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Enzyme-assisted and ultrasound-assisted extraction of phenolics from mulberry (*Morus alba*) fruit: comparison of kinetic parameters and antioxidant level

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Abstract

Nowadays, fruit juice with high antioxidant level has attracted great attention. In this study, pectinase preparation and ultrasound were alternatively used in the phenolic extraction from mulberry fruit. On the basis of kinetic model of second-order extraction, the extraction rate constant of total phenolics and anthocyanins in ultrasound-assisted extraction (UAE) increased approximately 16.9 and 21.5 times, respectively, in comparison with that in enzyme-assisted extraction (EAE). The level of total phenolics and anthocyanins in the UAE was 11.3% and 15.9%, respectively higher than that in the EAE. In addition, the antioxidant activity evaluated by Ferric Reducing Ability of Plasma (FRAP) and 2,2'-Azinobis-3-ethylBenzoThiazoline-6-Sulfonic acid (ABTS) in the UAE was statistically higher than that in the EAE.

Introduction

Fruit juice is a source of sugars, organic acids, vitamins, minerals and dietary fiber for human diet. In addition, certain fruit juices contribute phyto-chemicals with different biological and pharmacological effects to human (Song *et al*., 2009). Mulberry, the edible fruit of *Morus alba*, is rich in phenolic compounds with antioxidant activity. Among phenolic compounds in mulberry, anthocyanins are the most important antioxidants (Bae and Suh, 2007).

Extraction is a key operation in juice production. Conventionally, enzyme-assisted extraction (EAE) has been used in juice processing at the industrial scale. It was reported that application of pectinase preparations to fruit mash treatment significantly increased both juice yield and quality (Horvath-Kerkai and Steger-Mate, 2013). During the last few years, research on ultrasound-assisted extraction (UAE) in juice processing has attracted great attention. Sonication of fruit mash improved not only the juice yield (Lieu and Le, 2010) but also the level of bioactive compounds in fruit juice (Le and Le, 2012).

Recent studies showed that application of pectinase preparation (Phan *et al*., 2012) or ultrasound (Nguyen *et al*., 2012) to mulberry mash treatment improved not only the concentration of total phenolics and anthocyanins but also the antioxidant activity in the extract. However, kinetic parameters of antioxidant extraction from mulberry fruit in the EAE and UAE have not been reported in world literature. Moreover, the antioxidant level and activity in the extracts obtained from the EAE and UAE have not been compared. The objective of this study was to compare the kinetic parameters for antioxidant extraction from mulberry fruit by the EAE and UAE. In addition, the antioxidant level and activity in the extracts obtained from the both methods were also evaluated and compared.

Materials and Methods

Materials

Mulberry (*Morus alba*) fruits used in this study were originated from a farm in Dalat city (Vietnam). The fruits were crushed in a blender (Panasonic, Malaysia). The obtained mash was mixed with water (as a solvent) at the weight ratio of 1:1 and subsequently used for EAE and UAE. Pectinase preparation: Pectinex Ultra SP-L from *Aspergillus aculeatus* was purchased from Novo Nordisk Ferment (Switzerland). The activity of this preparation was approximately 4,190 polygalacturonase units per mL. The optimal pH and temperature were 4.0 and 50°C, respectively. Chemicals used in this study were purchased from Merck KGaA (Germany) and Sigma-

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Aldrich Co. (The United States).

Experimental methods

Comparison of kinetic parameters of the EAE and UAE from second-order kinetic model

In this experiment, total phenolics and anthocyanin in mulberry juice were selected as the target components in the extraction of antioxidants. The conditions of EAE and UAE for antioxidant extraction from mulberry fruit were previously optimized (Phan *et al*., 2012; Nguyen *et al*., 2012). Samples of 40 g mulberry mash were used in each assay. The samples were placed into 100 mL flasks.

For EAE, an amount of Pectinex Ultra SP-L of 0.08% v/w (volume of enzyme preparation to weight of fruit mash) was added into flasks of samples. The pH of mulberry mash was adjusted to 4.0. The extraction temperature was adjusted to 50°C using a thermostatic water bath (Memmert, Germany). The extraction time was changed from 0 to 140 min. After the period of incubation, the obtained mash was centrifuged (Sartorius, Switzerland) at 10,000 rpm and 4°C for 15 min and the supernatant was collected for further analysis.

