Australian Journal of Basic and Applied Sciences, 2(3): 632-642, 2008
ISSN 1991-8178

Characterization of Oyster Mushroom Mycelia as a Food Supplement

Hesham A. Moharram, Manal F. Salama and Azza. A. Hussien

Food Technology Department. National Research Centre, Dokki, Cairo, Egypt.

Abstract: The suitability of using oyster mushroom mycelia insoluble non starch polysaccharides (INSP's) as a food supplement is evaluated from the structural, functional and antioxidative properties point of views. The structure of these oyster INSP's were confirmed using FT-IR-FT Raman spectroscopy. This technique has indicated the presence of chitin which is covalently bound to β-glucan. It is suggested that this structure is considered to be the main matrix and fibrillar components of the cell wall polysaccharides in this oyster mycelia. The enhancement in the oyster mushroom INSP'S functional properties measured as water binding, fat binding and emulsification capacities were clearly noticed compared to the wheat bran dietary fibres. Also the mycelia methanolic extract exhibited marked antiradical activity when the DPPH- ESR technique was applied for detecting the antioxidant activity.

Key words: oyster mushroom mycelia- structural properties- FT-IR-FT Raman spectroscopy- thermal analysis- Insoluble Non-starch polysaccharides (INSPs)- Functional Properties- Antioxidative properties- DPPH-ESR technique

INTRODUCTION

Edible mushrooms have been widely utilized as a human foods for centuries and have been appreciated for texture and flavour as well as some medicinal and tonic attributes (Manzi *et al.,* 2001). However, the awarness of mushrooms as a healthy food and as an important source of biological active substances with medicinal value has only recently emerged (Cheung, 1997). Mushrooms are considered as healthy food because they are low in calories and fat but rich in proteins and dietary fibers (Manzi *et al.,* 1999).

Mushrooms are cooked with foods for preparing the highly nutritive soups, or it is used with other herbs as a strengthen agent. The soup or extract is consumed as a functional foods. It was shown that these extracts enhanced the phagocytic activity and improved the clearance of the injected foreign proteins (Salnlin, 1995). Mushroom concentrates, can also be freeze or spray dried to form granular powders for ease of handling and transportation, these mushrooms are available as a mixture of mushroom extracts and vitamin C crystals, or with other medicinal plants such as ginseng.

Other mushrooms can be used in the manufacture of snack cake for middle aged and old people. This cake is prepared with a mixture of mushroom powders, corn flour and soybean proteins (Minzuno *et al.,* 1995 and Salama, 2001).

From the nutritional point of view, mushrooms contain appreciable amounts of dietary fibre, particularly important for the regulation of physiological functions in human organism (Manzi *et al.,* 2001). These functional substances are able to lower cholesterolemia, modulate the immune system and inhibit tumor growth (Zhang *et al.,* 2001). In particular, chitin (N-acetyl D-glucosamine polymer) a nitrogen containing polysaccharide of the fungal cell walls and chitosan, their deactylated derivatives are responsible for decreasing the physiological cholesterol pool (Rebect *et al.,* 1999); Also β-glucan are also effective in lowering blood cholesterol levels and glycemic response(cheung, 1998). Recently, some studies have been carried out to evaluate the antioxidative activity of speciality commercial mushrooms(Mau *et al.,* 2004; Mau *et al.,* 2001 and Yang *et al.,* 2002). So, this study shed light on the evaluation of the quality of the oyster mushroom mycelia as an important food supplement to achieve a highly purified mushroom insoluble non starch polysaccharides. Functional properties such as water binding, oil binding and emulsification as well as thermal properties were measured also to get an idea about how these mushroom mycelia could be applied in various food systems as an excellent functional food with highly antioxidative properties.

Corresponding Author: Hesham A. Moharram, Food Technology Department. National Research Centre, Dokki, Cairo, Egypt.

MATERIALS AND METHODS

Materials:

Mycelia of the *pleuortus ostreatus* were obtained from the mushroom cultivation and production unit at National Research Centre, Dokki, Cairo.

Highly purified wheat bran were also obtained from the local market in a form of Bran tablets (Pharco, Egypt) and ground into fine powder.

Methods:

Preparation of the Mycelia Mushroom Powder:

The mycelia were cleaned with a minimum amount of distilled water to remove the dust and solids. The rind (outer covering) of the mycelia were removed to avoid any contaminant that might come from the compost and the plastic wrapping during cultivation, then air dried and were pulverized to pass through a mill. The milled mushroom powder were transferred to airtight plastic bags and stored at room temperature.

Preparation of the Ethanol Insoluble Residue:

Ethanol insoluble residue were prepared according to the method described by (Zivanovic *et al.,* 2000), and the residue were filtered and dried at 50^{o}C, then ground to a fine powder, sieved and stored in a dessicator.

Preparation of the Mushroom Insoluble Non-strach Polysaccharides (INSP's):

The INSP's in the mushroom was prepared by using the solvent extraction methods as outlined by (Cheung and Lee, 2000). The mixture in each extraction step was filtered through cheese cloth to separate the extracted solvent from the insoluble residue. The final insoluble residue was dialyzed and washed several times with distilled water and freeze dried.

