

Extraction of soluble dietary fiber and hemicellulose from *Cornus officinalis* residue and preparation of fiber drinking water

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Abstract Soluble dietary fiber (SDF) is an extract from spent residue of *Cornus officinalis* Sieb. et Zucc fruit by ultrasonic. The conditions of the experiment optimized with response surface methodology were ambient temperature of 70.3°C, the ratio of solvent to solid being 30 mL/g, and processing time of 110 min under the ultrasonic wave frequency of 40 kHz and ultrasonic power of 250 W. The best aggregative indicator, incorporating the yield of *Cornus* SDF (CSDF), the content of SDF, and light transmittance (% at 620 nm) of SDF, was predicted to be 12.7845 according to the fitting equation. After UV sterilization, 0.25% (w/v) dispersion of CSDF in mineral water presented a good light transmittance (94.9%) and shelf-life (over 2 months). It had no smell and tasted good. The left residue was extracted with caustic soda to get hemicellulose.

Keywords *Cornus officinalis*, soluble dietary fiber, response surface methodology, fiber drinking water, hemicellulose

Introduction

Dietary fiber includes soluble dietary fiber (SDF) and insoluble dietary fiber (IDF) that are considered to be important elements in human diet. In addition to xyloglucans and galactomanan hemicelluloses, SDF can also include β -glucans and pectic substances, gums, and mucilage (Jiménez-Escrig and Sánchez-Muniz, 2000). SDF exists widely in vegetables and food crops as an important part in plant cell wall and has a good effect on suppression of increasing serum cholesterol level (Arjmandi et al., 1992; Jensen et al., 1997; Schwesinger et al., 1999; Xu et al., 2010). A diet high in SDF is inversely associated with the risk of cardiovascular disease (Theuwissen and Mensink, 2008) and the decrease in ambulatory blood pressure in hypertensives (Burke et al., 2001). While viscous dietary fiber has a range of metabolic health benefits (Jenkins et al., 2004) and might be effective in reducing bodyweight gain (Artiss et al., 2006). The administration of SDF is useful for controlling spontaneous favorable bowel movement by improving symptoms of small intestinal mucosal atrophy and normalizing the intestinal flora (Nakao et al., 2002). Soluble fiber intake

with the corresponding increase in short-chain fatty acid production was proven to significantly contribute to the digesting energy. Thereby, the short-term benefits of soluble fiber consumption on potentially outweighing are reported (Isken et al., 2010). Adding thickening agents to traditional infant formulas based on SDF probably affects calcium, iron, and zinc availability in various ways (Bosscher et al., 2001; 2003).

The potential benefits of SDF have encouraged the consumption of fiber-rich products especially when there is a tendency of finer food processing and consequently lower dietary fiber. Some of the by-products of food processing, such as apples, citrus fruits, and *Brassica* vegetables, have already been used in the production of dietary fiber (Chantaro et al., 2008), which are subsequently added into baked products, staple food including noodles and buns, function food, dairy products, and beverage to improve product properties.

Cornus officinalis Sieb. et Zucc has been used as a traditional Chinese medicine for more than 2000 years. As health products, *Cornus* products, including *Cornus* wine, beverages, preserves, and oral liquid, enjoy a good reputation in China. It is also widely used as tonic, analgesic, and diuretic in Japan and Korea. Moreover, the semi-dried, pitted *Cornus* can treat symptoms associated with liver or kidney deficiencies, diabetes, and uterine bleeding (Wu et al., 2008). Iridoid total glycoside from *Cornus* is proven capable of

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inhibiting overformation of advanced glycation end-products and having an effect on partially recovering levels of some vasoactive factors and cytokines (Xu and Hao, 2004). One of the active principles (oleanolic acid) contained in *Cornus* can raise insulin secretion in Wistar rats (Hsu et al., 2006). However, the efficacy part of *Cornus* is almost concentrated in its pulp, whose by-products of extensive processing have always been discarded. It is a real economic loss since the by-products are still rich in fiber and can be further processed into dietary fiber.

Mineral water is the most available drink in our dairy life, supplying both water and mineral elements for body normal metabolism. In our study, we chose mineral water as solvent to prepare fiber drinking water.

