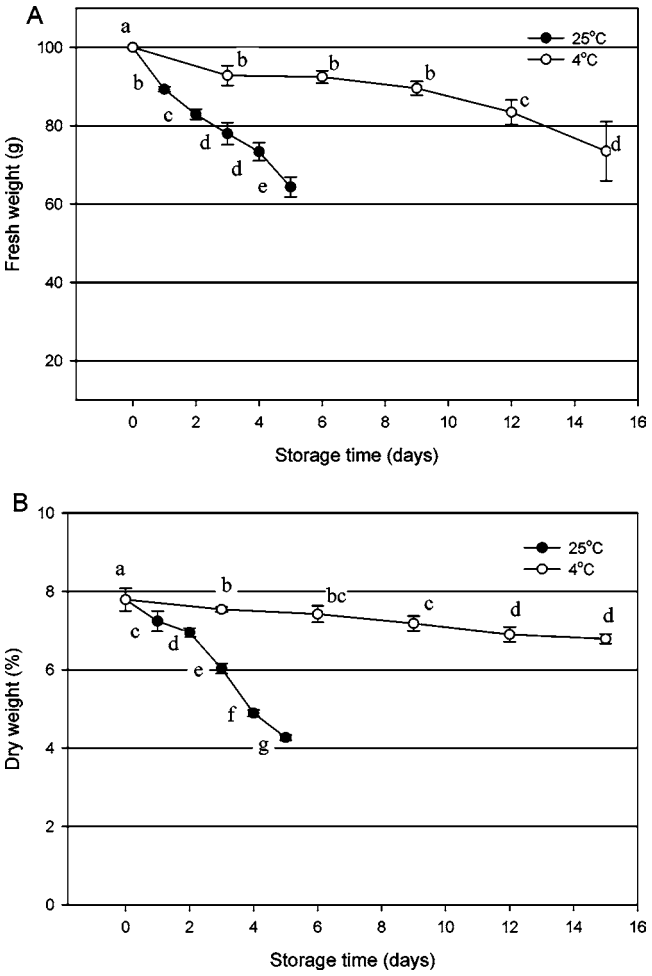

Figure 2. Fresh weight losses (**A**) and DW content (**B**) in mushroom stalks during storage at 4 and 25 °C. Error bars represent SDs of three replications.

Total Glucosamine Content. Chitin and chitosan are linear polysaccharides consisting of *N*-acetyl-D-glucosamine and D-glucosamine units present in different ratios in the polymers. Regardless of the method used for the determination of chitin and chitosan concentrations, a complete hydrolysis of the polymers is needed prior to the analysis. Acid hydrolysis results in cleavage of the glycoside bonds between (acetyl)glucosamine molecules and in deacetylation of *N*-acetyl-D-glucosamine monomers (*25*). The amount of end products, glucosamine residues, can be easily determined and used to estimate the amount of chitin and chitosan in the analyzed material. However, the type of material tested, the type and concentration of the acid, and the temperature and time of hydrolysis influence the recovery of glucosamine. According to Cousin (*34*), the optimum hydrolysis of chitinous material from *L. edodes* occurred with 6 M HCl, for 2 h at 110 °C. However, 2 h was insufficient for complete hydrolysis of the materials used in this study. To determine the optimum hydrolysis time for complete depolymerization of the fungal material, we examined glucosamine recovery during 18 h of hydrolysis in 6 M HCl at 110 °C. Because the extended crystallinity can reduce the accessibility of the polymers by acid molecules, commercial chitin and chitosan standards were included in the study. The results showed that the maximum recovery of glucosamine was achieved after 3 h of hydrolysis of chitosan and freeze-dried stalks and after 12 h of hydrolysis of chitin and AIM (**Figure 3**). With a prolonged time of hydrolysis, the amount of detected glucosamine decreased, apparently due to degradation of liberated monomers under harsh conditions of hydrolysis. Thus, the hydrolysis time for further analysis was 3 h for freeze-dried stalks and 12 h for AIM and fungal chitin. However, the absolute recovery of glucosamine from highly crystalline chitin was much lower than from chitosan. Analyzing commercial chitosan standards, we determined 93.02 ± 4.55% glucosamine after 3 h of hydrolysis and a maximum of 68.66 ± 2.42% from chitin standard although the hydrolysis lasted 12 h (**Table 1**).

