DIETARY SUPPLEMENTS

Measurement of β-Glucan in Mushrooms and Mycelial Products

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A robust and reliable method has been developed for the measurement of β-glucan in mushroom and mycelial products. Total glucan (plus free glucose and glucose from sucrose) was measured using controlled acid hydrolysis with H_2SO_4 and the glucose released specifically was measured using glucose oxidase/peroxidase reagent. α-Glucan (starch/glycogen) plus free glucose and glucose from sucrose were specifically measured after hydrolysis of starch/glycogen to glucose with glucoamylase and sucrose to glucose plus fructose with invertase and the glucose specifically measured with GOPOD reagent. β-Glucan was determined by the difference. Several acid and enzyme-based methods for the hydrolysis of the β-glucan were compared, and the best option was the method using H_2SO_4. For most samples, similar β-glucan values were obtained with both the optimized HCl and H_2SO_4 procedures. However, in the case of certain samples, specifically *Ganoderma lucidum* and *Poria cocus*, the H_2SO_4 procedure resulted in significantly higher values. Hydrolysis with 2 N trifluoroacetic acid at 120°C was found to be much less effective than either of the other two acids evaluated. Assays based totally on enzymatic hydrolysis, in general, yielded much lower values than those obtained with the H_2SO_4 procedure.

The medicinal properties of many species of mushroom have been valued and used in traditional Chinese medicine for centuries. More recent studies (1–7) have demonstrated that the key active compounds are triterpenoids, ergosterol, and, most importantly, 1,3:1,6-β-glucan. This β-glucan activates the immune system and might even have anticarcinogenic properties (1–7). The anticarcinogenic properties of the β-glucan from *Lentinula edodes* [Lentinan Shiitake (1, 4)], *Grifola frondosa* [Grifolan (2, 3)], *Ganoderma lucidum* [Reishi (6)], and others have been demonstrated throughout the past 3 to 4 decades. There is concern within the regulatory community regarding health claims relating to nutritional supplements as well as the identity and purity of these products (8), and this relates particularly to medicinal mushrooms where the key active components have been identified as 1,3:1,6-β-glucan, triterpenoids, and ergosterol.

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Fungal and yeast cell walls are composed of ≤50% 1,3:1,6-β-glucan, and numerous structural components have been identified (9). The 1,3:1,6-β-glucans of several mushroom species have been studied in considerable detail and the most predominant structural feature has been identified as a 1,3-β-glucan backbone with single D-glucosyl residues linked 1,6-β to every third (9) or fourth D-glucosyl unit in the 1,3-β-glucan backbone. However, much more complex structures have also been reported (10–14).

The structures of mushroom and fungal β-glucans are different from the cereal β-glucans (so-called mixed-linkage β-glucans) that are linear polysaccharides in which D-glucosyl residues are linked 1,3-β- and 1,4-β-, and the ratio of these linkage types varies with the source of the β-glucan (e.g., oats, barley, and wheat). Other β-glucans include cellulose (1,4-β-D-glucan) and curdlan (1,3-β-D-glucan).

A highly specific enzymatic procedure has been described for the measurement of cereal 1,3:1,4-β-D-glucans (15, 16). Enzymatic procedures have also been described for the measurement of 1,3:1,6-β-D-glucans in commercial yeast products (17, 18); however, although these procedures are useful for this particular application, they are less specific than the method that has been developed for the measurement of cereal β-glucan (15, 16). No quantitative enzymatic procedure has been described for the measurement of β-glucan in mushroom fruiting bodies or mycelium. Park et al. (19) measured the β-glucan content of *Agaricus blazei* by first extracting the nonstarch polysaccharide fraction according to the dietary fiber method of Prosky et al. (20, 21) using thermostable α-amylase and amyloglucosidase to hydrolyze starch/glycogen in the mushroom sample and recovering nonstarch polysaccharide by alcohol precipitation, washing, and drying. The recovered polysaccharide was subsequently acid hydrolyzed, and glucose was determined enzymatically. Rhee et al. (22), used a similar procedure to measure β-glucan content of *Inonotus obliquus* (Chaga). In this case, the polysaccharide recovered following incubation of the sample with α-amylase and amyloglucosidase under acid conditions and the resulting glucose was determined by HPLC. These authors also extracted polysaccharide in an alkaline buffer (pH 10). No enzyme treatment was included to remove α-glucan because this mushroom contains very little α-glucan. Synytsya et al. (23) used the Yeast and Mushroom β-glucan kit described in Megazyme technical booklet K-YBGL, in which total glucan is measured by hydrolysis with acid and α-glucan is specifically measured by enzymatic hydrolysis. Glucose was specifically measured with glucose oxidase/peroxidase reagent, and β-glucan is determined by the difference. Other interesting, but nonquantitative, methods have been described for the measurement of β-glucan in mushroom products, including the method of Mizuno et al. (24) using an ELISA and that of Molleken et al. (25) who used congo red dye.

Manzi and Pizzoferrato (26) measured the β-glucan content of a range of edible mushrooms using lichenase and β-glucosidase to hydrolyze the β-glucan. However, lichenase (a specific 1,3:1,4-*endo*-β-glucanase) has no action on 1,3:1,6-β-glucans, and the β-glucosidase has limited action on the polysaccharides, consequently the determined β-glucan values were greatly underestimated. Lichenase and β-glucosidase are used in the quantitative measurement of 1,3:1,4-β-glucans from cereal grains (15, 16).

Because complete enzymatic hydrolysis of β-glucan in mushroom products is very difficult, not only because of the array of β-glucan linkage types present but also as a result of the linkages to chitin and protein, the best approach to quantitative determination of this polysaccharide is complete acid hydrolysis to glucose, with subsequent measurement of glucose to measure total glucan. α-Glucan can either be removed before acid hydrolysis, or it can be measured separately and accounted for. Dallies et al. (27) measured β-glucan content of the yeast, *Saccharomyces cerevisiae*, by hydrolysis of the polysaccharide to glucose using a procedure described by Selvendran et al. (28) for plant cell walls, in which samples were first suspended in 72% w/w sulfuric acid at room temperature and then hydrolyzed at ~100°C in 2 M sulfuric acid according to Saeman (29). Under these conditions, the polysaccharide was completely hydrolyzed, and there was minimal loss of glucose through further degradation.

In the current study, acid hydrolysis and enzymatic procedures for hydrolysis of β-glucan in mushroom samples were compared, and a quantitative method for measurement of β-glucan in these products was developed.