Response surface methodology (RSM), an experimental strategy for seeking the optimum conditions of a multi-variable system, is a highly efficient technique for optimization. It can be used to evaluate the relative significance of several affecting factors even in the presence of complex interaction. This method can provide information about the interaction between variables, necessary information for design and process optimization, and give multiple responses at the same time, which has been successfully applied in the optimization of medium composition (Liew et al., 2005), conditions for hot water extraction (Lee et al., 2006), and analysis of photo-induced decoloration (Körbahti and Rauf, 2008). A central composite design was employed to construct the conditions adopted in the paper.

This study aimed at optimizing the conditions for the improvement of *Cornus* SDF (CSDF) output and purity, determining the chemical composition and functional properties of *Cornus*, and tentatively preparing CSDF drinking water with low-calorie and more capacity to create a sense of satiety.

Materials and methods

Experimental materials

Commercial *Cornus* was purchased from Hanzhong, Shaanxi Province, China and then washed with tap water. After the seeds were removed by fruit stone extractor, the *Cornus* juice was squeezed out, and the rest of the *Cornus* was dried in an electric dry oven, grounded to fine powder able to pass through 380 μm sieve, and finally stored at 5°C prior for analysis.

Methods

Pretreatment of material

The ground *Cornus* residue (50 g) was refluxed with 200 mL of 70% (v/v) ethanol at 70°C for 60 min to remove some of the polyphenols, flavonoids, monosaccharides, and saponin. The mixture was centrifuged at 3000 \times g for 10 min to obtain

the precipitate, which was later mixed with distilled water (1250 mL) at 80°C and soon placed in ultrasonic wave extractor at a wave frequency of 40 kHz and power of 250 W for 60 min. After that, the treated mixture was again centrifuged at 3000 \times g for 5 min. Afterwards, the residue was extracted twice with distilled water (500 mL) in the same process. Finally, all the supernatants were combined and concentrated to 100 mL under vacuum pressure.

Extraction of CSDF from concentrate

The concentrate was hydrolyzed with α -amylase (0.25 g) at 60°C for 4 h. The pH of reaction solution was adjusted to 6.5. The concentrate was centrifuged at 3000 \times g for 30 min. Then, to remove the protein from the supernatant, the Sevag method was adopted, with chloroform and normal butanol (v/v = 5:1) 30 mL, 2 h shaking in THZ-C and 10 min centrifugation at 3000 \times g. Repetitive operation will be performed when necessary. The supernatant was distilled under reduced pressure to remove the chloroform and normal butanol and then mixed with four times of volume of 95% (v/v) ethanol at 20°C for 12 h in oscillator and later centrifuged at 3000 \times g for 5 min. The precipitate was washed with hexane to remove fat, then washed with 95% ethanol twice, centrifuged (3000 \times g, 5 min), re-dispersed in water at the ratio of 1:5 (v/v), and finally dried in hot air to get CSDF powder (Fig. 1).

Alkali extraction of hemicellulose from residue

According to the method of Sun and others (Sun et al., 1999; Fang et al., 2000; Ren et al., 2007) with some modifications, the residue after extracting twice was dried in hot air circulating oven at 65°C overnight. Dried residue (20 g) was extracted twice with 10% NaOH (400 mL) at room temperature for 10 h in THZ-C and then centrifuged at 3000 \times g for 5 min. The supernatant was neutralized with acetic acid to pH 6.0 and centrifuged at 3000 \times g for 10 min to get precipitate (Part 1) and supernatant (Part 2). Part 1 was washed with 95% ethanol, shook in THZ-C for 30 min, centrifuged at 3000 \times g for 5 min, re-dispersed in water at ratio of 1:5 (w/v) in THZ-C for 1 h, and, after vacuum filtration, dried in hot air circulating oven at 65°C. Part 2 was concentrated to 1/3 of the original volume under vacuum pressure, precipitated in 4 vol ethanol for 12 h, and centrifuged at 3000 \times g for 5 min to remove supernatant. The precipitate was washed with 95% ethanol and re-dispersed in water at ratio of 1:5 (v/v) in THZ-C for 1 h. After vacuum filtration, the filtrate was dried in hot air circulating oven at 65°C (Fig. 1).

