

## Pectolytic treatment of non-sonicated or sonicated barbados cherry mash: alternative choice in juice processing

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**Abstract:** Traditionally, pectolytic treatment of fruit mash has been carried out in juice processing for improvement in extraction yield. In this study, barbados cherry mash was sonicated and subsequently treated by pectinase preparation. The efficiency of the pectolytic treatment of non-sonicated and sonicated barbados cherry mash in juice processing was evaluated and compared. Response surface methodology was used to optimize enzyme concentration and pectolytic time in the two treatment processes. The combination of ultrasound and pectinase increased extraction yield 9.2% as well as decreased enzyme concentration 27.2% and pectolytic time 24.1% in comparison with the conventional enzymatic treatment. In addition, the level of total phenolics, sugars, free amino nitrogen in the barbados cherry juice from the combined ultrasound and pectinase treatment was much higher than that in the juice from the pectolytic treatment.

**Keywords:** Juice, *Malpighia glabra*, pectinase, ultrasound

### Introduction

Barbados cherry or acerola (*Malpighia glabra*) is a tropical fruit native to West India, Central and South America (De Rosso and Mercadante, 2007). This fruit is rich in ascorbic acid and polyphenols (Mezadri *et al.*, 2008). Barbados cherry juice is an important industrial intermediate product because it has been further processed into jellies, punch bases and ice cream (Mustard, 1952), juice blends, concentrated juice (Brasil *et al.*, 2007).

Traditionally, juice extraction includes some consecutive steps such as pitting, crushing, heating, enzymatic treatment and pressing. The enzymatic treatment time usually varies from 1 to 2h (McLellan and Padilla-Zakour, 2005) and that augments the energy cost of the process (Kobus, 2005). Moreover, ascorbic acid in barbados cherry extract is thermo-sensitive and could be degraded during the extraction with high temperature (Belitz *et al.*, 2009).

From the last two decades, the application of ultrasound in extraction process has found increasing attention because of its practicability for large scale commercial applications (Patist and Bates, 2008). This technique increased the extraction yield of different components such as polysaccharides, aroma compounds, polyphenols, tartaric acid and amino acid (Vilkhu, 2008). In juice processing, simultaneous extraction of many water-soluble compounds in fruit flesh is essential for improvement in juice yield. Positive results of the ultrasonic extraction of grape juice was recently reported (Lieu and Le, 2010). However, there have been no studies on ultrasound assisted extraction in barbados cherry juice processing.

In this paper, pectinase preparation and ultrasound were used to increase the extraction yield in barbados cherry juice processing. The objective of this study was to determine optimal conditions of pectolytic treatment of the non-sonicated and the sonicated barbados cherry mash by using response surface methodology as well as to compare efficiency of the combined ultrasound and enzyme process with that of the conventional enzymatic process.

### Materials and Methods

#### *Pectinase preparation*

Pectinex Ultra SP-L from *A. aculeatus* was obtained from Novo Nordisk Ferment (Switzerland) and stored at 4°C. The activity of this preparation was approximately 2,335 polygalacturonase units (PGU)/mL (Doran *et al.*, 2000). The optimal pH and temperature were 4.0–5.0 and 55–60°C, respectively (Kashyap *et al.*, 2001).

#### *Barbados cherry mash*

Barbados cherry (*Malpighia glabra*) used in study was originated from a farm in Go Cong, Viet Nam. The fruits were harvested during the period from July to December, 2009. Only bright orange fruits without disease symptoms were selected. 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: 24.0; the total acidity was 48.9 mg of citric acid/g. Barbados cherry was destemmed, washed and crushed in a blender (Panasonic, MJ-70M, Malaysia). The pH of barbados cherry mash was then adjusted to 4.5. The obtained mash was used for further treatments.

