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Simultaneous treatment of acerola mash by ultrasound and pectinase preparation in acerola juice processing: optimization of the pectinase concentration and pectolytic time by response surface methodology

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Abstract: This study focused on the application of the simultaneous treatment of acerola mash by ultrasound and pectinase preparation to the juice processing. Firstly, the impacts of pectinase concentration and pectolytic time on the extraction yield and the chemical composition of the acerola juice were investigated. The response surface methodology was then used to optimize the conditions of pectolysis for maximizing the juice yield. When the pectinase concentration and pectolytic time in the combined ultrasonic and enzymatic treatment were 0.12% v/w and of 26.3 min, respectively, the extraction yield achieved maximum (87.4%). This value was 3.2% and 15.5% higher than that in the ultrasonic treatment and the enzymatic treatment, respectively. In addition, the simultaneous treatment of acerola mash by ultrasound and pectinase preparation significantly improved the nutritional quality of acerola juice.

Keywords: Acerola, extraction, pectinase, ultrasound

Introduction

Juice has been a popular beverage on the world because of high nutritional value. Extraction is one of the most important process in juice processing. This operation affects directly the juice yield as well as the economic efficiency of the production-line (McLelan and Padilla-Zakour, 2005). For improvement in juice yield, pectinase preparation has been widely used in the fruit mash treatment. Pectinases hydrolyze pectic substances in the fruit flesh tissue and facilitates juice release from the cellular cytoplasm (Doran *et al*., 2000). Another alternative method for enhancement in extraction yield is ultrasonic treatment. Many studies reported that acoustic cavitation produced by ultrasound increased extraction yield of different compounds from plant material (Vilkhu *et al*., 2008). Recently, the combined ultrasonic and pectinase extraction was introduced to grape juice processing as a potential method for enhancement of juice yield (Lieu and Le, 2010).

Acerola (*Malpighia emarginata* DC.) is a tropical fruit that is rich in ascorbic acid, phenolics, and sugars (Mezadri *et al*., 2006). This study focused on acerola juice processing. The aim of this research was to investigate the effects of pectinase concentration and pectolytic time in the simultaneous treatment of acerola mash by ultrasound and pectinase preparation on the extraction yield and the juice quality. Moreover, the conditions of enzymatic treatment were then optimized by response surface methodology for maximizing the juice yield.

Materials and Methods

Materials

Enzyme source

Pectinase preparation (Pectinex Ultra SP-L) used in this study was originated from Novo Nordisk Ferment (Switzerland). The activity was approximately 2,335 polygalacturonase units (PGU) per mL (Mutlu *et al*., 1999). The optimal pH and temperature were 4.0–5.0 and 55–60°C, respectively (Kashyap *et al*., 2001).

Acerola

Acerola (*Malpighia glabra*) was originated from a farm in Go Cong, Viet Nam. The fruits were harvested during the period from July to December, 2009. The main chemical composition (mg/g) of fruit flesh was as follows: ascorbic acid: 44.5, total phenolics: 21.9, sugars: 140, free amino nitrogen: 51.5, ash: 3.3, pectin: 24 and total acidity: 48.9.

Simultaneous treatment of acerola mash by ultrasound and pectinase preparation

Acerola was destemmed, washed and crushed in a blender (Panasonic, MJ-70M, Malaysia). The pH of acerola mash was then adjusted to value of 4.5. For

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each assay, samples of 100 g of acerola mash were taken and placed into 250 mL flasks. The weight ratio of water to acerola mash was fixed at 2:1.

Effect of pectinase concentration on extraction yield and juice quality

Different pectinase concentrations (0.05-0.25% v/w) were added to flasks of samples. The samples were sonicated with ultrasonic power of 150 W, temperature of 50°C and sonication time of 100 sec by a horn type ultrasonic probe (Sonics and Materials. Inc, VC750, USA). Subsequently, the samples were kept in the period of 30 min for pectolysis.

The sonication temperature was adjusted to 50°C by using a water bath (Memmert, WNB45, Indonesia). The pectolytic treatment temperature was adjusted to 50°C by using an incubation shaker (B. Braun Biotech. International, Certomat® BS-1, Germany). The agitation rate was 200 rpm. At the end of the pectolysis, enzymes in the sample were inactivated by heating the mash at 90°C for 5 min in a water bath. The mash was then filtered through a cheese cloth. The obtained suspension was centrifuged at 6,500 rpm for 10 min by a refrigerated centrifuge (Sartorius, Sigma 3K30, Switzerland) and the supernatant was collected for analysis of soluble extract, ascorbic acid, total phenolics and sugars.