Materials and Methods

Materials

(a) *Chemicals*.—Sulfuric acid, 95–98% (18.4 M; Cat. No. 258105-2.5L-D), hydrochloric acid, 37% (12 M, Cat. No. 258148-2.5L-D), trifluoroacetic acid (TFA, Cat. No. 299537-100G-D), potassium hydroxide, >85% (Cat. No. 221473-1KG-D), and lyticase (Cat. No. L2524-25KU; SLBL7091V) were obtained from Sigma-Aldrich (St. Louis, MO). Barley β-glucan (Cat. No. P-PGBM), yeast β-glucan (Cat. No. P-YBGL), curdlan (Cat. No. P-CURDL), *exo*-1,3-β-glucanase (100 U/mL) plus β-glucosidase (100 U/mL) (Cat. No. E-EXBGOS, Lot 140701), Total Starch assay kit (Cat. No. K-TSTA), Yeast and Mushroom β-Glucan assay kit (Cat. No. K-YBGL), enzymatic Yeast β-Glucan assay kit (Cat. No. K-EBHLG), and Sucrose/Glucose assay kit (K-SUCGL) were obtained from Megazyme International (Bray, County Wicklow, Ireland).

Sulfuric acid (72% w/w, ~12 M) was prepared by carefully adding 650 mL of concentrated sulfuric acid (98%, sp. gr. 1.835) to 300 mL of distilled water. The volume was then adjusted to 1 L. TFA (2 M) was prepared by adding 25 mL of concentrated acid to 162 mL of distilled water. Sodium acetate buffer (200 mM, pH 5) was prepared by adding 11.6 mL of glacial acetic acid (1.05 g/mL) to 900 mL of distilled water and adjusting the pH to 5.0 using 4 M (16 g/100 mL) sodium hydroxide solution. The volume was adjusted to 1 L.

(b) *Mushrooms and mycelial products*.—All of the pure mushroom fruiting bodies analyzed in this study were supplied by Jeff Chilton (Nammex, Gibsons, BC, Canada; Table 1).

Table 1. Details of the pure mushroom fruiting bodies supplied by Nammex

	Basidiomycete species	Lot number	Product code
1	*Polyporus umbellatus* (lumpy bracket)	MZ-PKPu1304	PuMuWp00
2	*Trametes versicolor* (turkey tail)	MZ-PKTv1412	TvMuWp00
3	*Inonotus obliquus* (Chaga mushroom)	MZ-FGlo1401	IoMuWp00
4	*Ganoderma lucidum* (Reishi or Lingzhi)	MZ-XTYGl1409-00	GlMuWp00
5	*Agaricus blazei*	MZ-ZFPAb1405	ABMuWp00
6	*Grifola frondosa* (hen of the woods)	MZ-QYZGf1408-00	GfMuWp00
7	*Ganoderma lucidum* (Reishi or Lingzhi)	MZ-QYZGl1407-00	GlMuWp00
8	*Poria cocos* powder (poria)	MZ-QYZPc1006-11	PcMuWp11
9	*Lentinula edodes* powder (Shiitake)	MZ-ZFPLe1309-P	LeMuWp11
10	*Cordyceps militaris* (ascomycete)	MZ-QYZCm1406	CmMuWp00
11	*Hericium erinaceus* (Lion's mane mushroom)	MZ-PKHe1304	HeMuWp11
12	*Agaricus bisporus* (button mushroom)	MZ-PKAbb1412	AbbMuWp00
13	*Pleurotus ostreatus* (oyster mushroom)	MZ-JCPo14112	PoMuWp00
14	*Tremella fuciformis* (white jelly mushroom)	MZ-PKTf1304	TfMuWp00
15	*Grifola frondosa* (hen of the woods)	MZ-PKGf1304	GfMuWp00
16	*Lentinula edodes* (Shiitake)	MZ-PKLe1412	LeMuWp00
17	*Pleurotus eryngii* (king trumpet mushroom)	MZ-PKPe1412	PeMuWp00
18	*Flammulina velutipes* (velvet shank)	MZ-PKFv1412	FvMuWp00
19	*Agaricus bisporus* (Portobello)	MZ-PKAbp1412	AbpMuWp00

Commercial capsules containing mushroom and mycelial products (fruiting bodies and mycelium) were purchased via the internet from Amazon.com. Details of these samples are provided in Table 2. The A. niger β-glucan control used in these studies was obtained from the Yeast and Mushroom β-Glucan assay kit (Megazyme Cat. No. K-YBGL). The concentration of this β-glucan was determined using both this kit and also enzymatically using the kit K-EBHLG.

Methods

(a) *Measurement of α-glucan (starch/glycogen)*.—Mushroom samples were milled to pass a 1.0 mm screen. Approximately 100 mg (weighed accurately) of the sample was added to a 20 × 125 mm Fisher Brand culture tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. A magnetic stirrer bar (5 × 15 mm) and 2.0 mL of ice-cold 2 M KOH was added to each tube, and the tube contents were stirred using a magnetic stirrer in an ice–water bath for 20 min to dissolve the starch/glycogen. Eight milliliters of 1.2 M sodium

Table 2. Details of mushroom products obtained commercially in capsule form[a]