For UAE, the mulberry mash was sonicated with a horn type ultrasonic probe (Sonics & Materials Inc., The United states). The ultrasonic power was 10.36 W/g of fruit mash. The sonication was carried out at 63°C. The sonication time was varied from 0 to 8 min. At the end of the sonication, the obtained mash was centrifuged at 10,000 rpm at 4°C for 15 min and the supernatant was collected for further analysis. To determine the extraction rate constant of the antioxidants, the second-order rate law was applied (Pan *et al.*, 2011). The general second-order model can be written as:

$$\frac{dC_t}{d_t} = k(C_e - C_t)^2 \qquad (1)$$

Where: k is the second-order extraction rate constant (L/g.min), C_e is the extraction capacity (the equilibrium concentration of antioxidants in the extract) (g/L), and C_t is the concentration of antioxidants in the extract at a given extraction time (g/L).

The integrated rate law for a second-order extraction, under the boundary conditions t = 0 to t and C_t = 0 to C_t, can be written as an equation (2) or a linearized equation (3):

$$C_t = \frac{C_e^2 kt}{1 + C_e kt} \qquad (2)$$

$$\frac{t}{C_t} = \frac{1}{kC_e^2} + \frac{t}{C_e} \qquad (3)$$

The initial extraction rate, h (g/L.min), as C_t/t when t approaches 0, can be defined as equation (4):

$$h = kC_e^2 \qquad (4)$$

The initial extraction rate, h, the extraction capacity, C_e, and the second-order extraction rate constant, k, can be determined experimentally from the slope and the intercept by plotting t/C_t vs. t.

Comparison of antioxidant level and activity in the extracts from the EAE and UAE

The EAE and UAE were carried out under optimal conditions. For EAE, the amount of Pectinex Ultra SP-L added to the mulberry mash was 0.08 % v/w (volume of enzyme preparation to weight of fruit mash); the pH value of fruit mash was 4.0; the extraction temperature and time was 50°C and 120 min, respectively (Phan *et al*., 2012). For UAE, the ultrasonic power was 10.36 W/g of fruit mash; the extraction temperature and time was 63°C and 6 min, respectively. After the extraction, the mixture was centrifuged for removal of solid residue and the extracts were used for further analysis. Control sample without enzymatic and ultrasonic treatment was also performed under the same conditions. The extraction time of the control sample was 120 min (Nguyen *et al*., 2012).

Analytical methods

Total phenolic level was evaluated by spectrophotometric method using Folin-Ciocalteu reagent (Ozgen *et al.*, 2009). The results were expressed as the equivalent to mg of gallic acid per litre of extract (mg GAE/L). Total anthocyanin content was determined by the pH-differential method (Ozgen *et al*., 2009). The 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). The results were presented as equivalent to millimole of Trolox per liter of extract (mM TE/L).

Statistical analysis

All experiments were performed in triplicate. Means were compared by Multiple range tests with $p < 0.05$. Analysis of variance was done using the software Statgraphics plus, version 3.0.

Results and Discussion

Comparison of kinetic parameters of the enzyme-assisted and ultrasound-assisted extraction from second-order kinetic model

Figure 1 presents the level of total phenolics and anthocyanins in the extract during the EAE and UAE. All samples treated with pectinase preparation demonstrated higher level of total phenolics and anthocyanins than the control without enzymatic treatment. Similar observation for phenolic extraction was reported by Oszmianski *et al.* (2011) who used pectinase preparations in the treatment of apple mash for the production of puree-enriched cloudy apple juice. According to these authors, pectinases degraded pectins in the middle lamella of fruit tissue and that enhanced the extraction of antioxidants from the cellular cytoplasm.

The level of total phenolics and anthocyanins in all sonicated samples was always higher than that in the control without ultrasonic treatment. Recently, ultrasound was applied to acerola mash treatment and enhanced the phenolic concentration in the acerola juice (Le and Le, 2012). Vilkhu *et al.* (2008) stated that cavitation generated by ultrasound led to surface erosion and particle breakdown in solid-liquid extraction. This phenomenon provided new surfaces and increased mass transfer. As a result, the extraction yield of antioxidants was improved.