Two control samples were realized. In control sample A, acerola mash was treated by Pectinex Ultra SP-L but was not sonicated; the pectinase concentration and pectolytic time were 0.15% v/w and 60 min, respectively. In control sample B, acerola mash was sonicated but was not treated by Pectinex Ultra SP-L; the ultrasonic power, temperature and time were 150 W, 50°C and 100 sec, respectively.

Effect of pectolytic time on extraction yield and juice quality

Pectinex Ultra SP-L (0.05% v/w) was added into flasks of samples. The samples were then sonicated with same conditions as mentioned in the experiment above. When the sonication treatment was completed, the samples were kept in a period ranged from 20 to 100 min for pectolysis. After the pectolysis, the following steps were similar to those in the previous experiment. As noted in the previous experiment, two control samples A and B were also carried out.

Optimization of pectinase concentration and pectolytic time by response surface methodology

A randomised, quadratic central composite circumscribe response surface design was used to optimize pectinase concentration and pectolytic time for the simultaneous treatment of acerola mash by ultrasound and pectinase preparation. The software Modde version 5.0 was also used to generate experimental planning and to process data. The amount of Pectinex SP-L was changed from 0.065% v/w to 0.135% v/w and the pectolytic time was varied from 5.86 to 34.14 min. Each factor in the design was studied at five different levels ($-\sqrt{2}, -1, 0, +1, +\sqrt{2}$). The treatment temperature was fixed at 50°C. At the end of the treatment, enzymes in the sample were also inactivated by heating the mash at 90°C for 5 min in a water bath and the following steps were similar to those in the previous experiment.

Analytical methods

Extraction yield was the ratio of the content of soluble extract in the obtained juice to the content of dry weight of material used in the treatment. It was calculated by the following formula:

$$Y = \frac{m_2 \times C}{m_1(100 - w)} \times 100$$

Where Y was the extraction yield (%) of the treatment method, m_1 and ω were the mass (g) and the moisture (%) of the initial acerola mash, respectively; and m_2 and C were the mass (g) and the total soluble extract content (%) in the obtained juice, respectively. The moisture and soluble extract content was quantified by the drying method (Robert, 1998). Ascorbic acid was quantified by titration method (Suntornsuk *et al*., 2002). Total phenolics were determined by spectrophotometric method using Folin-Ciocalteu reagent (Pyo *et al*., 2004). Sugars were evaluated by spectrophotometric method using 3,5-dinitrosalicylic acid reagent (Miller, 1959).

Statistical analysis

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

Results and Discussions

Effect of pectinase concentration on extraction yield and juice quality

Figure 1 shows that the extraction yield in the simultaneous treatment of acerola mash by ultrasound and pectinase was significantly higher than that in the ultrasonic or enzymatic treatments. This observation was in agreement with the findings of Lieu and Le

(2010), which used the combined ultrasound and pectinase extraction in grape juice processing. It can be explained that ultrasound generates collapsing cavitational bubbles the energy of which provides greater penetration of the solvent into the cellular material and enhances mass transfer to and from interfaces; in addition, acoustic cavitation can disrupt the cell walls and release the cellular materials for increase in extraction yield (Patist and Bates, 2008). On the other hand, pectinase preparation disintegrates the middle lamella in fruit tissue and that leads to an improvement in juice yield (Kashyap *et al*., 2001). Our results proved a synergic effect of sonication and pectolysis on extraction yield in acerola juice processing.

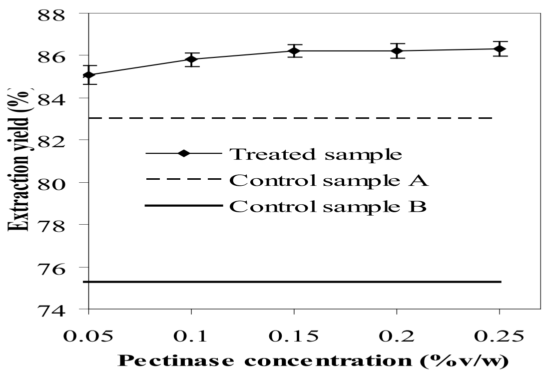

Figure 1. Effect of enzyme concentration on extraction yield of the simultaneous treatment of acerola mash by ultrasound and pectinase preparation
Control sample A: acerola mash was treated by Pectinex Ultra SP
Control sample B: acerola mash was treated by ultrasound

The juice yield achieved maximum when the Pectinex Ultra SP-L content was 0.1% v/w. The analysis of variance showed that increase in pectinase concentration from 0.1 to 0.25% v/w did not augment the extraction yield. The maximum extraction yield in the combined ultrasound and pectinase treatment was 12.7% and 2.8% higher than that in the ultrasonic treatment and enzymatic treatment, respectively.

