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Extraction of *Polygonatum odoratum* polysaccharides using response surface methodology and preparation of a compound beverage

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ABSTRACT

An ultrasonic procedure for the extraction of *Polygonatum odoratum* (Mill.) Druc (*P. odoratum*) polysaccharides was established. Response surface methodology (RSM) was applied to optimize the ultrasound-assisted extraction parameters (ultrasonic time (X_1), extraction times (X_2), and ratio of solvent to raw material (X_3)) for enhancing the forward extraction efficiency of polysaccharides. The optimum extraction conditions were found to be ultrasonic time 40 min, extraction times 3, and ratio of water to raw material 80. Under these conditions, the yield of *P. odoratum* polysaccharides can increase from 11.40% to 15.15%. The physicochemical properties of *P. odoratum* polysaccharides were characterised. The results of monosaccharide composition by gas chromatography (GC) showed that the polysaccharides consisted of fucose, mannose, galactose, with the molecular ratio of 4.72:3.90:1.00. Four factor, three-level designed orthogonal experiment was developed to better understand how flavor of compound beverage is affected by different factors. The optimal combination parameters of the processing technology were *P. odoratum polysaccharide* solution (45.4%), hawthorn juice (18.2%), apple juice (36.4%), glucose (4%) and citric acid (0.2%).

1. Introduction

Polygonatum odoratum (Mill.) Druc (*P. odoratum*, Yu Zhu in Chinese) belongs to Polygonatum, Liliaceae large family, which grows wildly and is cultivated in the southern area of China. In addition to China, *P. odoratum* grows in Thailand and Vietnam. It can also be found growing throughout the southern United States. *P. odoratum* has a long history of indigenous use such as a condiment and it has also been used as a crude medicinal agent in the treatment of analeptic (Tomoda, Yoshiko, Tanaka, & Uno, 1971). In China it has been used as functional foods and well-known Chinese traditional medicine with the functions of removing dryness, promoting secretion of fluid and quenching thirst, treatment of diverse diseases for example diabetes etc. (Liu, Fu, & Cui, 1998; Zhou, Tang, Gao, & Zhou, 2005). *P. odoratum* was reported to reduce significantly hyperglycemia caused by starch loading in normal and diabetic mice and the effect was similar to that of acarbose (Chen, Feng, Guo, Sun, & Jiang, 2001). In southern China, people like to cook it with meats or porridges as health foods. *P. odoratum* is attracting more and more attention for its healthy function value.

There are many types of compounds that exists in *P. odoratum*. Compounds that have been previously identified in *P. odoratum* include quercitol (Lazer, Gheta, & Grigorescu, 1971), flavonoids (Yang, Chen, Chen, Yang, & Liu, 2005), azetidine 2-carboxylic acid (Fowden, 1956), mucous polysaccharides (Tomoda et al., 1971), and steroidal compounds (Lin, Han, & Liao, 1994; Sugiyama, Nakano, Tomimatsu, & Nohara, 1984). Among them, polysaccharide is one of the main bioactivity components of *P. odoratum* with the hypoglycemic, antioxidant, antitumor and hypotense activities. There were some reports on the extraction and activities of polysaccharides of *P. odoratum* (POPS) in recent years. Ultrasound extraction is the new technology that attracts much more attention in the department of separation and extraction. The application of ultrasound-assisted extraction offers many advantages including the reduction of solvents, temperature and the time for extraction. But there was no information on ultrasonic assisted extraction of *P. odoratum* polysaccharide (POPS) using response surface methodology (RSM) and there was no report on the beverage about *P. odoratum* polysaccharide till now.

In the present study, RSM was employed to estimate effects of different extraction parameters on yields of polysaccharide from *P. odoratum*. At the same time, an orthogonal test was employed to estimate optimal combination parameters of three raw materials (hawthorn juice, *P. odoratum* polysaccharide juice, and apple juice) for the preparation of a compound beverage.

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2. Materials and methods

2.1. Materials

Rhizoma of *P. odoratum* was collected in November 2009 from natural habitat in Kuandian County (Liaoning, China) and authenticated by associated Professor Haixia Chen, School of Pharmaceutical Science and Technology, Tianjin University, Tianjin, where a voucher specimen (TJC2009003) has been deposited. Hawthorn and apple were obtained from the local fruit market of Tianjin, China. White granulated sugar and citric acid were food-grade. All other chemicals were of analytical grade.

