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Evaluation of antioxidant property and quality of breads containing *Auricularia auricula* polysaccharide flour

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Abstract

The polysaccharide in the fruit bodies of *Auricularia auricula* (commonly called black woody ear or tree ear) was extracted, lyophilized and ground. *Auricularia auricula* polysaccharide (AAP) flour blended bread was developed. Physical qualities and antioxidant activities of breads with different levels of substitution of AAP flour for wheat flour were analyzed.The results showed that up to 9% of AAP flour could be included in bread formulation without altering the sensory acceptance of the blended bread. The incorporation of AAP in bread markedly increased the antioxidant property of the bread as tested by DPPH free radical-scavenging method. Breads containing AAP flour can broaden the utilization of the fruit bodies of *Auricularia auricula* and may be regarded as possible health-promoting functional foods.

Keywords: *Auricularia auricula* polysaccharide (AAP); Extraction; Free radical-scavenging; AAP flour blended bread

1. Introduction

Free radicals can usually be generated by several biological reactions in the body, and these are capable of damaging crucial bio-molecules; if they are not scavenged effectively by cellular constituents, they lead to disease conditions (Aruoma, 1994; Benzie, 2000; Halliwells, Gutteridge, & Cross, 1992). The harmful action of free radicals can be blocked by antioxidant substances, which scavenge the free radicals and detoxify the organisms. Current researches into free radicals have confirmed that foods rich in antioxidants play an essential role in the prevention of cardiovascular diseases and cancers and neurodegenerative diseases, as well as inflammation and problems caused by cell and cutaneous aging (Finkel & Holbrook, 2000; Halliwells et al., 1992). Antioxidants are important in the control of degenerative diseases in which oxidative damage has been implicated. Natural antioxidants may inhibit lipid peroxidation in food and improve food quality and safety. Several plants extracts have been shown to have antioxidant activity (Amarowicz, Pegg, Rahimi-Moghaddam, & Barl, 2004; Duan, Zhang, Li, & Wang, 2006). The search for newer natural antioxidants has increased.

It is believed that polysaccharides from edible and medicinal mushrooms have numerous beneficial effects on health, and it has been suggested that quantity and frequency of intake of the mushroom is related to lower cancer death rates. A more detailed controlled study is ongoing. Some edible mushrooms have potential to prevent or treat such diseases (Ikekawa & Tetsuro, 2001).

The fruit bodies of *Auricularia auricula* have been traditionally used both as a food and a drug in China. Its fruit body is a kind of edible black-brown mushroom with high contents of carbohydrates (approximately 630 g kg^{-1} in dried fruit bodies) and protein, and the content of protein, minerals (Ca, P, and Fe) are higher than those in

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tremella (commonly called white fungus). The protein is plentiful in Lys and Leu, so it is acknowledged to be a healthy food.

The fruit bodies of *Auricularia auricula* are rich in polysaccharides. In recent years, AAP was found to have many biological activities, this compound possesses antioxidant activity, anticoagulant activity, decreases blood sugar, and could improve well-being (Acharya, Samui, Rai, Dutta, & Acharya, 2004; Finkel & Holbrook, 2000; Mizuno, Saito, Nishitoba, & Kawagishi, 1995; Pisuena, Mojica, & Merca, 2003; Takeuchi, Lau, & Mooi, 2004; Yoon, Yu, Pyun, Hwang, & Chu, 2003; Zhang Lina & Yang Liqun, 1995). People are paying increasing attention to its exploitation. Studies on its medicinal function, or as a healthy functional food, need to be done.

The harvest period of *Auricularia auricula* each year is short. It is not regarded as a staple food, and it is feasible to consider developing various new food products containing AAP, which will broaden the utilization of *Auricularia auricula* as a healthy functional food ingredient. The cachet of strengthening good health would enhance such food products.

