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Optimization of ultrasonic treatment of apple (*Malus domestica*) mash in the extraction of juice with high antioxidant content

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Abstract: - In this study, ultrasonic treatment of apple mash was used for improvement in antioxidant content in the fruit juice. Firstly, the effects of ultrasonic power and time on the ascorbic acid and phenolic content as well as the antioxidant activity of the apple juice were investigated. Response surface methodology was then applied to optimization of sonication conditions for maximizing the antioxidant activity of the juice. The optimal ultrasonic power and time were 20 W/g and 7.3 min, respectively under which the antioxidant activity of the apple juice achieved maximum of 13.18μmol Trolox equivalent antioxidant capacity per g dry weight (TEAC/g DW) using 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonic acid (ABTS) essay. This value was 67.5% higher than that in the control sample without ultrasonic treatment.

Keywords: - *Antioxidant activity, ascorbic acid, Malus domestica, phenolics, ultrasonic treatment*

I. INTRODUCTION

Apple is a rich source of antioxidants including ascorbic acid and phenolic compounds [1]. Apple juice is popular due to high nutritional value and attractive sensorial properties.

Enzyme-assisted extraction is a conventional method in apple juice production. However, phenolic compounds were reported to be oxidized during the prolonged enzymatic treatment [2]. Recently, ultrasound-assisted extraction of fruit juice has attracted great attention due to short time and high level of antioxidant yield [3]. There has been no report on optimization of ultrasonic treatment of apple mash for maximizing the antioxidant content in apple juice.

The objective of this study was: i) to evaluate the effects of sonication variables during the apple mash treatment on ascorbic acid and phenolic content in apple juice, ii) to optimize sonication conditions for maximizing antioxidant activity of apple juice.

II. MATERIALS AND METHODS

2.1. Materials and chemicals

Apples: Envy variety of apple (*Malus domestica*) fruits from New Zealand were used in this study. The main chemical composition (% of fresh fruit pulp) was as follows: moisture of 88.0±0.5%, total sugars of 10.3±1.2, total acidity of 0.45±0.08.

Chemicals were purchased from Sigma-Aldrich and Merck. All reagents were of analytical grade.

2.2. Experimentation

Apple fruits were cut into eight vertical segments, blanched in water at 95^{o}C for 5 min and then cooled to the ambient temperature. The fruit pieces were then crushed in a blender and the mash obtained was mixed with water – an eco-friendly solvent. Our preliminary study showed that the appropriate weight ratio of apple fruit and water was 1:1. Samples of 30 g dilute apple mash were put into 100 mL beakers, which were covered with aluminum foil for prevention of oxidative change from light.

2.2.1. Effect of sonication variables during the apple mash treatment on ascorbic acid and phenolic level in apple juice

Firstly, the ultrasonic power was change from 0 (control) to 25 W/g fruit mash while the sonication time was fixed at 4 min. The ultrasonic treatment was performed with a horn-type ultrasonic probe (Sonics and Materials Inc., USA) with frequency of 20 kHz and equal on/off time ratio. During the sonication, beakers with samples were put in a water bath with cooling water to keep the sample temperature inferior to 30^{o}C. Then, the ultrasonic time was varied from 0 (control) to 8 min; the sonication power was selected from the results of the previous experiment. At the end of the experiments, the mash was filtered through a cheese-cloth. The apple juice was separated by centrifugation at 10000×g for 10 min at 25^{o}C; the supernatant was used for further analysis.

2.2.2. Optimization of sonication conditions for maximizing the antioxidant activity of apple juice

Circumscribed central composite design was used to study the effects of ultrasonic power (X_1) and time (X_2) on the antioxidant activity of the apple juice (Y). Five levels of the independent variables and Modde

version 5.0 software were used to generate the experimental planning and to analyze data. A control without ultrasonic treatment was also performed.

2.3. Analytical methods

Total phenolic content was determined by spectrophotometric method using Folin-Ciocalteu reagent [4]. The results were expressed as the equivalent to milligrams of gallic acid per 100g of dry weight of apple fruit (mg GAE/100g DW).

Ascorbic acid content was determined with ascorbic acid test kit which was supplied from Merck KGaA (Germany).

Antioxidant activity was determined by ABTS method [5] and ferric reducing antioxidant potential (FRAP) method [6]. The results were expressed as µM TEAC per 1g of dry weight of apple fruit (µM TEAC/g DW).

2.4. Statistical analysis

All experiments were performed in triplicate and the results were expressed as average values. Means were compared by Multiple range test with $P<0.05$. Analysis of variance was done using the Statgraphics Centurion XV software.