Sample No.	*Basidiomyces* species	Product details from bottle labels
1	*Ganoderma lucidum*	Reishi Mushroom (*Ganoderma lucidum*) (fruiting bodies) 1.2 g/2 tablets. Other ingredients include: microcrystalline cellulose (plant fiber).
2	16 species blend	Maitake (*Grifola frondosa*) mycelium; Chaga (*Inonotus obliquus*) mycelium; Reishi (*Ganoderma lucidum var. resinaceum s.l.*) mycelium; Cordyceps (*Cordyceps sinensis s.l.)* mycelium; Royal sun Blazei (*Agaricus brasillensis f. blazei*) mycelium; Enokitake (*Flammulina volutipes*) mycelium; Mesima (*Phellinus linteus*) mycelium; Turkey Tails (*Trametes versicolor*) mycelium; Zhu Ling (*Polyporus umbellatus*) mycelium; Lions Mane (*Hericlum erinaceus*) mycelium; Maitake (*Grifola frondosa*) fruitbodies; Artists Conk (*Ganoderma applanatum s.l.*) mycelium; Oregon Reishi (*Ganoderma oregonense s.l.*) mycelium; Agarikon (*Fomitopsis officinalis*) mycelium; Amadou (*Fomes fomentarius*) mycelium; Shitake (*Lentinula edodes*) mycelium; Birch Polypore (*Piptoporus betulinus*) mycelium; Split Gill Polypore (*Schizophyllum commune*) mycelium.
3	7 species blend	Royal Sun Blazei (*Agaricus brasiliensis*) mycelium; Cordyceps (*Cordyceps sinesis s.l.*) mycelium; Reishi (*Ganoderma lucidum s.l.)* mycelium; Maitake (*Grifola frondosa*) mycelium; Lion's Mane (*Hericlum erinaceus*) mycelium; Chaga (*Inonotus obliquus*) mycelium; Mesima (*Phellinus linteus*) mycelium. Other ingredients freeze-dried mycelium, brown rice, pullulan.
4	*Ganoderma lucidum*	Reishi Mushroom fruiting body extract, 120 mg. Reishi mushroom mycelia powder 880 mg. Reishi mushroom fruiting body extract, 120 mg (standardized for 10% polysaccharides). Other ingredients; vegetable cellulose, rice flour, calcium silicate.
5	*Ganoderma lucidum*	Reishi mushroom powder without any additives. 480 mg capsules.
6	*Ganoderma lucidum*/ *Lentinula edodes*	Reishi Mushroom Extract Powder (10:1) (*Ganoderma lucidum*) 90 mg; Reishi mushroom powder (*Ganoderma lucidum*) 300 mg; Shitake Mushroom Extract Powder (4:1) (*Lentinula edodes*). 270 mg; per 2 capsules.
7	*Cordyceps* sp. (ascomycete)	Organic Cordyceps (mycelium). 1.5 g/2 capsules.
8	*Cordyceps* sp. (ascomycete)	Pure Cordyceps capsules. 525 mg each, 100% organic. Full Spectrum. *Cordyceps sinensis* in a nonorganic vegetarian capsule, nothing more, nothing less.
9	*Ganoderma lucidum*	Red Reishi. 100% Organic *Ganoderma lucidum*. 1500 mg.
10	*Cordyceps sinensis* (ascomycete)	*Cordyceps sinensis* (deep layer cultivated mycelia extract) 1.2 g. Other ingredients include microcrystalline cellulose (plant fiber).
11	*Cordyceps sinensis* (ascomycete)	*Cordyceps* dried extract (mycelium). 1000 mg. 10% cordycepic acid. Other ingredients; rice powder.
12	*Inonotus obliquua*	Chaga Mushroom (mycelium) 400 mg. Other ingredients include: brown rice flour

[a] Bottled products containing encapsulated mycelium/mushroom powder. Bottles were purchased from online retailers. Most of the products studied are "mycelium propagated on grain."

acetate buffer (pH 3.8) was added to each tube with mixing on a vortex stirrer. A total of 0.2 mL of a mixture of amyloglucosidase (1630 U/mL) plus invertase (500 U/mL) (from Megazyme assay kit, Cat. No. K-YBGL) was immediately added, the contents were mixed well, and the tubes were incubated at 40°C for 30 min. For samples containing <10% starch, this solution (10.3 mL final volume) was analyzed directly. For samples containing 10–100% starch/glycogen, the tube contents were quantitatively transferred to a 100 mL volumetric flask and adjusted to volume with deionized water, and the contents were mixed thoroughly. In both cases, 1.0 mL of the solution was centrifuged at 11 000 *g* for 3 min in a microfuge, and 0.1 mL of the supernatant solutions was analyzed for glucose with glucose oxidase/peroxidase reagent.

(b) *Measurement of total glucan*.—(*1*) *Hydrolysis with sulfuric acid*.—Mushroom samples were milled to pass a 1.0 mm screen. Approximately 100 mg (weighed accurately) of the sample was added to a 20 × 125 mm Fisher Brand culture tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. A total of 2.0 mL of ice-cold 12 M sulfuric acid was added to each tube, and the tubes were capped and stirred on a vortex mixer. Tubes were placed in an ice–water bath and left for 2 h. During this time, the tube's contents were vigorously stirred (for 10–15 s) several times on a vortex mixer to ensure complete dissolution/dispersion of the sample. Twelve milliliters of water was added to each tube, and the tubes were capped and vigorously stirred on a vortex mixer for 10 s. The caps were loosened and the tubes were placed in a boiling-water bath (~100°C). After 5 min, the caps were tightened and the incubation was continued at 100°C for 2 h. The tubes were cooled to room temperature, and the caps were carefully loosened. Six milliliters of 10 M KOH was added, and the tube contents were mixed well. The contents of each tube were quantitatively transferred to 100 mL volumetric flasks using a wash bottle containing 200 mM sodium acetate buffer (pH 5), and the volume was adjusted to 100 mL with 200 mM sodium acetate buffer (pH 5). The contents were mixed thoroughly, and an aliquot (~1.2 mL) of the solution was centrifuged at 11 000 × *g* for 3 min in a microfuge; alternatively, a 5 mL aliquot of the solution was centrifuged at ~1500 × *g* for 10 min in a bench centrifuge. The content of glucose in the solutions was analyzed by incubating an aliquot (0.1 mL) of the supernatant with 3.0 mL of GOPOD reagent at 40°C for 20 min. Absorbance was measured at 510 nm. Concurrently, a 0.1 mL aliquot of glucose standard solution (1 mg/mL), was incubated in quadruplicate (standard) with GOPOD reagent; also, 0.1 mL of acetate buffer (200 mM, pH 5) was incubated with 3.0 mL of GOPOD reagent (reagent blank). Alternatively, 0.1 mL of the sample solution was incubated with 0.1 mL of a mixture of *exo*-1,3-β-glucanase (20 U/mL) plus β-glucosidase (4 U/mL) at 40°C for 60 min, and the glucose was determined with GOPOD reagent as previously described (all of the reagents used are available in the Megazyme test kit Cat. No. K-YBGL).

In preliminary experiments, samples were stored in 12 M sulfuric acid for 30, 60, and 120 min; effect of stirring or intermittent shaking in the 12 M sulfuric acid was evaluated; effect of sample size (25–100 mg) was also evaluated. The effect of time of incubation at 100°C on determined glucan content and stability of released glucose was determined by incubating a series of samples [glucose, wheat starch, barley β-glucan, Actigum (scleroglucan), purified yeast β-glucan, and *Cordiceps militaris* mushroom sample] in 12 M sulfuric acid for 2 h at ~0°C followed by incubation in 2 M sulfuric acid at 100°C for 0, 30, 60, 90, and 120 min.

(*2*) *Hydrolysis with hydrochloric acid*.—Mushroom samples were milled to pass a 1.0 mm screen. Approximately 100 mg (weighed accurately) of the sample was added to a 20 × 125 mm Fisher Brand culture tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. A total of 1.5 mL of 37% v/v (12 M) hydrochloric acid was added to each tube, and the tubes were capped and stirred on a vortex mixer. The tubes were placed in a water bath at 30°C for 60 min and the contents were stirred for 15 s on vortex mixer. Ten milliliters of water was added to each tube, the tubes were capped, and the contents were vigorously stirred on a vortex mixer for 10 s. The caps were loosened and the tubes were placed in a boiling-water bath (~100°C). After 5 min, the caps were tightened and the incubation was continued for 2 h. The tubes were cooled to room temperature, and the caps carefully loosened. Ten milliliters of 2 M KOH was added, and the contents were mixed well. The contents of each tube were quantitatively transferred to 100 mL volumetric flasks using a wash bottle containing 200 mM sodium acetate buffer (pH 5), and the volume was adjusted to 100 mL with 200 mM sodium acetate buffer (pH 5). The contents of each flask were mixed thoroughly. Samples were then treated in the same manner as those from H_2SO_4 hydrolysis.