From these experimental data, the extraction rate reciprocal at various extraction times was calculated and Figure 2 illustrates the linearized forms of the second order model for the EAE and UAE. Table 1 shows the kinetic parameters of antioxidant extraction from mulberry fruit. The extraction capacity C_e for phenolics and anthocyanins was nearly similar in both EAE and UAE. However, the values of extraction rate constant k were found approximately 16.9 times for phenolics and 21.5 times for anthocyanins as fast in UAE as in EAE. Similarly, the initial extraction rate h for phenolics and anthocyanins in UAE was 17.8 times and 27.9 times, respectively higher than that in EAE. Consequently, the UAE significantly facilitated the extraction of phenolics and anthocyanins from mulberry fruit and highly shortened the extraction time in comparison with the EAE. Similar kinetic parameters were recently reported by Khan *et al.* (2010) and Pan *et al.* (2011) for polyphenol extraction from orange peel and pomegranate peel, respectively. However, the values and the increase in the kinetic parameters estimated by these authors were much lower than those in our study. According to Le and Le (2012), phenolic compounds could be extracted from fruit easier than from peel. In both extraction methods,

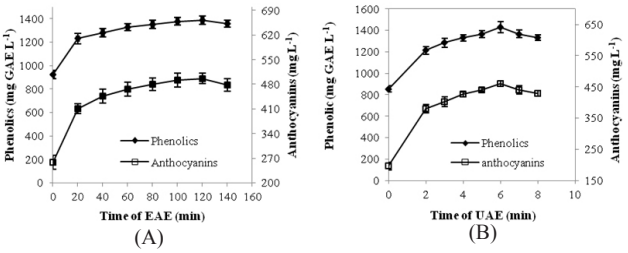
Table 1. Comparison of the second-order kinetic parameters of antioxidant extraction in the enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE)

Components	Extraction method	Extraction capacity, C_e (g L^{-1})	Initial extraction rate, h (g $L^{-1}min^{-1}$)	Extraction rate constant, k (L $g^{-1}min^{-1}$)	R^2
Phenolics	UAE	1.39	11.76	6.09	0.996
	EAE	1.36	0.66	0.36	0.999
Anthocyanins	UAE	0.45	2.79	13.76	0.995
	EAE	0.39	0.10	0.64	0.998

Various superscripts in each column indicate significant differences ($p < 0.05$)

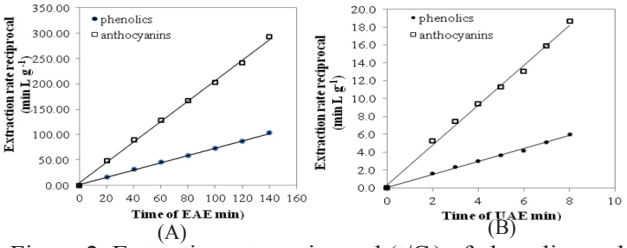
Table 2. Antioxidant level and activity in the extracts from enzyme-assisted extraction (EAE) and ultrasound-assisted extraction (UAE)

Extraction method	Level of		Antioxidant activity	
	Phenolics (mg GAE/L)	Anthocyanins (mg/L)	FRAP (mM TE/L)	ABTS (mM TE/L)
EAE	1375±35^b	477±20^b	13.62±0.19^b	11883±245^b
UAE	1530±38^c	553±14^c	14.04±0.17^c	12328±174^c
Control sample	910±24^a	234±18^a	8.19±0.12^a	7097±155^a



(A) (B)

Figure 1. Change in phenolic and anthocyanin level in the extract from mulberry fruits during the enzyme-assisted extraction (A) and ultrasound-assisted extraction (B)



(A) (B)

Figure 2. Extraction rate reciprocal (t/C_t) of phenolics and anthocyanins at different extraction times (t) in enzyme-assisted extraction (A) and ultrasound-assisted extraction (B)

the coefficient of determination R^2 for both phenolics and anthocyanins was quite high ($R^2 > 0.99$). It can be concluded that the second order kinetic model fitted perfectly the experimental results.

Comparison of antioxidant level and activity in the extracts from the enzyme-assisted and ultrasound-assisted extraction

Table 2 confirmed that both EAE and UAE improved the antioxidant level in the extract. It can be noted that the concentration of total phenolics and anthocyanins in the sonicated sample was 11.3% and 15.9%, respectively higher than that in the sample treated with pectinase preparation. Similar results were reported when acerola mash treatment with ultrasound and cellulase preparation were compared (Le and Le, 2012). Ultrasonic treatment of fruit mash resulted in a higher level of antioxidants in the obtained extract in comparison with the conventional

enzymatic treatment. Due to the increase in total phenolics and anthocyanin contents, the increase in antioxidant activity of the extract obtained in UAE was also higher than that in EAE.

Conclusions

Both EAE and UAE improved the antioxidant content and activity in the extract from mulberry fruit. The UAE exhibited some advantages such as shorter extraction time and higher extraction yield for total phenolics and anthocyanins in comparison with the EAE. Application of UAE to fruit juice processing was therefore potential for enhancement of product quality.

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