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Research Article

OPTIMIZATION OF ULTRASOUND-ASSISTED EXTRACTION CONDITIONS OF FLAVONOIDS FROM *TARTARY BUCKWHEAT*

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ABSTRACT

The objective of this study was to establish the optimum ultrasound-assisted extraction (UAE) conditions for flavonoids from *tartary buckwheat* (FTB). Single factor experiments were performed to determine the appropriate range of extraction conditions and orthogonal experiment were performed to obtain the optimum extraction conditions. The results showed that the optimum UAE conditions for FTB were as followings: ethanol concentration of 65%, solid:liquid ratio of 1:40 g/ml, extraction time of 35 min and extraction temperature of 65 °C. UAE method is an alternative extraction technique for fast extraction of FTB.

Keywords: Ultrasound-assisted extraction, Single factor experiments, Orthogonal experiment, Flavonoids from *tartary buckwheat*

INTRODUCTION

Tartary buckwheat (*Fagopyrum tataricum Gaertn.*) is a dry fruit that belongs to the *Polygonaceae* family[1]. It is an important crop in the remote mountainous and upland areas inhabited by the minorities of southwest China[2]. In previous reports, *tartary buckwheat* was studied as an important functional food material, was shown to contain proteins with high biological value and balanced amino acid composition, relatively high crude fiber content, vitamins B_1, B_2, and B_6, and more flavonoids than present in common *buckwheat*[3]. Flavonoids from *tartary buckwheat* (FTB) including the predominant flavonol rutin and minor flavonols quercetin 3-O-rutinoside-3′-O-β-glucopyranoside, kaempferol 3-O-rutinoside and quercetin[4,5]. many studies have demonstrated that flavonoids is the most main biologically active ingredient of *tartary buckwheat*, which possess antihypertensive, antioxidant, anti hypercholesterolemia, anticancer, hypoglycemic, preventing cardiovascular or cerebrovascular sclerosis, promoting digestion and raising immunity[6,7,8,9].

Conventional methods for the extraction of flavonoids from plant material, eg Soxhlet, liquid-liquid, and solid-liquid extractions are characterized by the consumption of large volumes of solvent and energy, lengthy extraction procedures, and the potentially deleterious degradation of labile compounds[10,11]. In recent years, new extraction methods which include supercritical carbondioxide extraction, subcritical water extraction, ultrasonic assisted extraction (UAE) and microwave assisted extraction (MAE) have also become of interest as alternatives for the conventional methods[12]. Among these, UAE method has been found to enhanced extraction efficiency and extraction time. In addition, UAE method can be carried out at lower temperatures which avoid thermal damage. It has been suggested that improvement of solvent extraction from material by ultrasound is due to mainly the mechanical effects of acoustic cavitations, which enhances mass transfer and solvent penetration into the plant material by disrupting the cell walls[13,14]. Nevertheless, few researches regarding UAE of flavonoids from *tartary buckwheat* (FTB) have been reported. The objective of this study was to establish the optimum UAE conditions for FTB for the development and application of the plant resource.

MATERIALS AND METHODS

Materials and chemicals:

The air-dried *tartary buckwheat* grains were purchased from Liangshan Agricultural Institution (Sichuan, China). A voucher specimen was deposited at the Herbarium of the Xichang College (Sichuan, China). The dried sample was

ground in a blender (FW177, Taisite Instrument Company Limited Tianjin, China) for 10 s to produce powder with an approximate size of 1 mm. The powders of *tartary buckwheat* grains were stored at -20°C before the experiment. Authentic standard rutin (>97%) was purchased from Guoyao Group of Chemical Reagents Ltd. (Shanghai, China). All other chemicals and reagents were of analytical grade and were obtained from the usual commercial sources. Distilled water was used to prepare all solutions and in all experiments.

Ultrasound-assisted extraction (UAE):

For the ultrasound-assisted extraction experiments, an ultrasonic bath was used as an ultrasound source. The bath (KQ-5200B, Kunshan Ultrasound Co. Ltd., China) was a rectangular container (300×240×150 mm), to which 40 kHz transducers were annealed at the bottom. The bath power rating was 200 W. The extraction of flavonoids was performed by adding 2.0 g of powders of *tartary buckwheat* grains in a 100 mL flask. The flask was then partially immersed into the ultrasonic bath and the extraction was carried out with different extraction conditions. The extract was filtered and the filtrate was collected and freeze-dried for determination of flavonoids.

Experimental design:

The experimental design for this study was divided into two major parts. Firstly, single factor experiments were performed to determine the appropriate range of extraction conditions for flavonoids from *tartary buckwheat* (FTB), namely, ethanol concentration (40, 50, 60, 70, 80 %), solid:liquid ratio (1:10, 1:20, 1:30, 1:40, 1:50 g/mL), extraction time (10, 20, 30, 40, 50 min) and extraction temperature (40, 50, 60, 70, 80 °C) by varying one independent variable at a time while keeping the others constant. Secondly, the optimum extraction conditions for FTB were obtained by using orthogonal experiment design based on single factor experiments.

