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Effects of ultrasonic treatment of rumduol mash on the antioxidant level of fruit juice

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Abstract

Rumduol (*Sphaerocoryne affinis* (Teijsm. and Binn.) Ridl) fruit is rich in antioxidants including ascorbic acid and phenolic compounds. In this study, ultrasonic treatment of rumduol mash was applied to extraction of fruit juice with high antioxidant level. Firstly, the effects of ultrasonic power and time on the antioxidant content and activity of the rumduol juice were investigated. Response surface methodology was then used to optimize the ultrasonic treatment conditions. The optimal ultrasonic power and time were 14.8 W/g and 4.4 min, respectively, under which the content of ascorbic acid and total phenolics achieved 179.7 mg/100 g dry matter and 15.62 g gallic acid equivalent/100 g dry matter, respectively. In the ultrasound-assisted extraction, the rumduol juice showed maximum antioxidant activity of 34.3 mM Trolox equivalent/100 g dry matter (estimated by 2,2-Azinobis-3-ethylBenzoThiazoline-6-Sulfonic acid ABTS assay) which was 59.7% higher than that in the control without ultrasonic treatment.

Introduction

Rumduol (*Sphaerocoryne affinis* (Teijsm. and Binn.) Ridl) is a species of flowering plant in the Annonaceae family (Ridley, 1917). The fruits in the Annonaceae family contain high level of antioxidants including ascorbic acid and phenolic compounds (Vasco *et al*., 2008). In Vietnam, rumduol is known as "Duong Duong" and the fruit has been used to produce juice and non-distilled alcoholic beverage at small scale.

Extraction is an important step in fruit juice production. Conventionally, enzyme-assisted extraction has been applied for improvement in juice yield since pectinase and cellulase preparations could degrade the middle lamella and cell wall in fruit pulp for juice release. However, the time of enzymatic treatment can last for 2 h (Kashyap, 2001) and the loss thermo-sensitive compounds could be observed (Le and Le, 2012). Recently, ultrasound has been used in fruit mash treatment for juice extraction. Ultrasound generates cavitation which includes the formation, growth and violent collapse of small bubbles in liquid due to pressure fluctuation. Collapse of the bubbles causes shock wave that passes through the solvent. This phenomenon results in reduction in fruit particle size as well as improvement in mass transfer within the extraction systems. Ultrasound-assisted extraction has many benefits including improved extraction efficiency and reduced extraction time in comparison with the enzyme-assisted extraction (Lieu and Le, 2010). Many studies showed that short sonication time resulted in high antioxidant level of fruit juice from guava (Nguyen *et al*., 2011), acerola (Le and Le, 2012), red raspberry (Golmohamadi *et al.*, 2013), mulberry (Nguyen *et al.*, 2014a), rose myrtle (Vo and Le, 2014), ambarella (Nguyen *et al.*, 2014b). However, ultrasonic treatment of rumduol fruit mash for juice extraction has never been reported.

Many variables affect ultrasound-assisted extraction; ultrasonic power and time were the important technological factors (Chittapalo and Noomhorm, 2009). The objectives of this research were to (i) investigate the effects of ultrasonic power and time of the rumduol mash treatment on the content of phenolic compounds and ascorbic acid as well as the antioxidant activity of the fruit juice, (ii) optimize the conditions of the ultrasonic treatment for maximizing the antioxidant activity of the rumduol juice.

Materials and Methods

Materials

Rumduol (*Sphaerocoryne affinis* (Teijsm. and Binn.) Ridl) fruits were originated from a farm in

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Phu Quoc, Vietnam. The (185±3) day fruits were harvested during the period from May to June in 2015. After harvesting, the fruits were transported to the laboratory in the same day.

Chemicals including Folin-Ciocalteu reagent, gallic acid, potassium persulfate ($K_2S_2O_8$), anhydrous sodium carbonate (Na_2CO_3), iron (III) chloride hexahydrate ($FeCl_3.6H_2O$), sodium acetate trihydrate ($CH_3COONa.3H_2O$), 2,4,6-Tri(2-pyridyl)-1,3,5-triazine (TPTZ) were obtained from Merck (Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 2,2'-azinobis-(3-ethylbenzo-thiazoline-6-sulphonic acid) (ABTS), acetic acid (CH_3COOH) were purchased from Sigma-Aldrich (Singapore); ethanol was supplied from Chemos (Vietnam). All chemicals were at analytical grade.