Confirming the Structure by Using FT-IR-FT Raman Spectroscopy:

Infra red spectrum was measured by preparing potassium bromide disks containing both the mushroom ethanol insoluble residue and the purified mushroom insoluble non-strach polysaccharides in addition to Bran. FT- Raman spectra of the previously mentioned samples were obtained using a Nicolet 870 spectrometer with the Nicolet Raman moudule 32B(Madison WI, USA) and ND-YAG laser source operated at 1064nm with a maximum powder at 0.7w. The samples were used without further preparation in a NMR glass tube. The calibration was carried out in two possible means. 1st mean by internal calibration using He-Ne laser beam and 2nd mean by external calibration using polystryene reference sample. Spectra was obtained in Raman shift range between 200 and 4000 cm^{-1}. The system was operated using Omnic 5.1 software and the experiments were replicated three times.

Measurement of the Antioxidative Properties of the Dried Mycelia Mushroom Using ESR-DPPH Technique:

Methanolic extract was prepared according to the method described by (Yang et al, 2002) and adjusted to 50 mg.ml^{-1}, prior running out ESR Experiments.

The previously prepared methanolic extract (4ml) was mixed with (1ml) of methanolic solution containing DPPH 0.2 mM[15]: The resulted DPPH-H adduct was detected with electron spin resonance (ESR). Bruker-Flex-sys 5000, operated at X-band. Frequency samples were measured in a pure silica liquid tube. The ESR spectrometer set at the following conditions: 2480 to 4480 +G magnetic filed. 0.001 field modulation amplitude, 9.77537e $^{+99}$ microwave frequency (Hz), 0.0202637 microwave power (ω) at 60 receiver gain at 0.00512 (s).

Chemical Analysis:

Total nitrogen, fat and ash contents were determined using AOAC official methods as described by (AOAC, 1990).

Functional Properties:

Water holding, oil binding and emulsion stabilizing capacities were determined as outlined by (Sosulski, 1962), Thammaki *et al.,* 2004, Lin and Humbert , 1974 and Smiles *et al.,* 1989). and calculated as follows:-

- Water-holding capacity was calculated as a percentage of the amount of additional water held by a 100g sample on a 14% moisture.

- Oil binding capacity was expressed as a percentage of the amount of corn oil bound by a 100g sample of 14% moisture. And finally, emulsion stability was also estimated as a volume of emulsion remaining un-separated after centrifugation as a percentage of the original weight.

Thermal Properties:

Differential scanning colorimetery shimadzu DSC-50 was used to measure thermal parameters. The heating rate was $10C^{o}$. min^{-1} and the hold temp at $300^{o}C$. the melting temp (Tm) and enthalpy (ΔH) were determined from the thermogram.

RESULTS AND DISCUSSION

The quality of the oyster mushroom non-starch polysaccharides as a food supplement is evaluated in this research, due to their unique structure and functional properties as well as the high level of antioxidative properties.

IR and Raman Structural Features of Mushroom Mycelia:

Data in Figures (1) and (2) illustrated the structure of the cell wall components of the mushroom mycelia of both the ethanol insoluble residue and the insoluble non starch polysaccharides after purification. The obtained data could be explained in comparison with the wheat bran.

In figure(1a), the presence of IR peaks at (1031),(1112),(1236) and ($1652cm^{1}$) suggested the presence of starch, cellulose, hemicellulose and amide I group, respectively. These bands are considered to be the main characteristics bands of wheat bran (Yu *et al.,* 2004). On contrast, as seen in figures (1b) and (1c), the presence of IR adsorption bands at (1381-1383) and (897) cm^{1} suggested the prevelance of the β- glycosidic linkage in these two extracted forms of mushroom. Moreover the IR spectra had also a band near (1631-1621), (1524-1522) cm^{-1} which are the characteristics of chitin (Michell and Scurfield, 1970). Also the presence of the peaks at (1746 and 1631) cm^{-1} indicated the existence of the ester and carbohydrate functional groups respectively (Pressena *et al.,* 2004). From the above facts, it is suggested that β-glucan is covalently linked to other polymers such as chitin, this structure is common in a different number of fungi since chitin is considered to be the main element which provides the structural features for the cell wall of mushrooms(Grauda, 2005).

To confirm this structure, Raman was also investigated in both the two insoluble forms of the mushroom in comparison with the wheat bran as seen in Fig (2a). The data confirmed what is previously discussed. The spectra of the bran is characterized by three bands at (520), (1379) and (1457) cm^{-1}. These bands could be related to S-S stretch, CH-oH bend and aliphatic residue respectively (Zhao *et al.,* 2004 and Mrozek *et al.,* 2004).

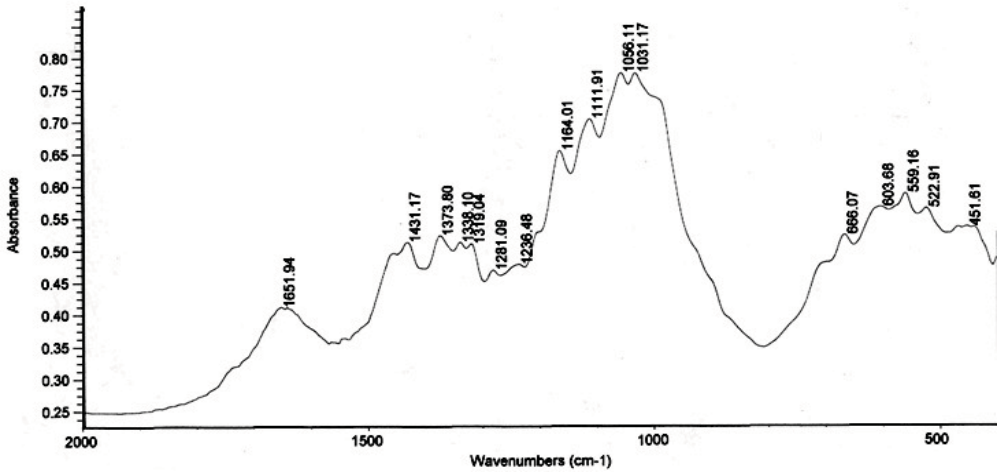
In both the two mushroom insoluble residues, the data revealed six major peaks at (1459-1457), (1372-1374), (1266-1268), (893-882), (800-807) and (498-493)cm^{-1}. The assignments of these peaks are shown in table (1). The presence of the peaks at (800-807)cm^{-1} are derived from symmetric stretching vibration of (o-p-o) of phosphodiester and confirms the presence of N-acetyl glucosamine- phosphate residue in the glycoprotein (Twaxdowski and Anzenbacher, 1994). Also during extraction and purification, a drastic change in the amide I group was also visualized. Denaturation effect was also noticed due to changes in the structure of N-H band, therby conformational changes in the residual amino acid is taken place.