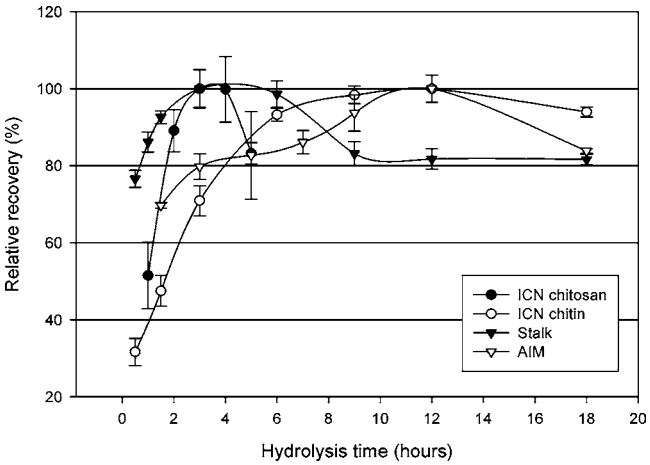

Figure 3. Effect of hydrolysis time on recovery of glucosamine from commercial chitin and chitosan (ICN), freeze-dried mushroom stalks obtained at harvest, and AIM from stalks stored for 5 days at 25 °C. All samples were hydrolyzed in triplicate in 6 M HCl at 110 °C. Glucosamine recovery represents the ratio of detected glucosamine at a particular point and maximum detected glucosamine in the sample. Error bars represent SDs of three replications.

Table 1. Effects of Hydrolysis Time on Glucosamine Extracted from Mushroom Sources

	glucosamine (%)			
time (h)	chitosan[a]	chitin[b]	stalks[c]	AIM[d]
0.5	*e*	21.74 ± 2.42 d	5.36 ± 0.16 d	
1.0	47.93 ± 7.98 b[f]		6.03 ± 0.18 bc	
1.5		32.64 ± 2.75 c	6.48 ± 0.12 ab	37.33 ± 0.37 c
2.0	82.87 ± 5.07 a			
3.0	93.02 ± 4.55 a	48.69 ± 2.68 b	7.00 ± 0.35 a	42.74 ± 1.78 b
4.0	92.88 ± 7.93 a			
5.0	83.22 ± 2.54 a			44.31 ± 6.12 ab
6.0		64.03 ± 1.17 a	6.90 ± 0.24 a	
9.0		67.54 ± 1.56 a	5.82 ± 0.22 cd	50.21 ± 2.54 a
12.0		68.66 ± 2.42 a	5.72 ± 0.19 cd	53.59 ± 0.12 a
18.0		64.51 ± 0.88 a	5.71 ± 0.10 cd	44.90 ± 0.34 ab

[a] ICN chitosan. [b] ICN chitin. [c] Freeze-dried stalks obtained at harvest. [d] AIM extracted from stalks stored for 5 days at 25 °C. [e] Not determined. [f] Mean values within the column followed by the same letter are not significantly different ($p < 0.05$).

The glucosamine content in mushroom stalks increased from 7.14% DW at day 0 (at harvest) to 11.00 and 19.02% DW after 15 days of storage at 4 °C and 5 days of storage at 25 °C, respectively ($p < 0.0001$; **Figure 4B**). Calculated on a fresh weight basis, the maximum glucosamine content of 0.88% was reached in the stalks stored at 25 °C after 4 days (**Figure 4A**). More chitinous material was produced at 25 °C than during storage at 4 °C (maximum 0.75%). However, the content of chitinous material on fresh basis decreased after 4 days at 25 °C due to extensive DW loss and deterioration, either by endogenous enzymes and/or spoilage microorganisms.

