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Extraction of BaChu mushroom polysaccharides and preparation of a compound beverage

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Abstract

Mushroom is attracting more and more attention for its medical foods and antitumour value. The three extraction parameters (extraction temperature, extraction time, and ratio of solvent to raw material) were determined for the highest yield of polysaccharides. To better understand how flavour of compound beverage is affected by different variable factors, a three-factor, three-level designed orthogonal experiment was developed. Factors include hawthorn juice, mushroom polysaccharides solution, and apple juice. Flavour coefficient of the compound beverage was found to depend significantly on hawthorn juice. The optimal combination parameters of the processing technology were $A_2B_3C_1$, namely, mushroom juice (36.4%), hawthorn juice (45.4%), and apple juice (18.2%).

Keywords: Single-factor test; Orthogonal test; Polysaccharides; BaChu mushroom; Beverage

1. Introduction

Fungus is also called mushroom by the populace. In recent years, it has been very popular in the north American and Asian markets (Zhang, Cheung, Chiu, Wong, & Ooi, 2006). Because of its high value in nutrition and medicine, it has attracted more and more attention. Mushroom is featured with crisp meat, delicious taste, unique fragrance, reasonable nutrition composition, rich in essential amino acids, vitamin, and mineral contents. Test results reveal that 100 g of the dry product of mushroom contains 20.85 g protein, 25.42 g amino acids, vitamins C, B1, and B2, and organic selenium (Song et al., 2003). Mushroom has many medication and healthcare functions: inhibit cancer with 86.3% inhibiting rate after oral intake (Lin, Li, Lee, & Kan, 2003); slow down aging and promote the gonad function; prevent and treat diabetes (Gallagher, Flatt, Duffy, & Abdel-Wahab, 2003); inhibit obesity and regulate blood pressure in a two-way fashion for the treatment of arteriosclerosis and cerebral embolism (Strausfeld, Strausfeld, Stowe, Rowell, & Loesel, 2006); enhance facial beauty and moisten the skin to postpone the appearance of age pigment; increase appetite, promote growth, build up immunity, and improve memory (Kuo, Weng, Ha, & Wu, 2006).

In recent years, mushroom polysaccharides have drawn the attention of both chemists and immunobiologists due to their multipurpose medicinal activities that include immunomodulating and antitumour properties (Borchers, Stern, Hackman, Keen, & Gershwin, 1999; Carbonero et al., 2006; Huie & Di, 2004; Wong, Wong, Chiu, & Cheung, 2007). But of all the polysaccharides isolated from mushroom origin, glucans are the most important due to their potent antitumour properties. Various linear (1,3)-β-glucans and branched (1,3)(1,6)-linked β-glucans isolated from different mushroom origins are well known (Daba & Ezeronye, 2003; Gonzaga, Ricardo, Heatley, & Soares, 2005; Lim et al., 2005; Sasaki, Abiko, Sugino, & Nitta, 1978). On the genetic level, it has powerful inhibition of mutation caused by chemicals. It can be used in the comprehensive treatment of lassitude, leukocytopenia, and reduced immunity due to chronic hepatitis and radio-chemotherapy for malignant tumours. It also has certain preventive role

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for AIDS (Miao et al., 2004). Oral intake of mushroom polysaccharide is both safe and effective. Wild edible BaChu mushrooms grow in northern edge of Tarium basin in Xinjiang Province, China. It is named because it is produced in BaChu county in Xinjiang Province, China. BaChu mushrooms belonging to the genera *helvella*, was reported to possess antitumour, antioxidant, cholesterol reducing, and immunomodulating activities (Zhu et al., 1998) and have been used as traditional medicines for the treatment of gastrointestinal cancer, cerebral arteriosclerosis, cardiovascular disease, tuberculosis, liver or heart diseases, fester, bellyache, hypercholesterolemia, hyperlipidemia, stomach ailments, and diabetes (Zhang & Xiao, 1993). Recently, BaChu mushrooms are reported to efficiently enhance engulfing ability of leucocytes, lymphocytes conversion ratio, and antibody titer (Meng, Zhang, & Hu, 2005). In this region, the climate is mild and rainy. The seasons are normally high day–night temperature difference. The climate during the year, especially, in spring and autumn, is ideal for wild mushroom growth (Chrysayi-Tokousbalides, Kastanias, Philippoussis, & Diamantopoulou, 2007; Özçelik & Pekşen, 2007).

