Comparative Chemical Analysis of Fiber Material Prepared by Enzymatic and Chemical Methods from Two Mushrooms (*Pleurotus sajor-caju* and *Pleurotus tuber-regium*)

Peter C. K. Cheung* and Man Yi Lee

Department of Biology, The Chinese University of Hong Kong, Shatin, New Territories, Hong Kong

The fiber material of *Pleurotus sajor-caju* (oyster mushroom) and *Pleurotus tuber-regium* (tiger milk mushroom) was prepared by two protocols: the enzymatic approach based on the Association of Official Analytical Chemists (AOAC) total dietary fiber (TDF) method and the nonenzymatic method based on the cell wall material (CWM) isolation by the sequential use of chemical solvents. The gravimetric yield of the TDF of the two mushrooms obtained by the enzymatic method was higher than that of the CWM prepared according to the chemical method. The chemical method was more effective than the enzymatic one in removing the mushroom protein. However, some polymeric materials (including fiber material) were solubilized in the chemical solvents used to remove protein and starch from the mushroom. The nonstarch polysaccharide content and composition of the mushroom fiber obtained from the above varied depending on the morphology and types of cell wall polysaccharides present in the two *Pleurotus* species.

Keywords: *Mushroom; fiber material; Pleurotus; enzyme; chemical solvent*

INTRODUCTION

The principal components of fiber material of plants are mainly nonstarch polysaccharides (NSP) and some minor noncarbohydrate substances such as polyphenols (lignin), cutin, and proteins (Selvendran, 1983). In the preparation of fiber material from food plants, the conventional method involves a nonenzymatic method that is usually based on the removal of nonfiber material (mainly protein and starch) by chemical solvents (Selvendran and O'Neill, 1987). The treatment of plant material by alcohol to produce an alcohol-insoluble residue (AIR) is a relatively easy and quick method for obtaining cell wall material (CWM). Moreover, this method is quite efficient for the inactivation of enzymes but avoids significant degradation of cell wall polysaccharides. However, the AIR contains much coprecipitated intracellular proteins, nucleic acids, polyphenols, and their condensed products with proteins and starch (Newcomb, 1963; Loomis and Battaile, 1966; Selvendran and O'Neill, 1987). To minimize such coprecipitation effects, the use of aqueous solvents that have a strong affinity for intracellular compounds, particularly proteins, phenolics, and oil, is preferred. Common chemical extractants include sodium dodecyl sulfate (SDS), phenol/acetic acid/water (PAW), and dimethyl sulfoxide (DMSO) (Selvendran and O'Neill, 1987).

An alternative approach is to use specific enzymes to remove starch and proteins from the plant materials followed by either a gravimetric or chemical quantification of the fiber residues in terms of its carbohydrate and noncarbohydrate components (Prosky, 1988; Theander et al., 1994). Sources of commonly used enzymes to remove starch and proteins include both microbial (bacterial α-amylases and protease) and animal (pancreatin and trypsin) origins.

Owing to the different mechanisms, the chemical and enzymatic treatments of the plant materials may cause different extents of degradation to the fiber materials, especially cell wall polysaccharides, during the removal of the nonfiber material. Hence, it is envisaged that fiber materials obtained by these two methods may have different chemical and physical characteristics that may eventually affect their technological properties when applied to the food industry as well as their nutritional functions in human health (Edwards, 1995; McDougall et al., 1996).

In recent years, most research work on plant fiber has mainly been focused on fruits, vegetables, cereals, and grains (Englyst et al., 1988; Potty, 1996; Thebaudin et al., 1997). Lower plants such as mushroom (edible fungi) as an alternative source of plant fiber have been little studied (Cheung, 1997). In general, the CWM of mushrooms contain NSP such as chitin [β(1−4)-*N*-acetylglucosamine] and hemicelluloses (β-glucans and mannans), which are the major constituents of mushroom fiber (Bartnicki-Garcia, 1968). Although *Pleurotus sajor-caju* and *Pleurotus tuber-regium* are two edible mushrooms from the same genus, their edible forms have very contrasting morphological differences. Unlike the conventional edible form of a soft and fleshy fruiting body (cap) produced in *P. sajor-caju*, *P. tuber-regium* forms very hard and solid sclerotia, which have many culinary uses as well as medicinal purposes (Oso, 1977). In this study, the fiber materials of the edible forms of these two mushrooms were prepared according to the chemical extraction method (Selvendran and O'Neill, 1987) and enzymatic procedures (Prosky et al., 1988). The yields, content, and composition of the NSP in the mushroom fiber obtained by these two methods were compared.

