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Optimization of ultrasonic treatment of rose myrtle mash in the extraction of juice with high antioxidant level

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Abstract

Phenolic compounds and ascorbic acid are main antioxidants in rose myrtle (*Rhodomyrtus tomentosa*) fruit. In this study, ultrasound was applied to the treatment of fruit mash for juice extraction. The effects of ultrasonic power and time on the level of total phenolics and ascorbic acid in the rose myrtle juice were investigated. Ultrasonic treatment significantly improved both antioxidant content and activity of the extract. Response surface methodology was then used to optimize the sonication conditions for maximizing the antioxidant activity of the juice. The optimal ultrasonic power and time were 25 W/g and 6.5 min, respectively under which the concentration of total phenolics and ascorbic acid in the extract achieved 6,067 mg gallic acid equivalent (GAE)/L and 516 mg/L, respectively. In the ultrasound-assisted extraction, the maximal antioxidant activity of the rose myrtle juice evaluated by 1,1-diphenyl-2-picrylhydrazyl (DPPH) method was 24.76 mmol Trolox equivalent antioxidant capacity (TEAC)/L and this value was 86% higher than that in the control sample.

Introduction

Rose myrtle (*Rhodomyrtus tomentosa* (Ait.) Hassk) is a shrub of the *Myrtaceae* family, native to tropical and subtropical countries. This fruit is rich in antioxidants including phenolic compounds and ascorbic acid (Liu *et al*., 2012). The sweet ripen fruits are consumed fresh or made into jam, non-alcoholic and alcoholic beverages (Li, 2008). Extraction is a key operation in fruit juice processing. Conventionally, enzyme-assisted extraction has been used for improvement in juice yield and quality (Kashyap *et al*., 2001). Nevertheless, this technique demonstrates disadvantages to some extent such as relatively high temperature and long extraction time. These inconveniences could reduce the level of thermo-sensitive compounds in the fruit juice (Le and Le, 2012).

From the last decade, ultrasound-assisted extraction has shown high extraction efficiency, short extraction time and low energy consumptions (Pan *et al*., 2011). Thus, ultrasonic treatment of fruit mash has recently been used in the extraction of antioxidant-rich juice from strawberry (Herrera and Luque de Castro, 2005), grape (Lieu and Le, 2010), guava (Nguyen *et al*., 2011), acerola (Le and Le, 2012) and mulberry (Phan *et al*., 2013). However, the application of ultrasound to the extraction of antioxidant-rich rose myrtle juice has not been reported yet. The objectives of this research were to (i) investigate the effects of ultrasonic power and time on the content of phenolic compounds and ascorbic acid as well as the antioxidant activity of the rose myrtle juice; (ii) optimize the conditions of the ultrasound-assisted extraction for maximizing the antioxidant activity of the fruit juice.

Materials and methods

Materials

Rose myrtle (*Rhodomyrtus tomentosa*) fruits: The fruits were originated from a farm in Phu Quoc, Vietnam. The ripened fruits were harvested during the period from July to August in 2013. Chemicals: Folin–Ciocalteu reagent, methanol, ethanol, gallic acid, oxalic acid, potassium persulfate ($K_2S_2O_8$) and anhydrous sodium carbonate (Na_2CO_3) were obtained from Merck (Germany); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were purchased from Sigma-Aldrich (Singapore).

Experimental methods

Rose myrtle fruits were washed with potable water and crushed in a blender (Panasonic, Malaysia). The rose myrtle mash was stored at -36^oC until use. Before use, the fruit mash was defrosted and then

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mixed with water as solvent; the weight ratio of fruit mash to water was 1:2. For each assay, samples of 30 g diluted rose myrtle mash were taken and placed into 250 mL beakers which were covered with aluminium-foil papers to prevent the oxidative change from light.

Effects of ultrasonic power on the content of total phenolics, ascorbic acid and antioxidant activity of the rose myrtle juice

The rose myrtle mash was sonicated with a horn type ultrasonic probe (Sonics & Materials Inc., The United states). The ultrasonic power was adjusted to 0 (control sample), 15, 20, 25, 30, 35 W/g of the diluted fruit mash, respectively. The ultrasonic time was fixed at 2 min. During the sonication, the temperature of all treated samples was adjusted to be inferior to 30^{o}C by using a water bath (Memmert, Germany) with cold water. At the end of the ultrasonic treatment, the mash was filtered through a cheese cloth. The filtrate was centrifuged at 3,500 rpm for 20 min at 20^{o}C by a refrigerated centrifuge (Sartorius, Switzerland). The supernatant was obtained for further analysis. For the control sample, the rose myrtle mash was pressed with a screw press (Oekotec, Germany) to express out the extract. The extract was then centrifuged at 3,500 rpm for 20 min at 20^{o}C and the supernatant was used for further analysis.