The amount of chitin determined in stalks stored at 25 °C for 5 days expressed on a DW basis was 19.02% (**Figure 4B**)

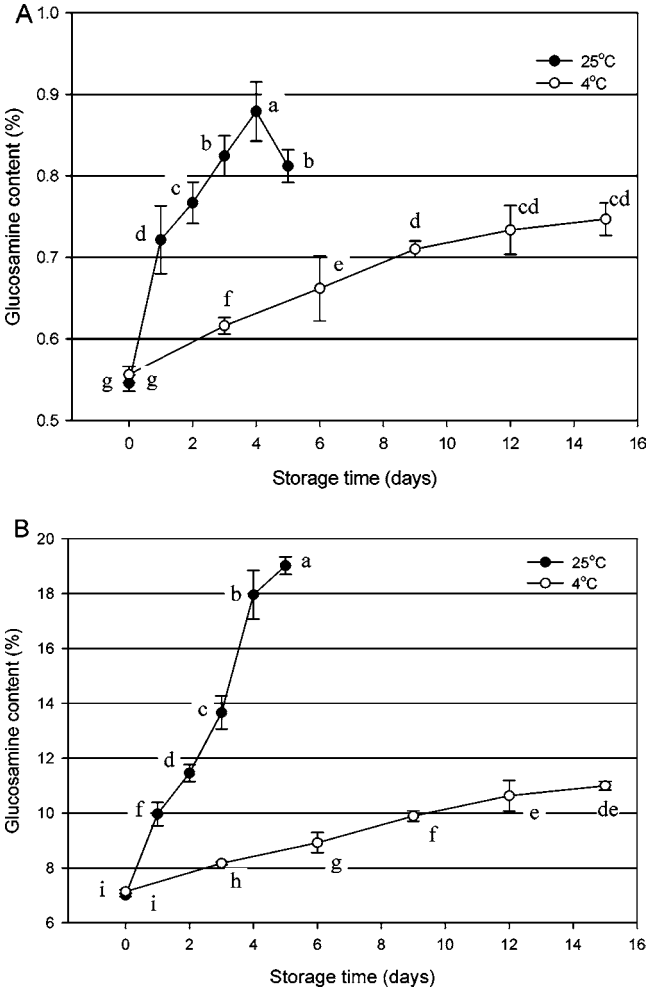

Figure 4. Total glucosamine content in mushroom stalks stored at 4 and 25 °C. The results are expressed on a fresh weight (**A**) and DW (**B**) basis. Error bars represent SDs of three replications.

and was consistent with 21% in *A. bisporus*, 19.0% in mushroom *Lactarius vellereus*, and 18.5% in *Penicillium notatum* (*9*, *35*). However, quantity and solubility of wall polymers largely fluctuate with the development stage of mycelia (*13*). Experiments with filamentous fungi *Absidia* spp., *M. rouxii*, *R. oryzae*, and mushroom *L. edodes* have indicated that the highest accumulation of chitosan in hyphae walls was in the late exponential phase (*18*, *27*, *36*, *37*). Thus, although *M. rouxii* is the most commonly explored fungi for chitosan extraction, the content of chitosan reported by different authors ranges from 8.8 to 44.5% on a dry cell wall weight basis (*8*, *9*, *11*, *38*). The total glucosamine level of 19.02% on a DW basis of *A. bisporus* stalks is significant for commercial production considering the amount of waste generated during mushroom production.

Extraction and Characterization of Chitinous Material. The freeze-dried samples obtained from stalks stored at 25 °C for 5 days were used for the extraction and characterization of chitin and chitosan. The yield of AIM was 32.03% DW and contained 43.84% glucosamine and 17.92% β-glucan (**Table 2**). Alkali extraction has usually been carried out in 1 N NaOH by autoclaving a mycelia suspension at 121 °C for 15 min (*15*, *18*, *19*, *39*). McGahren et al. (*13*) used milder conditions of alkali extraction but reported that protein impurities were still present. The AIM in our experiments contained no residual proteins, and extraction under atmospheric pressure is more practical for commercial applications. Boiling acetic acid further removed impurities, and the treatment resulted in 27.00% crude chitin but did not yield any chitosan. Chitosan is commonly extracted from AIM by aqueous acids at temperatures from 25 to 121 °C during 1−14 h, with higher yields achieved with higher temperatures and longer treatments (*11*, *14*, *16*, *20*, *39*). Nevertheless, the acid extraction of *A. bisporus* stalks did not yield any chitosan. This result was consistent with the results of Mol and Wessels (*40*) who found that chitinous material in the *A. bisporus* (phylum Basidiomycota) cell wall is mainly in the acetylated form as chitin, contrary to *M. rouxii* (phylum Zygomycota) where it is present in the deacetylated, acid soluble, chitosan form.