Composition analysis

Moisture content was determined by oven-drying sample at 105°C to constant weight. Ash content was measured by ignition at 550°C using a muffle furnace up to constant

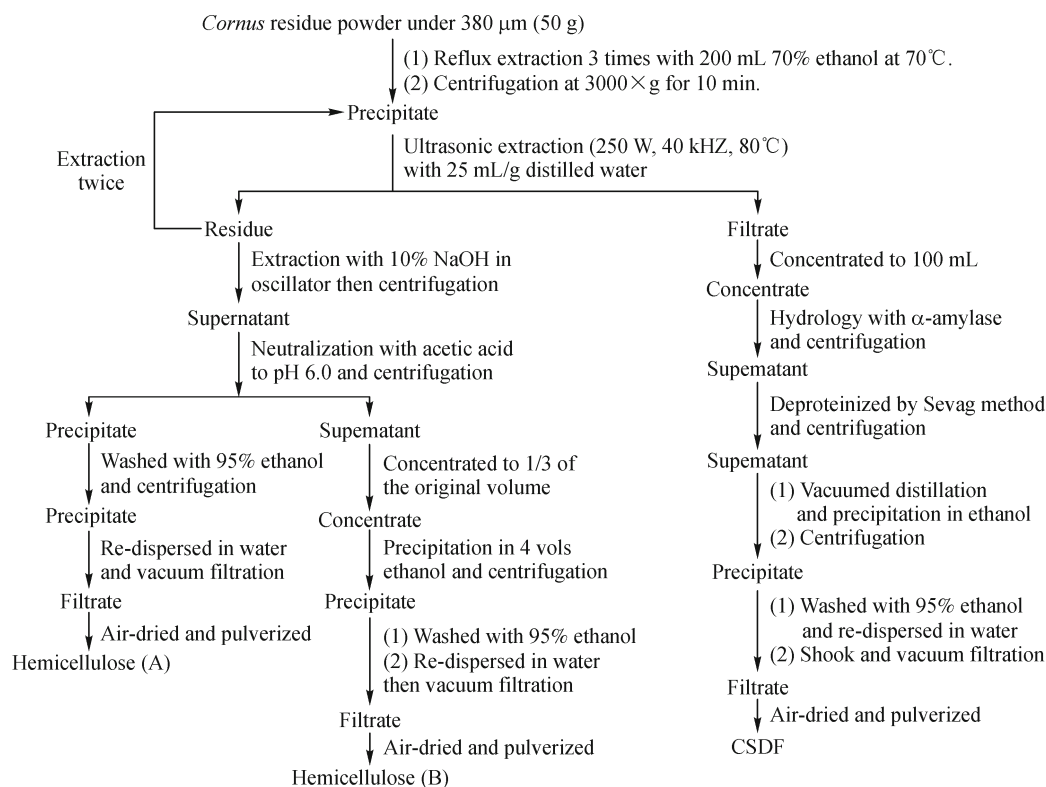


Figure 1 Scheme for extraction of CSDF from *Cornus* powder.

weight, according to the Association of Official Agricultural Chemists (AOAC) (1995) Kjeldhal procedure. The SDF was analyzed in accordance with the enzymatic-gravimetric (AACC method 32–06, 2000). The determination of crude fat content was conducted when the sample was weighed after extraction in Soxhlet apparatus for 12 h with petroleum ether (40–60°C) and subsequent evaporation.

Solubility

Solubility was determined in triplicate according to the method described by Wang et al. (2008) with slight modifications. CSDF suspensions (1%, w/w) were prepared in flasks and then heated to 40°C. The flasks were shook every 5 min, then cooled to room temperature, and later centrifuged at 3000 × g for 30 min. The supernatant was then decanted onto a culture dish, dried in an oven for 2 h at 105°C, and then weighted, with its solubility finally calculated.

Transparency determination of CSDF drinking water

After being prepared in mineral water in THZ-C for 30 min at 25°C, 0.25% (w/v) dispersion of CSDF was filtered in a vacuum filtrator. The transparency of dispersion was evaluated at 25°C under percent transmittance at 620 nm in a 1-mm-thick cell (window: silica glass) against a mineral water blank in the UV-visible recording spectrophotometer UV-752. Every sample was determined in triplicate.