(*3*) *Hydrolysis with TFA*.—Mushroom samples were milled to pass a 1.0 mm screen. Approximately 100 mg (weighed accurately) of the sample was added to a 20 × 125 mm Fisher Brand culture tube, and the tube was tapped to ensure that the entire sample fell to the bottom of the tube. Five milliliters of 2 M TFA was added to each tube, the tubes were capped, and the contents were stirred vigorously on a vortex mixer. The caps were loosened, and the tubes placed in an oil bath at 120°C. After 2 min, the caps were tightened and incubation was continued for 40 min. The contents of the tubes were stirred after 10 min intervals for approximately 10 s. After 40 min, the tubes were cooled to room temperature and the caps carefully loosened. Five milliliters of 2 M KOH was added, and the contents were mixed well. The contents of each tube were quantitatively transferred to 100 mL volumetric flasks using a wash bottle containing 200 mM sodium acetate buffer (pH 5), and the volume was adjusted the to 100 mL with 200 mM sodium acetate buffer (pH 5). The contents of the flasks were mixed thoroughly. Samples were then treated in the same way as those from H_2SO_4 hydrolysis.

Enzymatic Methods for Measurement of β-glucan in Mushroom Samples

(a) *Glucan enzymatic method (GEM) assay*.—This assay was performed as described by Danielson et al. (17). Under these conditions, lyticase incubation was performed at pH 5, which is not optimal (pH optima for lyticase is 7.0–7.5).

(b) *Modified GEM assay*.—In a modified incubation, mushroom sample (~20 mg, weighed accurately) was suspended in 0.4 mL of 2 M KOH and stirred for 20 min in an ice–water bath. The solution was neutralized by adding 1.2 mL of 0.6 M sodium acetate buffer (pH 3.8). Tris buffer (1 mL, 10 mM, pH 7.1) containing 1 mM EDTA, 20 mM NaCl, and 6000 U of lyticase (units as defined by Sigma Chemical Co.) was added, and the suspension was incubated at 50°C for 16 h. The incubation mixture was centrifuged (11 000 × *g*, 3 min) and aliquots (130 µL) were removed and incubated with 650 µL of 200 mM sodium acetate buffer (pH 5.0) containing *exo*-1,3-β-glucanase (8 U) plus β-glucosidase (1.6 U) for 1 h at 40°C. Aliquots (50 µL) were removed for determination of glucose using GOPOD reagent.

(c) *Assay using exo-β-glucanase/endo-β-glucanase/β-glucosidase/chitinase mix*.—This assay was performed according to the method described in a commercial kit procedure for the enzymatic measurement of yeast β-glucan (Megazyme Cat. No. K-EBHLG). Samples (~20 mg, weighed accurately) were suspended in 0.4 mL of 2 M KOH and stirred in an ice–water bath for 30 min. Sodium acetate buffer (1.6 mL, 1.2 M, pH 3.8) was added with mixing followed by 40 µL of Glucazyme™ enzyme mixture (containing *exo*-1,3-β-glucanase, *endo*-1,3-β-glucanase, β-glucosidase, and chitinase), and the mixture was incubated at 40°C for ~16 h. Water (10 mL) was added to the tube with mixing and the tubes were centrifuged at 3000 rpm for 10 min. Aliquots (0.1 mL) were removed for determination of glucose using GOPOD reagent (4 mL). Glucose standards and reagent blanks were run concurrently.

Phenol-sulfuric assays of carbohydrate concentration were performed according to the procedure of Dubois et al. (30).

Results and Discussion

In these studies, two approaches for the measurement of β-glucan in mushroom and mushroom products have been evaluated. In the first approach, controlled acid hydrolysis was evaluated for the measurement of total glucan. α-Glucan (starch/glycogen) was specifically measured using an enzymatic procedure (AOAC Method **996.11**), and β-glucan was calculated by the difference. In a second approach, methods were evaluated for the specific determination of β-glucan using a mixture of β-glucan degrading enzymes, devoid of enzymes active on starch and glycogen.

Methods based on acid hydrolysis aim to achieve complete hydrolysis of both α- and β-glucan to glucose, while minimizing the loss of glucose through secondary reactions (e.g., formation of hydroxymethylfurfural). The effect of incubation time in 2 M H_2SO_4 and 1.6 M HCl at 100°C on the level of glucose released on hydrolysis of samples is shown in Table 3. Samples (~100 mg weighed accurately) were suspended in 2 mL of ice-cold 12 M H_2SO_4 and stored on ice for 2 h. Tube contents were stirred vigorously for ~20 s every 15 min. Solutions were diluted to 2 M H_2SO_4 by the addition of deionized water and then were incubated in a boiling-water bath for ≤2 h. On addition of water, the contents of one tube were immediately neutralized with KOH and adjusted to 100 mL with 200 mM sodium acetate buffer (pH 5.0) for further analysis. Other samples were incubated in the boiling-water bath for 30, 60, 90, and 120 min before removal and neutralization. From the results in Table 3, it is evident that glucose is stable under these

Table 3. Determined glucose (and calculation of total glucan) on incubation of glucose, wheat starch, various β-glucans, and *Cordyceps militaris* mushroom in either 1.6 M HCl or 2 M H_2SO_4 at 100°C for 0–120 min

		Total glucan, g/100 g (dry weight basis)			
Sample and incubation time		1.6 M HCl hydrolysis		2 M H_2SO_4 hydrolysis[a]	
Sample[b]	Incubation time at 100°C	No enzymes	Plus enzymes	No enzymes	Plus enzymes
Glucose	0	90	92	42	48
	30	96	94	93	95
	60	99	98	98	98
	90	98	98	97	98
	120	97	97	97	97
Wheat starch	0	25	25	1	4.1
	30	87	85	80.1	83.3
	60	92	90	88	89.1
	90	89	88	89.4	91.2
	120	89	89	88.7	89.9
Barley β-glucan	0	2.4	84	12.1	13
(Megazyme lot 90801)	30	49	87	74.8	80.5
	60	84	86	80.2	86.3
	90	86	86	80.1	86.2
	120	86	85	79.3	85.3
Scleroglucan	0	8.7	86	27.8	31.3
(Actigum CSII)	30	70	89	74.5	83.4
	60	85	86	75.8	84.9
	90	86	85	75.2	84.0
	120	85	84	75.3	84.2
Cordyceps militaris	0	2	8.9	6.5	7.2
Mushroom	30	12.8	17	20.9	23.2
	60	23.4	27	26.9	29.8
	90	26.6	30	30.2	33.6
	120	27	30	31.1	34.5
Purified yeast β-glucan	0	7.4	76	1.8	28.7
(Megazyme lot 20301)	30	61	79	59.6	77.4
	60	77	79	76.6	78.9
	90	77	79	78.8	80.5
	120	77	77	77.3	79.1

[a] Samples were either preincubated in 12 M HCl at 30°C for 1 h, or samples were preincubated in 12 M H_2SO_4 at ~0°C for 2 h.