2.2. Experimental design

A three-level-three-factor, Box-Behnken factorial design (BBD) was employed in this optimization study. Ultrasonic time (X_1), extraction times (X_2), and ratio of water to raw material (X_3) were the independent variables selected to be optimized for the extraction of *P. odoratum*. Extraction yield (Y) was taken as the response of the design experiments. Seventeen experiments were augmented with three replications were carried out at the center points to evaluate the pure error.

Once the experiments are performed, the response variable (extraction yield) was fitted a second-order model in order to correlate the response variable to the independent variable. The general form of the second-order polynomial equation is as follows:

$$Y = \beta_0 + \sum_{i=1}^{3} \beta_i X_i + \sum_{i=1}^{3} \beta_{ii} {X_i}^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j$$

where Y is the predicted response; X_i and X_j are input variables which influence the response variable Y; β_0 is a constant; β_i is the linear coefficient; β_{ii} is the quadratic coefficient and β_{ij} $(i \neq j)$ is the linear- by-linear interaction between X_i and X_j. The test variables were transformed to range between −1 and 1 for the appraisals of factors.

2.3. Ultrasonic extraction (UAE) of crude polysaccharides (UPS)

Dried ground samples (10 g) were extracted with water at the corresponding ultrasonic conditions. The water extraction solutions were obtained by centrifugation (2000 × *g* for 10 min, at 20 °C), and then concentrated and precipitated by the addition of dehydrated alcohol to a final concentration of 75% (v/v). The precipitates collected by centrifugation (2000 × *g* for 10 min, at 20 °C) were washed by dehydrated alcohol for three times and freezing dried. The sugar content was measured by phenol–sulfuric method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) using D-glucose as a standard (R^2 = 0.9949), the purity (%) was calculated as the glucose content of extraction/dried crude polysaccharide weight.

2.4. Hot water extraction (HWE) of crude polysaccharides (PS)

Dried ground *P. odoratum* samples (10 g) were extracted with water at 100 °C (1:56 (w/v), 3 h, 3 times). The water extraction solutions were separated by centrifugation (2000 × *g* for 10 min, at 20 °C), and then were concentrated and precipitated by the addition of dehydrated alcohol to a final concentration of 75% (v/v). The precipitates were collected by centrifugation (2000 × *g* for 10 min, at 20 °C) and were washed by dehydrated alcohol for three times and then were freezing dried.

2.5. Molecular weight distribution

The molecular weight of polysaccharides was determined using gel permeation chromatography (GPC) on Sephadex G-150 column (60 × 2.5 cm, i.d.). The column was eluted by 0.02 M PBS at a flow rate of 8.0 mL/h. Fraction was collected for every 4 mL. The total carbohydrate of each fraction was determined by using phenol–sulfuric acid method. The molecular weight of polysaccharides was obtained from the regression line of the standard molecular weight compared with fraction number plot. The calibration curve was made with dextran standards of different molecular weighs (Dextran T-500, T-70, T-40, and T-10) (Fu, Chen, Dong, Zhang, & Zhang, 2010).

2.6. Determination of the monosaccharide composition

The composition of neutral monosaccharide of UPS and PS was measured by gas chromatography after converting them into acetylated derivatives (Chaplin & Kennedy, 1994). Briefly, 30 mg of different samples were hydrolyzed in a sealed glass tube with 2 M trifluoroacetic acid (TFA) at 120 °C for 4 h. The hydrolysate was evaporated to dryness. The acid was removed under reduced pressure by repeated coevaporations with methanol. The hydrolysate was then converted into alditol acetates according to conventional procedures. Gas chromatography was performed on a Shimadzu GC-14B instrument with capillary column (HP-5, 30 m × 0.32 mm × 0.5 μm). The operation was performed in the following conditions: injection temperature: 250 °C; detector temperature: 260 °C; column temperature programmed: 150–210 °C increasing at 10 °C/min for 6 min; then increasing to 255 °C at 15 °C/min for 3 min; and finally increasing to 260 °C at 1 °C/min for 5 min. Nitrogen was used as the carrier gas and maintained at 1.0 mL/min. Arabinose, xylose, galactose, glucose, rhamnose, mannose and fructose were used as the standards.