Effects of ultrasonic time on the content of total phenolics, ascorbic acid and antioxidant activity of the rose myrtle juice

In this experiment, the ultrasonic time was changed from 0 (control sample) to 2, 4, 6, 8 and 10 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. The control sample was performed according to the similar procedure in the previous section.

Optimization of the ultrasonic treatment conditions of rose myrtle mash for maximizing the antioxidant activity of the fruit 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 the rose myrtle mash. The ultrasonic power (X_1) and time (X_2) were the input variables while the antioxidant activity of the rose myrtle juice was the output variable (Y). The complete design consisted of 13 experimental points including 4 factorial points, 4 axial points and 5 centre points. The software Modde version 5.0 was used to generate the experimental planning and to process data.

Analytical methods

Total phenolic content was determined by spectrophotometric method using Folin–Ciocalteu reagent (Luque-Rodrıguez *et al*., 2007). The results were expressed as grams of Gallic Acid Equivalent per liter (g GAE/L). Ascorbic acid content was measured by the enzymatic method using ascorbic acid kit with a reflectometer model 116970 (MercK KgaA, Germany). The results were expressed as milligrams of ascorbic acid per liter (mg/L). Antioxidant activity: DPPH assay was performed according to the procedure described by Brand-Williams *et al.* (1995). ABTS assay was carried out by the procedure described by Re *et al*. (1999). For both assays, the results were expressed as mmol Trolox Equivalent Antioxidant Capacity per liter (mm TEAC/L).

Statistical analysis

All experiments were performed in triplicate. The experimental results obtained were expressed as means ± standard deviation. Mean values were considered significantly different when P values < 0.05. Analysis of variance was performed by using the software Statgraphics plus, version 7.0 (Manugistics, The United states).

Results and discussion

Effects of ultrasonic power on the content of phenolic compounds, ascorbic acid and antioxidant activity of the rose myrtle juice

Table 1 shows that all samples treated with ultrasound always exhibited higher antioxidant content and activity than the control. As the ultrasonic power increased from 0 to 25 W/g, the concentration of total phenolics and ascorbic acid in the rose myrtle juice enhanced 62% and 88%, respectively. Although ascorbic acid has been considered as a power antioxidant in juice (Li, 2008), its level in rose myrtle juice was strongly lower than that of phenolic compounds. At the ultrasonic power of 25 W/g, the antioxidant activity of the fruit juice achieved maximum; the value evaluated by DPPH and ABTS method was 58% and 52%, respectively higher than that in the control. Similar observation was previously reported by Le and Le (2012) who applied ultrasonic treatment of acerola mash to the extraction of high antioxidant juice. It was due to acoustic cavitation generated from ultrasound in solid-liquid extraction. The implosion of cavitation bubbles improved diffusion in the system. In addition, cavitation within the proximity of solid surface promoted surface erosion and particle breakdown. Consequently, mass transfer was improved and the extraction

Table 1. Effects of ultrasonic power on the content of phenolic compounds, ascorbic acid and antioxidant activity of the rose myrtle juice

Ultrasonic power (W/g)	Total phenolics (mg GAE/L)	Ascorbic acid (mg/L)	Antioxidant activity (mmol TEAC/L)	
			DPPH	ABTS
0	3545 ± 55[a]	228 ± 4[a]	13.33 ± 0.32[a]	17.34 ± 0.21[a]
15	4767 ± 126[b]	405 ± 4[b]	16.96 ± 0.23[b]	20.53 ± 0.27[b]
20	5467 ± 200[c]	414 ± 8[b]	19.72 ± 0.26[c]	24.04 ± 0.21[c]
25	5750 ± 144[d]	429 ± 4[c]	21.05 ± 0.17[d]	26.27 ± 0.21[e]
30	5633 ± 150[d]	417 ± 4[bc]	20.35 ± 0.44[cd]	25.25 ± 0.26[d]
35	5550 ± 161[cd]	405 ± 4[b]	19.91 ± 0.22[c]	24.85 ± 0.12[d]