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### *Pectolytic treatment of non-sonicated barbados cherry mash*

In this experimental section, samples of 100 g barbados cherry mash were taken for each assay. The samples were placed into 250 mL flasks. First series: Different amounts of water were added into flask of samples to obtain various ratios of water to material such as 0:1; 0.5:1, 1:1, 1.5:1; 2:1; 2.5:1; 3:1; 3.5:1 (w/w). Pectinex Ultra SP-L with concentration of 233.5 PGU/100g of fruit mash was supplemented into each sample. All samples were then kept in the period of 30 min.

Second series: Pectinase preparation with concentration varied from 0 to 700.5 PGU/100g of fruit mash was added into flask of samples. The weight ratio of water to barbados cherry mash in all samples was 2:1 (w/w). The treatment time was fixed at 30 min. Third series: The weight ratio of water to material was fixed at 2:1 (w/w). Pectinex Ultra SP-L (350.3 PGU/100g of fruit mash) was added into flask of samples. The treatment time was changed from 30 to 120 min. In all series, control samples were prepared with weight ratio of water to material of 2:1 (w/w) and pectinase preparation was not used in the treatment. The 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 process, 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 further analysis.

A randomised, quadratic central composite circumscribe response surface design was then used to optimize the pectinase concentration and the pectolytic time. The software Modde version 5.0 was used to generate the experimental planning and to process data. A determined amount of Pectinex Ultra SP-L (from 267.7 to 432.8 PGU/100g of fruit mash) was used and the pectolytic time was ranged from 38.8 to 81.2 min. Each factor in the design was studied at five different levels ( $-\sqrt{2}$ ,  $-1$ ,  $0$ ,  $+1$ ,  $+\sqrt{2}$ ). The temperature was maintained 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.

### *Pectolytic treatment of sonicated barbados cherry mash*

A preliminary study was carried out to determine

the appropriate conditions of sonication of barbados cherry mash (unpublished data). In this experimental section, for each assay, samples of 100g barbados cherry mash were taken and placed into 250 mL flask. The weight ratio of water to material was fixed at 2:1 (w/w). Each sample was sonicated with ultrasound power of 150 W, temperature of 70 °C and treatment time of 100 sec by a horn type ultrasonic probe (Sonics and Materials. Inc, VC750, USA). This equipment operated at frequency of 20 kHz. The sonication temperature was regulated by placing the flasks containing the samples in a thermostatic water bath (Memmert, WNB45, Indonesia). All samples were subsequently divided into two series.

First series: Different amounts of Pectinex Ultra SP-L were added into flasks of samples. Enzyme concentration was varied from 0 to 583.8 PGU/100g of fruit mash. The samples were then kept in the period of 30 min. Second series: Pectinex Ultra SP-L (233.5 PGU/100g of fruit mash) was added into flasks of samples. The pectolytic time was ranged from 20 to 100 min.

In both series, temperature was maintained at 50°C, agitation rate during the pectolysis was fixed at 200 rpm. The following steps were similar to those in the previous experimental section. Control sample was carried out without pectinase and sonication treatment.

A randomised, quadratic central composite circumscribe response surface design was used to optimize the pectinase concentration and the pectolytic time of the sonicated barbados cherry mash. The software Modde version 5.0 was also used to generate the experimental planning and to process data. A determined amount of Pectinex SP-L (from 151.0 to 316.0 PGU/100g of fruit mash) was used and pectolytic time was varied from 11.7 to 68.3 min. Each factor in the design was examined 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.

### *Comparison of physico-chemical characteristics of barbados cherry juice obtained from pectolytic treatment of non-sonicated and sonicated fruit mash*

Different components such as ascorbic acid, total phenolics, total acidity, sugars, free amino nitrogen of barbados cherry extracts obtained in the appropriate conditions in the two previous experimental sections were measured and compared. Control sample without any treatment was also carried out.

Analytical methods

Extraction yield is the ratio of the content of soluble extract in the obtained juice to the 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  $w$  were the mass (g) and the moisture (%) of initial barbados cherry mash, respectively;  $m_2$  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).