[b] All values are reported on a dry weight basis. Glucose concentration was determined accurately using GOPOD reagent before incubation with acid.

conditions, with a recovery of 97% after 1 and 2 h at 100°C. The lower determined glucose at zero time in the presence of sulfuric acid (and to a lesser extent, hydrochloric acid) is a result of Fischer glycosylation (31). The purified polysaccharides, starch, barley β-glucan, yeast β-glucan, and scleroglucan are completely hydrolyzed after 60 min, with little loss of glucose on subsequent incubation for ≤2 h. In contrast, complete hydrolysis of β-glucan in the *Cordyceps militaris* mushroom sample requires incubation at 100°C for 2 h. Incubation for 3 h produced no further increase in determined glucose values (data not shown). Hydrolysis with 1.6 M HCl (Table 3) was also near complete for the samples analyzed (except *Cordyceps militaris*) within 1 h, and there was little loss of glucose under the incubation conditions for ≤2 h. Incubation of aliquots of the 1–2 h acid hydrolysates of *Cordyceps militaris* samples with a mixture of *exo*-1,3-β-glucanase plus β-glucosidase increased determined glucan values by 2–3%. The resistant oligosaccharides were shown as mainly laminaribiose and laminaritriose (data not shown).

The total glucan (including free glucose and glucose in sucrose), α-glucan (including free glucose and glucose in sucrose), and β-glucan (by difference) values obtained for a number of mushroom samples on hydrolysis with HCl and H_2SO_4 under the defined incubation conditions are shown in Table 4. Total glucan was determined by suspending the samples in either 12 M HCl with stirring at 30°C for 1 h, or in 12 M sulfuric acid in an ice–water bath with occasional stirring throughout 2 h. The HCl was diluted to 1.6 M and H_2SO_4 to 2 M with distilled water, and samples were incubated at 100°C for 2 h. Samples were neutralized and adjusted to 100 mL, and 0.1 mL aliquots were analyzed for glucose, with and without preincubation with enzymes (*exo*-1,3-β-glucanase plus β-glucosidase). α-Glucan (including free glucose and glucose from sucrose) in the original sample was specifically determined by incubation with amyloglucosidase and invertase with specific determination of glucose with GOPOD reagent, and β-glucan was determined by the difference. Free glucose was low in all samples, and no sucrose could be measured in any of the mushroom samples. α-Glucan values were <1% w/w for most samples, but a few samples had ≤3–4% w/w α-glucan content. For certain samples, the value determined for β-glucan with the HCl hydrolysis was 1–2% higher than with the H_2SO_4 procedure, but for many samples, the value determined with the H_2SO_4 procedure was significantly higher than that obtained when HCl was used. The one clear example is *Ganoderma lucidum* in which the value obtained with H_2SO_4 was approximately twice that obtained with HCl. Also, the determined β-glucan content of *Poria cocus* powder, *Agaricus blazei*, and *Cordyceps militaris* are higher with H_2SO_4 hydrolysis. In an attempt to understand why much higher β-glucan values were obtained for *Ganoderma lucidum* samples with H_2SO_4 than with HCl, the effect of time of preincubation (dissolution) in 12 M HCl and 12 M H_2SO_4 was studied, and the results of this study are shown in Table 5. Increasing the preincubation time with 12 M HCl from 30 to 120 min increased the determined total glucan content of curdlan from 58.5 to 75.2% and of *Poria cocus* glucan from 69.7 to 74.7, values very much in line with those obtained with the optimized H_2SO_4 procedure. However, the total glucan content of *Ganoderma lucidum* mushroom only increased from 26.7 to 31.4%, or much lower than the value obtained with the H_2SO_4 procedure. So, in summary, the method that most consistently yields an accurate measurement of β-glucan across the range of samples studied was that using H_2SO_4, making this the method of choice, especially if unknown mushroom products are being analyzed. With some samples, slightly higher total glucan (and thus β-glucan) was obtained with the HCl hydrolysis method. The reason for this greater amount is not clear, because the H_2SO_4 is clearly more effective in dissolution and hydrolysis of β-glucan across the range of samples studied (as seen from the more rapid rate of dissolution of curdlan in H_2SO_4), and glucose

Table 4. Total glucan, α-glucan, and β-glucan contents of a range of pure mushroom samples[a]

	Total glucan + free glucose (g/100 g, dwb)				α-Glucan + free glucose (g/100 g, dwb)	Free glucose (g/100 g, dwb)	β-Glucan (g/100g, dwb)	
Mushroom sample	1.6 M HCl	1.6 M HCl + enzymes	2 M H_2SO_4	2 M H_2SO_4 + enzymes			1.6 N HCl + enzymes	2 M H_2SO_4 + enzymes
Polyporus umbellatus	52.7	53.4	50.9	51.1	0.6	0.4	52.8	50.5
Trametes versicolor	51.5	53.5	46.2	47.3	0.2	0.2	53.3	47.1
Inonotus obliqus	7.5	8.7	7.9	8.1	0.2	0.4	8.5	7.9
Ganoderma lucidum (sample 1)	21.6	23.8	53.6	54.2	0.2	0.2	23.6	54.0
Agaricus blazei	11.9	12.3	14.6	16.5	3.4	0.4	8.9	13.1
Grifola frondosa	32.4	33.4	34.5	36.4	1.3	0.4	32.1	35.1
Ganoderma lucidum (sample 2)	28.9	26.8	55.4	55.4	0.6	0.4	26.2	54.8
Poria cocus (powder)	60.2	67.7	74.3	74.7	0.8	0.8	66.9	73.9
Lentinula edoses (powder)	40.1	40.6	38.3	39.4	3.2	0.2	37.4	36.2
Cordyceps militaris	30.0	30.8	35.4	36.5	2.2	0.3	28.6	34.3
Hericium erinaceus	37.4	38.5	36.2	37.1	3.2	0.1	35.3	33.9
Agaricus bisporus (button)	8.1	8.8	7.6	7.3	1.3	0.5	7.5	6.0
Pleurotus ostreatus	32.9	33.7	32.1	32.7	0.4	0.2	33.3	32.3
Tremella fuciformis	15.8	16.8	14.8	16.1	1.2	0.1	15.6	14.9
Grifola frondosa	32.2	34.3	32.4	33.3	1.8	0.3	32.5	31.5
Lentinula edodes	27.9	28.3	23.5	24.4	0.9	0.2	27.4	23.5
Pleurotus eryngii	38.7	39.8	37.9	37.5	0.4	0.1	39.4	37.1
Flammulina velutipes	21.1	21.7	19.9	20.7	0.7	0.2	21.0	20.0
Agaricus bisporus (portobella)	10.3	11.3	9.8	9.8	4.1	0.8	7.2	5.7
Aspergillus niger mycelium (control 49%)	49.0	49.8	50.8	51.3	0.7	—	50.6	50.6

[a] All samples were analyzed in duplicate and results are reported on a dry weight basis (dwb).