* Author to whom correspondence should be addressed (telephone 8520 26096144; fax 8520 26035646; e-mail petercheung@cuhk.edu.hk].

10.1021/jf980561y CCC: $15.00
Published on Web 12/04/1998

MATERIALS AND METHODS

Materials. Fruiting bodies of *P. sajor-caju* were cultivated in the laboratory by using cotton wastes as the compost materials. The fresh mushroom was cleaned to remove any residual compost and freeze-dried. The dried samples were finely ground (0.5 mm in size) in a Cyclotech mill (Tecator, Hoganas, Sweden). Sclerotia of *P. tuber-regium* were cultivated in the Sanming Mycological Institute in the Fujian Province of Mainland China. The sclerotia were rinsed to remove the soil and dust adhered on their outer covering, dried, and milled similarly as *P. sajor-caju*.

Enzymatic Preparation. The fiber materials in the mushroom samples were prepared according to the AOAC total dietary fiber (TDF) method (Prosky et al., 1988). In brief, aliquots of samples (1 g of dry matter) were treated with two amylases, a heat-stable bacterial α-amylases (EC 3.2.1.1 from *Bacillus licheniformis*, catalog no. A3306, Sigma Chemical Co., St. Louis, MO) for 30 min in a boiling water bath and a fungal amyloglucosidase (EC 3.2.1.3 from *Aspergillus niger*, catalog no. A3513, Sigma) for 30 min at 60 °C to remove starch and a bacterial protease (from Subtilisin Carlsberg, catalog no. P3910, Sigma) to solubilize protein. The amylase enzymes used had been tested to be free of β-glucanase. The enzyme-treated mixture containing the buffer solution and nondigestible materials was precipitated with 78% ethanol. The ethanol-insoluble residue recovered was oven-dried and weighed to give the gravimetric yield of the mushroom fiber material or TDF.

Chemical Preparation. In the chemical extraction method, a modified procedure from Selvendran and O'Neill (1987) was followed. Basically, the mushroom fiber material was obtained after sequential treatment of the mushroom sample (4 g) with 1% aqueous SDS (40 mL) at room temperature for 2 h (two times), PAW (2:1:1, w/v/v) (100 mL) at room temperature for 1 h (two times), and 90% aqueous DMSO (200 mL) at room temperature for 16 h. The final residue was washed with distilled water (six times), dialyzed against water, and freeze-dried to give the mushroom fiber material or CWM. Polymeric materials solubilized in the SDS (PMS) was recovered from the SDS fraction after extensive dialysis against water and freeze-drying.

Chemical Composition. The NSP content and composition of the mushroom fiber material prepared according to the above two methods including the PMS material were determined by gas chromatography. The samples were subjected to Saeman hydrolysis (Saeman et al., 1963) with the following modified conditions: 72% sulfuric acid for 1 h at 35 °C followed by dilution to 2 M sulfuric acid for 1 h at 100 °C. Alditol acetates of the neutral and amino sugars in the acid hydrolysates were prepared according to the method as described by Blakeney et al. (1983) with β-D-allose as the internal standard. The alditol acetate derivatives of the polysaccharide sugars were quantified by an HP6890 gas chromatograph (GC) using an Alltech DB-225 capillary column (15 m × 0.25 mm i.d., 0.25 μm film) and an oven temperature program of initial temperature 170 °C, followed by a temperature rise of 2 °C/min to 220 °C with a final hold of 10 min. The carrier gas was helium, and detection was by flame ionization. Individual sugars were corrected for losses during hydrolysis and derivatization and for the differences in their responses to the GC detector by a molar correction factor determined from the recovery of sugar standards subjected to the same treatment as the mushroom fiber samples. The values for monosaccharides were expressed as polysaccharide residues (anhydrosugars) by multiplying the amounts of pentoses and deoxypentoses with a factor of 0.88 and of hexoses with a factor of 0.90. The uronic acid content was determined colorimetrically using 3,5-dimethylphenol with D-galacturonic acid monohydrate as the standard and expressed as a polysaccharide residue by multiplying by a factor of 0.89 (Scott, 1979). The carbohydrate content (NSP) in the mushroom fiber preparation was calculated as the sum of neutral, amino, and uronic acid polysaccharide residues. Residual protein content in the mushroom fiber material was estimated by multiplying the nitrogen (N) content by a factor of 6.25, which was determined by a CHNS/O Analyzer (Perkin-Elmer 2400, Norwalk, CT,) after correction for chitin N (Cheung, 1997). The noncarbohydrate content of the mushroom fiber material was calculated by difference.