Sugar content of barbados cherry juice was determined by spectrophotometric method using 3,5-dinitrosalicylic acid reagent (Miller, 1959). Total phenolics were evaluated by spectrophotometric method using Folin-Ciocalteu reagent (Pyo *et al.*, 2004). Ascorbic acid was quantified by titration method (Suntornsuk *et al.*, 2002). Free amino nitrogen was measured by spectrophotometric method using ninhydrin reagent (Millio and Loffredo, 1995). Total acidity expressed in equivalent of citric acid content (g/L), was determined by diluting a 10 mL aliquot of each sample with 90 mL distilled water and subsequently titrating the sample with 0.1 N NaOH to a pH endpoint of 8.1 (Robert, 1998).

Statistical analysis

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

Results and Discussion

Pectolytic treatment of non-sonicated barbados cherry mash

The extraction yield in the pectolytic treatment of non-sonicated barbados cherry mash is showed in Figure 1. The weight ratio of water to material of 2:1 w/w, the pectinase concentration of 350.3 PGU/100g of fruit mash and the pectolytic time of 60 min were the appropriate conditions for the enzymatic treatment.

High level of solvent (water) facilitated the diffusion of soluble extract from the material into the solvent and that led to an improvement in extraction yield (Fennema, 1996). In addition, pectinases in

Ultra Pectinex SP-L preparation degraded pectic substances in the cell wall matrix and in the middle lamella of barbados cherry pulp into smaller units and eventually into galacturonic acid which in turn allowed maximal juice levels to be expelled and enhanced the extraction yield (Doran *et al.*, 2000).

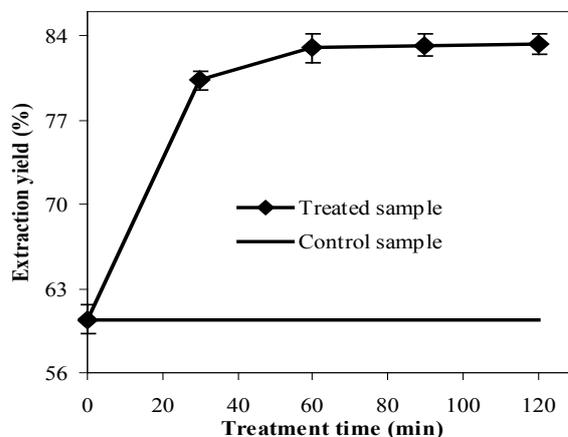
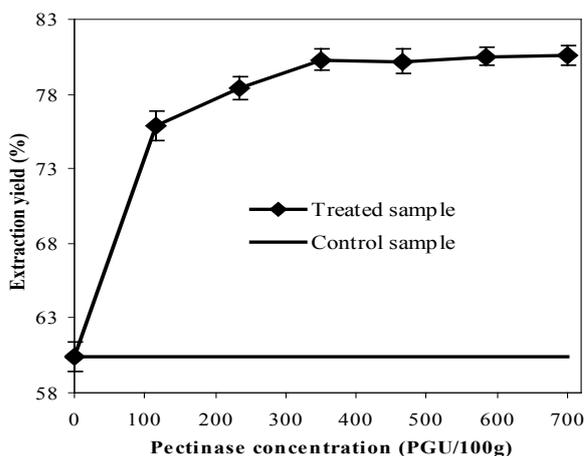
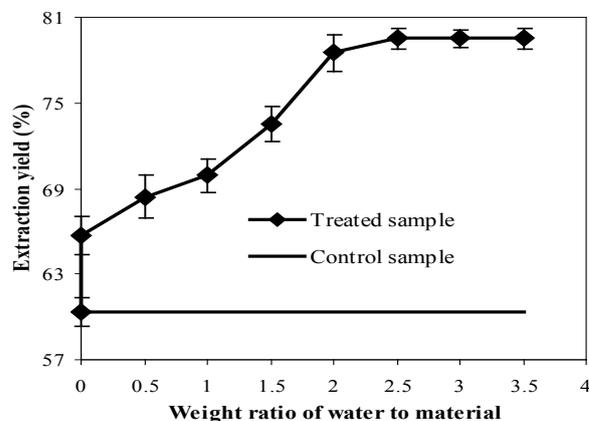