Bread is an important staple food; it is mainly made of wheat flour, salt, margarine and yeast, and it is consumed all over the world. Many food ingredients (food additives or functional food additives) have been included in bread formulation to increase its diversity, nutrition, or product appeal. AAP flour has been successfully manufactured and its antioxidant properties and other biological activities were proven by a series of experiments in our laboratory, but this type of flour is not convenient for people to consume abundantly and regularly. To promote the usage of AAP flours, it may be possible to include AAP flour in bread or other food formulations. Thus, this present research was aimed at establishing the feasibility of manufacturing breads supplemented with AAP flour and to determine the influence of AAP flour on bread quality and to study the antioxidant property of breads containing AAP flour.

2. Materials and methods

2.1. Materials

The samples of dried *Auricularia auricula* fruit bodies were purchased in local supermarkets, and cultured at Fang city in Hubei province in China. The high gluten wheat flour, sugar, salt, margarine, bread improver, rapid-rise yeast and milk powder used in the formulation of bread were purchased from a local food ingredient company.

2.2. Preparation of *Auricularia auricula* polysaccharides (AAP) flour

The procedure for *Auricularia auricula* polysaccharide extracts, as shown in Fig. 1, was carried out following the scientific literature on this subject (Pisuena et al., 2003; Shigeo & Shoichi, 1982).

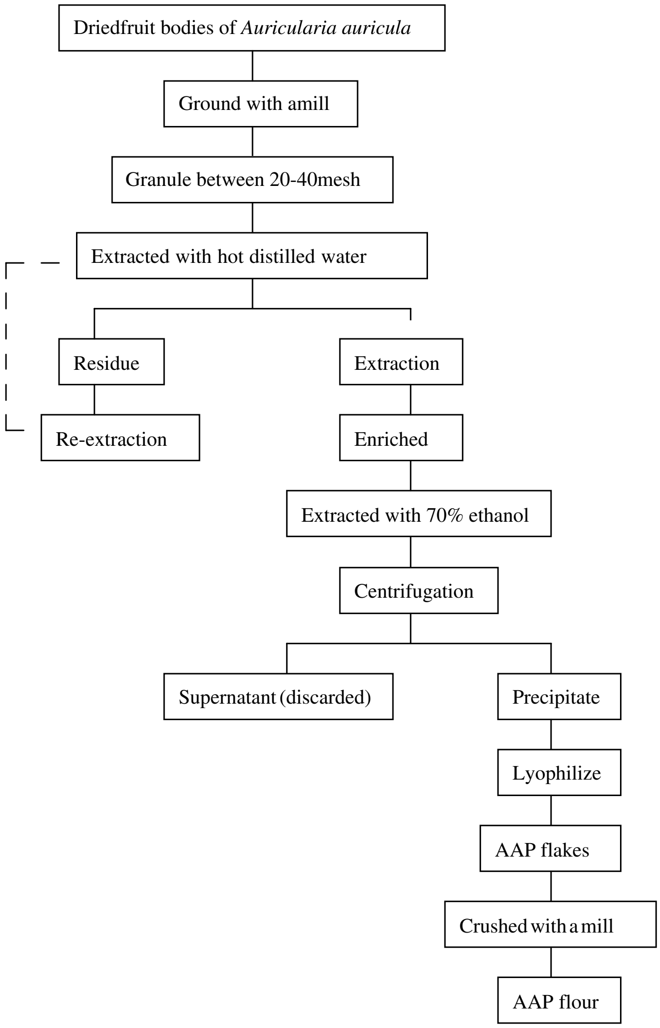

Fig. 1. Preparation of AAP flour.

After the fruit bodies of *Auricularia auricula* were cleaned, they were ground in an electric mill, and sieved through screens; only those granules which were sieved between 20-mesh and 40-mesh were used as material samples to extract polysaccharides.