Table 5. Effect of time of preincubation in 12 M H_2SO_4 or 12 M HCl on determined total glucan content of samples, with and without subsequent incubation with *exo*-1,3-β-glucanase plus β-glucosidase[a]

		Total glucan, g/100 g, dwb			Total glucan, g/100 g, dwb	
Sample	12 M H_2SO_4 Preincubation time (min)	12 M H_2SO_4 no enzyme incubation	12 M H_2SO_4 with enzyme incubation	12 M HCl preincubation time (min)	12 M HCl no enzyme incubation	12 M HCl with enzyme incubation
G. lucidum	30	48.2	52.4	45	25.1	26.7
G. lucidum	60	52.2	53.8	90	27.6	29.9
G. lucidum	120	56.2	56.3	120	29.7	31.4
Poria cocus	30	56.8	67.3	45	61.5	69.7
Poria cocus	60	64.5	71.2	90	69.9	73.3
Poria cocus	120	74.4	75.9	120	73.1	74.7
Curdlan	30	58.7	72.3	45	48.4	58.5
Curdlan	60	65.8	76.1	90	53.7	65.0
Curdlan	120	77.7	81.5	120	66.8	75.2
Alpha-cellulose	30	14.9	16.0	45	15.5	16.3
Alpha-cellulose	60	14.1	15.1	90	21.8	22.5
Alpha-cellulose	120	14.6	15.4	120	24.5	25.0

[a] Sample size was ~100 mg (weighed accurately). After preincubation in 12 M H_2SO_4 in an ice bath for 30–120 min, samples were diluted to 2 M H_2SO_4 and incubated at 100°C for 2 h. Alternatively, samples were preincubated in 12 M HCl at 30°C for 45–180 min and then diluted to 1.6 M HCl and incubated at 100°C for 2 h. All samples were analyzed in duplicate. All values are given on a dry weight basis (dwb).

appears to have similar stability in both HCl and H_2SO_4 at the acid concentrations used.

TFA is commonly used in the hydrolysis of polysaccharide samples in preparation for derivitization for gas liquid chromatography. Hydrolysis is usually performed with 2 M TFA at 120°C in an oil bath for 1 h. Hydrolysis patterns for a Mitake mushroom sample and regular maize starch and waxy maize starch are shown in Figure 1a and b. Clearly, the optimal time of hydrolysis (maximum production of free glucose) of the Mitake sample is 40 min (Figure 1a). However, starch samples

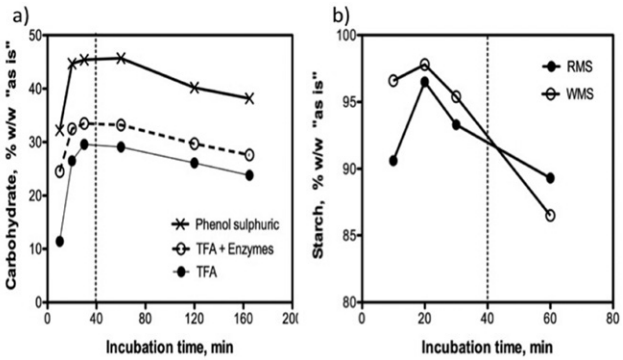

Figure 1. Time course hydrolysis of (a) Maitake mushroom powder and (b) regular maize starch (RMS) and waxy maize starch (WMS) by 2 M TFA at 120°C as measured by glucose release.

are hydrolyzed much more rapidly, and by 40 min of incubation, a significant percentage of the released glucose is further degraded (Figure 1b). Total glucan values obtained for five mushroom samples, four β-glucans, and wheat starch are shown in Table 6. Results are compared to those obtained with H_2SO_4 hydrolysis. For all of the mushrooms analyzed, except *Agaricus blazei*, the total glucan values obtained with TFA hydrolysis were much lower than those obtained with H_2SO_4 hydrolysis. Consequently, TFA hydrolysis was not studied further.

Ideally, the best procedure for quantitation of β-glucan in mushroom products would involve specific hydrolysis of the 1,3:1,6-β-glucan to glucose with no hydrolysis of starch or other β-glucans such as 1,3:1,4-β-glucans from cereals or 1,4-β-glucan (cellulose). Two enzymatic procedures have been described for the measurement of the 1,3:1,6-β-glucans in commercial yeast products. The GEM procedure (17) uses lyticase followed by a mixture of β-glucosidase and β-glucanases. The second procedure uses incubation with a β-glucosidase/β-glucanase/chitinase mixture (K-EBHLG procedure, 18). Results obtained for four mushroom samples are shown in Table 7. In the GEM procedure

Table 6. Comparison of TFA and H_2SO_4 hydrolysis conditions for the determination of total glucan in mushroom samples and purified glucans

	Total glucan, g/100 g (dwb)			
	2 M H_2SO_4 hydrolysis		2 M TFA hydrolysis	
Sample[a] and incubation time	No enzymes	Plus enzymes	No enzymes	Plus enzymes
Trametes versicolor	46.2	47.3	32.1	40.4
Ganoderma lucidum	53.6	54.2	16.7	20.3
Agaricus blazei	14.6	16.5	13.0	16.9
Grifola frondosa	34.5	36.4	23.2	27.5
Cordyceps militaris	35.4	36.5	25.3	28.0
Scleroglucan	74.3	75.5	15.4	33.2
Curdlan	82.0	85.3	76.8	78.0
Yeast β-glucan (20701)	77.3	79.1	70.6	76.9
Barley β-glucan	79.3	85.3	78.5	86.1
Wheat starch (Sigma Lot SS127-5Kg)	88.7	89.9	89.4	90.7

[a] All values are reported on a dry weight basis (dwb). Glucose concentration was determined accurately using GOPOD reagent before incubation with acid.