Experimental method

Rumduol fruits were washed with potable water to remove dust and crushed in a blender (Panasonic, Malaysia). The rotation rate of the blades and crushing time were 500 rpm and 1 min, respectively. The fruit mash was then stored at -18°C for further experiments. Before use, the rumduol mash was defrosted and then mixed with water as solvent; the rumduol mash/ solvent ratio was 1:2 by weight. For each assay, samples of 50 g diluted rumduol mash were taken and added into 250 mL beakers which were covered with aluminium-foil papers to prevent the oxidative change from light.

Effects of ultrasonic power on the phenolic and ascorbic acid content and antioxidant activity of the rumduol juice

The ultrasonic treatment of rumduol mash was performed with a horn type ultrasonic probe (Sonics and Materials Inc., The United states). The ultrasonic power was varied: 0 (control sample), 4, 8, 12, 16, 20, 24 and 28 W per gram of the fruit mash. The sonication time was fixed at 2 min. During the ultrasonic treatment, the temperature of all samples was adjusted to be inferior to 30°C by using a cooling water bath (Memmert, Germany). At the end of the ultrasonic treatment, the mash was vacuum-filtered (Vida, Vietnam) through a cheese cloth. The juice obtained was used for further analysis.

Effects of ultrasonic time on the phenolic and ascorbic acid content and antioxidant activity of the rumduol juice

In this experiment, the ultrasonic time was changed: 0 (control sample), 1, 2, 3, 4, 5, 6 and 7 min. The ultrasonic power was selected from the result of the previous experimental section. The other steps were similar to those in the previous section.

Optimization of ultrasonic treatment conditions for maximizing antioxidant activity of the rumduol juice

The quadratic central composite circumscribed response surface design with 2 factors and 5 levels was used to optimize the conditions of ultrasonic treatment of rumduol mash. Ultrasonic power (X_1) and ultrasonic time (X_2) were independent variables while the antioxidant activity of rumduol juice was dependant variable (Y). The design consisted of 13 experimental points including 4 factorial points, 4 axial points and 5 central points. Modde 5.0 software was used to generate the experimentation planning and to process data.

Analytical methods

Total phenolic content was determined by spectrophotometric method using Folin-Ciocalteu reagent (Dewanto *et al*., 2002). The results were expressed as grams of Gallic Acid Equivalent per 100 g dry matter (g GAE/100 g dry matter). Ascorbic acid content was measured by the enzymatic method using ascorbic acid kit with a reflectometer (MercK KgaA, Germany). The results were expressed as milligrams of ascorbic acid per 100 g dry matter (mg/100 g dry matter). Antioxidant activity was evaluated by Ferric Reducing Ability of Plasma (FRAP) method (Benzie and Strain, 1996) and 2,2'-Azinobis-3-ethylBenzoThiazoline-6-Sulfonic acid (ABTS) method (Re *et al*., 1999). For both assays, the results were expressed as mmol Trolox Equivalent Antioxidant Capacity per 100 g dry matter (mM TE/100 g dry matter).

Statistical analysis

All experiments were performed in triplicate. The experimental results obtained were expressed as means ± standard deviation. Multiple range test with the least significant difference was applied in order to determine which means were significantly different from which others ($P < 0.05$). Analysis of variance was performed by using the software Statgraphics Centurion XV (Manugistics Inc, The United states).