2.7. Morphological analysis

Scanning electron micrographs were obtained with an environmental scanning electron microscope (ESEM, Philips XL-30, Philips-FEI Co., Eindhoven, The Netherlands). The polysaccharide samples of were placed on a specimen holder with the help double-sided adhesive tapes and coated with gold powder (Yu, Wang, Jin, Sun, & Yu, 2009). Each sample was observed with 5000-fold magnification at an accelerating potential of 20 kV during micrography.

2.8. Beverage formulation

2.8.1. Preparation of hawthorn juice

The hawthorn berry extract was prepared in accordance with conventional method by using water. In brief, fresh fruit was washed, cleaned, and crushed into particles of 90 mesh, and infiltrated with hot water (90 °C, hawthorn berry-water (g/mL) ratio 1:6) for 30 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 was 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 was filtrated and concentrated to a formation of a concentrated hawthorn berry extract.

2.8.2. Preparation of apple juice

Good-quality apple juice is made from a blend of apple varieties. Briefly, after selected and washed, fresh apples were directly

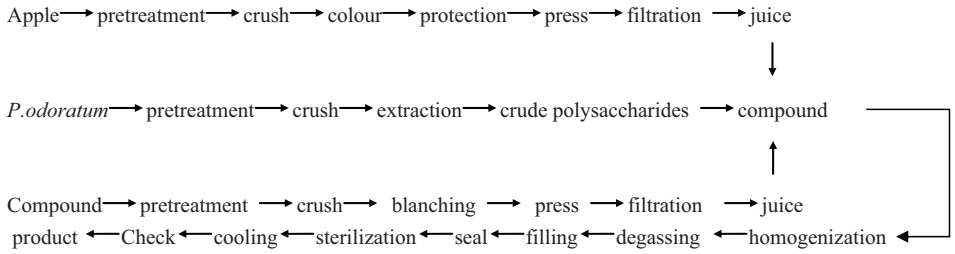

Fig. 1. Processing flow chart of compound beverage.

crushed in to particles, pre-soaked for 10 min in 1% (w/w) Vitamin C. The particles were further ground by a gum machine into a size less than 15 μm. Apple jam was filtered with nylon cloth. The filtrate was sterilized to get apple juice.

2.8.3. Preparation of compound beverages

The *P. odoratum* polysaccharides solution was concentrated for a final concentration. *P. odoratum* polysaccharide solution, hawthorn juice, apple juice were mixed to make fumet. Then, sugar and citric acid were incorporated into fumet for the purpose of making compound beverages (Fig. 1).

2.8.4. Descriptive sensory analysis

Eleven panelists were selected based on interest, time availability, and sensitivity to basic tastes. Each panelist (3 male, 8 female) had at least 40 h of previous descriptive sensory analysis training using basic tastes and the Spectrum technique (Meilgaard, Civille, & Carr, 1999). During subsequent sessions, practice with references and products was conducted to determine the final ballot. Final terms included on the ballot were color intensity, fruit aroma and sweet (Table 1). Standard references for flavor (universal scale) and taste (basic) attributes were used. Panelists evaluated nine samples per 2 h session for 8 sessions. Attributes were scored using a 15 cm unstructured line scale. The steps in each evaluation session included reference review, anchor sample review and evaluation of experimental samples. Panelists cleared the palate between samples with unsalted crackers and spring water. A 10 min rest period was used between samples. The anchor sample was evaluated by the panel prior to data collection and given consensus scores for each descriptive attribute. These consensus scores were marked on the ballot for reference by the panelists. During the data collection phase, the sample was tasted before each experimental sample and used along with reference standards for anchoring attributes on the scale. A randomized complete block design was used for sensory testing with session as a block. Three replications of each design point were completed. The scores were first analyzed using orthogonal test with color intensity, fruit aroma and sweet included as the main effects (Baron & Hanger, 1997).

Table 1
Sensory language for descriptive sensory analysis of beverages.