Table 7. A comparison of enzymatic and acid hydrolysis procedures for the measurement of β-glucan in four mushroom species

	β-Glucan, g/100 g (dry weight basis)				
Mushroom sample	H_2SO_4 procedure[a]	HCl procedure[a]	GEM assay as published (lyticase at pH 5)[b]	Modified GEM assay (lyticase at pH 7.1)[c]	Enzymatic procedure (Kit K-EBHLG)[d]
Trametes versicolor	50.5	52.8	3.9	7.4	29.5
Ganoderma lucidum	54.0	23.6	6.8	25.4	8.8
Grifola frondosa	32.8	29.8	6.0	8.3	15.9
Cordyceps militaris	34.3	28.6	13.6	15.8	12.3

[a] Acid hydrolysis procedures were performed as described in materials and methods and β-glucan was calculated by subtracting determined α-glucan from the total glucan value (*see* Table 4).

[b] The glucan enzymatic method (GEM) assay performed exactly as described by the authors (16) where the pH of incubation with lyticase is ~5.0.

[c] The GEM assay performed at a pH more suitable for lyticase activity (pH 7.1, information from Sigma Chemical Co.).

[d] Assays performed according to a commercial test kit for yeast β-glucan (Megazyme Cat. No. K-EBHLG).

as published (17), incubation with lyticase was performed at pH ~5.0, whereas the pH optima for this enzyme is 7.0–7.5. Consequently, Table 7 also includes data for the GEM assay in which the lyticase step was performed at pH 7.1. Also shown in Table 7 are the β-glucan values obtained for these samples using the H_2SO_4 and HCl methods. For each of the samples analyzed, much higher total glucan (and thus β-glucan) values were obtained with the H_2SO_4 procedure than with any of the enzymatic procedures, so enzymatic determination was not studied further. Each of the enzymatic methods provides quantitative hydrolysis of pachyman, curdlan, and scleroglucan, but also completely hydrolyzes barley 1,3:1,4-β-glucan; so analytical specificity is not possible if cereal β-glucans are present with the mushroom products.

The reproducibility of HCl and H_2SO_4 acid hydrolysis methods across seven samples is shown in Tables 8 and 9. Samples were analyzed in duplicate during 4 days, and average values were determined together with standard deviations and coefficient of variation. The preincubation time used with 12 M HCl (at 30°C) was 1 h and with the 12 M H_2SO_4 (in an ice–water bath), it was 2 h. All samples were then diluted to the appropriate acid concentration and incubated at 100°C for 2 h. The values obtained with both of the acid hydrolysis procedures demonstrate that within-day repeatability and between-day reproducibility is very good. The highest standard deviation (6.2) and coefficient of variation (14.0%) was obtained with *Ganoderma lucidum* mushroom with the HCl hydrolysis procedure (Table 9). The most likely reason for this is that with HCl, the glucan in this sample is not completely hydrolyzed and, thus, slight differences in incubation times or water-bath temperature are likely to have a more significant effect on determined values. With H_2SO_4 hydrolysis, the measured total glucan is doubled, and coefficient of variation is reduced to 1.6%.

Having settled on the analytical method of choice (H_2SO_4 hydrolysis format), the method was applied to the measurement of the total glucan, α-glucan (starch/glycogen), and β-glucan

Table 8. Repeatability of H_2SO_4 acid hydrolysis procedure for the measurement of total glucan in mushroom products

	Total glucan, % (w/w),[a] mean[b] ±2 SD (CV,[c] %)				
Sample	Day 1	Day 2	Day 3	Day 4	Interday mean ±2 SD (CV, %)
Trametes versicolor	45.5 ± 2.8	45.8 ± 3.2	48.3 ± 4.2	49.1 ± 4.4	47.2 ± 4.3
	(3.0%)	(3.4%)	(4.4%)	(4.5%)	(4.6%)
Ganoderma lucidum	52.4 ± 2.6	51.8 ± 2.4	52.7 ± 0.2	53.1 ± 0.5	52.5 ± 1.7
	(2.5%)	(2.3%)	(0.2%)	(0.4%)	(1.6%)
Agaricus blazei	16.2 ± 1.4	16.2 ± 0.9	16.9 ± 0.6	16.7 ± 0.2	16.5 ± 0.9
	(4.4%)	(2.8%)	(1.6%)	(0.6%)	(2.9%)
Grifola fondosa	35.6 ± 1.3	35.1 ± 1.6	37.8 ± 0.9	37.1 ± 0.5	36.4 ± 2.5
	(1.8%)	(2.2%)	(1.1%)	(0.7%)	(3.4%)
Cordyceps militaris	36.8 ± 1.7	36.8 ± 1.8	37.4 ± 0.3	37.3 ± 0.2	37.1 ± 1.1
	(2.2%)	(2.5%)	(0.4%)	(0.2%)	(1.5%)
Purified yeast β-glucan (Megazyme Lot No. 20301)	75 ± 5.7	75.5 ± 3.3	77 ± 0.1	77.4 ± 0.1	76.2 ± 3.3
	(3.8%)	(2.2%)	(0.1%)	(0.04%)	(2.2%)
Aspergillus niger mycelium (49% β-glucan Megazyme Lot No. 130905a)	53.7 ± 0.4	53.3 ± 1	54 ± 0.6	54.8 ± 0.9	54 ± 1.2
	(0.4%)	(0.9%)	(0.5%)	(0.8%)	(1.1%)

[a] All results are presented as total glucan as a percentage of dry weight of sample. α-Glucan and glucose contents of these samples are low and can be seen in Table 4.

[b] On each day, samples of each material were analyzed in duplicate.

[c] SD = Standard deviation; CV = coefficient of variation.

Table 9. Repeatability of HCl acid hydrolysis procedure for the measurement of total glucan in mushroom products