Figure 1. Effect of weight ratio of water to material (A), pectinase concentration (B) and treatment time (C) on extraction yield of pectolytic treatment of non-sonicated barbados cherry mash

Maximum extraction yield obtained in the pectolytic treatment was 22.5% higher than that of the control sample. Many previous studies reported that the use of pectinase preparation improved juice yield from grapes, apples, pears, strawberry, raspberry and blackberry (Lieu and Le, 2010; Kashyap *et al.*, 2001). Base on these preliminary investigations, a concentration of Pectinex Ultra SP-L of 350.3 PGU/100g of fruit mash and a time of 60 min were chosen as the central conditions of randomised, quadratic central composite circumscribe response surface design. Table 1 shows extraction yield of each run according to the experimental planning.

**Table 1.** Experimental planning and results of extraction yield for pectolytic treatment of non-sonicated barbados cherry mash

| Run | X <sub>1</sub> - Pectinase concentration (PGU/100g of fruit mash) | X <sub>2</sub> - Pectolytic time (min) | Y <sub>1</sub> - Yield <sup>a</sup> (%) | Predicted yield <sup>b</sup> (%) | Error <sup>c</sup> (%) |
|-----|---|--|---|----------------------------------|------------------------|
| 1   | 291.9   | 45                                     | 77.7                                    | 77.5                             | 0.2                    |
| 2   | 408.6   | 45                                     | 79.9                                    | 79.8                             | 0.1                    |
| 3   | 291.9   | 75                                     | 79.0                                    | 79.7                             | 0.7                    |
| 4   | 408.6   | 75                                     | 83.2                                    | 84.0                             | 0.8                    |
| 5   | 267.7   | 60                                     | 78.3                                    | 78.0                             | 0.3                    |
| 6   | 432.8   | 60                                     | 83.1                                    | 82.8                             | 0.3                    |
| 7   | 350.3   | 38.8                                   | 77.4                                    | 77.8                             | 0.4                    |
| 8   | 350.3   | 81.2                                   | 83.2                                    | 82.3                             | 0.9                    |
| 9   | 350.3   | 60                                     | 83.5                                    | 83.2                             | 0.3                    |
| 10  | 350.3   | 60                                     | 83.4                                    | 83.2                             | 0.2                    |
| 11  | 350.3   | 60                                     | 83.2                                    | 83.2                             | 0                      |
| 12  | 350.3   | 60                                     | 82.8                                    | 83.2                             | 0.4                    |
| 13  | 350.3   | 60                                     | 83.1                                    | 83.2                             | 0.1                    |

<sup>a</sup> Observed values (or experimental values), <sup>b</sup> Predicted values, <sup>c</sup> Absolute prediction error = |observed - predicted|.

The best-fitting quadratic model was then determined by multiple regression and backward elimination. The coefficients of the model were evaluated for significance with a Student t-test. The insignificant coefficients were eliminated, and the model was finally refined. Quadratic equation (1) obtained was as follows:

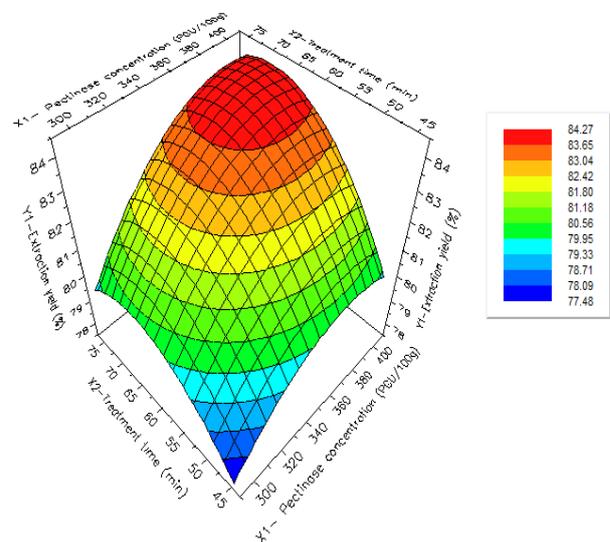
$$Y_1 = 83.1 + 1.68X_1 + 1.61X_2 - 1.36X_1^2 - 1.60X_2^2 \quad (1)$$

where Y<sub>1</sub> is the extraction yield in cherry mash (%), the enzyme concentration (PGU/100g of fruit mash) and the pectolytic time (min), respectively.