Around 25 g of those powder material with 500 ml distilled water were put in a vessel, heated and kept boiling for 5 h and stirred regularly. Then the extraction (liquid fraction) and the residue were collected separately; the residue was also re-extracted twice more to obtain polysaccharide. The thrice extracted liquid fraction was collected and enriched, extracted with 70% ethanol for 12 h, after centrifugation (3000 g for 5 min) to remove the supernatant portion. The precipitates of those extractions were lyophilized, then AAP flakes were obtained and crushed with an electric mill. AAP flour was obtained and was verified to be polysaccharide by a series of traditional physicochemical tests, (IR, GC–MS) sealed and kept at room temperature(21 ± 1 °C) for later usage.

2.3. Analyses of proximate compositions of AAP flour and wheat flour

The analyses of moisture, crude protein ($N \times 6.25$), crude fat, ash, and crude fibre in products were conducted according to the methods of AOAC 15.950.01, 15.976.05, 15.920.39, 15.955.03 and 15.962.09, respectively (AOAC, 1990). Nitrogen-free extract (NFE) was calculated by subtracting the total content of moisture, crude protein, crude fat, ash, and crude fibre from 100.

Ten grams of each sample was suspended in 90 ml of sterile distilled water and homogenized. The mixture was allowed to stand for 30 min before being filtered. The pH values of the filtrates were then determined by a pH meter (pH211C, Hanna Co., Italy). Three readings were taken per sample. The values reported were means of triplicate samples with SD.

2.4. Bread manufacture

2.4.1. General

The AAP substitution levels were 0%, 3%, 6%, 9% and 12%. The raw materials for bread-baking were weighed according to the formula proportions listed in Table 1. Each substitution level experiment was carried out in triplicate. The baking procedure was as follows.

2.4.2. Preparation of dough

Dough was prepared by using a straight method. First of all, yeast was dissolved in water at 30 °C, and the dry ingredients were mixed. Margarine was melted and added to the dry ingredients in the liquid phase, together with dissolved yeast. All the ingredients were mixed by a mixer (Dierks & Sohne, Germany) for 2 min at low speed, followed 6 min of mixing at high speed. After complete mixing of the dough, it was placed in the incubator (Nuve EN300, China) at 30 °C and 85% RH for fermentation; the total duration of the fermentation was 120 min. After the first 70 min, the dough was taken out of the incubator, punched and placed in the incubator again. A second punch took place after a further 35 min. The dough was divided into the same weight pieces after fermentation. Each piece was shaped and put into the incubator for the last 15 min under the same incubator conditions.

Table 1
Formulation of AAP flour incorporated bread

Ingredients (g)	The substitution of AAP flour				
	0%	3%	6%	9%	12%
Wheat flour	200	190	180	170	160
AAP flour	0	10	20	30	40
Sugar	6	6	6	6	6
Salt	3	3	3	3	3
Yeast	2.2	2.2	2.2	2.2	2.2
Margarine	2	2	2	2	2
Milk powder	2.4	2.4	2.4	2.4	2.4
Improver	2.4	2.4	2.4	2.4	2.4
Water	125	125	125	125	125
Total	343	343	343	343	343

2.4.3. Conventional baking

Conventional baking was performed at 220 °C for 20 min in a rotating oven (Type Bex1.0, Japan).The oven was preheated to set temperatures before placing the dough samples into it. Afterwards the bread was taken out of the bread case, cooled to room temperature, and weighed to calculate the baking loss by the following formula:

$$\text{Baking loss}(\%) = [1 - (\text{bread weight}/\text{total material weight})] \times 100$$

The loaf volume was determined by the rapeseed displacement method (AACC, 1988). The values reported were averages of triplicate samples with SD.