Data processing

All the measured values were expressed as mean arithmetical value of several determinations. Fitting equation and 3D response plots were followed by the Statistics Analysis System (SAS 8.01), and the max value of fitting equation was obtained by MATLAB 7.0.

Results and discussion

Effects of ambient temperature, solvent-to-solid ratio, and time on CSDF extraction

A sample of 5.0 g *Cornus* residue powder and distilled water were put into a triangular flask in the ratio of solid to solvent 1:20 (g/mL) and then treated in an ultrasonic extracting apparatus with a ultrasonic power of 250 W and ultrasonic wave frequency of 40 kHz at 20°C for 100 min. The extraction liquid was precipitated in 4 vols ethanol (95%) when concentrated to 30 mL. The obtained precipitate was weighed. The same process was repeated at 40°C, 60°C, 80°C, 90°C, and 100°C, respectively. The results showed that, around 80°C, CSDF was heavier than that at the other temperatures.

The above procedure was conducted again to determine the effect of the ratio of solvent to solid on CSDF extraction. The ambient temperature was set at 80°C, while the selected ratios of solvent to solid were set at 5:1, 10:1, 15:1, 20:1, 25:1,

30:1, and 35:1, respectively. The results suggested that the heavier CSDF was around the ratio of 30:1.

An analogous operation was followed to test the effect of time on CSDF extraction in the ratio of 30:1 at 80°C. The results indicated that the weight of CSDF barely increased over the 90 min (Fig. 2).

Central composition design to optimize extraction conditions

The key factors must be precisely determined in order to find out the best conditions for CSDF preparation. Based on the single factor results, the major ranges of variables were decided. A total of 20 experiments were performed by a

central composite design with five levels for all three factors: ambient temperature (X_1), solvent-to-solid ratio (X_2), and time (X_3), whose results were shown as follows (Table 1).

According to the experiment results, SAS 8.01 was employed to a fit equation. The data were shown to be fitter to the second-order polynomial equation. The values of regression coefficients were calculated and the fitted equation for predicting aggregative indicator value (Y_p) was given as follows:

$$Y_p = -6.55049 + 0.21402X_1 + 0.42337X_2 + 0.10059X_3 - 0.00170X_1X_1 - 0.00667X_2X_2 - 0.00044737X_3X_3 + 0.00030891X_1X_2 + 0.00014404X_1X_3 - 0.00042463X_2X_3$$

($R^2 = 0.8441$, $R = 0.919 > R_{0.001} = 0.647$, P -value = 0.0049).

Coefficient of determination (R^2) was defined as the ratio of

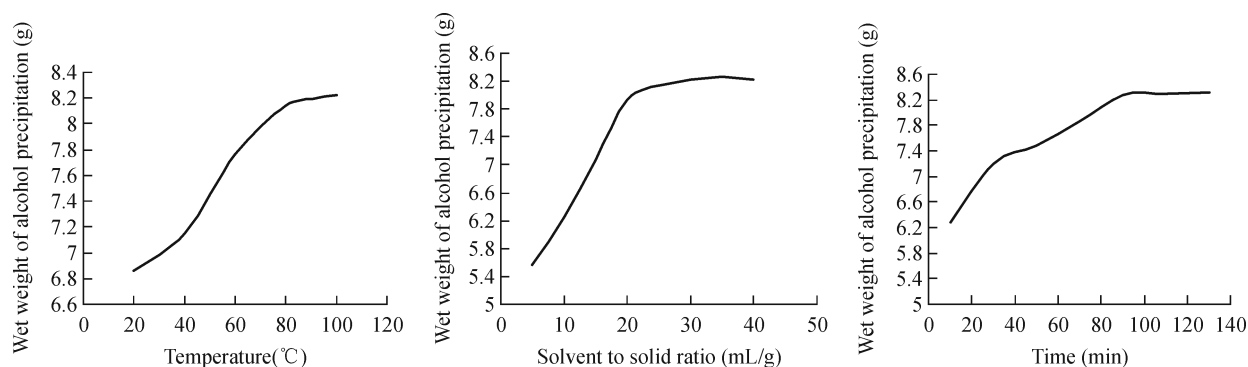


Figure 2 Effects of ambient temperature, solvent-to-solid ratio, and time on wet weight of alcohol precipitation.