Determination of flavonoids:

The contents of flavonoids were determined by the $NaNO_2$-$Al(NO_3)_3$-NaOH colorimetric assay and by reference to Rutin, and wavelenth in spectrophotometer was set at 510 nm[15,16]. Extraction yield of flavonoids (%) was calculated using the following formula.

$$Y = \frac{C \times V \times D}{M \times 100} \times 100\%$$

Where; Y is the extraction yield of flavonoids (%), C is the flavonoids content of test solution calculated by standard curve (mg/mL); V is the volume of test solution (mL); D is total dilution value; M is the mass of *tartary buckwheat* (g).

Statistical analysis:

Every experiment was performed three times in duplicate. Data of interest were analyzed with LSD (least significant data test) method. DPS (V3.01) soft-ware was used to design and process experiment in this study.

RESULTS AND DISCUSSION

Effect of the ethanol concentration on the extraction yield of flavonoids:

The correct selection of the extraction solvent was fundamental for obtaining optimal extraction conditions of flavonoids. It is known that flavonoids are compounds containing hydroxyl groups conferring a high solubility in methanol and ethanol[17,18]. However, methanol was not tested in this study, because it is highly toxic and is not practical for use in food and pharmaceutical processing. Fig. 1 showed the effect the different ethanol concentration (40, 50, 60, 70, 80 %) on the extraction yield of FTB. In these extractions, other experimental conditions were as follows: solid:liquid ratio of 1:30 g/mL, extraction time of 30 min and extraction temperature of 60 °C. With the increase of ethanol concentration from 40 % to 60 %, the extraction yield of FTB quickly increased from 3.29 % to 6.25 % and reached the peak value. However, with the increase of the ethanol concentration from 60% to 80%, extraction yield quickly decreased from 6.25 % to 3.51 %. This maybe because the extraction efficiency of water-soluble flavonoids and alcohol-soluble flavonoids is best when extracted with 60% ethanol[19]. Thus, 60% ethanol was chosen as the appropriate ethanol concentration.

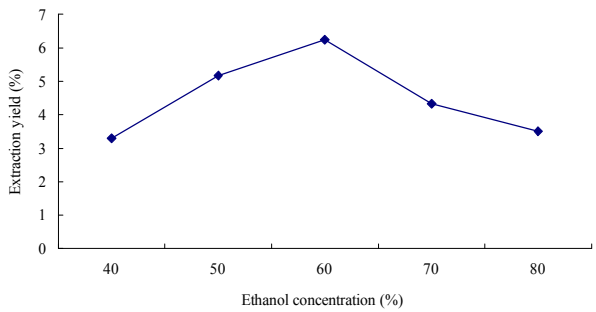

Figure 1: Effect of the ethanol concentration on the extraction yield of flavonoids

Effect of the solid:liquid ratio on the extraction yield of flavonoids:

Fig. 2 showed the effect the different solid:liquid ratio (1:10, 1:20, 1:30, 1:40, 1:50 g/mL) on the extraction yield of FTB. In these extractions, other experimental conditions were as

follows: ethanol concentration of 60%, extraction time of 30 min and extraction temperature of 60 °C. With the increase of solid:liquid ratio from 1:10 to 1:40 g/mL, the extraction yield of FTB quickly increased from 2.36 % to 6.94 % and reached the peak value. However, with the increase of solid:liquid ratio from 1:40 to 1:50 g/mL, extraction yield decreased from 6.94 % to 5.47 %. This was probably due to the larger volume of extraction solvent causing excessive swelling of the material by water and absorbing the effective constituent[20, 21]. Thus, 1:40 g/mL was chosen as the appropriate solid:liquid ratio.

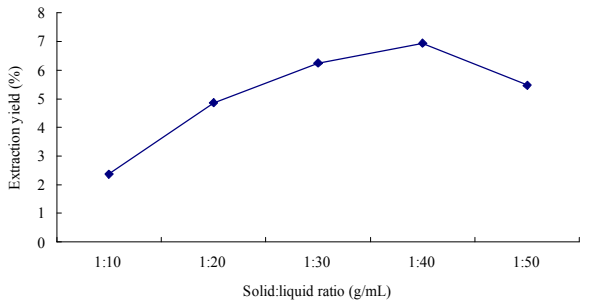

Figure 2: Effect of the solid:liquid ratio on the extraction yield of flavonoids

Effect of the extraction time on the extraction yield of flavonoids:

Fig. 3 showed the effect the different extraction time (10, 20, 30, 40, 50 min) on the extraction yield of FTB. In these extractions, other experimental conditions were as follows: ethanol concentration of 60 %, solid:liquid ratio of 1:30 g/mL and extraction temperature of 60 °C. With the increase of extraction time from 10 to 30 min, the extraction yield of FTB quickly increased from 3.03 % to 6.25 %. However, with the increase of extraction time from 30 to 50 min, extraction yield increased slowly from 6.25 % to 6.43 %. Because longer extraction time could delay and lengthen production cycle, 30 min was chosen as the appropriate extraction time.