So, it is noticed that the major composition of the fungal cell wall polysaccharides in mushroom mycelia could be reflected from the monosaccarides constituents of the insoluble dietary fibre, the predominant presence of glucose together with glucosamine in these fractions suggested that β-glucan and chitin would be the main matrix and fibrillar components of the fungal cell wall polysaccharide in the mycelia. In this matrix, both, chitin and the majority of the β-glucan were insoluble(Wong *et al.,* 2003).

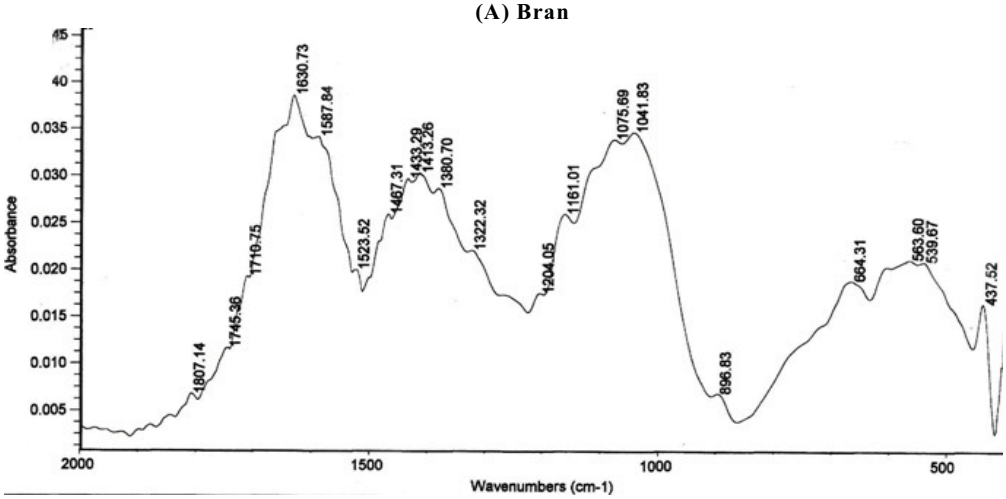
Chemical Composition and Functional Properties of the Mushroom Mycelia:

Table (2), showed the major chemical composition of both the oyster mushroom ethanol insoluble residue and insoluble non-starch polysaccharide (INSP's) in comparison with the wheat bran dietary fibre. The data were calculated on dry weight basis. Protein was found to be in the range of 12.47-14.64%. Fat was varied between 3.99 to 5.00%. in both the two previously mentioned oyster mushroom forms, respectively. These data were in accordance with those previously mentioned(Sonmee, 2004 and Diez and Alverez, 2001).

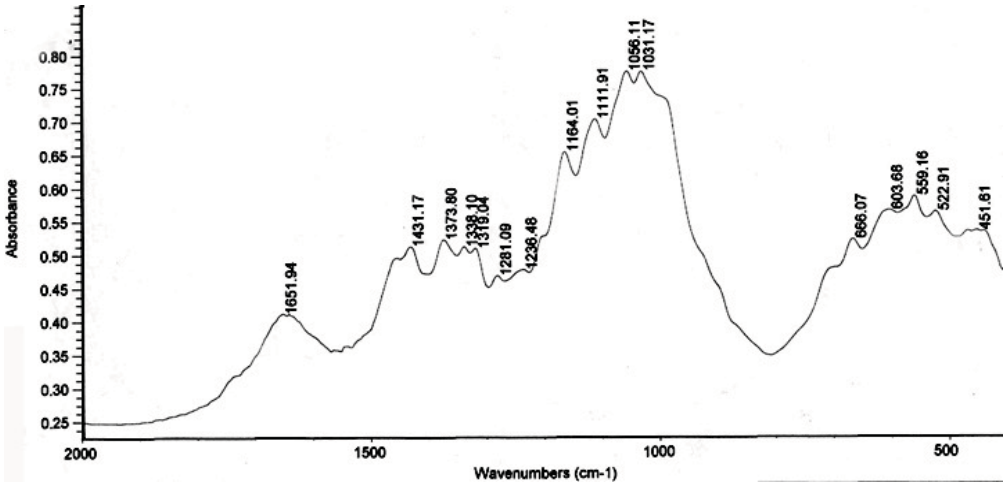
The water-holding capacities of oyster mushroom mycelia in the forms of ethanol insoluble residue and insoluble non-starch polysaccharide are presented in Figure (3a). INSP's were significantly ($p>0.05$) the