	Total glucan, % (w/w),[a] mean[b] ±2 SD (CV,[c] %)				
Sample	Day 1	Day 2	Day 3	Day 4	Interday mean ±2 SD (CV, %)
Trametes versicolor	55.2 ± 2	52 ± 4.7	53.2 ± 1.3	53.6 ± 0.3	53.5 ± 3.2
	(1.8%)	(4.6%)	(1.2%)	(0.3%)	(3.0%)
Ganoderma lucidum	20.7 ± 5.8	25.6 ± 1.4	21.3 ± 7.2	20.7 ± 6.6	22.1 ± 6.2
	(14.0%)	(2.7%)	(16.9%)	(16.0%)	(14.0%)
Agaricus blazei	11.7 ± 1.8	11.7 ± 0.7	11.9 ± 0.8	12 ± 0.1	11.9 ± 0.8
	(7.9%)	(2.9%)	(3.3%)	(0.6%)	(3.6%)
Grifola fondosa	31.5 ± 2.4	34 ± 1	32.2 ± 0.7	31.8 ± 1.5	32.4 ± 2.4
	(3.7%)	(1.4%)	(1.0%)	(2.4%)	(3.7%)
Cordyceps militaris	30.5 ± 0	31.9 ± 0.7	30.1 ± 0.1	31 ± 0.5	30.9 ± 1.5
	(0.04%)	(1.0%)	(0.2%)	(0.8%)	(2.4%)
Purified yeast β-glucan (Megazyme Lot No. 20301)	76.7 ± 0.8	76.8 ± 0.5	75.8 ± 0	76.9 ± 1.5	76.6 ± 1.2
	(0.5%)	(0.3%)	(0.01%)	(1.0%)	(0.8%)
Aspergillus niger mycelium (49% β-glucan Megazyme Lot No. 130905a)	47.9 ± 3.2	50.2 ± 1.6	49 ± 0.5	50.3 ± 1.3	49.4 ± 2.5
	(3.3%)	(1.6%)	(0.5%)	(1.3%)	(2.6%)

[a] All results are presented as total glucan as a percentage of dry weight of sample. α-Glucan and glucose contents of these samples are low and can be seen in Table 4.

[b] On each day, samples of each material were analyzed in duplicate.

[c] SD = Standard deviation; CV = coefficient of variation.

(by difference) contents of a range of commercial mushroom/mycelium products sold in capsule form. Results obtained are shown in Table 10. Clearly, for most of these products, the major component is α-glucan. Commercially, mushroom products are produced in two ways: grown and harvested as mushroom fruiting bodies (Figure 2a and b) or alternatively grown as mushroom mycelium over a sterilized cereal grain base (Figure 2c and d). In the latter process, the standard procedure is to harvest the mycelium-infested grain, dry the product, and mill ready for inclusion in capsules or tablets. Clearly, based on the analytical data, it is apparent that the bulk of the starch in the grain remains intact and is by far the major glucan present in the final product. The β-glucan content in these types of products is much lower than that found in most mushrooms (Table 4). α-Glucan in most of the mushroom species analyzed was <4% w/w, and in many it was <1% w/w.

Hydrolysis of a number of pure β-glucans by H_2SO_4 is shown in Table 3. Cereal β-glucan is completely hydrolyzed and

Table 10. Total glucan,[a] α-glucan,[a] and β-glucan contents of a range of encapsulated mushroom and mycelium based products

	Sample details	Total glucan (g/100 g)	α-Glucan (g/100 g)	β-Glucan (g/100 g)
1	*Ganoderma lucidum*	74.3	29.2	45.1
2	16 *Basidiomycete* species blend	69.5	66.4	3.2
3	7 *Basidiomycete* species blend	73.7	72.5	1.3
4	*Ganoderma lucidum*	44.6	22.6	22.0
5	*Ganoderma lucidum*	87.7	83.2	4.3
6	*Ganoderma lucidum*/*Lentinula edodes*	59.9	41.9	18.0
7	*Cordyceps* sp. (ascomycete)	64.8	53.9	10.9
8	*Cordyceps* sp. (ascomycete)	65.5	64.0	1.5
9	*Ganoderma lucidum*	52.5	45.2	7.3
10	*Cordyceps sinensis* (ascomycete)	13.9	3.0	10.9
11	*Cordyceps sinensis* (ascomycete)	29.3	24.1	5.2
12	*Inonotus obliquua*	69.8	70.0	~0.0
13	Control (*A. niger* mycelium) 49% β-glucan	51.9	1.0	50.9

[a] Total glucan and α-glucan include any free glucose in the sample and glucose derived from the hydrolysis of sucrose.

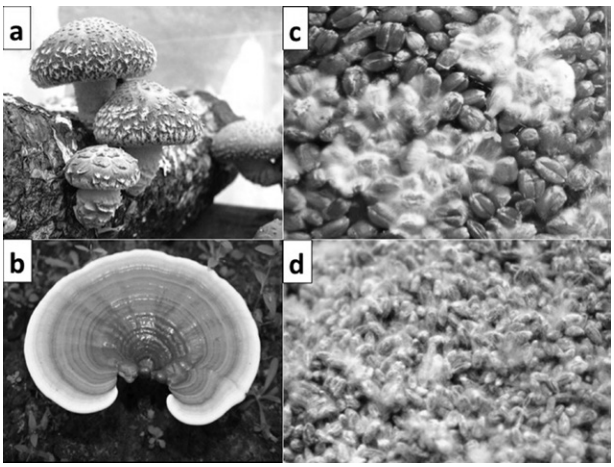

Figure 2. Mushroom samples and mushroom mycelium infested grain. (a) *Lentinula edode* (Shitake) mushrooms; (b) *Ganoderma lucidum* (Reishi) mushroom; (c) wheat infested with mushroom mycelium (early stage); and (d) wheat infested with mushroom mycelium (late stage).

there is substantial hydrolysis of α-cellulose. This means that contamination of mushroom β-glucan with cereal β-glucan or cellulose cannot be detected with either an HCl or H_2SO_4 acid hydrolysis procedure. However, because cereal β-glucan can be specifically determined enzymatically, the presence of this in mushroom products is easily determined (15). Adulteration with cellulose is more difficult to determine, but procedures based on the use of *endo*-1,4-β-glucanase (cellulase) could be developed if required.

Conclusions

Several procedures for the measurement of β-glucan in mushroom samples and products have been evaluated in this study. Enzymatic procedures using lyticase and 1,3-β-glucanases are not suitable, as they significantly underestimate the β-glucan content of all of the mushroom samples analyzed. Acid hydrolysis with TFA also results in underestimation of the β-glucan content of most of the samples analyzed. This result is partly due to ineffective solubilization of some glucan material as well as further degradation of a proportion of the released glucose during the acid hydrolysis step. For most samples, HCl and H_2SO_4 behave similarly, however, for certain samples such as *Ganoderma lucidum*, significantly higher values for β-glucan content are obtained with H_2SO_4. In most cases, the additional incubation with a mixture of *exo*-1,3-β-glucanase plus β-glucosidase increases the measured glucose by approximately 2% w/w, and thus this step is recommended. The current studies are consistent with the work of Selvendran et al. (28) on the analysis of plant cell walls and of Dallies et al. (27) for yeast cell walls, indicating that the preferred acid for hydrolysis of polysaccharides is H_2SO_4. For the measurement of total glucan in mushroom products, we thus recommend hydrolysis with H_2SO_4 as described here, followed by incubation with *exo*-1,3-β-glucanase/β-glucosidase to ensure complete hydrolysis of laminari-oligosaccharides to glucose. α-Glucan is measured specifically using a starch assay procedure, and β-glucan is determined by the difference.

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