In the present experiment, a single-factor test was employed to estimate effect of different extraction parameters on yield of polysaccharides. At the same time, an orthogonal test was employed to estimate optimal combination parameters of three raw materials (hawthorn juice, mushroom polysaccharides juice, and apple juice) for the preparation of a compound beverage.

2. Materials and methods

2.1. Materials

The edible wild BaChu mushrooms were collected from BaChu county in northern edge of Tarium basin in Xinjiang Province, China. Hawthorn and apple were picked from an orchard in Alar city, Xinjiang Province, China.

White granulated sugar and citric acid were Food-grade; ethanol, anthrone, sulphate acid, and glucan were of analytical grade. All other chemicals were of analytical grade.

2.2. Equipment

The electronic analytical balance was produced by Shimadzu Co. in Japan; the grinder and blender were made in China; the high pressure autoclave was produced by Astell Co. in England; the EmulsiFlex-B3 high-pressure homogenizer was purchased from Avestin Inc. (Ottawa, Ont.); the speed centrifuger was made in Germany; the vacuum deaerator was purchased from BeiJing TaiKeRuo Science Technology Co., Ltd.

2.3. Extraction of crude polysaccharides

Dried BaChu mushrooms (20 g) were ground in a high speed disintegrator (Model SF-2000, Chinese Traditional Medicine Machine Works, Shanghai, China) to obtain a fine powder, then were extracted in a Soxhlet apparatus with ether (20–40 °C), and pretreated with 80% ether twice to remove some coloured materials, oligosaccharides, and some small molecule materials. The organic solvent was volatilized and pretreated dry powder was obtained, as described previously (Yang, Qu, & Cheng, 2004; Zykwinska, Rondeau-Mouro, Garnier, Thibault, & Ralet, 2006). The pretreated dry powder (20.0 g) was extracted with deionized water (water–mushroom (ml/g) ranging from 1:1 to 6:1) at pH 6.5–7.5 (adjusting the suspension pH by 0.1 mol/l NaOH or HCl), while the temperature of the water bath ranged from 20 to 90 °C and was kept steady (within ±1.0 °C). The water–mushroom slurry in a 2.0 L stainless steel boiler in the water bath was stirred with an electric mixing paddle for a given time (extraction time ranging from 2 to 10 h) during the entire extraction process. The mixture was centrifuged (2000*g*, 20 min), then the supernatant was separated from insoluble residue with nylon cloth (pore diameter: 38 μm). The extracts were then defatted by the method of Sevag (Sevag, Lackman, & Smolens, 1938), precipitated by the addition of ethanol to a final concentration of 75% (v/v), and the precipitates were collected by centrifugation (2000*g*, 20 min), then solubilized in deionized water and lyophilized to get the crude polysaccharides.

2.4. Preparation of hawthorn juice

The hawthorn berry extract may be prepared in accordance with any conventional method by using suitable solvents, such as water. In brief, fresh fruit was washed, cleaned, and crushed into particles of 90 mesh, and infiltrated with warm water (60 °C) for 20 min. The particles were further ground by a gum machine into a size less than 15 μm. After enough amount of water was added (based on the amount of the solid content in fresh hawthorn) and uniformly stirred at a temperature lower than 50 °C. The mixture is kept at a temperature ranging from 30 to 70 °C for a period ranging from 1 to 6 h and was then hydrolyzed for 6 h and filtered. The filtrate was ultra-filtered and sterilized with ultrasonication (25 kHz, 1500 W, power density 100 W/cm^2, and flowing speed 4 m/s). The resulting extract is filtrated and concentrated to a formation of a concentrated hawthorn berry extract.

2.5. Preparation of apple juice

Good quality apple juice is made from a blend of apple varieties by processing fresh apples. In brief, after selected and washed, 15 kg of apples were directly crushed into less than 170 mesh, pre-soaked for 20 min in oxygen isolation, finely ground into less than 5 μm, added with the biological enzymes, and mixed slowly at a temperature lower than 55 °C. Refrigerate juice for 24–48 h. Without mixing, carefully pour off clear liquid and discard sediment. If desired, strain clear liquid through a paper coffee filter or double layers of damp cheesecloth. Heat quickly, stirring occasionally, until juice begins to boil. Pour immediately into sterile pint or quart jars, or pour into clean half-gallon jars, leaving 1/4-in. headspace. The juice productivity was 98.4%. The type of the raw material can be distinguished by watching, smelling, and tasting, respectively, the colour, flavour, and content of the juice so prepared. Nourishment value of the juice is higher than the edible part of the raw material, and much higher than that of the products made by the traditional process.