2.5. DPPH free radical-scavenging activity test

Radical-scavenging activity against the stable radical DPPH$^\cdot$ (1,1-diphenyl-2-picryl-hydrazyl radical (Sigma, USA)) was determined spectrophotometrically following the scientific literature (Hsu, Hurang, Chen, Weng, & Tseng, 2004; Duan et al., 2006). The bread was cut into slices (1 cm thickness) and dried in an electric convection drying oven (40 °C) for 30 h.The dried bread slices were ground and sieved through a 60-mesh screen to obtain the bread flour for the following tests. AAP flour or ground bread flour (2.5, 5.0, 7.5, or 10.0 g) and methanol (40 ml) were placed in flasks, kept in a magnetic agitator overnight, and then filtered (Whatman No. 1 filter paper). The volume of the filtrate was adjusted to 50 ml with methanol and stored at −20 °C for further tests. Then the methanolic extracts represented 50, 100, 150 or 200 mg of sample per millilitre, respectively.

The solution of DPPH in methanol (0.1 mM) was prepared daily, before UV measurements. Methanolic extract (1 ml) was thoroughly mixed with 5 ml of freshly prepared DPPH and kept in the dark for 30 min at room temperature (21 ± 1 °C), and then the absorbance was measured (U-2000, Hitachi, Japan) at 517 nm. The experiment was carried out in triplicate. The ability to scavenge the DPPH radical was calculated by the following formula:

$$\text{Scavenging effect}(\%) = [1 - (A_{\text{sample}} - A_{\text{sampleblank}})/A_{\text{control}}] \times 100$$

where the A_{control} is the absorbance of the control (DPPH solution without sample), the A_{sample} is the test sample (DPPH solution plus test sample), and the $A_{\text{sample blank}}$ is the absorbance of the sample only (sample without DPPH solution).

2.6. Sensory evaluation

The sensory evaluation was carried out on the bread samples within 24 h of baking. The samples were evaluated by a ten-member trained panel of judges using the hedonic scaling method (9–1 scoring): 1 = extremely dislike (or lowest quality), 5 = neither like nor dislike (or medium quality), and 9 = extremely like (or highest quality), respectively. The samples were sliced into equally sized pieces (1.5 cm thick) and served as coded randomized duplicates. All sensory evaluations were conducted under white fluorescence light at a room temperature of 21 ± 1 °C. The judges were required to rinse their mouth with distilled water between samples. Internal and external characteristics of appearance of texture (the size of air space and the evenness of air space distribution), colour, aroma, taste, and mouthfeel, as well as general acceptability, were measured.

2.7. Data analysis

Statistical analysis was evaluated using the SAS System (1999 edition; SAS Institute Inc., Cary, NC, USA). Results were considered significantly different for $P < 0.05$.

3. Results and discussion

3.1. Proximate compositions of AAP flour and wheat flour

The proximate compositions of AAP flour and wheat flour were determined and are shown in Table 2. It is noteworthy that lyophilized AAP flour contained much less moisture than the commercial wheat flour in Table 2. The protein content of the wheat flour was as high as expected, and AAP flour has a low fat content and high carbohydrate content. The fruit bodies of *Auricularia auricula* contain relatively high levels of minerals (Fe, Ca, P and Mg), so it was found that the ash content in AAP flour was sevenfold that of wheat flour. The crude fibre content was also higher than that of wheat flour.

Table 2
Proximate compositions of AAP flour and wheat flour

Parameters* (%)	AAP flour	Wheat flour
Moisture content	2.76 ± 0.06^{b}	10.98 ± 0.05^{a}
Crude protein	12.0 ± 0.31^{b}	17.6 ± 0.29^{a}
Crude fibre	1.65 ± 0.03^{a}	0.32 ± 0.09^{b}
Crude fat	0.19 ± 0.25^{b}	1.28 ± 0.06^{a}
Ash content	3.92 ± 0.11^{a}	0.55 ± 0.02^{a}
Nitrogen free extract	79.5 ± 0.21^{a}	70.3 ± 0.19^{b}
pH	6.15 ± 0.08^{a}	6.03 ± 0.05^{a}

Values bearing different superscripts in the same row are significantly different ($P < 0.05$).

* Dry basis except for the moisture content. Reported values are the means ± SD ($n = 3$).