(A) Bran

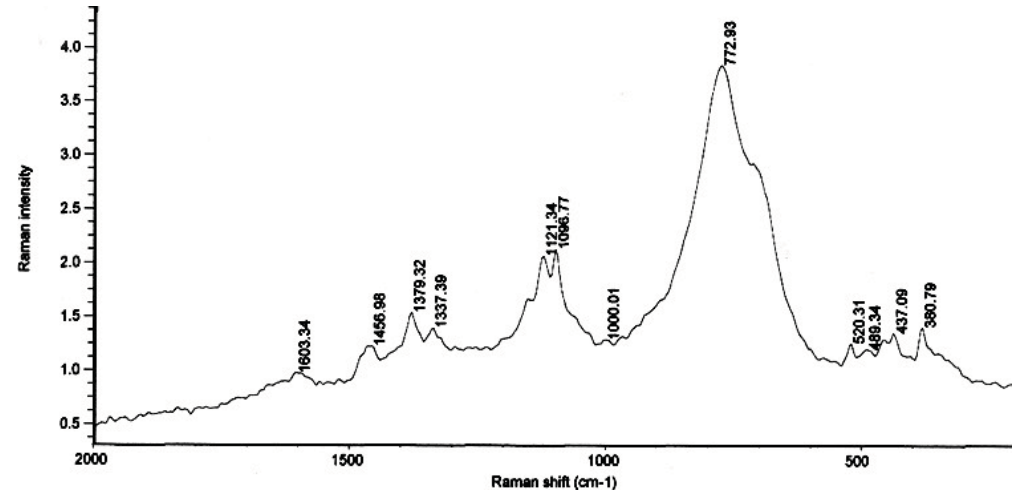


(B) mushroom ethanol insoluble residue

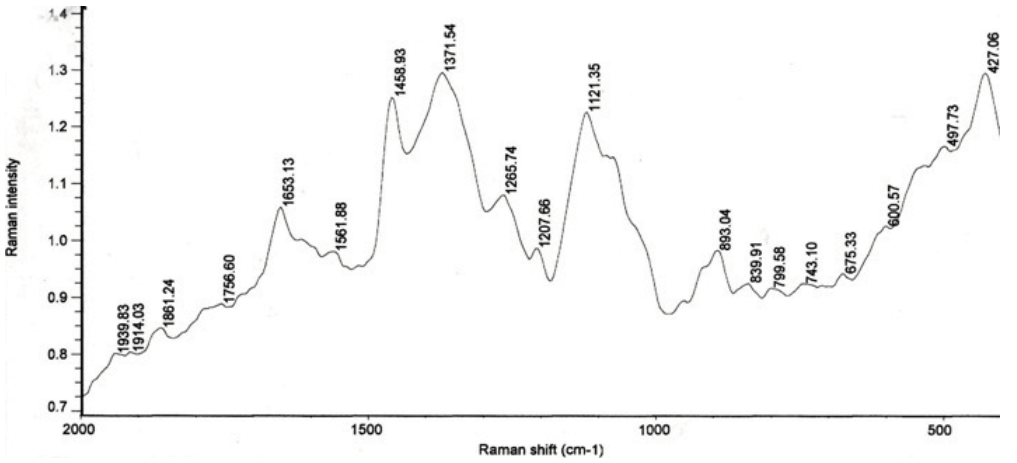


(C) mushroom insoluble Non-strach polysaccharide

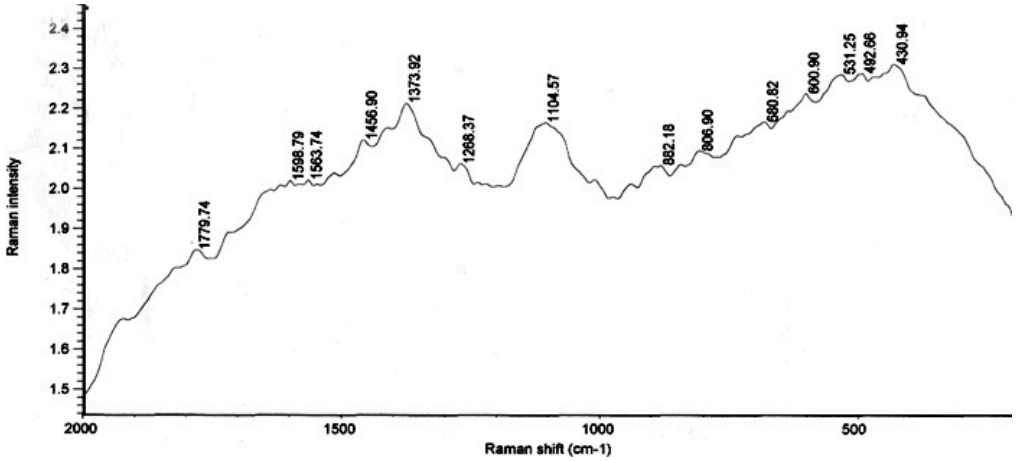
Fig. 1: IR spectra of different preparations from mushroom mycelia in comparison with bran as standard



(A) Bran



(B) mushroom ethanol insoluble residue



(C) mushroom insoluble Non-starch polysaccharide

Fig. 2: Raman spectra of different preparations from mushroom mycelia in comparison with bran as standard