The effect of each variable on the response was evaluated for a 95% confidence level. X<sub>1</sub> and X<sub>2</sub> were considered as dominative effects. Moreover, two variables X<sub>1</sub><sup>2</sup> and X<sub>2</sub><sup>2</sup> were estimated as significant

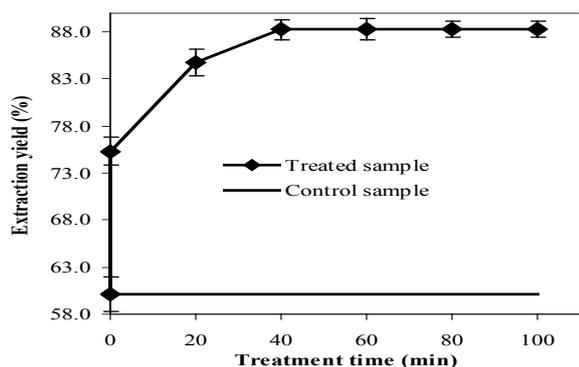
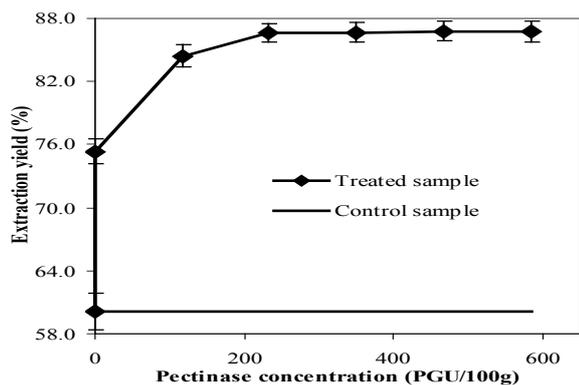
effects. However, the interaction term of X<sub>1</sub> x X<sub>2</sub> was an insignificant factor. The coefficient of determination (R<sup>2</sup>) of the model for the response was 0.967. Therefore, the model was found to be adequate in representing the response data of the juice yield and can be further used for analysis and prediction purposes (Sharma *et al.*, 2005). Predicted results were close to the observed, and all absolute prediction errors were less than 0.9. The analysis of variance showed that the F value (40.8) was much higher than the F-listed value (6.4). Consequently, the regression model was significant (P < 0.05).

Figure 2 shows the plot of response surface of the pectolytic treatment of non-sonicated barbados cherry mash. The optimal conditions were the enzyme concentration of 399.3 PGU/100g of fruit mash and the pectolytic time of 68.5 min, at which the model predicted a maximum extraction yield of 84.1%. Consequently, the extraction yield was 23.7% higher than that of the control sample. In order to verify the accuracy of the model, three independent replicates were conducted for measuring extraction yield under the optimal conditions. The extraction yield was 83.7 ± 0.3%. The experimental values were therefore similar to the predicted value from quadratic function (1).



**Figure 2.** Response surface plot for extraction yield in the pectolytic treatment of non-sonicated barbados cherry mash

*Pectolytic treatment of sonicated barbados cherry mash*



**Figure 3.** Effect of pectinase concentration (A) and treatment time (B) on extraction yield of pectolytic treatment of sonicated barbados cherry mash.

Figure 3 shows that the pectinase concentration of 233.5 PGU/100g of fruit mash and the time of 40 min were the appropriate conditions for the pectolytic treatment of sonicated barbados cherry mash. The combination of sonication and conventionally enzymatic treatment increased the extraction yield about 29.7% in comparison with the control sample. Moreover, the combined ultrasonic and pectolytic process increased the extraction yield approximately 5.8 % more than the enzymatic process.