The acid insoluble residue, crude chitin, was apparently a chitin−glucan complex composed of 46.07% glucosamine polymers and 20.94% glucan (**Table 2**). The total amount of glucosamine in acid insoluble residue was lower than expected. The total glucosamine content in mushroom stalks after 5 days of storage at 25 °C was 19.02% DW (**Figure 4B**), but only 12.44% was obtained after alkali and acid treatments. The loss of (acetyl)glucosamine and its oligomers during extraction was excluded as the potential reason for the difference since no glucosamine was detected in either alkali or acid soluble material (data not shown). More likely, the difference may be attributed to crystallinity of chitin alone and/or within the chitin−glucan complex (*40*). Crystallinity would prevent accessibility of chitin molecules by hydrochloric acid and result in incomplete hydrolysis. If the maximum recovery of glucosamine from chitin standards is taken into account (68.66%; **Table 1**), the detected glucosamine in crude fungal chitin would represent 67.10% chitin. In other words, out of 19.02% of total glucosamine originally present in *A. bisporus* stalks, 18.11% DW was extracted in the crude chitin fraction.

The coexistence of glucan in crude fungal chitin has been reported (*9*, *40*). The presence of β-glucan provides an additional benefit to the crude fungal chitin since the β-glucan has been

Table 2. Yield and Composition of Material Obtained during Extraction of Chitinous Material from *A. bisporus*

sample	treatment	yield[a]	glucosamine[b]	glucans[c]
freeze-dried stalks	fresh[d]	(100)	7.14 ± 0.01 c[e]	18.99 ± 1.16 a
	15 days at 4 °C	(100)	11.00 ± 0.16 b	14.03 ± 2.88 a
	5 days at 25 °C	(100)	19.02 ± 0.32 a	15.90 ± 2.92 a
AIM	fresh	12.65 ± 0.53 c	38.17 ± 2.12 a	34.99 ± 1.18 a
	15 days at 4 °C	17.47 ± 1.09 b	45.76 ± 1.89 a	22.07 ± 3.74 b
	5 days at 25 °C	32.03 ± 0.42 a	43.84 ± 5.96 a	17.92 ± 2.24 b
acid insoluble residue (crude chitin)	fresh	8.30 ± 0.33 c	43.36 ± 1.61 b	46.27 ± 2.21 a
	15 days at 4 °C	13.90 ± 1.24 b	48.07 ± 1.86 a	26.79 ± 2.88 b
	5 days at 25 °C	27.00 ± 0.39 a	46.07 ± 3.02 ab	20.94 ± 1.75 c

[a] Yield expressed as % of DW of stalks. [b] Cell wall chitin was hydrolyzed and determined as glucosamine (% of analyzed material). [c] Cell wall neutral polysaccharides were determined by anthrone reagent (% of analyzed material). Results are presented as mean values of three determinations ± SDs. [d] Mushrooms were freeze-dried and tested before any extraction treatments. [e] Mean values within the column and the same sample type followed by the same letter are not significantly different ($p < 0.05$).

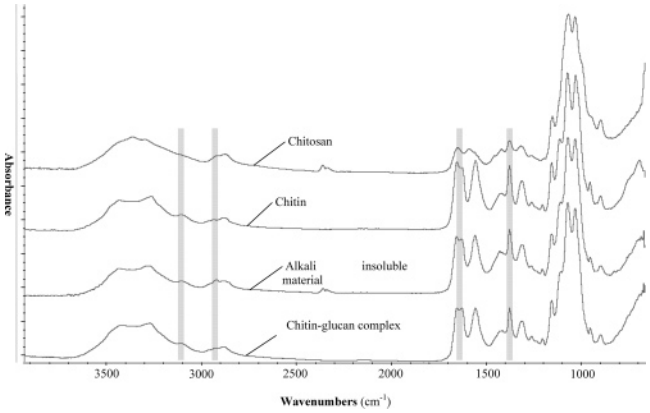

Figure 5. FT-IR spectra of commercial chitin and chitosan and AIM and acid insoluble residue (chitin–glucan complex) from *A. bisporus*.

shown to act as a potent elicitor that induces defense responses in several plants (*4*). Currently, only shrimp chitin has been registered as a nematocide (*41*) but the applications of fungal chitin−glucan complex as a biopesticide or plant growth regulator are promising, and this research is under way in our laboratories.