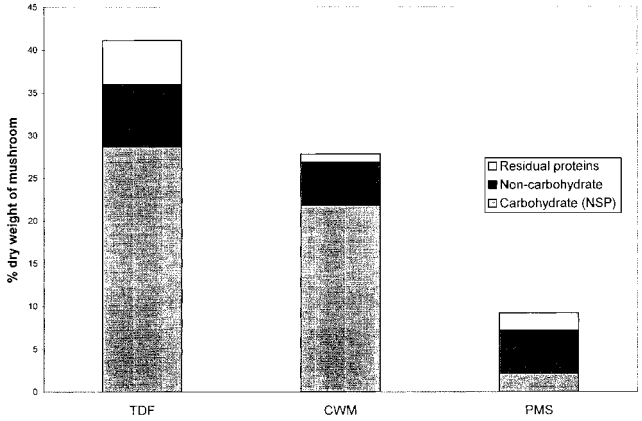

Figure 1. Chemical composition of the fiber material obtained by enzymatic and chemical extraction protocols from *P. sajor-caju*.

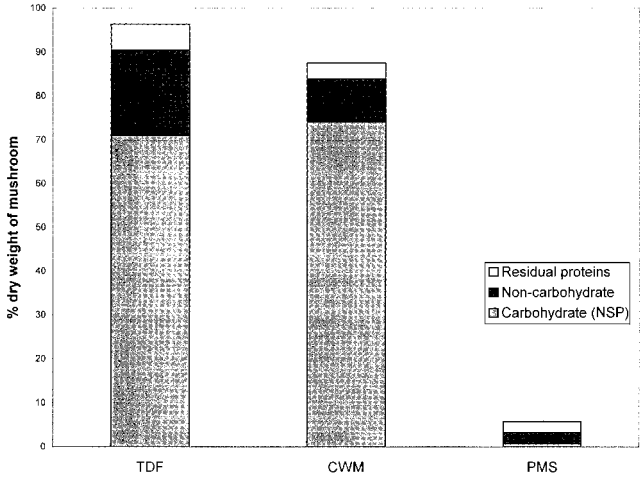

Figure 2. Chemical composition of the fiber material obtained by enzymatic and chemical extraction protocols from *P. tuber-regium*.

Statistical Analysis. All determinations were carried out in triplicate. The results were analyzed by Student's *t* test ($p < 0.05$) to determine the significance of differences between the mean values obtained from the two fiber methods.

RESULTS AND DISCUSSION

Figures 1 and 2 show the chemical composition of the fiber material obtained by the enzymatic method (TDF) and the chemical extraction method (CWM and PMS) from *P. sajor-caju* and *P. tuber-regium*, respectively. The yield of fiber material obtained from *P. sajor-caju* by the enzymatic method (TDF) (41.4 ± 0.94% dry weight) appeared to be much higher than that prepared from the chemical method (CWM) (27.8 ± 0.01% dry weight). A substantial amount of polymeric materials from *P. sajor-caju* solubilized in the SDS (PMS) was also recovered (9.19 ± 0.83% dry weight). Although a similar trend was observed in *P. tuber-regium*, the difference between the yields of the TDF (96.3 ± 0.46% dry weight) and the CWM (87.5 ± 1.74% dry weight) was not as great as in the *P. sajor-caju*. Moreover, relatively less PMS (5.75 ± 0.32% dry weight) was found *in P. tuber-regium* as compared to *P. sajor-caju*. In terms of the amount of fiber material, our results on *P. sajor-caju* were in agreement with the fiber content of

Table 1. Monosaccharide Composition of the Nonstarch Polysaccharides in the Fiber Material of *P. sajor-caju* Obtained by Enzymatic and Chemical Extraction Protocols Expressed as Percent of Total Polysaccharide Sugars[a]

monosaccharide	TDF	CWM	PMS
fucose	0.38 ± 0.04	1.07 ± 0.62	ND
rhamnose	0.18 ± 0.01	ND[b]	1.49 ± 0.09
ribose	0.59 ± 0.42	0.98 ± 0.15	ND
xylose	0.76 ± 0.02	0.73 ± 0.07	ND
mannose	4.51 ± 0.03	1.81 ± 0.10	18.7 ± 0.43
galactose	3.09 ± 0.02	ND	36.9 ± 0.62
glucose	77.8 ± 0.70	80.1 ± 1.42	33.0 ± 2.18
glucosamine	10.6 ± 0.18	12.2 ± 1.22	10.1 ± 1.04
uronic acids	2.04 ± 0.09	3.19 ± 0.19	ND