Values with different letters in the same column are not significantly different at the level of $p = 0.05$

Table 2. Effects of ultrasonic time on the content of phenolic compounds, vitamin C and antioxidant activity of the rose myrtle

Ultrasonic time (min)	Phenolics (mg GAE/L)	Ascorbic acid (mg/L)	Antioxidant activity (mmol TEAC/L)	
			DPPH	ABTS
0	3545 ± 55[a]	231 ± 4[a]	13.33 ± 0.32[a]	17.34 ± 0.21[a]
2	5333 ± 144[bc]	429 ± 4[b]	21.08 ± 0.23[b]	26.27 ± 0.21[b]
4	5717 ± 76[c]	444 ± 0[c]	23.30 ± 0.33[e]	29.92 ± 0.25[d]
6	5983 ± 104[d]	480 ± 8[d]	23.86 ± 0.12[f]	32.87 ± 0.23[e]
8	5850 ± 153[c]	441 ± 4[c]	22.39 ± 0.27[d]	29.16 ± 0.56[c]
10	5683 ± 104[b]	417 ± 4[bc]	21.75 ± 0.16[c]	27.07 ± 0.11[b]

Values with different letters in the same column are not significantly different at the level of $p = 0.05$

Table 3. Experimental planning and result of total antioxidant activity of the rose myrtle juice from the ultrasound-assisted extraction

Number	X_1	X_2	Ultrasonic power (W/g)	Ultrasonic time (min)	Y - Antioxidant activity (µM TEAC/L)
1	-1	-1	20	4	20047
2	1	-1	30	4	21822
3	-1	1	20	8	21972
4	1	1	30	8	22237
5	-1.414	0	17,93	6	18613
6	1.414	0	32,07	6	21142
7	0	-1.414	25	3,17	20047
8	0	1.414	25	8,83	22690
9	0	0	25	6	24862
10	0	0	25	6	24910
11	0	0	25	6	24896
12	0	0	25	6	24904
13	0	0	25	6	24865

yield was increased. However, when the ultrasonic power increased from 25 to 35W/g, the level of total phenolics and ascorbic acid and the antioxidant activity of the rose myrtle juice were reduced. Mason and Lorimer (2002) reported that high ultrasonic power could generate hydroxyl radicals that would react with phenolic compounds and ascorbic acid. As a result, the antioxidant content and activity of the fruit juice decreased. Recently, Phan *et al.* (2012) demonstrated a reduction in antioxidant level in mulberry juice at an elevated ultrasonic power of the fruit mash treatment.

It can be noted that a positive correlation between the content of antioxidants and the antioxidant activity of rose myrtle juice was observed. The increase in biologically active compounds may lead to enhance the antioxidant activity of the extract. Nevertheless, the antioxidant activities determined by DPPH method were always lower than those measured by ABTS method. According to Liu *et al.* (2012), anthocyanins were main compounds of phenolics in rose myrtle fruit. The absorbance of anthocyanins achieved maximum at 520 nm (Zanatta *et al*., 2005) and that would interfere with the DPPH

Table 4. Multiple regression analysis of the model representing antioxidant activity of rose myrtle juice

Y	Coeff. SC	Std. Err.	P value	Conf. int (±)
Constant	24847.30	248.08	2.6064E-012	586.627
X_1	702.11	196.14	0.00898064	463.804
X_2	759.77	196.14	0.00610335	463.804
X_1*X_1	-2261.13	210.37	1.3268E-005	497.445
X_2*X_2	-1515.41	210.37	0.00017689	497.445
X_1*X_2	-377.50	277.36	0.215692	655.869
N = 13	$Q^2 = 0.733$		Cond. No. = 2.8971	
DF = 7	$R^2 = 0.962$		Y-miss = 0	
	R^2 adj = 0.936		RSD = 554.7270	
			Conf. lev. = 0.95	

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

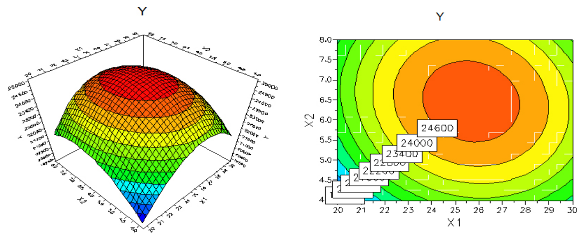

Figure 1. Effects of ultrasonic power (X_1) and time (X_2) on the antioxidant activity (Y) of the rose myrtle juice

chromogen, which had maximum absorbance at 515 nm. This would lead to the results in the relatively lower activity measured by DPPH method. Similar color interference of DPPH with anthocyanins was previously reported in the research on acerola juice (Le and Le, 2012) and black sorghum bran extract (Awika *et al*., 2003).