The DA of crude chitin was determined as liberated acetic acid after hydrolysis of insoluble residue in sulfuric and oxalic acid (*28*). The DA of crude chitin was 36.7 ± 1.7%. However, when the β-glucan content was considered, the DA value of fungal chitin was calculated to be 79.7 ± 3.6%, which is in the range of DA values of commercial crustacean chitin (70−90%). Furthermore, the high DA value confirms that chitinous material of *A. bisporus* is insoluble acetylated chitin rather than the deacetylated polymer, chitosan.

The FT-IR spectra of chitin and chitosan standards, fungal AIM, and chitin−glucan complex are shown in **Figure 5**. The spectra of AIM, chitin−glucan complex, and crustacean chitin were similar but the chitosan spectrum was different. Several important characteristics in the spectra of AIM and chitin−glucan complex were observed. According to Focher et al. (*42*), the split of the amide I vibration band occurring at 1655 cm^{-1} indicates that the fungal chitin is in an antiparallel, α-conformation. Furthermore, the weakening of the band occurring at 1655 cm^{-1} and absence of the band at 3100 cm^{-1} was associated with deacetylation and clearly differentiated chitosan standards from chitin and fungal chitinous materials in our experiment. These results further confirmed that glycosaminoglycans of *A. bisporus* were exclusively in a form of highly acetylated chitin molecules. The ratio of intensities of the bands at 1379 and 2900 cm^{-1} has been suggested as the crystallinity index for chitin and chitosan (*29*). As shown in **Table 3**, the crystallinity increased in the following order: commercial chitosan < fungal AIM < fungal chitin < commercial chitin. These results were expected because of the high DA of crude *Agaricus* chitin (79.7%). The high DA of chitin molecules (>70%) was associated with extended crystallinity while the low DA of chitosan molecules (<30%) resulted in a random distribution of the acetyl groups that did not allow significant development of the crystalline regions. The crystallinity index increased with purification of fungal material (fungal chitin > fungal AIM) mainly due to removal of interfering amorphous components. Similarly, Mol and Wessels (*40*) used X-ray diffraction to evaluate the crystallinity of untreated, alkali-, and acid-treated hyphae walls and found that X-ray patterns sharpened with purification and characteristic crystallinity peaks of α-chitin appeared after acid treatment of alkali insoluble material.

Table 3. Crystallinity of Chitinous Materials[a] Determined by FT-IR

	peak area		
chitinous material	$A_{1379cm^{-1}}$	$A_{2920cm^{-1}}$	$A_{1379cm^{-1}}/A_{2920cm^{-1}}$
chitin-1	0.15	0.12	1.25
chitin-2	0.18	0.15	1.20
chitin–glucan complex-1	0.22	0.29	0.76
chitin–glucan complex-2	0.12	0.16	0.75
AIM-1	0.18	0.33	0.55
AIM-2	0.20	0.39	0.51
chitosan-1	0.08	0.30	0.27
chitosan-2	0.05	0.25	0.20
chitosan-3	0.05	0.19	0.26
chitosan-4	0.08	0.40	0.20
chitosan-5	0.07	0.31	0.23

[a] Chitinous material included crustacean chitin (ICN and Primex), crustacean chitosan (Aldrich, Fluka, ICN, Primex), AIM, and crude chitin (chitin–glucan complex) from *A. bisporus* stalks stored for 5 days at 25 °C.

We found that the chitin content in *A. bisporus* stalks reached 19.02% DW during postharvest storage and could be efficiently extracted. Given the composition and amount of mushroom waste annually accumulated by growers, the proposed procedure could yield about 1000 metric tons of crude fungal chitin per year. The crude chitin from *A. bisporus* is composed of a chitin−glucan complex that has the potential to be used as a biopesticide, plant growth regulator, and feed additive. This research was the first step in our attempts to utilize the waste material from mushroom production as a value-added product. Future research will focus on production of fungal chitin from the waste and evaluation of its bioactive effects.

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