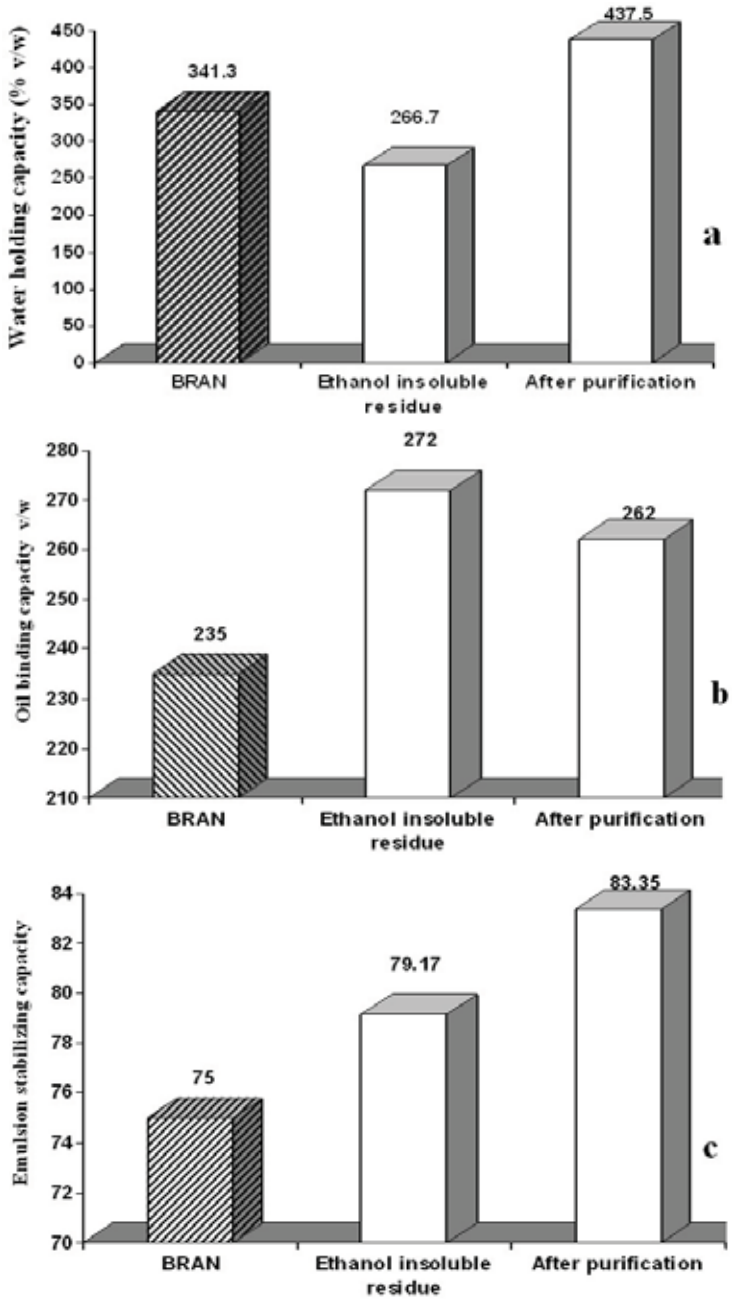


Fig. 3: Water holding (a), oil binding (b) and emulsification (c) capacities of the mushroom mycelia in the forms of ethanol insoluble residue and insoluble non starch polysaccharides in comparison with bran as a standard.

highest, their value were 438% compared to bran which was 341.3%. This enhancement in the water holding capacity in the purified form could be related to the presence of semi-pure glucomanan which is obtained by alkali treatment.

Also, the presence of chitin which is cross linked to various glucans had also an essential influence in affecting the solubility of these extracts. It is also noticed in these insoluble fractions that the highest magnitude of swelling is found in the purified form since swelling is enhanced at pH (5) and pH (9), where loosing of the protein matrix occur.

Table 1: Functional groups and vibrational modes obtained for the FT- Raman spectra of both mushroom ethanol insoluble residue and insoluble non-starch polysaccharides in comparison with the bran

	Band position in spectra cm^{-1}		
Bran	Mushroom ethanol insoluble residue	Insoluble non-starch polysaccharides	Assignment
520	498	493- 531	S-S group
1379	1372	1374	CH (bend) OH (bend)
1457	1459	1457	Aliphatic residue
-	1653	-	Amide I C=O stretch N-H wag α. Helix
-	1266	1268	Amide III N-H in plane bend C-N stretch β. Helix
-	893	882	β- glycosidic linkage
-	800	807	N-actyl –glucose amine

Table 2: Chemical composition of the Bran and mushroom mycelia in the forms of ethanol insoluble residue and insoluble non-starch polysaccharides

	Moisture %	Ash%	Protein %	Fat %
Bran	4.114	9.206	5.96	1.267
Mushroom mycelia				
Ethanol insoluble residue	8.483	7.608	12.47	3.998
Insoluble non-starch				
Polysaccharides	15.090	14.66	14.64	5.008

Data in figure (3b) showed the changes that occurred in oil binding capacities of both the oyster mushroom ethanolic residue and (INSP's). A slight enhancement in these residues were found compared to bran. It is also thought that products with high oil binding capacity contain non-polar side chain which binds to fat.

Results of the emulsion capacity is clearly illustrated in fig (3c). The emulsion stabilizing capacity of Bran dietary fibre was used as control. All the tested mushroom samples gave higher ($p>0.01$) emulsion stabilizing capacity than control. It seems that INSP's had the highest emulsifying capacity compared to the ethanol insoluble residue. Alkaline extraction takes a major role in excessive hydrolysis which results in smaller peptides, facilitates the formation of the emulsion (Tamelli and Burkus, 2000).