2.6. Preparation of compound beverages

A given quantity of BaChu mushroom polysaccharides was dissolved in water for a final concentration of 8.7 mg/ml. Polysaccharides solution (36.4%), hawthorn juice (45.4%), and apple juice (18.2%) were mixed to make fumet. Then, white granulated sugar and citric acid were incorporated into fumet according to a ratio of 3 and 0.15 g/100 ml for the purpose of making compound beverages (see Fig. 1).

3. Results and discussion

3.1. Effect of temperature on extraction yield of polysaccharides

As far as the extraction temperature is concerned, the higher the temperature, the higher the extraction yield of polysaccharides. As shown in Fig. 2, extraction yield increased from 4.17% to 6.65% with the increasing temperature. This is because an increase in temperature (from 70 to 95 °C) could enhance the extraction efficiency as a result of an increased diffusivity of the solvent into cells and an enhanced desorption of the components from cells (Amnuaykanjanasin, Epstein, & Labavitch, 2003; Ferreira, Mafra, Rosário Soares, Evtuguin, & Coimbra, 2006). However, on the other hand, a relatively high extraction temperature (at 100 °C) was detrimental to the extraction yield. Only a bit of the extraction yield was increased as the temperature was higher than 95 °C because of destruction of enzyme activity at high temperature in this reaction system (Shogren, Fanta, & Felker, 2006). Meanwhile, as the temperature increased, speed of polysaccharides entering and

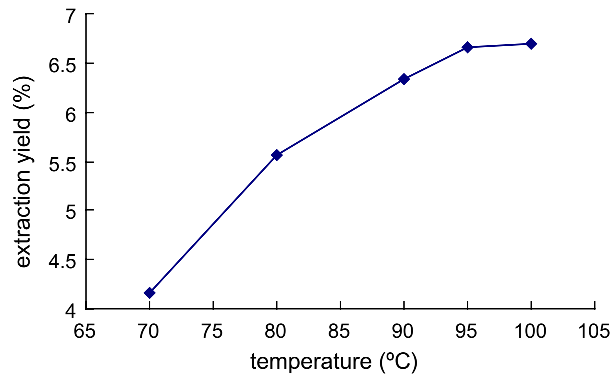

Fig. 2. Effect of extraction temperature on extraction yield of polysaccharides.

leaving from plant cells reached a balance (Singh & Malviya, 2006) which leads to the insignificant increase of the extraction yield. Combined with the above effects, the suitable temperature for highest total yield of the polysaccharides was considered to be 95 °C.

3.2. Effect of extraction time on extraction yield of polysaccharides

Extraction time is another factor that would influence the extraction efficiency and selectivity of the fluid (Ray et al., 2004). With the increase of the extraction time from 60 to 120 min in the extraction system, the extraction yield quickly increased from 4.92% to 7.42% (Fig. 3). When the extraction time continued to lengthen, the extraction yield increased little, probably because the polysaccharides in plant cells are already sufficiently extracted up. Because longer extraction time could delay and lengthen production cycle, 120 min of extraction time was adopted in the present work.

3.3. Effect of ratio of solvent to raw material on extraction yield of polysaccharides

Ratio of solvent to raw material was another factor affecting extraction yield of polysaccharides (Sun & Tomkinson, 2002). On the basis of our previous experiment (Hou & Chen (2008)), the ratio of solvent to raw material