III. RESULTS AND DISCUSSION

3.1. Effects of sonication variables during the apple mash treatment on ascorbic acid and phenolic level in apple juice

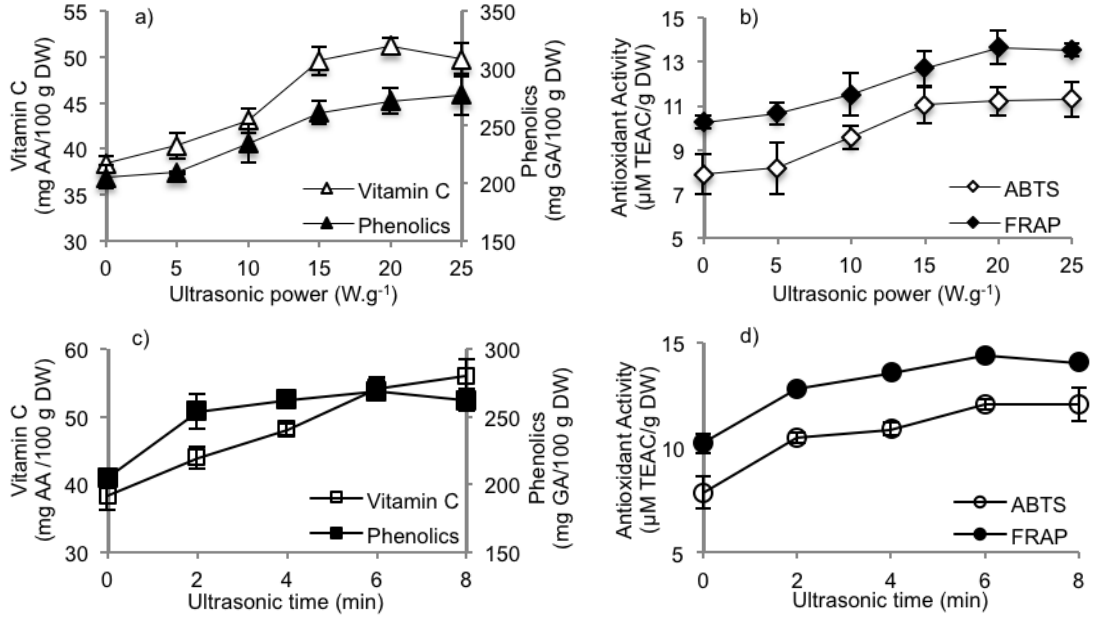

Figure 1. Effects of ultrasonic power and time on the content of ascorbic acid, total phenolics and antioxidant activity of apple juice

Fig. 1a and 1b show that the increase in ultrasonic power from 0 to 15W/g augmented the concentration of ascorbic acid (29.3%), total phenolics (27.6%) as well as the antioxidant activity of the apple juice (39.8% by ABTS method and 24.0% by FRAP method). That was due to acoustic cavitation which reduced material size, increased mixing rate and improved mass transfer during the extraction [7]. However, when the ultrasonic power was raised from 15 to 25W/g, the antioxidant content and activity of the apple juice remained constant. It can be explained that the antioxidant level in apple mash was limited. Previously, Pan et al. (2011) noted that the extraction yield of antioxidants from pomegranate peel did not increase more as the ultrasonic power exceeded a determined value [8].

From Fig. 1c and 1d, when the sonication time lasted from 0 to 6 min, the ascorbic acid and phenolic content increased 41.5 % and 31.2 %, respectively; in addition, the antioxidant activity of the apple juice augmented 53.5% by ABTS essay and 40.9% by FRAP essay. Longer treatment time did not enhance both antioxidant content and activity of the apple juice. Similar observation was reported by Le and Le (2012) who applied ultrasound to the acerola juice extraction [3].

Based on the results obtained, the ultrasonic power of 15W/g and time of 6 min were chosen as central conditions for the following optimization. Under these conditions, the antioxidant activity of the apple juice evaluated by ABTS achieved 12.08µM TEAC/g DW.

3.2. Optimization of sonication conditions for maximizing the antioxidant activity of apple juice

Table 1 presents the experimental design and results.

Multiple regression analysis was performed on the experimental data and the coefficients of the model were evaluated for their significance using Student t-test. Table 2 shows that all coefficients of the model were significant (P<0.05). As a consequence, both ultrasonic power and time affected the antioxidant activity of apple juice. The coefficient of determination (R^2) was calculated as 0.98 indicating good agreement between the experimental and the predicted values.