Thermal Properties of the Mushroom Mycelia:

Fig. (4) showed the changes that occurred in DSC thermograph of both the ethanol insoluble residue and the insoluble non-starch polysaccharides of the mushroom mycelia. The data in figure (4c) showed a very sharp exothermic peak equivalent to two ΔH points corresponding to-127.82 $J.g^{1}$ and -13.44 $J.g^{-1}$ at both temperatures 109.64 and 125.14°C. These narrow ranges of temperatures, indicate the purity of these insoluble non-starch polysaccharides. On contrast to the data presented in figures (4b), ethanol insoluble residue was less pure, since these transition temperatures are far away in their range, one at 244.19°C and the other at 60.43°C corresponding to ΔH 44.66 $j.g^{-1}$ for the former and-92.95 $j.g^{-1}$ for the latter. It was found that multiple transitions in the DSC thermograph may be caused by different conformations and interactions among amylose, amylopectin, protein, and lipids (Vasanthan and Bhatty, 1996).

Antioxidative Properties of the Oyster Mushroom INSP's:

As indicated in fig (5), the scavenging activity of mushroom methanolic extract was measured compared to bran methanolic extract. Strong DPPH radical scavenging activity was found in the mushroom extract (80.09%) compared to bran extract (77.47%). Similar results were also obainted in various varieties of mushrooms (Cheung *et al,* 2003). This remarkable results revealed that oyster mushrooms were free radical inhibitors or scavengers. Since they act as primary antioxidant (Frankel, 1991). These primary antioxidant terminate the free-radical chain reaction by donating hydrogen or electrons to free radicals and converting them to more stable products; also these antioxidant have also a chain breaking properties and strong activity at very low concentration. As indicated during our ESR experiments with a concentration less than (50mg. ml^{-1}) an attainable effectiveness could be obtained (Rajalakshml and Varasimhan, 1996).

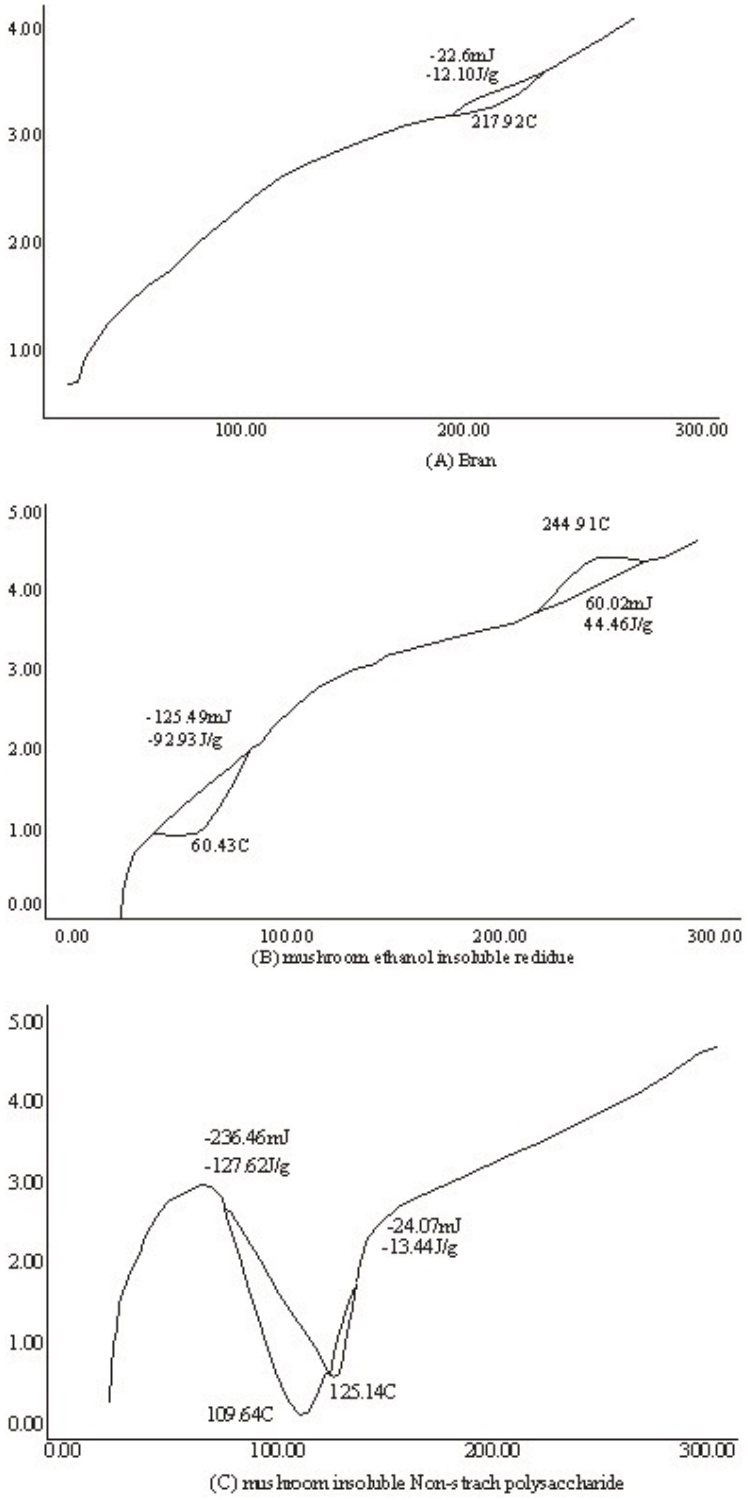
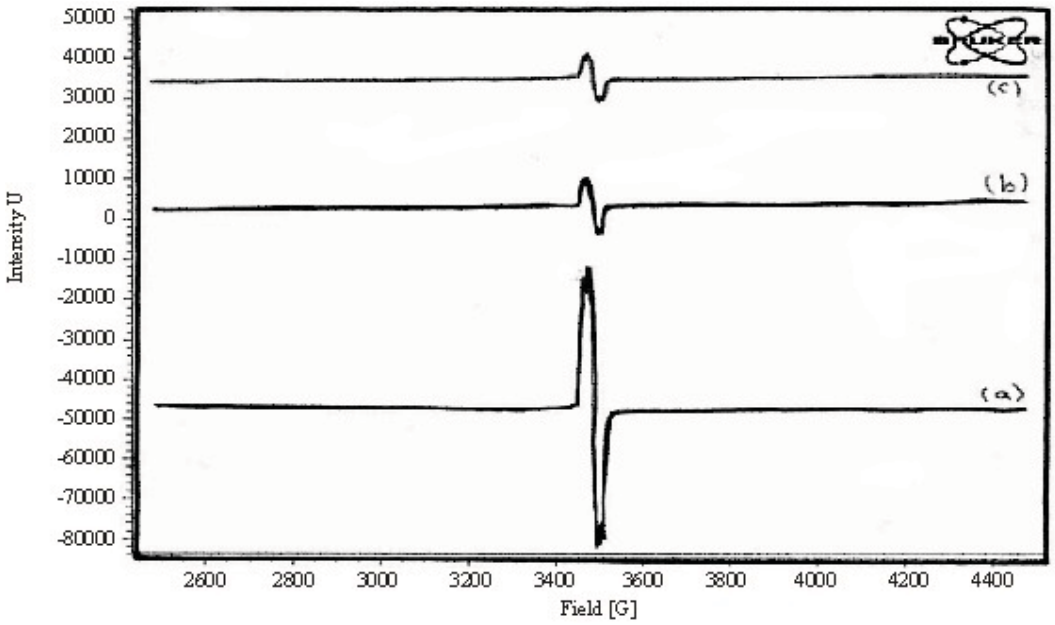