From a botanical standpoint, vegetal tissues are composed of cells surrounded by middle lamella which consists of pectic compounds similarly to the concrete between bricks in the brick wall (Toma *et al.*, 2001). During the ultrasonic treatment, the ultrasound intensity created the micro-bubbles in the liquid in the expansion cycle. Once formed, these bubbles would absorb the energy from the sound waves, grow during the expansion cycles and recompress during the compression cycle. The increase in pressure and temperature caused by the compression leads to the collapse of the bubbles (Palma and Barroso, 2002). Acoustic cavitation provides greater penetration of the solvent molecules into the material, improves mass transfer to and from interfaces. As a result, the juice yield was increased. However, disruption of plant cell

walls by cavitation released macromolecules such as pectin substances (Patist and Bates, 2008) which blocked the drainage channels in the pulp through which the juice pass (Kashyap *et al.*, 2001). During the subsequent enzymatic treatment, the pectinases degraded pectin, facilitated extract release and enhanced juice yield. Thus, ultrasound and pectinase preparation had a synergistic effect on extraction yield in barbados cherry juice processing. Moreover, the enzyme concentration and the pectolytic treatment time in the combined ultrasound and enzyme process decreased significantly in comparison with those in the conventional enzymatic process.

**Table 2.** Experimental planning and results of extraction yield for pectolytic treatment of sonicated acelora mash

| Run | X <sub>1</sub> - Pectinase concentration (PGU/100g of fruit mash) | X <sub>2</sub> - Pectolytic time (min) | Y <sub>2</sub> - Yield <sup>a</sup> (%) | Predicted yield <sup>b</sup> (%) | Error <sup>c</sup> (%) |
|-----|---|--|---|----------------------------------|------------------------|
| 1   | 175.1   | 20                                     | 77.0                                    | 75.8                             | 1.2                    |
| 2   | 291.9   | 20                                     | 88.4                                    | 86.5                             | 1.9                    |
| 3   | 175.1   | 60                                     | 80.0                                    | 81.5                             | 1.5                    |
| 4   | 291.9   | 60                                     | 90.6                                    | 91.4                             | 0.8                    |
| 5   | 151.0   | 40                                     | 76.3                                    | 76.0                             | 0.3                    |
| 6   | 316.0   | 40                                     | 90.0                                    | 90.7                             | 0.7                    |
| 7   | 233.5   | 11.7                                   | 78.4                                    | 80.5                             | 2.1                    |
| 8   | 233.5   | 68.3                                   | 89.7                                    | 88.0                             | 1.7                    |
| 9   | 233.5   | 40                                     | 88.5                                    | 88.5                             | 0.0                    |
| 10  | 233.5   | 40                                     | 88.5                                    | 88.5                             | 0.0                    |
| 11  | 233.5   | 40                                     | 88.5                                    | 88.5                             | 0.0                    |
| 12  | 233.5   | 40                                     | 88.4                                    | 88.5                             | 0.1                    |
| 13  | 233.5   | 40                                     | 88.5                                    | 88.5                             | 0.0                    |

<sup>a</sup> Observed values (or experimental values), <sup>b</sup> Predicted values,

<sup>c</sup> Absolute prediction error = |observed - predicted|.