Effects of ultrasonic time on the content of phenolic compounds, ascorbic acid and antioxidant activity of the rose myrtle juice

Table 2 presents that at the ultrasonic time of 6 min, the maximal concentration of total phenolics and ascorbic acid of the rose myrtle was 69% and 108%, respectively higher than those of the control. In addition, the antioxidant activity evaluated by DPPH and ABTS method also achieved maximum as the ultrasonic time was 6 min. Longer sonication time reduced both antioxidant level and activity of the fruit juice. Similar observation was mentioned when the sonication of mulberry mash prolonged (Phan *et al.*, 2012). Long ultrasonic time generated high level of hydroxyl radicals and that led to a negative impact on the juice quality.

Optimization of the ultrasonic treatment conditions of rose myrtle mash for maximizing the antioxidant activity of the fruit juice

According to the results of the previous sections, an ultrasonic power of 25 W/g and ultrasonic time of 6 min were selected as the central conditions of the optimization experiment. The ultrasonic power and time were varied from 17.93 to 32.07 W/g and from 3.17 to 8.83 min, respectively. The antioxidant activity of the rose myrtle juice was evaluated by DPPH method. The experimental design and results are presented in Table 3. Multiple regression analysis was performed on the experimental data and the results are given in Table 4.

The coefficients of the model were evaluated for significance with a Student t-test. The insignificant coefficients were eliminated. The final predictive equation was as follow:

$$Y = 24847.3 + 702.109 X_1 + 759.766 X_2 - 2261.13 X_1^2 - 1515.41 X_2^2 \quad (1)$$

where Y, X_1, X_2 were the total antioxidant activity of rose myrtle mash (μmol TEAC/L), the ultrasonic power (W/g) and the ultrasonic time (min), respectively. The R^2 value of this model was determined to be 0.962, which proved that the regression model was significant. The results showed that linear coefficients (X_1, X_2) and pure quadratic coefficients (X_1^2, X_2^2) were significant, but the interaction coefficient ($X_1 \times X_2$) was not ($P > 0.05$).

Our results proved that ultrasonic power and time had significantly positive effects on the antioxidant activity of the fruit juice, while their obvious quadratic effects were also observed, but were negative. Surface response graph, obtained by using the fitted model above, is presented in Figure 1. According to the model, the optimal ultrasonic power and time for rose myrtle mash treatment were 25 W/g and 6.5 min, respectively; the antioxidant activity of the fruit juice achieved maximum of 24.98 mmol TEAC/L. This value was 4.7% higher than that in the non-optimization experiment. In comparison with the ultrasonic treatment conditions of acerola mash, the ultrasonic power used for rose myrtle mash treatment was higher while the sonication time was nearly similar. It can be suggested that for complete degradation of fruit pulp tissue, different fruits required different ultrasonic powers for the treatment.

In order to verify the accuracy of the model, three independent replicates were carried out for measuring the antioxidant activity under optimal conditions. The average antioxidant activity obtained was 24.86 mmol TEAC/L. The experimental value was therefore similar to the predicted value from the equation obtained. Moreover, the control without ultrasonic treatment was also performed. The antioxidant activity in the control was 13.36 mmol TEAC/L. Consequently, the antioxidant activity of the fruit juice in the ultrasound-assisted extraction was 86% higher than that of the control sample. Under optimal conditions, the concentration of total phenolics and ascorbic acid in the juice achieved 6,067 mg gallic acid equivalent (GAE)/L and 516 mg/L, respectively.

Conclusions

Ultrasonic treatment of rose myrtle mash has been shown to be an efficient method for the extraction of fruit juice with high phenolic and ascorbic acid level. Further study will be performed to clarify the effects of sonication variables on the level of important phenolic compounds in rose myrtle juice. Short extraction time seems to be suitable for the extraction of bioactive compounds in fruit juice. Ultrasound-assisted extraction can be considered as an alternative technique in the extraction of antioxidant-rich juices.

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