Results and Discussion

Effects of ultrasonic power on the phenolic and ascorbic acid level and antioxidant activity of the rumduol juice

Figure 1 shows the effects of ultrasonic power on the antioxidant content and activity of the rumduol juice. When the ultrasonic power increased from 0 to

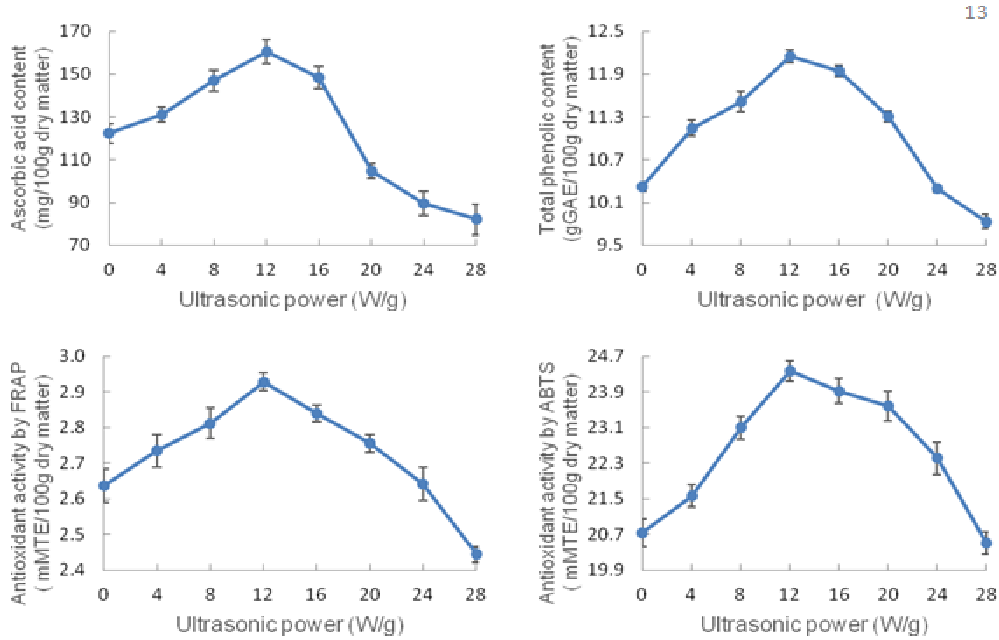

Figure 1. The effect of ultrasonic power on the antioxidant content and activity of the rumduol juice

12 W/g, the ascorbic acid and total phenolic content was improved by 21.3% and 17.7%, respectively. It can be noted that the content of phenolic compounds in rumduol fruit was much higher than that of vitamin C. In addition, gradual increase in antioxidant activity of the rumduol juice was also observed as the ultrasonic power was augmented from 0 to 12 W/g. At the ultrasonic power of 12 W/g, the antioxidant activity evaluated by FRAP and ABTS method was 11.1% and 22.3%, respectively higher than that in the control sample. Similar results were reported for the ultrasonic treatment of acerola (Le and Le, 2012) and rose myrtle mash (Vo and Le, 2014) in juice production. According to these authors, ultrasound generated cavitation in solid-liquid extraction which facilitated reduction in size of material particles. As a result, the extraction yield of the ascorbic acid and total phenolics was enhanced.

However, when the ultrasonic power increased from 12 to 28 W/g, both antioxidant content and activity of the rumduol juice were reduced. Yue *et al.* (2012) showed that high ultrasonic power could generate hydroxyl radicals which can react with vitamin C and phenolic compounds. Treatment of ambarella mash with high ultrasonic power also reduced vitamin C and phenolic level in the fruit juice (Nguyen *et al.*, 2014b).

A good correlation between the content of ascorbic acid and total phenolics and the antioxidant activity of the rumduol juice was observed in Figure 1. Increase in level of biologically active compounds may result in improvement in the antioxidant activity of the rumduol juice. Nevertheless, the radical scavenging activities evaluated by FRAP assay were always lower than those determined by ABTS assay. This observation was probably due to different types of reactions of different antioxidants in the rumduol juice on various free radicals. Based on the results obtained, the ultrasonic power of 12 W/g was selected for the next experiment.

Effects of ultrasonic time on the phenolic and ascorbic acid content and antioxidant activity of the rumduol juice

Figure 2 presents that increase in ultrasonic time from 0 to 4 min augmented the ascorbic acid and total phenolic content in rumduol juice by 42.4% and 38.9%, respectively. The antioxidant activity of the rumduol juice estimated by both FRAP and ABTS assays also achieved maximum when the ultrasonic time was 4 min. Longer sonication time reduced antioxidant level as well as antioxidant activity in the fruit juice. Similar observation was noted when the ultrasonic treatment of mulberry (Nguyen *et al.*, 2014a) and rose myrtle mash was prolonged (Vo and Le, 2014). Long sonication time could generate high level of hydroxyl radicals in the extract (Feng *et al.*, 2011); consequently, the antioxidant content and activity of the fruit juice could be reduced. The appropriate ultrasonic time for rumduol mash treatment was therefore 4 min. Short extraction time was an important advantage in the extraction of fruit juice since the loss of thermally labile compounds could be prevented.