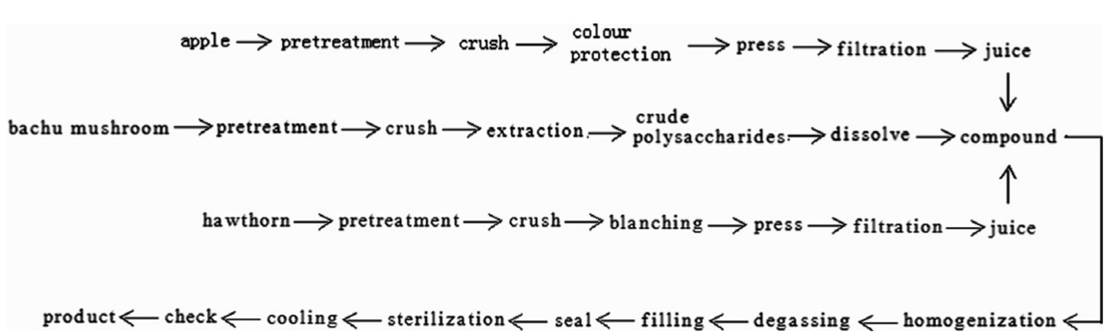

Fig. 1. Processing flow chart of compound beverage.

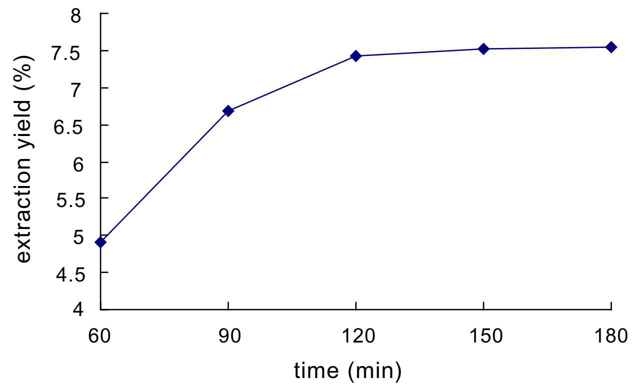

Fig. 3. Effect of extraction time on extraction yield of polysaccharides.

was set at 10, 20, 30, 40, and 50, respectively. With the increase of the ratio of solvent to raw material from 10 to 40 in the extraction system, the extraction yield quickly increased from 5.73% to 8.7% (Fig. 4). It can be seen from Fig. 4 that extraction yield of polysaccharides in the extraction system gives the highest value when the ratio of solvent to raw material was 40. This is due to the fact that the increasing ratio of solvent to raw material could decrease solution concentration difference inside and outside plant cells, which consequently prompted diffusion rate of solute particles and made more polysaccharides molecules enter solution. In addition, the extraction yield of polysaccharides increased little when the ratio of solvent to raw material surpassed 50. Combined with above effects, the suitable ratio of solvent to raw material for highest total yield of the polysaccharides was considered to be 40.

3.4. Comparison of two kinds of mushroom polysaccharides solution

Table 1 showed flavour comparison of mushroom polysaccharides solution prepared by two kinds of different methods. The squeezed juice was found to be deeper colour and worse flavour than the polysaccharides extract. The polysaccharides extract displayed a light colour and better

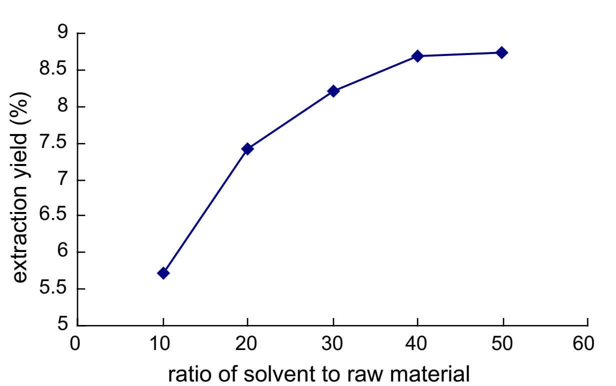

Fig .4. Effect of ratio of solvent to raw material on extraction yield of polysaccharides.

Table 1
Compare of flavour of squeezed juice and polysaccharides extract

	Solute content	Flavour	Colour
Squeezed juice	8.7	Bad	Brown
Polysaccharides extract	8.7	Average	Light amber

flavour. A possible explanation was that adverse flavour components were removed due to ethanol precipitation reaction. Therefore, it was assumed that the beverage made by latter method is better. That is to say, crude polysaccharide in BaChu mushroom was first extracted, and then mixed with water and fresh juice for the preparation of mushroom beverage.