3.2. The effects of AAP flour substitution on physical qualities of breads

Breads with different substitution levels of AAP flour for wheat flour were made by a bread-making machine; the weight, loaf height, and loaf volume of breads made with different contents of AAP flour are shown in Table 3. All raw materials were added to the baking machine at the beginning of the process and no intermediate products were taken out during the process; therefore, the weight loss from raw materials was all caused by water evaporation. Table 3 shows that the loaf weight of bread made with 3% AAP flour did not show a significant difference when compared with the loaf weight of the bread made without AAP flour. The same is true at 6%. However, there was a significant difference in loaf weight of the bread made with 9% and 12% AAP flour compared to the control; this was mainly because of the relatively low moisture content of the freeze-dried AAP flour (Table 2). The higher ratio of freeze-dried AAP flour in the recipes means a lower moisture content in the bread, so the evaporation water is lower and the loaf weight and volume are relatively higher. Bread made with 3% AAP flour did not show a significant difference in the loaf height when compared with the control bread without AAP flour. Bread with 9% AAP flour substitution had a loaf height that was reduced to 76% of bread without AAP flour. Moreover, the 12% substitution not only reduced the loaf height to 68% but also resulted in irregularly shaped bread. The effects of the ratio of AAP flour on the loaf volume were similar to effects on loaf height. While 3% substitution showed no significant difference, 12% substitution reduced loaf volume to 73% of that shown by bread without AAP flour. As less wheat gluten in the formulation may retrain less fermentation gas, this may be the main reason for the decline in both loaf height and volume in bread with high contents of AAP flour.

Table 3
Effect of substitution level on loaf weight, volume and height of AAP flour-incorporated breads

Substitute level(%)	Loaf weight		Loaf height		Loaf volume	
	Weight^{A} (g)	Loss^{B} (%)	Height^{A} (cm)	Decrease^{C} (%)	Volume^{A} (cm^{3})	Decrease^{C} (%)
0	273.1 ± 1.2^{b}	19.9	11.07 ± 0.10^{a}	0	1225 ± 21^{a}	0
3	275.2 ± 2.3^{b}	19.3	10.13 ± 0.32^{a}	8.49	1218 ± 33^{a}	0.57
6	277.5 ± 2.0^{b}	18.6	8.54 ± 0.21^{b}	22.85	1066 ± 18^{b}	12.98
9	288.5 ± 1.6^{a}	15.4	8.36 ± 0.40^{b}	24.48	1057 ± 28^{b}	13.71
12	283.5 ± 2.6^{a}	16.9	6.25 ± 0.22^{c}	43.54	890 ± 16^{c}	27.35

[A] Reported values are the mean ± SD ($n = 3$). Values bearing different superscripts in the same column are significantly different ($P < 0.05$).

[B] Weight loss calculation based on the total ingredient weight of 343 g used for each loaf of bread.

[C] Compared with the bread without AAP flour.

Table 4
Sensory evaluation of AAP flour-incorporated breads

Substitute level (%)	Color	Aroma	Texture	Taste	Mouth feel
0	7.33 ± 0.62^{a}	6.90 ± 1.41^{a}	6.19 ± 1.52^{a}	5.88 ± 0.91^{a}	6.80 ± 1.62^{a}
3	7.12 ± 1.32^{a}	7.01 ± 1.35^{a}	6.21 ± 1.22^{a}	6.01 ± 1.14^{a}	5.93 ± 1.51^{a}
6	7.01 ± 0.98^{a}	6.90 ± 1.06^{a}	7.00 ± 1.43^{a}	5.83 ± 1.21^{a}	6.12 ± 0.97^{a}
9	6.61 ± 1.61^{ab}	7.21 ± 1.31^{a}	6.45 ± 1.64^{a}	4.90 ± 1.02^{a}	5.89 ± 1.08^{a}
12	5.37 ± 1.52^{b}	7.33 ± 1.58^{a}	6.11 ± 1.52^{a}	6.11 ± 1.52^{a}	6.33 ± 1.22^{a}

Reported values are the means ± SD ($n = 10$). Values bearing different superscripts in the same column are significantly different ($P < 0.05$).