[a] All values are on percent of the total polysaccharide sugars and are means ± SD of triplicate measurements. [b] ND, not detected.

the fruiting bodies of other *Pleurotus* species, which ranged from 30 to 40% dry weight (Kurasawa et al., 1982). Although the exceptionally high fiber content found in *P. tuber-regium* in our study was much higher than the previous result (crude fiber content of 10% dry weight) (Fasidi and Ekuere, 1993), it was similar to that of other mushroom sclerotia, such as those from *Poria cocos* (Cheung, 1997).

The apparent higher yield of the TDF compared to the CWM found in the two *Pleurotus* species was partially due to the presence of a larger amount of residual proteins in the former. This could be due to the protein coprecipitation during the ethanol precipitation step in the AOAC method and the inaccessibility of protein by the protease due to the presence of the plant fiber. It has been shown in plant foods that high fiber content can lead to low enzyme digestibility of protein (Mongeau et al., 1989; Baer et al., 1997). Our results suggested that the chemical method seemed to be more effective in removing mushroom protein than the enzymatic ones. The incomplete removal of intracellular proteins from both the mushroom TDF and CWM could be due to the problems of oxidation and dehydration during the sample preparation, especially the freeze-drying process in *P. sajor-caju*. Moreover, the physically dehydrated form of *P. tuber-regium* would possibly make deproteination more difficult.

During the preparation of CWM, small amounts of detergent-soluble cell wall polymers (including protein and NSP) solubilized in the SDS fraction (PMS) had been recovered after dialysis to remove the low molecular weight compounds. The PMS fraction from *P. sajor-caju* contained quite a significant amount of NSP, which was ~10% of the total NSP found in the CWM. Apart from the carbohydrate (NSP) and protein components, all of the mushroom fiber materials including TDF, CWM, and PMS contained substantial amounts of noncarbohydrate polymeric substances (Figures 1 and 2), which might consist of polyphenolic materials such as lignin and tannins.

Tables 1 and 2 show the monosaccharide composition of the fiber material obtained by the enzymatic method (TDF) and the chemical method (CWM) for *P. sajor-caju* and *P. tuber-regium*, respectively. In general, the dominant sugar residues present in the NSP of both fiber materials obtained by the two methods were glucose (78−89%) and glucosamine (7−12%). This result was consistent with the major polysaccharides (β-glucans and chitin) known to be present in mushroom (Bartnicki-Garcia, 1968). Other minor sugar residues

Table 2. Monosaccharide Composition of the Nonstarch Polysaccharides in the Fiber Material of *P. tuber-regium* Obtained by Enzymatic and Chemical Extraction Protocols Expressed as Percent of Total Polysaccharide Sugars[a]

monosaccharide	TDF	CWM	PMS
fucose	0.38 ± 0.01	0.46 ± 0.05	ND
arabinose	0.14 ± 0.03	ND[b]	ND
mannose	0.68 ± 0.02	ND	22.9 ± 3.81
galactose	0.15 ± 0.04	ND	6.55 ± 0.84
glucose	89.2 ± 0.26	87.6 ± 0.11	36.8 ± 7.08
glucosamine	7.33 ± 0.12	10.1 ± 0.05	33.8 ± 8.10
uronic acids	2.08 ± 0.17	1.84 ± 0.16	ND

[a] All values are on percent of the total polysaccharide sugars and are means ± SD of triplicate measurements. [b] ND, not detected.

Table 3. Monosaccharide Content of the Nonstarch Polysaccharides in the Fiber Material of *P. sajor-caju* Obtained by Enzymatic and Chemical Extraction Protocols Expressed as Percent Dry Weight of Mushroom Sample[a]

monosaccharide	TDF	CWM + PMS
fucose	0.11 ± 0.01	0.24 ± 0.16
rhamnose	0.05 ± 0.00[x]	0.03 ± 0.00[y]
ribose	0.17 ± 0.12	0.21 ± 0.02
xylose	0.22 ± 0.01[x]	0.16 ± 0.01[y]
mannose	1.29 ± 0.05[x]	0.80 ± 0.01[y]
galactose	0.88 ± 0.02[x]	0.80 ± 0.01[y]
glucose	22.3 ± 0.53[x]	18.2 ± 0.84[y]
glucosamine	3.05 ± 0.13	2.88 ± 0.45
uronic acids	0.58 ± 0.04[x]	0.69 ± 0.03[y]
total	28.7 ± 0.89[x]	24.0 ± 1.46[y]

[a] All values are means ± SD of triplicate measurements. Means in rows with different superscripts (x, y) are significantly different ($p < 0.05$, t test).