3.5. The preparation of compound beverage

The orthogonal test with three factors and three levels is designed to analyze the optimal process parameters of the compound beverage (Table 2). And the L_9 $(3)^4$ table was designed to detect the effects of combination ratio of three raw materials (mushroom juice, hawthorn juice, and apple juice) on the flavour of compound beverage (Table 3). Furthermore, the score reflecting the flavour results were also listed. According to the value of range R in Table 3, the hawthorn juice (factor A) exerted the most significant effect on the flavour of compound beverage, and the order of importance that influenced the flavour of compound beverage was found to be hawthorn juice (B) > mushroom juice (A) > apple juice (C). The optimal combination parameters of the processing technology were $A_2B_3C_1$, namely, mushroom juice (36.4%), hawthorn juice (45.4%), and apple juice (18.2%). The percent ratio of three components in fumet were in turn hawthorn juice (B) > mushroom juice (A) > apple juice (C). The beverage product made by the optimal technology was good taste and odour and reddish brown.

This juice recipe has become our favorite drink and stunningly refreshing and full of flavour. It has more than 14,000 IU of vitamin A and over three times the vitamin C content of an apple. It also contains myoinositol, a lipid which helps with anxiety, insomnia, and in battling hardening of the arteries. It also contains the greatest amount of digestive enzymes. Mushroom is recommended by the American Cancer Society as powerful agents in the fight against intestinal and skin cancer (Milano et al., 2005). Hawthorn contains approximately 105 cal, yet it is dense in nutrients. This makes hawthorn a perfect food for healing and weight loss. In conclusion, the compound beverage is delicious, filling, and low in calories.

3.6. Quality index evaluation of the product

3.6.1. Sensory index

Reddish brown; light mushroom flavour, soft and refreshing taste, and attractive fruit flavour; uniform liquid.

Table 2
Factors and levels of the orthogonal experiment

No.	Factor		
	A (mushroom polysaccharides solution) (ml)	B (hawthorn juice) (ml)	C (apple juice) (ml)
1	30	30	20
2	40	40	30
3	50	50	40

Table 3
$L_9\ (3)^4$ orthogonal test result

No.	A (mushroom polysaccharides solution) (ml)	B (hawthorn juice) (ml)	C (apple juice) (ml)	Score
1	1	1	1	8.0
2	1	2	2	8.1
3	1	3	3	8.6
4	2	1	2	7.9
5	2	2	3	8.3
6	2	3	1	9.2
7	3	1	3	7.2
8	3	2	1	8.3
9	3	3	2	8.2
K1	8.24	7.70	8.33	
K2	8.60	8.20	8.10	
K3	7.77	8.70	8.04	
R	0.83	1.00	0.29	

3.6.2. Physical index

The soluble solid content (calculated with refractometer) > 9%; total acid content ⩾ 0.4%; polysaccharides content ⩾ 0.2%.

3.6.3. Microbial index

Bacteria count ⩽ 100 cfu/ml; *Escherichia coli* count ⩽ 5 cfu/100 ml; pathogenic bacteria count = 0.

4. Conclusion

This paper presents an experimental investigation upon the effect of different extraction parameters on yield of BaChu mushroom polysaccharides and optimal parameters of processing technology of a compound beverage.

(1) On the basis of the single-factor experiment employed in this study, it can be concluded that the optimal extraction parameters of BaChu mushroom polysaccharides are as followings: extraction temperature 95 °C, extraction time 120 min, and ratio of solvent to raw material 40.

(2) The squeezed juice was found to be deeper colour and worse flavour than the polysaccharides extract. The polysaccharides extract displayed a light colour and better flavour. It was assumed that the beverage made by latter method is better. That is to say, crude polysaccharide in BaChu mushroom was first extracted, and then mixed with water and fresh juice for the preparation of mushroom beverage.

(3) The orthogonal experiment employed in this study shows that the hawthorn juice (factor A) exerted the most significant effect on the flavour of compound beverage, and the order of importance that influenced the flavour of compound beverage was found to be hawthorn juice (B) > mushroom juice (A) > apple juice (C). The optimal combination parameters of the processing technology were A2B3C1, namely, mushroom juice (36.4%), hawthorn juice (45.4%), and apple juice (18.2%). The percent ratio of three components in fumet were in turn hawthorn juice (B) > mushroom juice (A) > apple juice (C). The beverage product made by the optimal technology was of good taste and odour, reddish brown.

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