Attribute	Definition	Reference
Color intensity (20%)	The intensity or strength of a color from light to dark	Dark = 0–12
		Medium = 12–15
		Light = 15–20
Fruit aroma (20%)	Aromatics associated with different fruits evaluated orthonasally	Sole < 12
		Light = 12–15
		Mix = 16–20
Sweet (60%)	Taste sensation associated with sugar	Supersour or supersweet < 36
		Slightly acidic or slightly sweet = 36–45
		Moderate = 46–60

3. Results and discussion

3.1. Model fitting

Orthogonal testing is applied extensively in the extraction of polysaccharides. Orthogonal test design focuses on arranging reasonable experiments that can consider several factors simultaneously and find optimal factor levels, but it fails to give a regression equation for the whole parameter space tested. Response surface optimization, on the other hand, establishes a high precision regression equation, details the interactions between several factors, and is more advantageous than the traditional orthogonal test design because of its highly efficient, time saving design pattern (Liang, 2008; Qiao et al., 2009). A total of 17 runs for optimizing the three individual parameters in the current BBD were applied in the production of POPS. The values of the independent process variables (X_1, X_2 and X_3) considered, as well as the measured and predicted values for response (extraction yields of polysaccharides), are given analytically in Table 2. The POPS yields ranged from 9.29% to 15.03%. By applying multiple regression analysis on the experimental data, the response variable and the test variables can be related using the following polynomial equation:

$$Y = 12.74667 + 1.40625X_1 + 0.38X_2 - 0.12875X_3 - 0.395833X_1X_1 + 1.3325X_1X_2 + 0.175X_1X_3 + 0.311667X_2X_2 + 0.2775X_2X_3 - 0.015833X_3X_3$$

where Y was the polysaccharide yield and X_1, X_2, and X_3 were the values for the ratio of water to ultrasonic time, extraction times, and ratio of solvent to raw material, respectively.

Table 2
Three-factor, three levels central composite design and experimental data of the investigated responses of POPS extracts ($n = 2$).

	Factor 1(X_1) extraction times	Factor 2(X_2) extraction time (minute)	Factor 3(X_3) liquid–material ratio	Yield (%)
1	−1(1)	0(30)	−1(1:80)	11.53
2	−1(1)	−1(20)	0(1:90)	13.00
3	−1(1)	0(30)	+1(1:100)	9.29
4	−1(1)	+1(40)	0(1:90)	10.55
5	0(2)	−1(20)	−1(1:80)	11.98
6	0(2)	−1(20)	+1(1:100)	12.80
7	0(2)	0(30)	0(1:90)	12.79
8	0(2)	0(30)	0(1:90)	13.17
9	0(2)	0(30)	0(1:90)	12.28
10	0(2)	+1(40)	+1(1:100)	14.66
11	0(2)	+1(40)	−1(1:80)	12.73
12	+1(3)	0(30)	+1(1:100)	13.49
13	+1(3)	−1(20)	0(1:90)	12.11
14	+1(3)	+1(40)	0(1:90)	14.99
15	+1(3)	0(30)	−1(1:80)	15.03

Table 3
Predicted and experimental values of responses under optimal conditions.

Extraction method	Sample of quantity (g)	Ratio of water to raw material (mL/g)	Extraction time (min)	Extraction times	Yield of polysaccharides (%)
UAE (predicted)	1.00	56	27	3	15.44
UAE (experimental)	1.00	56	27	3	15.15
HWE	1.00	56	180	3	11.40

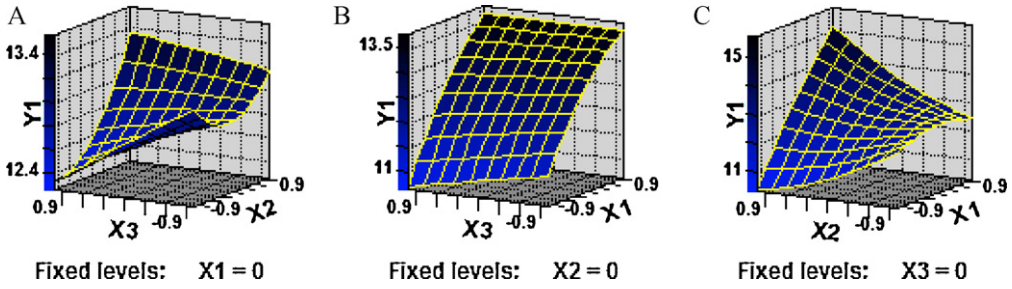

Fig. 2. Response surface of the effects of extraction times, ultrasonic time and liquid–material ratio on the yield of POPS extracts.