Table 1 Central composite design consisting of experiments for the study of three factors in coded and levels with experimental results

Trial no.	Variable			Y			Y_A	Y_P
	X_1 (Temperature (°C))	X_2 (Solvent to solid (mL/g))	X_3 (Time (min))	Y_1 (%)	Y_2 (%)	Y_3 (%)		
1	(-1) 66.3	(-1) 27.1	(-1) 98.5	3.646	83.5	95.4	12.58441	12.6731
2	(1) 83.7	(-1) 27.1	(-1) 98.5	3.992	79.0	91.4	12.29368	12.3548
3	(-1) 66.3	(1) 32.9	(-1) 98.5	3.792	81.2	95.3	12.56038	12.6831
4	(1) 83.7	(1) 32.9	(-1) 98.5	4.236	77.9	90.2	12.31984	12.3960
5	(-1) 66.3	(-1) 27.1	(1) 121.5	3.810	80.7	95.3	12.60467	12.6778
6	(1) 83.7	(-1) 27.1	(1) 121.5	4.246	78.0	90.3	12.34188	12.4172
7	(-1) 66.3	(1) 32.9	(1) 121.5	3.848	79.6	94.8	12.54301	12.6312
8	(1) 83.7	(1) 32.9	(1) 121.5	4.374	77.3	89.6	12.34110	12.4018
9	(-1.732) 60	(0) 30	(0) 110	3.732	85.0	95.7	12.79320	12.6270
10	(1.732) 90	(0) 30	(0) 110	4.438	76.4	88.5	12.24063	12.1549
11	(0) 75	(-1.732) 25	(0) 110	4.132	79.6	94.2	12.70907	12.6088
12	(0) 75	(1.732) 35	(0) 110	4.230	78.6	93.8	12.70478	12.6041
13	(0) 75	(0) 30	(-1.732) 90	4.024	80.0	94.7	12.68920	12.5897
14	(0) 75	(0) 30	(1.732) 130	4.240	78.7	93.6	12.69688	12.5989
15	(0) 75	(0) 30	(0) 110	4.182	79.9	94.4	12.78142	12.7733
16	(0) 75	(0) 30	(0) 110	4.168	79.6	94.5	12.76773	12.7733
17	(0) 75	(0) 30	(0) 110	4.166	79.7	94.5	12.77030	12.7733
18	(0) 75	(0) 30	(0) 110	4.178	79.7	94.4	12.76987	12.7733
19	(0) 75	(0) 30	(0) 110	4.176	79.5	94.5	12.76992	12.7733
20	(0) 75	(0) 30	(0) 110	4.178	79.9	94.4	12.77822	12.7733

Note: All the measured values were expressed as mean arithmetical value of three determinations. Y_1 , Y_2 , and Y_3 are yield of CSDF, content of SDF, and light transmittance at 620 nm, respectively. Y_A is aggregative indicator ($Y_A = Y_1 * Y_2 + Y_3 * 10$). Y_P is predicted value.

the explained variation to the total variation, namely, the measure of the degree of fitness (Nath and Chattopadhyay, 2007). The value of R^2 value was always between 0 and 1 and the closer the R^2 was to 1.0, the stronger the model was and the better it predicted the response (Ghodke et al., 2009). A small value of R^2 indicated a poor relevance of the dependent variables in the model, which could fit well with the actual data when R^2 approached unity. By analysis of the variances in this experiment, the R^2 value of this model was determined to be 0.8441, which proved that the regression model well defined the true behavior of the system.

Response surface optimization and prediction

The 3D response plots are generally the graphical representations of the regression equation from which the values of aggregative indicator for different setting of variables can be predicted. Each contour curve represents an infinite number of combinations of two variables with the other maintained at zero level.

The response surface plots based on the model are depicted

in Figs. 3 to 5, which present that the “mound-shaped” surface is near the center. The contour line indicates that the value of aggregative indicator augments with the increase of the three factors to a certain value, however, thereafter decreases.

The best independent variables, the ambient temperature (70.3–9°C), the solvent-to-solid ratio (29.8775 mL/g), the time (109.5628 min), and the predicted aggregative indicator (12.8077), can be sketched out by MATLAB. Another study was performed to confirm the data, employing the statistics of the MATLAB (70.3°C, 30 mL/g, and 110 min). In the study, the yield of CSDF was 4.097%, the content of SDF was 80.9%, and the light transmittance (% at 620 nm) was 94.7%. Accordingly, the aggregative indicator was calculated to be 12.7845, which was not significantly different from the indicator value within 95% confidence interval.