Table 1 presents the level of ascorbic acid, total phenolics and sugars in the obtained acerola juice. In general, the simultaneous treatment of the fruit mash by sonication and pectolysis significantly enhanced the nutritional value of the final product in comparison with the ultrasonic or enzymatic treatment. Ascorbic acid is an important component in acerola juice (Mezadri *et al*., 2006). The higher the pectinase concentration used in the treatment, the higher the ascorbic acid content in the acerola juice. The ascorbic acid content achieved 99.5 mg/g of juice when the pectinase concentration was 0.25% v/w.

Maximal increase in level of total phenolics and sugars in the acerola juice achieved when the pectinase concentration was 0.1 and 0.05% v/w, respectively. Higher increase in concentration of pectinase preparation did not lead to a higher level of these nutritional compounds in the obtained juice.

Effect of pectolytic time on extraction yield and juice quality

Figure 2 illustrates that 20 min was an appropriate time for pectolysis of the fruit mash. The analysis of variance reveals that increase in pectolytic time from 20 to 100 min did not improve the extraction yield. Many previous studies reported that the time for pectinase treatment of fruit mash varied from 60 to 120 min (Kashyap *et al*., 2001). In this study, the pectolytic time for acerola mash treatment was significantly shorter. It was due to synergic effect of ultrasound and pectinase preparation on juice extraction. Sonication during the first 100 sec of the fruit mash treatment broke down the flesh tissue and released the pectin which was simultaneously hydrolyzed by Pectinex Ultra SP-L. The release of pectic substrates from the material by sonication facilitated the pectolysis in the acerola mash and shortened the pectolytic time.

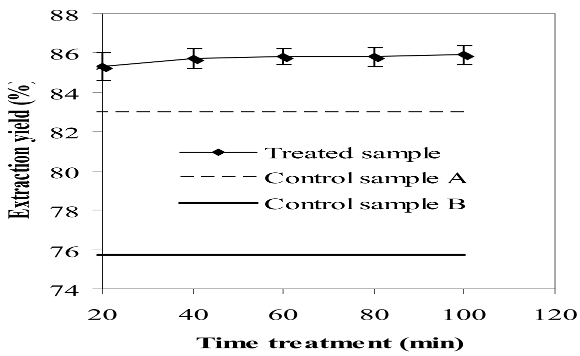

Figure 2. Effect of pectolytic time on extraction yield of the simultaneous treatment of acerola mash by ultrasound and pectinase preparation
Control sample A: acerola mash was treated by Pectinex Ultra SP
Control sample B: acerola mash was treated by ultrasound

Table 2 demonstrates the main chemical composition of the acerola juice obtained from different times of pectolytic treatment. Maximum concentration of ascorbic acid achieved when the pectolytic time was 40 min. A longer pectolytic time reduced ascorbic acid concentration in the obtained juice. Ascorbic acid is a thermo-sensitive compound (Mezadri *et al.*, 2006). The prolonged pectolysis carried out at 50°C facilitated oxidative reaction and that led to a low level of ascorbic acid in the final product. However, the ascorbic acid in the acerola juice from the combined ultrasound and pectinase treatment was obviously higher than that in the two

Table 1. Effect of pectinase concentration in the simultaneously ultrasonic and enzymatic treatment on main chemical composition of the acerola juice
Control sample A: acerola mash was treated by Pectinex Ultra SP
Control sample B: acerola mash was treated by ultrasound

Pectinase concentration (% v/w)	Control A	Control B	0.05	0.1	0.15	0.2	0.25
Ascorbic acid (mg/g)	72.2^{b}	45.8^{a}	89.5^{c}	92.3^{d}	94.8^{e}	97.7^{f}	99.5^{g}
Total phenolics (mg/g)	34.0^{b}	25.7^{a}	58.2^{c}	60.2^{d}	60.3^{d}	60.5^{d}	60.8^{d}
Reducing sugars (mg/g)	191.7^{b}	169.2^{a}	204.9^{c}	204.7^{c}	205.3^{c}	205.3^{c}	205.4^{c}

Different letters in each row indicate statistically significant difference at the level of p= 0.05

Table 2. Effect of pectolytic time in the simultaneously ultrasonic and enzymatic treatment on main chemical composition of the acerola juice
Control sample A: acerola mash was treated by Pectinex Ultra SP
Control sample B: acerola mash was treated by ultrasound

Pectolytic time (min)	Control A	Control B	20	40	60	80	100
Ascorbic acid (mg/g)	74.3^{b}	47.6^{a}	91.2^{c}	93.8^{d}	91.1^{c}	87.8^{f}	83.7^{g}
Total phenolics (mg/g)	36.2^{b}	26.5^{a}	59.5^{c}	59.4^{c}	59.2^{c}	58.7^{d}	57.9^{e}
Reducing sugars (mg/g)	198.7^{b}	171.6^{a}	204.3^{c}	205.3^{c}	205.6^{c}	205.6^{c}	205.7^{c}

Different letters in each row indicate statistically significant difference at the level of p= 0.05

control samples.