Three ultrasonic factors used for the incorporated network were listed below: ultrasonic time (20–40 min), extraction times (1–3), and ratio of water to raw material (80–100). Coefficient (R^2) of determination was defined as the ratio of the explained variation to the total variation and was a measurement of degree of fitness (Nath & Chattopadhyay, 2007). The small value of R^2 indicates the poor relevance of the dependent variables in the model. The model can fit well with the actual data when R^2 approaches unity (Sin, Yusof, Hamid, & Rahman, 2006). Analysis of variance, the R^2 values of the model for the yield of POPS was determined to be 0.7517.

Through these three-dimensional plots and their respective contour plots, the optimal values of the tested variables for obtaining a POPS yield of 15.44% can be predicted as follows: ratio of water to raw material, 55.83 mL/g; extraction time, 27.24 min; and extraction times, 2.56. However, considering the operability in actual production, the optimal conditions can be modified as follows: ratio of water to raw material, 56 mL/g; extraction time, 27 min; and extraction times, 3. To validate the adequacy of the model equations, a verification experiment is carried out under the optimal conditions mentioned above. Under these conditions, the experimental yield of polysaccharides was 15.15% ($n = 3$), which was agreed closely with the predicted value (Table 3). The results demonstrated the validation of the extraction model.

3.2. Effects of ultrasonic time, extraction times, and ratio of solvent to raw material on the yield of POPS

The effects of ultrasonic time, extraction times, and ratio of solvent to raw material on the yield of POPS as well as their interactions are shown in Fig. 2A–C. Each factor exhibited a complicated correlation with the yield. As shown in Fig. 2A, at a constant extraction times and in the range of ratio of solvent to raw material under investigation, the yield of POPS increased with the extension of ultrasonic time. There was an increase in the POPS yield when the yield reaches its maximum at a fixed extraction time, with no significant further improvement (Fig. 2B). The ultrasonic time showed a positive effect on the yield of POPS when the extraction time was relatively high (Fig. 2C).

3.3. Molecular weight distribution of polysaccharides

The molecular weight distribution of polysaccharides UPS, PS were determined by gel permeation chromatography (GPC) on Sephadex G-150 column (2.0×60 cm, i.d.). The elution cure of the 2 polysaccharides is shown in Fig. 3. The gel permeation chromatographic profiles showed that the carbohydrate fraction of the polysaccharides eluted over a wide range of molecular size, indicating that the two polysaccharides were highly polydispersed. The molecular weight distribution of the main two fractions of the polysaccharides UPS were 2.3×10^6, 5.4×10^4 and 5.3×10^3, respectively. The weight percentage of the three peaks were 43.3%, 39.4% and 17.2%. While the molecular weight distribution of the main two fractions of the polysaccharides PS were 1.0×10^6 and 0.77×10^4. The weight percentages of two peaks were 73.6% and 26.4%. The results showed that the molecular weight of the ultrasonic treatment polysaccharides was higher than that of extraction by hot water, which suggested that ultrasound can facilitate swelling and hydration and so cause an enlargement in the pores of the cell wall. This will improve the diffusion process and therefore enhancing mass transfer (Vinatoru, 2001), some large molecular size polysaccharides were extracted. However, the weight percentage of large molecular size polysaccharides by ultrasonic treatment was lower than that of extraction by hot water, which suggested that a small portion of large molecular size polysaccharides was shifted to small ones after ultrasonic treatment, ultrasonic wave could degrade high molecular weight