Besides, 0.25% (w/v) CSDF drinking water was prepared. After UV sterilization, the water presented good light transmittance of 94.9% and long shelf-life over 2 months, without obvious change in subzero treatment, smell and precipitate after 2 months later.

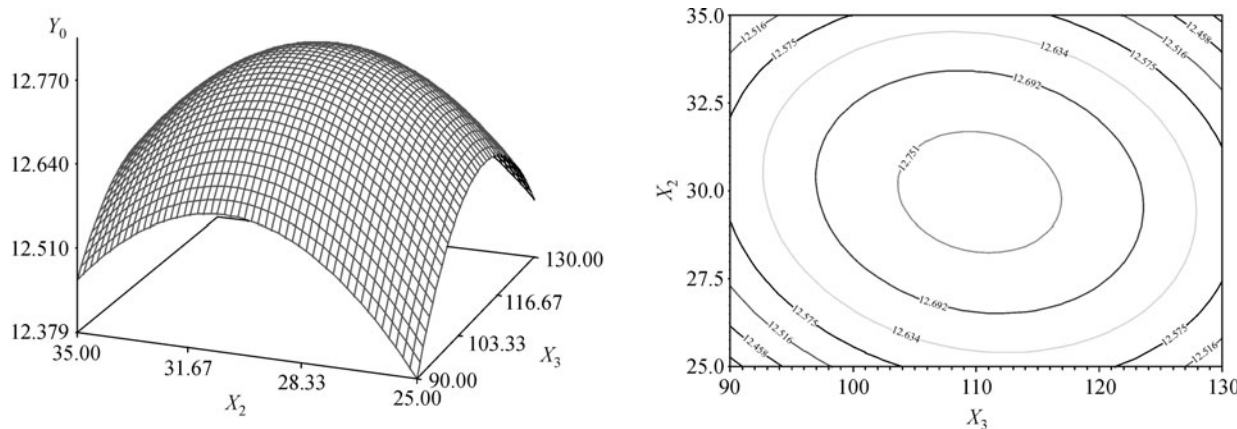


Figure 3 Response surface plot of the relation between the ratios of solvent to solid and time to the aggregative indicator of CSDF at constant ambient temperature of 75°C.

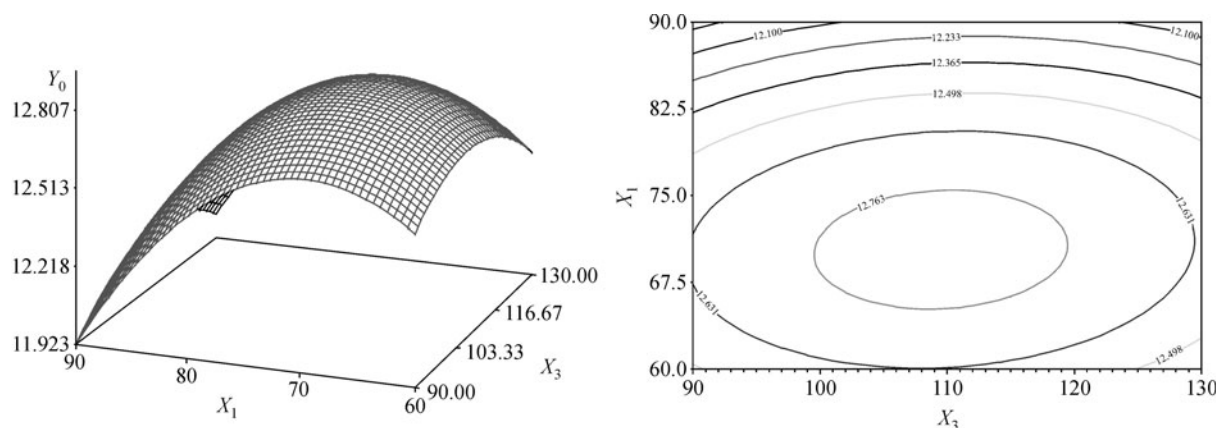


Figure 4 Response surface plot of the relation between ambient temperature and time to the aggregative indicator of CSDF in the constant ratio of solvent to solid at 30 mL/g.