Fig. 4: DSC- thermograph of the different preparations from mushroom mycelia in comparison with bran as standard

Antioxidative properties of the Oyster mushroom INSP's.



(a): mushroom methanolic extract
(b): BRAN
(c): DPPH

Fig. 5: Antioxidative pattern of mushroom methanolic extract compared with the bran methanolic extract measured by ESR technique.

Conclusion:

In conclusion, this study indicated the suitability of using mushroom insoluble non starch polysaccharides (INSP's) after purification from the oyster mushroom mycelia as a food supplement.

The FT-IR-FT Raman spectroscopy illustrated properly the structure of oyster mushroom non-starch polysaccharides (INSP's). The predominant presence of glucose with glucosamine in the insoluble fraction, suggested that β-glucan and chitin would be the main matrix and fibrillar component of the oyster cell wall polysaccharide in the mycelia.

During thermal analysis by using DSC technique, the purified insoluble non- starch polysaccharides, showed a very sharp exothermic peak and a narrow ranges in temperature indicating the purity of these insoluble polysaccharides.

The presence of chitin which is cross-linked to glucan in the oyster mushroom insoluble non-starch polysaccharides has an essential influence in changing the functional properties measured as water binding, oil binding and emulsification capacities compared to the high purity wheat bran. Water binding capacity was found to be the highest in the non-starch polysaccharide derived from the oyster mushroom. Also, the presence of non-polar side chain in this insoluble non-starch polysaccharide affected the oil binding capacity to be reached to the maximum. High emulsion capacity was also attained, since excessive hydrolysis during purification results in smaller peptides; facilitated the formation of emulsion. Finally, ESR studies revealed the superiority of mushroom extracts as a free radical scavenger compared to bran as standard.

REFERENCES

AOAC. 1990 "Association of official analytical chemists". Official methods of analysis 15th edition. Association of official analytical chemists Inc. Arlington Virgina USA.

Bobeck, P. and S. Goldbavy, 1999. Hypocholestrolemic and antiathrogenic effect of oyster mushroom. *(Pleurotus ostreatus)* in Rabbit. Nahrung, 43(5): 339-342.

Cheung, L.M, P.C.K Cheung and V.E.C. OOI, 2003. Antioxidant activity and total phenolics of edible mushroom extracts. Food Chemistry, 249-255.

Cheung, P.C.K., 1998. Plasma and hepatic cholesterol levels and fecal neutral sterol excretion are altered in hamsters fed straw mhsuroom diets. Journal of Nutrition, 1512-1516.

Cheung, S.T., 1999. World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on lentinus edodes (berk). Sing in China. Journal of Medicinal Mushrooms, I: 291-300.

Cheung, R.C.K, and M.Y. LEE, 2000. Fractionation and characterization of mushroom dietary fiber (Non starch polysaccharides) as a potential nutraceuticals from mycelia of *pleurotus tuber- regium* (fries) Singer. Journal of Agriculture and Food Chemistry, 48: 3148-3151.

Diez, V.A and M. Alvarez, A.2001. Compositional and nutritional studies on two wild edible mushrooms from northwest Spain. Food Chemistry, 75: 417-442.

Frankel, E.N., 1991. Recent advances in Lipid oxidation. Journal Science Food and Agriculture, 54: 495-511.

Garuda International, INC.2005. Product Overview- medicinal mushrooms. http://www.cowcium. com/omush.htm.