Bread physical qualities were influenced by many factors, for example, wheat flour compositions, additives and dough fermentation conditions (Chen & Chiang, 1984; Havet, Mankai, & Le Bail, 2000). With regard to the loaf weight, loaf height and loaf volume, the highest ratio of AAP flour in the bread formulation should not exceed 9%.

3.3. Antioxidant properties of incorporated breads

DPPH is a useful reagent for investigating the free radical-scavenging activities of materials. In the DPPH test, the antioxidants were able to reduce the stable DPPH· to the yellow-coloured diphenylpricrylhydrazine. In this research, the breads made with varying levels of substitution of AAP flour were tested for their free radical-scavenging activity.

The DPPH free radical-scavenging activities of breads with different levels of substitution of AAP flour for wheat flour are shown in Fig. 2. As can be seen in Fig. 2, the AAP flour alone exhibited a rather strong concentration-dependent DPPH radical-scavenging activity. It was noted that, when the substitution levels of AAP flour increased in the bread, the antioxidant activity increased. Normally, Maillard reaction products possess free radical-scavenging activities (Jing & Kitts, 2000; EL-massry, Farouk, EL-ghorab, & Volatile, 2003); therefore, the bread without AAP flour also showed some degree of free radical-scavenging activities.

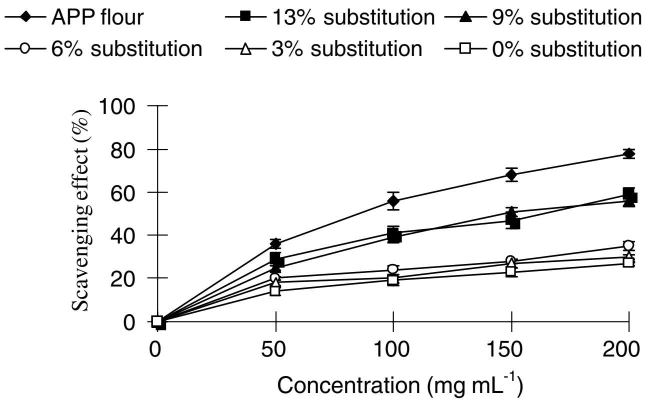

Fig. 2. DPPH free radical-scavenging effect of AAP flour-incorporated breads. Results are the means ± SD of triplicates.

3.4. Sensory evaluation of breads containing AAP flour

The sensory evaluations of breads with different substitution levels of AAP flour are shown in Table 4. The colour of bread with 12% AAP flour had the lowest evaluation score. The colour of lyophilized AAP flour was shallow grey, so, 3–5% substitution of AAP flour did not interfere with the original colour of the bread made with wheat. However, the colour rating increased when the substitution ratio increased. For other sensory characteristics (aroma, texture, taste and mouth feel), no statistically significant difference was observed for breads made with AAP flour at substitution levels of 3–12%. The results suggested that substituting AAP flour in a wheat bread formulation would not interfere with bread acceptability, with the exception of the very high substitution ratios.

4. Conclusions

During this study, we extracted the polysaccharides from the fruit bodies of *Auricularia auricula*, developed AAP blended bread, analyzed physical qualities and antioxidant activity of breads with different levels of substitution of AAP flour, and observed that up to 9% AAP flour could be included in a bread formulation without interfering with the sensory acceptance of the bread. Incorporation of AAP flour markedly increased its antioxidant activity. The manufacture of AAP flour bread can broaden the utilization of the polysaccharides from the fruit bodies of *Auricularia auricula*, because of its higher ability to scavenge free radicals, and it could be regarded as a potential health-promoting functional food. However, the ability of the bread components to scavenge free radicals after ingestion and partial or full digestion is unknown. Researches to demonstrate its health giving-properties in vivo, after consumption, are being conducted.

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