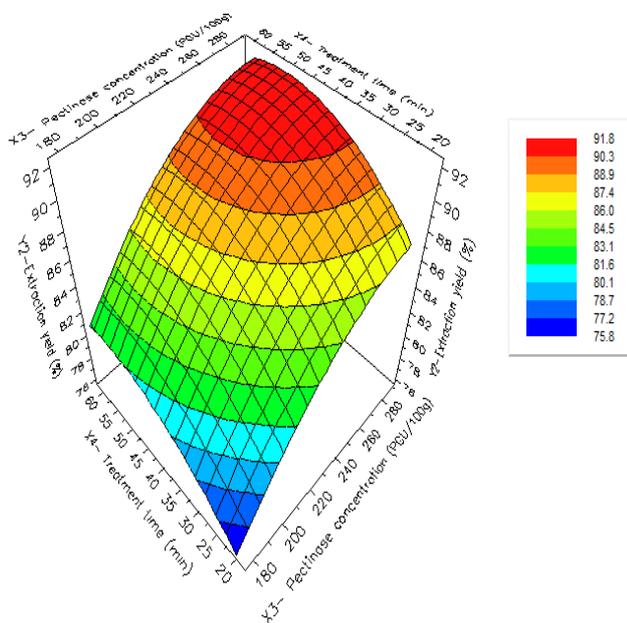
Table 2 shows the extraction yield for each run in the optimization of the enzymatic treatment of sonicated barbados cherry mash. In order to establish fitted model, multiple regression analysis was performed on the experimental data and final predictive equation (2) is as given below:

$$Y_2 = 88.45 + 5.16X_3 + 2.64X_4 - 2.56X_3^2 - 2.10X_4^2 \quad (2)$$

Where Y<sub>2</sub>, X<sub>3</sub>, X<sub>4</sub> were the extraction yield in the pectolytic treatment of sonicated barbados cherry mash, the enzyme concentration (PGU/100g of fruit mash) and the pectolytic time (min), respectively. The effect of each variable on the response was evaluated for a 95% confidence level. X<sub>3</sub> was considered as dominative effect. In addition, three variables X<sub>4</sub>, X<sub>3</sub><sup>2</sup> and X<sub>4</sub><sup>2</sup> were estimated as significant effects but the interaction term of X<sub>3</sub> x X<sub>4</sub> was an insignificant factor.

The regression model was significant (P < 0.05) because the coefficient of determination (R<sup>2</sup>) 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 2.1. According to analysis of variance, the F- value was 4.7 times more than the F listed value. In order to determine optimal

levels of the variables for the juice extraction, the plot of response surface was constructed according to equation (2) (Figure 4). The optimal conditions were the enzyme concentration of 290.8 PGU/100g of fruit mash and the time of 52 min, at which the model predicted a maximum extraction yield of 91.8 %. The extraction yield was therefore 31.9 % higher than that of the control sample. Moreover, the extraction yield in the combined ultrasound and enzymatic treatment was 9.2% higher than that in the enzymatic treatment. The enzyme concentration and the treatment time in the pectolytic treatment of sonicated barbados cherry mash were decreased by 27.2% and 24.1%, respectively in comparison with those of the pectolytic treatment of non-sonicated barbados cherry mash.



**Figure 4.** Response surface plot for extraction yield in the pectolytic treatment of sonicated barbados cherry mash

In order to verify the accuracy of the model, three independent replicates were carried out for measuring extraction yield under the optimal conditions. The obtained extraction yield was  $91.7 \pm 0.3\%$ . Thus, the experimental values were nearly similar to the predicted values from quadratic equation (2). Similar result was recently observed when the combined pectinase and ultrasound treatment was used in grape juice processing. The researchers reported that the extraction yield in the enzymatic treatment of sonicated grape mash was 7.3% higher than that in the conventional enzymatic treatment (Lieu and Le, 2010).

*Comparison of physicochemical characteristics of barbados cherry juice obtained from pectolytic*

*treatment of non-sonicated and sonicated fruit mash*

Some physicochemical characteristics of barbados cherry juice obtained from the pectolytic treatment of non-sonicated and sonicated fruit mash are given in Table 3. The level of phenolic compounds in the barbados cherry juice from the pectolytic treatment of non-sonicated fruit mash increased 31.1% in comparison with that in the control sample.