Lin M.J.Y and F.S. Humbert, 1974. Certain functional properties of sunflower meal products. Journal of Food Science, 39: 368-370.

Manzi, P., P Aguzi and L. Pizzoferrato, 2001. Nutritional value of mushroom widely consumed in Italy. Food Chemistry 73: 321-325.

Manzi, P., L. Gambelli, S. Marconi, V Vivanti and L. Pizzoferatto, 1999. Nutrients in edible mushroom: An inter species comparative study. Food Chemistry, 65: 477-482.

Mau, J.L, G.R Chao and WU.K. Tzer, 2001. Antioxidant properties of methanolic extracts from several ear mushroom. Journal Agric. Food Chemistry, 49: 4561-4567.

Mau, J.L, P.N Huang, S.J Huang and C.C. Chen, 2004. Antioxidant properties of methanolic extracts from two kinds of Antrodia comphorota mycelia. Food Chemistry, 86: 25-31.

Michell, J.A. and G. Scurfield, 1970. An Assessment of infrared spectra as indicators of fungal cell wall composition. Aust. J. Biol. Sci., 23: 345-360.

Mizuno, T, T Sakai, and G. Chihara, 1995. Health Foods and medicinal usages of mushrooms. Food Review International, 11(1): 69-81.

Mrozek, M.F, D Zhang and D.B. Amotz, 2004. Oligosaccharide identification and mixture quantification using Raman spectroscopy and chemometric analysis. Carbohydrate Research, 339: 141-145.

Prasenna, V, T.N Prabha and R.N. Tharanthan, 2004. Pectic polysaccharide of mango (*Mangifera indica. L*) structural studies. J. Sci. Food Agric, 84: 1731-1735.

Rajalakshml, D and S. Varasimhan, 1996. Food antioxidant, sources and methods of evaluation. In food antioxidants, technological, toxicological and health perspectives. Maldhavi. D. L, desphane S. S and solunkke. Marcel Dekker Inc.

Salama, F. Manal, 2001. Effect of natural antioxidant on the baking quality and stability of cakes. Egypt. J. Food. Sci, 30(2): 135-154.

Sanlin I. Robert, 1995. Phytochemicals and antioxidants in food antioxidants, technological, toxicological and health prospectives ed. Madhavi, D. L., Deshpande S. S and Salunthe. D.K.

Smiles A., Y. Kakuda and D.E. Macdonald, 1989. Effect of degumming reagents on the composition and emulsifying properties of canola, soybean and sunflower acetone insoluble. Journal of the American oil chemist 66: 348-352.

Sonmee, R. B Dell, P Lumyong, K Izumori and S. Lumyong, 2004. Nutritive value of popular wild edible mushroom from northern Thailand. Food Chemistry, 527-532.

Sosulski, F.W., 1962. The centrifuge method for determining flour absorption in hard and spring wheats. Cereal Chemistry, 39: 368-370.

Temelli, F and Z. Burkus, 2000. Stabilization of emulsions and foams using barly. B-glucan Food Research International, 33: 27-33.

Thammakii, S., M Suphantharika, T Phaesuwan and C. Verduyn, 2004. Preparation of spent brewer's yeast B-glucans for potential applications in the food industry. International Journal of Food Science and Technology, 39: 21-29.

Travillas, P, C.A Calliste, D.P ALLAIS, A Simon, A Marfak, C Delage and J.L. Dwoux, 2003. Antioxidant, anti-inflammatory and anti proliferative properties of sixteen water plant extracts used in limousine countryside as herbal teas. Food Chemistry, 80: 399-407.

Twardowski, J and Anzenbacher, 1994. Raman and IR spectroscopy in Biology and Biochemistry M. Masson ed. Polish scientific publisher. PWN Warsaw.

Vasanthan, T and R. Bhatty, S.1996. Physiochemical Properties of small and large-granule starches of waxy, regular and high amylose barleys. Cereal Chemistry, 73(2): 199-207.

Wong K.A.H, P.C.K Cheung and J.Z. WU, 2003. Biochemical and microstructural characteristics of Insoluble and soluble dietary fiber prepared from mushroom scleortia of *plurotus tuber- regium,* polyporus rhinocerus and wolfiporia cocos. J. Agric. Food Chem., 7197-7202.

Yang J.H, H.C LIN and J.L. Mau, 2002. Antioxidant Properties of several commercial mushrooms. Food Chemistry, 77: 229-235.

Yu. P, J.J Mckinnon, C.R Christenen and D.A. Christenen, 2004. Using Synchrotron transmission FTIR micro spectroscopy as a rapid, direct and Non- destructive analytical technique to reveal molecular microstructure- chemical features within a tissue of Grain Barley. J. Agric. Food Chemistry, 52: 1484-1494.

Zhang, M., P.C.K Cheung and L. Zangh, 2001. Evaluation of mushroom dietary fibre (Non starch polysaccharides) from mycelia of *pleurotus tuber- regium (fries)* singer as a potential antitumor agent. Journal Agriculture and Food Chemistry, 49: 5059-5062

Zhao, Y, MA Ching-Yung, S.N Yuen and D.L. Phillips, 2004.Raman spectroscopic determination of the extent of O-esterification in acetylated soy protein isolates. Food Chemistry, 87: 477-481.

Zivanovic, S., R.W Buescher and K.S. Kim, 2000. Textural Changes in mushrooms (*Agaricus bisporus*) associated with tissue ultrastructure and composition. Journal of Food Science, 8(65): 1404-1408.