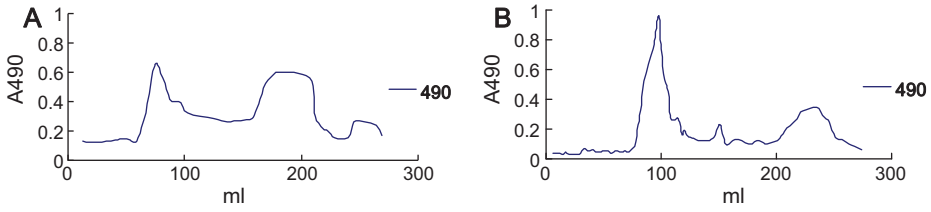

Fig. 3. Gel permeation chromatography of polysaccharides on Sephadex G-150 column. (A, UPS, the polysaccharide obtained from *Polygonatum odoratum* at the ultrasonic treatment condition; B, PS, the polysaccharide extracted from *Polygonatum odoratum* by hot water.)

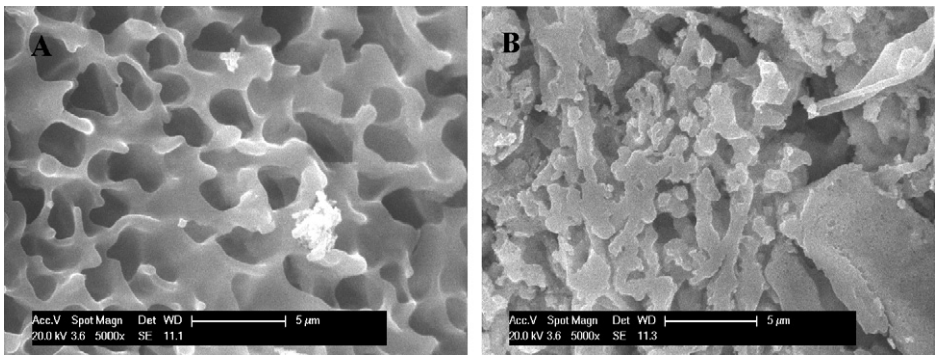

Fig. 4. Scanning electron micrographs of UPS and PS (5000×; A, UPS, the polysaccharide obtained from *Polygonatum odoratum* at the ultrasonic treatment condition; B, PS, the polysaccharide extracted from *Polygonatum odoratum* by hot water).

polysaccharides to small ones. Therefore, degradation on ultrasonic treatment was responsible for the change in the molecular weight of polysaccharides (Mislovicova, Masarova, Bendzalova, Soltes, & Machova, 2000; Zhou & Ma, 2006).

3.4. Monosaccharide composition of polysaccharides POPS

The monosaccharide composition of the two kinds of POPS (UPS and PS) were determined by gas chromatography (GC). Both UPS and PS were found to be composed of fucose, mannose and galactose. UPS was composed of fucose, mannose and galactose with the molecular ratio of fucose, mannose, galactose was 4.72:3.90:1.00, while PS was composed of fucose, mannose and galactose with the molecular ratio of fucose, mannose and galactose was 4.72:3.90:1.00. The results showed that ultrasonic treatment during the extraction process induced the changes of the monosaccharides composition of the polysaccharides.

3.5. Morphological analysis of polysaccharides

The image of POPS extracted by hot water contains clear and small particles distributed discretely (Fig. 4A). After UAE, a more significant impact on the structural changes caused by ultrasonic cavitation was observed (Fig. 4B). A spongy structure with a defined fracture can also be seen more clearly, suggesting that ultrasonic vibration increases the number of cavitation formed as well as the mass transfer rates to prompt the migration of target compounds from the material to the surroundings and to bring about a higher extraction efficiency of polysaccharides. These results were accord with those of the molecular weight distribution.

In the continual search for improved degrading or reassociating pathways, recent emphasis has been given to ultrasound. It has been pointed out that the sonochemical method were potentially useful in carbohydrate chemistry. Firstly, the mechanical effect of ultrasonic wave improves heterogeneous reactions of polysaccharides in terms of smoother experimental conditions. Secondly, due to the easy formation of transient reactive species new transformation can be designed (Kardos & Luche, 2001).