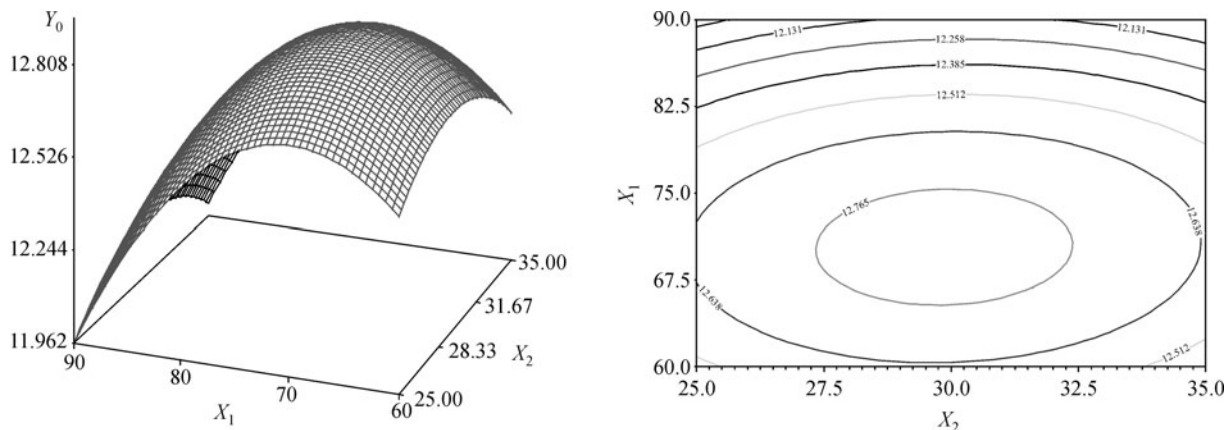


Figure 5 Response surface plot of the relation between ambient temperature and ratio of solvent to solid to the aggregative indicator of CSDF with constant time of 110 min.

Table 2 Chemical composition, solubility, and light transmittance of product (%)

Sample	Moisture	Ash	Fat	SDF	Solubility	Light transmittance
CSDF	5.68	4.56	0.66	80.2	95.5	94.9

Note: All the measured values are expressed as mean arithmetical value of three determinations.

CSDF chemical composition, solubility, and light transmittance

The chemical composition, solubility, and light transmittance of CSDF were determined according to the method described above. The results are shown below (Table 2).

Hemicellulose

The hemicellulose (A) 7.4219 g and hemicellulose (B) 7.8288 g were prepared from *Cornus* residue powder 50.0 g. The light transmittance (% at 620 nm) of 0.25% (w/v) hemicellulose in mineral water was 6.5%. The solubility of suspensions (1%, w/w) was near to 100% in mineral water almost insoluble in ethanol.

Conclusions

From the obtained results, each of three variables (ambient temperature, solvent-to-solid ratio, and time) showed a significant effect in the CSDF extraction. The mathematical model gave an R^2 of 0.8441 and P -value less than 0.005, implicating a good agreement between the predicted values and the actual ones. The optimum conditions were thus determined as temperature of 70.3°C, solvent-to-solid ratio of 30 mL/g, time of 110 min, ultrasonic power of 250 W, and ultrasonic wave frequency of 40 kHz. The predicted aggregative indicator was 12.8077, which is in accord with the actual value ($P < 0.005$). The weight of water extraction CSDF from 20.0 g raw material was 0.8194 g with an extraction yield of 4.097%. Water-insoluble fraction was extracted twice in 10% NaOH with the ratio of solvent to

solid 20 mL/g at room temperature in THZ-C for 10 h, and its extraction yield was 26.9%.

Fortunately, 0.25% (w/v) CSDF drinking water possessed good solubility, nice transparency, and long shelf-life. It may become healthy daily drinking water and desirable water for diabetes patients, cardiovascular patients, obese patients, and patients with intestinal disease.

The further research can focus on the structural characteristics and other physiological activities of CSDF, which will help us to gain a broader understanding of the physical and chemical properties of CSDF.

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