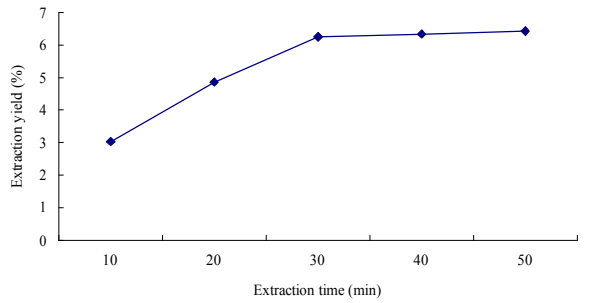

Figure 3: Effect of the extraction time on the extraction yield of flavonoids

Effect of the extraction temperature on the extraction yield of flavonoids:

Fig. 4 showed the effect the different extraction temperature (40, 50, 60, 70, 80 °C) on the extraction yield of FTB. In these extractions, other experimental conditions were as follows: ethanol concentration of 60 %, solid:liquid ratio of 1:30 g/mL and extraction time of 30 min. With the increase of extraction temperature from 40 to 70°C, the extraction yield of FTB quickly increased from 3.74 % to 7.18 % and reached the peak value. However, with the increase of extraction temperature from 70 to 80°C, extraction yield quickly decreased from 7.18 % to 5.22%. This was probably due to the decrease of number of acoustic cavitation bubbles created by ultrasound and the thermal degradation of flavonoids[22,23]. In addition, high extraction temperature might result in improvement of energy cost and enhancement of impurities. Thus, 70 °C was chosen as the appropriate extraction temperature.

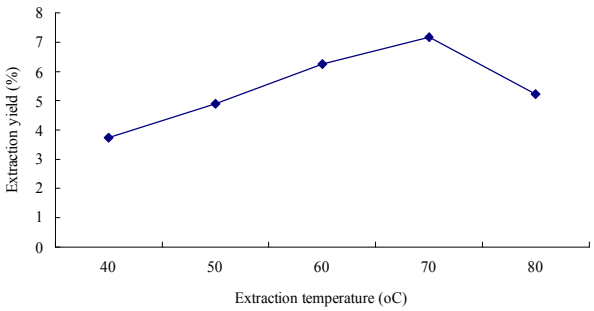

Figure 4: Effect of the extraction temperature on the extraction yield of flavonoids

Orthogonal experiments:

The optimum extraction conditions for FTB were obtained by using orthogonal experiment design ($L_9 3^4$) based on single factor experiments. Table 1 lists the selected four factors and three levels. Table 2 shows factors at different levels in nine experiments conducted and the statistical analysis results.

The order of the effect of factors on FTB extraction was found to be: D>C>A>B. The optimum extraction conditions obtained from the statistical analysis were $A_3B_2C_3D_1$. It means that ethanol concentration of 65 %, solid:liquid ratio of 1:40 g/mL, extraction time of 35 min and extraction temperature of 65 °C were the optimum UAE conditions for FTB. The above conditions were tested later to ascertain the dependability of the results in this study, the extraction yield of FTB was 7.98 %. So the hypothesis of the orthogonal experiment was valid.

Table 1: Factors and levels of orthogonal experimental

Levels	Factor			
	A. Ethanol concentration (%)	B. Solid: liquid ratio (g/mL)	C. Extraction time (min)	D. Extraction temperature (°C)
1	55	1:35	25	65
2	60	1:40	30	70
3	65	1:45	35	75

Table 2: Results and analysis of orthogonal experimental

Test no.	Factor				Extraction yield of polysaccharides (%)
	A. Ethanol concentration (%)	B. Solid:liquid ratio(g/mL)	C. Extraction time (min)	D. Extraction temperature (°C)	
1	1	1	1	1	7.34
2	1	2	2	2	7.18
3	1	3	3	3	7.01
4	2	1	2	3	6.94
5	2	2	3	1	7.86
6	2	3	1	2	7.19
7	3	1	3	2	7.53
8	3	2	1	3	7.13
9	3	3	2	1	7.51
X_1	7.177	7.270	7.220	7.570	
X_2	7.330	7.390	7.210	7.300	
X^3	7.390	7.237	7.467	7.027	
R	0.213	0.153	0.257	0.543	

CONCLUSION

The present study indicated that UAE method is an alternative extraction technique for fast extraction of flavonoids from tartary buckwheat. Extraction yield of FTB were affected by ethanol concentration, solid:liquid ratio, extraction time and extraction temperature. The optimum UAE condition through single factor experiments and orthogonal experiment was determined as followings: ethanol concentration of 65%, solid:liquid ratio of 1:40 g/ml, extraction time of 35 min and extraction temperature of 65 °C.

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