Table 4. Monosaccharide Content of the Nonstarch Polysaccharides in the Fiber Material of *P. tuber-regium* Obtained by Enzymatic and Chemical Extraction Protocols Expressed as Percent Dry Weight of Mushroom Sample[a]

monosaccharide	TDF	CWM + PMS
fucose	0.27 ± 0.00	0.34 ± 0.03
arabinose	0.10 ± 0.02	ND[b]
mannose	0.48 ± 0.01[x]	0.15 ± 0.01[y]
galactose	0.11 ± 0.03	0.04 ± 0.00
glucose	63.3 ± 0.99	65.1 ± 2.90
glucosamine	5.20 ± 0.18[x]	7.70 ± 0.30[y]
uronic acids	1.48 ± 0.14	1.37 ± 0.12
total	71.0 ± 1.29	74.8 ± 3.23

[a] All values are means ± SD of triplicate measurements. Means in rows with different superscripts (x, y) are significantly different ($p < 0.05$, t test). [b] ND, not detected.

found in the mushroom NSP such as mannose and uronic acids indicated the presence of small amounts of mannans and polyuronides in the mushroom fiber materials (Tables 1 and 2). Galactose was not found in the CWM of *P. sajor-caju* but was present in significant amount in its PMS fraction (Table 1). This was due to the preferential solubilization of galactans in the SDS solvent (PMS) during the preparation of the mushroom CWM. Moreover, the low levels of uronic acid, the absence of arabinose, and the extractability of galactose in SDS would suggest that pectic polysaccharides were not present in the mushroom fiber material and that most galactans present would probably be intracellular. The uronic acid would most likely be glucuronic acid, present as the major component of hemicelluloses, which contain other neutral sugars such as fucose,

mannose, and galactose (Ruiz-Herrera, 1992). Overall, the composition of the NSP in *P. sajor-caju* differed from that of *P. tuber-regium* in that the former contained more mannans and galactans but less β-glucans than the latter.

As mentioned earlier, NSP in general is the major component of fiber material (Selvendran, 1983); therefore, its content would be a good indicator of the "purity" of the fiber material isolated from the mushroom. Tables 3 and 4 show the content of monosaccharides and NSP found in the fiber material obtained from *P. sajor-caju* and *P. tuber-regium*, respectively. Whereas the NSP content of the TDF was significantly higher ($p < 0.05$) than that of the combined CWM and PMS in *P. sajor-caju*, there were no such differences found in *P. tuber-regium*. It was found that the amounts of rhamnose, xylose, galactose, glucose, and uronic acids in the combined CWM and PMS of *P. sajor-caju* were significantly less ($p < 0.05$) than those in the TDF (Table 3). In *P. tuber-regium*, the monosaccharide contents between the TDF and the combined CWM and PMS were very similar except for mannose and glucosamine (Table 4). This implied that during the preparation of fiber material in *P. sajor-caju*, relatively greater loss of NSP might have occurred in the chemical solvent treatment than in the enzymatic ones. It is known that PAW and DMSO treatment can also solubilize small amounts of NSP including β-glucans in plant material (Selvendran and O'Neill, 1987).

CONCLUSION

Although the mushroom fiber material prepared according to the enzymatic treatment (TDF) had a higher gravimetric yield than the chemical method (CWM), the TDF had comparatively more noncarbohydrate type fiber materials (such as lignin) and more enzyme-resistant protein. The difference in the NSP content between the TDF and CWM depended on the morphological features (fleshy fruiting bodies versus solid sclerotia) and the NSP composition in individual mushroom species. Further work is being carried out to fractionate and characterize the NSP in these mushroom fiber preparations by column chromatography and methylation study.

LITERATURE CITED

Baer, D. J.; Rumpler, W. V.; Miles, C. W.; Fahey, G. C., Jr. Dietary fiber decreases the metabolizable energy content and nutrient digestibility to mixed diets fed to humans. *J. Nutr.* **1997**, *127*, 579−586.