With regards to other nutritional components in the acerola juice, the concentration of total phenolics and sugars reached maximum when the pectolytic time was 20 min. Longer pectolytic time did not affect the sugar level but reduced slightly the total phenolic level probably due to oxidative reactions.

Optimization of pectinase concentration and pectolytic time by reponse surface methodology

Table 3 shows the extraction yield (Y) for each run according to the experimental planning. In order to establish the fitted model, multiple regression analysis was performed on the experimental data and the final predictive function obtained is as given below:

$$Y = 85.22 + 3.29X_1 + 2.76X_2 - 2.14X_1^2 - 2.24X_2^2 \qquad (1)$$

Where Y, X_1, X_2 were the extraction yield in the simultaneous treatment of acerola mash by ultrasound and pectinase preparation, the enzyme concentration (% v/w) and the pectolytic time (min), respectively.

The effect of each variable on the response was determined for a 95% confidence level. Four variables X_1, X_2, X_1^2 and X_2^2 were estimated as significant effects but the interaction term of X_1 x X_2 was insignificant factor. The regression model was significant ($P < 0.05$) because the coefficient of determination (R^2) of the model for the response was 0.955; the predicted values were close to the observed values, and all absolute prediction errors were less than 1.5. According to analysis of variance, the F-value was 4.65 times more than the F listed value.

In order to determine optimal levels of the variables for the extraction yield, three dimensional surface plots were constructed according to function (1) (Figure 3). The optimal conditions were the pectinase concentration of 0.12% v/w and the pectolytic time of 26.3 min, at which the model predicted a maximum response of 87.4%. The extraction yield in the combined ultrasound and pectinase treatment was 3.2% and 15.5% higher than that in the ultrasonic treatment and the enzymatic treatment, respectively. In order to verify the accuracy of the model, three independent replicates were conducted for measuring extraction yield under the optimal conditions. The average extraction yield was 88.1 ± 0.3%. The experimental values were therefore nearly similar to the predicted value from quadratic function (1).

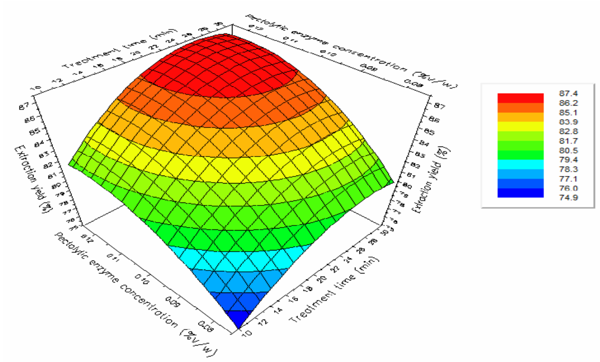

Figure 3. Response surface plot for extraction yield in the simultaneous treatment of acelora mash by ultrasound and pectinase preparation

Conclusion

The simultaneous treatment of acerola mash by ultrasound and pectinase preparation enhanced the extraction yield, shortened the pectolytic time, reduced the pectinase concentration and improved the nutritional quality of acerola juice in comparison

Table 3. Experimental planning and results of extraction yield for the simultaneously combined pectolytic and ultrasonic treatment of acelora mash

Run	X_1 - Enzyme concentration (% v/w)	X_2 – Pectolytic time (^{0}C)	Y - Yield[a] (%)	Predicted yield[b] (%)	Error[c] (%)
1	0.075	10	75.9	74.9	1.0
2	0.125	10	82.3	81.3	1.0
3	0.075	30	79.2	80.3	1.1
4	0.125	30	85.8	86.9	1.1
5	0.065	20	76.3	76.3	0.0
6	0.135	20	85.7	85.6	0.1
7	0.1	5.86	75.4	76.8	1.4
8	0.1	34.14	86.2	84.7	1.5
9	0.1	20	85.7	85.2	0.5
10	0.1	20	84.9	85.2	0.3
11	0.1	20	85.3	85.2	0.1
12	0.1	20	85.5	85.2	0.3
13	0.1	20	84.7	85.2	0.5

[a] Observed values (or experimental values). b Predicted values. cAbsolute prediction error = |observed − predicted|.

with the sonication treatment or the pectolytic treatment. Application of the combined sonication and pectinase treatment to juice processing is therefore very potential.

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