3.6. Descriptive evaluation of the compound beverage

The orthogonal test with four factors and three levels is designed to analyze the optimal process parameters of the compound beverage. And the L9 $(3)^4$ table was designed to detect the effects of combination ratio of three raw materials (*P. odoratum polysaccharide* solution, hawthorn juice, and apple juice) on the flavor of compound beverage. Furthermore, the score reflecting the flavor results were also listed. According to the value of range *R* in Table 4, the hawthorn juice (factor *B*) exerted the most significant effect on the flavor of compound beverage, and the order of importance that influenced the flavor of compound beverage was found to be hawthorn juice (*B*) > apple juice (*C*) > *P. odoratum polysaccharide* solution (*A*). The optimal combination parameters of the processing technology were $A_3B_1C_3$, namely, PO juice (45.4%), hawthorn juice (18.2%), apple juice (36.4%). Then, sugar and citric acid were incorporated into fumet according to a ratio of 4.0 and 0.2 g/100 mL for the purpose of making compound beverages. The beverage product made by the optimal technology was good taste and odor, light orange.

Table 4
L9 $(3)^4$ orthogonal test result.

No.	*A* (POPS solution) (mL)	*B* (hawthorn juice) (mL)	*C* (apple juice) (mL)	Score
1	30	20	20	66.0
2	30	30	30	65.8
3	30	40	40	67.8
4	40	20	30	75.5
5	40	30	40	66.8
6	40	40	20	61.0
7	50	20	40	82.5
8	50	30	20	62.0
9	50	40	30	61.5
K1	199.60	224.00	189.00	
K2	203.30	194.60	202.80	
K3	206.00	190.30	217.10	
R	2.13	11.23	9.37	

This juice recipe has become our favorite drink and stunningly refreshing and full of flavor. It also contains many bioactive nutrients such as polysaccharides, polyphenols, flavonols, vitamins, which helps with anxiety, insomnia, and in battling hardening of the arteries. It also contains the greatest amount of digestive enzymes. In conclusion, the compound beverage is delicious, filling, and low in calories.

3.7. Physical index

The soluble solid content (calculated with refractometer) >9.0%; total acid content ≥0.4%; polysaccharides content ≥0.2%.

3.8. Microbial index

Bacteria count ≤100 cfu/mL; *Escherichia coli* count ≤5 cfu/100 mL; pathogenic bacteria count = 0.

4. Conclusion

This paper presented an experimental investigation upon the effect of different extraction parameters on yield of *P. odoratum*

polysaccharides and optimal parameters of processing technology of a compound beverage. On the basis of the response surface methodology test employed in this study, the optimal extraction parameters of *P. odoratum* polysaccharides were as followings: extraction times 3, ultrasonic time 40 min, liquid–material ratio 80. The orthogonal experiment employed in this study showed that the hawthorn juice (factor *B*) exerted the most significant effect on the flavor of compound beverage, and the order of importance that influenced the flavor of compound beverage was found to be hawthorn juice (*B*) > *P. odoratum polysaccharide* solution (*A*) > apple juice (*C*). The optimal combination parameters of the processing technology were $A_3B_1C_3$, namely, PO juice (45.4%), hawthorn juice (18.2%), and apple juice (36.4%). The percent ratio of three components in fumet were in turn hawthorn juice (*B*) > apple juice (*C*) > *P. odoratum polysaccharide* solution (*A*). The beverage product made by the optimal technology was of good taste and odor, light orange.

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References

Baron, R. F., & Hanger, L. Y. (1997). Using acid level, acesulfame potassium/aspartame blend ratio and flavor type to determine optimum flavor profiles of fruit flavored beverages. *Journal of Sensory Studies*, *13*, 272–274.

Chaplin, M. F., & Kennedy, J. F. (1994). *Carbohydrate analysis: A practical approach*. Oxford University Press.

Chen, H. B., Feng, R. Z., Guo, Y., Sun, L. H., & Jiang, J. L. (2001). Hypoglycemic effects of aqueous extract of rhizoma *Polygonati odorati* in mice and rats. *Journal of Ethnopharmacology*, *74*, 225–229.

Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical Chemistry*, *28*, 350–356.

Fowden, L. (1956). Azetidine-2-carboxylic acid: A new cyclic imino acid occurring in plants. *Biochemical Journal*, *64*, 323–332.

Fu, L. L., Chen, H. X., Dong, P., Zhang, X., & Zhang, M. (2010). Effects of ultrasonic treatment on the physicochemical properties and DPPH radical scavenging activity of polysaccharides from mushroom *Inontus obliquus*. *Journal of Food Science*, C323–C328.