Bartnicki-Garcia, S. Cell wall chemistry, morphogenesis and taxonomy of fungi. *Annu. Rev. Microbiol.* **1968**, *22*, 87−108.

Blakeney, A. B.; Harris, P. J.; Henry, R. J.; Stone, B. A. A. A simple and rapid preparation of alditol acetates for monosaccharide analysis. *Carbohydr. Res.* **1983**, *113*, 291−299.

Cheung, P. C. K. Dietary fiber content and composition of some edible fungi determined by two methods of analysis. *J. Sci. Food Agric.* **1997**, *73*, 255−260.

Edwards, C. A. The physiological effects of dietary fiber. In *Dietary Fiber in Health and Disease*; Kritchevsky, D., Bonfield, C., Eds.; Eagan Press: St. Paul, MN, 1995; pp 58−71.

Englyst, H. N.; Bingham, S. A.; Runswick, S. A.; Collinson, E.; Cummings, J. H. Dietary fiber (non-starch polysaccharides) in fruit, vegetables and nuts. *J. Hum. Nutr. Diet.* **1988**, *1*, 247−286.

Fasidi, I. O.; Ekuere, U. U. Studies on *Pleurotus tuber-regium* (Fries) Singer: cultivation, proximate composition and mineral contents of sclerotia. *Food Chem.* **1993**, *48*, 255−258.

Kurasawa, S. I.; Sugahara, T.; Hayashi, J. Studies on dietary fiber of mushrooms and edible wild plants. *Nutr. Rep. Int.* **1982**, *26*, 167−173.

Loomis, W. D.; Battaile, J. Plant phenolic compounds and the isolation of plant enzymes. *Phytochemistry* **1966**, *5*, 423−438.

McDougall, G. J.; Morrison, I. M.; Stewark, D.; Hillman, J. R. Plant cell walls as dietary fiber: range, structure, processing and function. *J. Sci. Food Agric.* **1996**, *70*, 133−150.

Mongeau, R.; Sarwar, G.; Peace, R. W.; Brassard, R. Relationship between dietary fiber levels and protein digestibility in selected foods as determined in rats. *Plant Foods Hum. Nutr.* **1989**, *39*, 45−51.

Newcomb, E. H. Cytoplasm-cell wall relationships. *Annu. Rev. Plant Physiol.* **1963**, *14*, 43−64.

Oso, B. A. *Pleurotus tuber-regium* from Nigeria. *Mycologia* **1977**, *69*, 271−279.

Potty, V. H. Physio-chemical aspects, physiological functions, nutritional importance and technological significance of dietary fibers—A critical appraisal. *J. Food Sci. Technol.* **1996**, *33*, 1−18.

Prosky, L.; Asp, N.-G.; Schweizer, T. F.; DeVires, J. W.; Furda, I. Determination of insoluble, soluble and total dietary fiber in foods and food products: interlaboratory study. *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 1017−1023.

Ruiz-Herrera, J. Chemical composition of the fungal cell wall. In *Fungal Cell Wall: Structure, Synthesis, and Assembly*; CRC Press: Boca Raton, FL, 1992; pp 5−22.

Saeman, J. F.; Moore, W. E.; Millet, M. A. Sugar unit present. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Ed.; Academic Press: New York, 1963; Vol. 3, pp 54−69.

Scott, R. W. Colorimetric determination of hexuronic acids in plant materials. *Anal. Chem.* **1979**, *51*, 936−941.

Selvendran, R. R. The chemistry of plant cell walls. In *Dietary Fiber*; Birch, G. G., Parker, K. J., Eds.; Applied Science: London, 1983; pp 95−147.

Selvendran, R. R.; O'Neill, M. A. Isolation and analysis of cell walls from plant material. In *Methods of Biochemical Analysis*; Glick, D., Ed.; Springer-Verlag: New York, 1987; Vol. 32, pp 25−153.

Theander, O.; Aman, P.; Westerlund, E. Enzymatic/chemical analysis of dietary fiber. *J. Assoc. Off. Anal. Chem. Int.* **1994**, *77*, 703−709.

Thebaudin, J. Y.; Lefebvre, A. C.; Harrington, M.; Bourgeois, C. M. Dietary fibers: nutritional and technological interest. *Trends Food Sci. Technol.* **1997**, *8*, 41−48.

Received for review May 26, 1998. Revised manuscript received August 18, 1998. Accepted August 18, 1998. This work is financially supported by the Research Grant Council of the Hong Kong SAR government.

JF980561Y