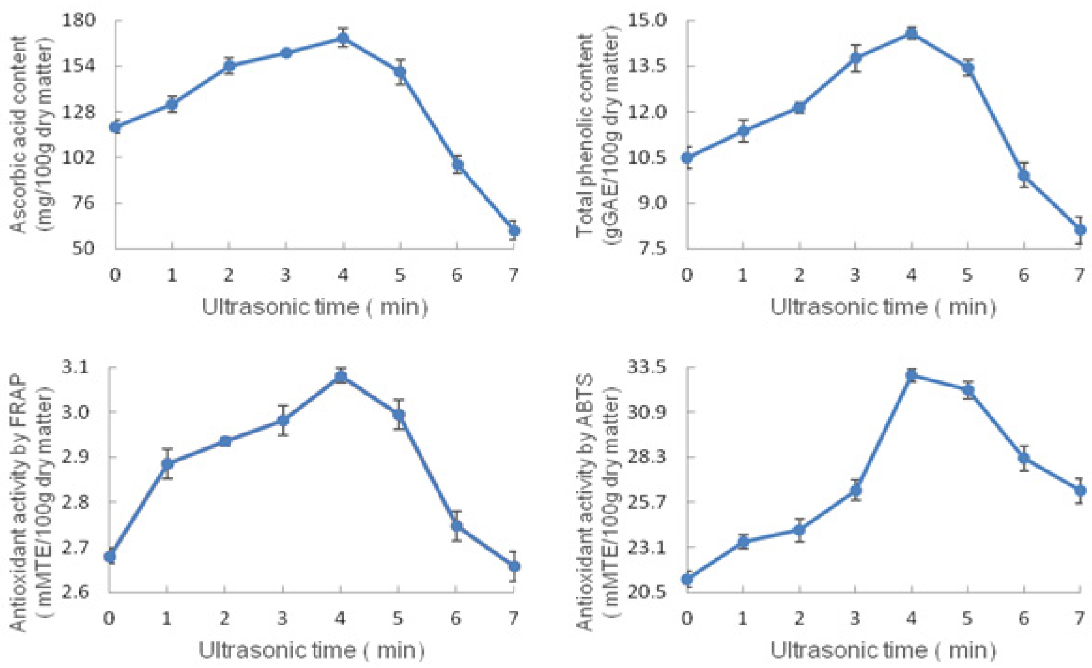

Figure 2. The effects of ultrasonic time on the antioxidant content and activity of the rumduol juice

Table 1. Experimental planning and result of total antioxidant activity of the rumduol juice from the ultrasound-assisted extraction (Antioxidant activity was evaluated by ABTS assay)

Number	Ultrasonic power (W/g)	Ultrasonic time (min)	Antioxidant activity (mM TE/ 100 g dry matter)	Number	Ultrasonic power (W/g)	Ultrasonic time (min)	Antioxidant activity (mM TE/ 100 g dry matter)
1	8	3	25.70	7	12	2.59	24.74
2	16	3	28.45	8	12	5.41	29.08
3	8	5	26.02	9	12	4	33.35
4	16	5	34.04	10	12	4	33.98
5	6.34	4	25.77	11	12	4	32.67
6	17.66	4	31.93	12	12	4	33.61
				13	12	4	33.75

Optimization of ultrasonic treatment conditions for maximizing antioxidant activity of the rumduol juice

In this experiment, an ultrasonic power of 12 W/g and an ultrasonic time of 4 min were chosen as central conditions of the optimization. The ultrasonic power and time were varied from 6.35 to 17.66 W/g and 2.59 to 5.41 min, respectively. The antioxidant activity of the rumduol juice was evaluated by ABTS assay. Table 1 shows the experimental design and the results. Based on the experimental data, multiple regression analysis was conducted and the results are visualized in Table 2. All coefficients were significant ($P < 0.05$). The regression model was also significant since the R^2 value was high (0.985). The final predictive equation was as follow:

$$Y = 33.467 + 1.995X_1 + 1.234X_2 - 1.435X_1^2 - 2.063X_2^2 + 0.881X_1X_2 \quad (1)$$

where Y, X_1, X_2 were the total antioxidant activity of rumduol juice (mM TE/100 g dry matter), the ultrasonic power (W/g) and the ultrasonic time (min), respectively.