Table 1. Experimental design and results

(Y: antioxidant activity of apple juice; The antioxidant activity was evaluated by ABTS essay)

Run order	X_1, Ultrasonic power (W/g)	X_2, Ultrasonic time (min)	Y	Run order	X_1, Ultrasonic power (W/g)	X_2, Ultrasonic time (min)	Y
1	7.93	6	10.98	8	15	3.172	11.47
2	20	4	11.89	9	15	6	12.69
3	20	8	13.24	10	10	8	11.285
4	15	8.828	12.02	11	15	6	12.58
5	15	6	12.625	12	22.07	6	13.05
6	15	6	12.56	13	10	4	11.01
7	15	6	12.65				

Table 2. Analysis of variance for the model representing the antioxidant activity of apple juice (Y)

	DF	SS	MS (variance)	F	p	SD
Total	13	1928.6	148.354			
Constant	1	1921.52	1921.52			
Total Corrected	12	7.08105	0.590088			0.768172
Regression	5	6.97817	1.39563	94.9582	0	1.18137
Residual	7	0.102881	0.014697			0.121233
Lack of Fit	3	0.091862	0.030621	11.1147	0.021	0.174987
R^2 = 0.985						
R^2 Adj. = 0.975						

SS: Sum of squares; DF: Degrees of freedom; MS: Mean square; F: F-value at 95% of confidence level; SD: Standard deviation

The final predictive equation for natural variable is as follow:

$$Y = 5.29 + 0.35X_1 + 1.07X_2 – 0.01X_1^2 – 0.11X_2^2 + 0.027X_1X_2 \quad (1)$$

in which Y, X_1 and X_2 are antioxidant activity of apple juice by ABTS essay (µM TEAC/g DW), ultrasonic power (W/g) and time (min), respectively.

Surface response graph of the equation (1) is illustrated in Fig. 2. According to the model, the antioxidant activity achieved maximum of 13.22 µM TEAC/g DW at the ultrasonic power of 20W/g and time of 7.3 min. The experiments showed that the average antioxidant activity of apple juice was 13.18µM TEAC/g DW under optimal conditions. The predicted and experimental values were nearly similar. A control sample without ultrasonic treatment was also performed. The antioxidant activity of apple juice in the ultrasound-assisted extraction was 67.5% higher than the control. The maximal antioxidant activity of apple juice evaluated by ABTS essay in the optimization experiment was 9.4% higher in comparison with that in section 3.1.

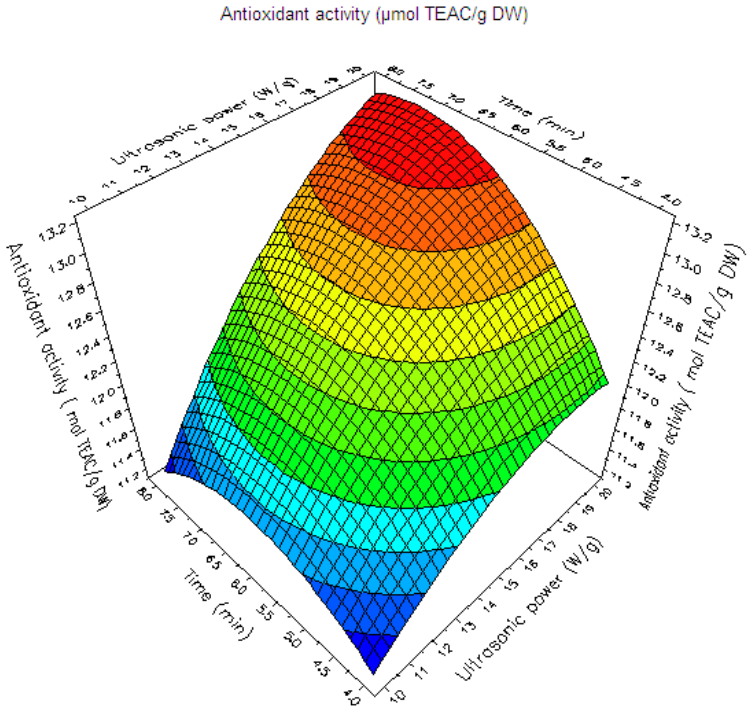

Figure 2. Effects of ultrasonic power and time on the antioxidant activity of apple juice.

IV. CONCLUSION

Ultrasound-assisted extraction increased both ascorbic acid and phenolic content in the extract as well as the antioxidant activity of the apple juice. At the optimal ultrasonic power of 20W/g and time of 7.3 min, the antioxidant activity of apple juice was 67.5% higher than that in the control without ultrasonic treatment. The application of ultrasound to the extraction of juice with high antioxidant activity was therefore potential.

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