Kardos, N., & Luche, J. L. (2001). Sonochemistry of carbohydrate compounds. *Carbohydrate Research*, *332*, 115.

Lazer, M., Gheta, D., & Grigorescu, E. (1971). Contributii la studiul fitochimic al plantei *Polygonatum officinale*. *Farmacia*, *19*, 31–38.

Liang, R. J. (2008). Optimization of extraction process of *Glycyrrhiza glabra* polysaccharides by response surface methodology. *Carbohydrate Polymers*, *74*, 858–861.

Lin, H. W., Han, G. Y., & Liao, S. X. (1994). Studies on the active constituents of the chinese traditional medicine *Polygonatum odoratum* (Mill.) Druce. *Yao Hsueh Hsueh Pao*, *29*, 215–222.

Liu, Y. P., Fu, G. F., & Cui, H. (1998). Current advances on pharmacological researches of Huang Jing, Yu Zhu and their preparations. *Li Shizhen Medicine and Materia Medica Research*, *4*, 371–373.

Meilgaard, M., Civille, G. V., & Carr, B. T. (1999). Descriptive analysis techniques. In M. Meilegaard, G. V. Civille, & B. T. Carr (Eds.), *Sensory evaluation techniques* (3rd ed., pp. 173–183). Boca Raton, FL: CRC Press.

Mislovicova, D., Masarova, J., Bendzalova, K., Soltes, L., & Machova, E. (2000). Sonication of the chitin-glucan, preparation of water-soluble fractions and characterization by HPLC. *Ultrasonics Sonochemistry*, *7*, 63–68.

Nath, A., & Chattopadhyay, P. K. (2007). Optimization of oven toasting for improving crispness and other quality attributes of ready to eat potato-soy snack using response surface methodology. *Journal of Food Engineering*, *80*, 1282–1292.

Qiao, D. L., Hu, B., Gan, D., Sun, Y., Ye, H., & Zheng, X. X. (2009). Extraction optimized by using response surface methodology, purification and preliminary characterization of polysaccharides from *Hyriopsis cumingii*. *Carbohydrate Polymers*, *76*, 422–429.

Sin, H. N., Yusof, S., Hamid, N. S. A., & Rahman, R. A. (2006). Optimization of enzymatic clarification of sapodilla juice using response surface methodology. *Journal of Food Engineering*, *73*, 313–319.

Sugiyama, M., Nakano, K., Tomimatsu, T., & Nohara, T. (1984). Five steroidal components from the rhizomes of *Polygonatum odoratum* var Pluriflorum. *Chemical and Pharmaceutical Bulletin*, *32*, 1365–1372.

Tomoda, M., Yoshiko, Y., Tanaka, H., & Uno, M. (1971). Plant mucilages. II. Isolation and characterization of a mucous polysaccharide, odoratan, from *Polygonatum odoratum* var japonicum rhizomes. *Chemical and Pharmaceutical Bulletin*, *19*, 2173–2177.

Vinatoru, M. (2001). An overview of the ultrasonically assisted extraction of bioactive principles from herbs. *Ultrasonics Sonochemistry*, *8*, 309.

Yang, X. G., Chen, S. B., Chen, S. L., Yang, D. J., & Liu, T. S. (2005). Studies on TLC fingerprint of flavonoids in rhizome of *Polygonatum odoratum*. *China Journal of Chinese Materia Medica*, *30*, 104–106.

Yu, J. L., Wang, S. J., Jin, F. M., Sun, L., & Yu, J. G. (2009). The structure of C-type rhizoma Dioscorea starch granule revealed by acid hydrolysis method. *Food Chemistry*, *113*, 585–591.

Zhou, C., & Ma, H. (2006). Ultrasonic degradation of polysaccharide from a red algae (*Porphyra yezoensis*). *Journal of Agricultural and Food Chemistry*, *54*, 2223–2228.

Zhou, Y., Tang, C., Gao, X., & Zhou, R. L. (2005). Programs of studies on Materia Medica Yu Zhu. *Journal of Tianjin Medical University*, *11*, 328–330.