Equation (1) shows that the ultrasonic power, the ultrasonic time and the interaction between these two input variables impacted on the antioxidant activity of rumduol juice. The linear coefficients (X_1 and X_2) and the interaction coefficient ($X_1 \times X_2$) were positive while the pure quadratic coefficients (X_1^2 and X_2^2) were negative. Figure 3 presents the effects of ultrasonic power and ultrasonic time on the antioxidant activity (evaluated by ABTS assay)

Table 2. Multiple regression analysis of the model representing antioxidant activity of the rumduol juice

Y	Coeff. SC	Std. Err.	P value	Conf. int(±)
Constant	33.4665	0.265167	5.1707e-013	0.627029
X_1	1.99514	0.171165	7.72596e-006	0.404746
X_2	1.23381	0.171165	0.000176176	0.404746
X_1*X_1	-1.43497	0.149881	2.8483e-005	0.354416
X_2*X_2	-2.0629	0.149881	2.52122e-006	0.354416
X_1*X_2	0.881202	0.197614	0.00293887	0.467289
N = 13	Q^2 = 0.746		Cond. no. = 3.3541	
DF = 7	R^2 = 0.985		Y-miss = 0	
Comp. =2	$R^2_{Adj.}$ = 0.974		RSD = 0.5929	
			Conf. lev. = 0.95	

Coeff. SC: Scaled and Centered coefficient; Std. Err.: Standard error; Conf. int: Confidence interval.

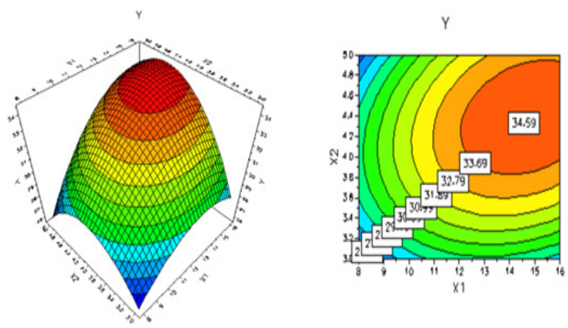

Figure 3. Effects of ultrasonic power and time on the antioxidant activity of the rumduol juice; Y, X_1, X_2 were the total antioxidant activity of rumduol juice (mM TE/100g dry matter), the ultrasonic power (W/g) and the ultrasonic time (min), respectively

of the rumduol juice. According to the equation (1), maximum antioxidant activity was 34.6 mM TE/100 g dry matter as the ultrasonic power and time were 14.8 W/g and 4.4 min, respectively.

In order to evaluate the accuracy of the model, three replicates were conducted for determining the antioxidant activity of the rumduol juice under optimal conditions of the ultrasonic treatment of fruit mash. The average antioxidant activity was 34.3 mM TE/100 g dry matter. The difference between the experimental value and the predicted value from equation (1) was therefore 1.28%. The antioxidant activity of the rumduol juice in the control was 21.5 mM TE/100 g dry matter. As a result, the antioxidant activity of rumduol juice in the ultrasound-assisted extraction increased by 59.7% compared the control without ultrasonic treatment. Under optimal conditions, the ascorbic acid and total phenolic content in the rumduol juice were 179.7 mg and 15.62 g GAE per 100 g dry matter, respectively.

Conclusions

Ultrasonic treatment of fruit mash increased both antioxidant level and activity of the rumduol juice. Short extraction time was an important advantage which could prevent the loss of thermally labile compounds in fruit juice. Ultrasonic treatment has been shown to be an efficient method for the extraction of fruit juice with high content of antioxidant compounds.

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