**Table 3.** Comparison in physico-chemical characteristics of Barbados cherry juice obtained from pectolytic treatment of non-sonicated and sonicated fruit mash.

| Physico-chemical characteristics (mg/g dry weight) | Control sample                     | Pectolytic treatment of non-sonicated fruit mash | Pectolytic treatment of sonicated fruit mash |
|--|------------------------------------|--|--|
| Phenolics  | $21.2 \pm 2.7^a$                   | $27.8 \pm 2.5^b$                                 | $62.4 \pm 2.4^c$                             |
| Ascorbic acid                                      | $44.5 \pm 0.3^a$                   | $48.9 \pm 0.2^b$                                 | $47.6 \pm 0.2^c$                             |
| Sugars   | $134.9 \pm 0.1^a$                  | $172.2 \pm 0.2^b$                                | $207.5 \pm 0.1^c$                            |
| Free amino nitrogen                                | $51.5 \pm 0.4^a$                   | $110.0 \pm 0.2^b$                                | $128.7 \pm 0.3^c$                            |
| <b>Total acidity</b>                               | <b><math>48.9 \pm 0.2^a</math></b> | <b><math>61.5 \pm 0.1^b</math></b>               | <b><math>51.6 \pm 0.2^c</math></b>           |

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

This observation was in agreement with the findings of Bagger-Jørgensen *et al.* (2004) who used Pectinex Ultra SP-L in the treatment of blackcurrant mash; however, the phenolic content in the blackcurrant juice treated by pectinase preparation was just 14-15% higher than that in the control sample. Variation in the phenolic yield from different fruits is due to difference in amount and localization of phenolics in the plant tissue (Haizhou *et al.*, 2004).

Many studies reported that ultrasound assisted extraction increased the phenolic yield from vegetables and fruits in comparison with conventional extraction (Vilkhu *et al.*, 2008). In this research, the amount of phenolic compounds in the barbados cherry juice obtained from the combined ultrasound and pectinase treatment was approximately 2.2 times higher than that from the pectolytic treatment. Polyphenols possess antiulcer, anti-mutagenic, and anti-inflammatory activities (Flamini, 2003). Consequently, high level of polyphenols in barbados cherry extract improved nutritional quality of the final product.

The highest ascorbic acid content in barbados cherry juice was observed when the fruit mash was treated by pectinase preparation. Contradictory results were reported by Carvalho *et al.* (2006) who used pectinase preparation in lemon juice processing. According to these authors, decrease in ascorbic acid level in lemon juice was due to a prolonged exposure of the fruit mash to oxygen at an elevated temperature during the enzymatic treatment.

It was reported that ultrasound enhanced chemical reaction rate and yield (Ashokkumar *et al.*, 2008). The oxidative reaction, which induced ascorbic acid loss during the treatment of barbados cherry mash, was also accelerated by ultrasound. Moreover,

ultrasound released more the hydroxyl radicals which promoted the decomposed reaction of ascorbic acid (Hu *et al.*, 2008). Adekunle *et al.* (2010) observed the loss of ascorbic acid when sonication was used as a pasteurization method in tomato juice processing. In our study, the level of ascorbic acid in the combined ultrasonic and pectolytic treatment was 2.7% lower than that of pectolytic treatment. However, ascorbic acid concentration in the barbados cherry juice from the combined ultrasound and enzyme process was still 7% higher than that in the control sample.

Both sugar and free amino nitrogen contents in the combined ultrasound and enzyme treatment were 20.5% and 17.0%, respectively, higher than those in the enzymatic treatment. It can be noted that the levels of sugar and free amino nitrogen in the two treatment methods were considerably higher than those of the control sample because both pectinase preparation and ultrasonic waves could degrade plant tissue in the fruit flesh for releasing sugars and amino acids from cellular cytoplasm (El-Sharnouby *et al.*, 2009). Accordingly, the combined physical and biochemical treatment improved sugar and free amino nitrogen extraction more than the biochemical treatment.

The total acidity in the barbados cherry juice from the enzymatic treatment increased 25.8% in comparison with that of the control sample. An increase in total acidity in grape juice was also observed when pectinase preparation was applied to the fruit grape mash treatment (Lieu and Le, 2010). However, Matta *et al.* (2000) reported that the treatment of West Indian cherry pulp by Pectinex Ultra SP-L had no effect on the total acidity in the obtained juice.

The total acidity in the barbados cherry juice from the pectolytic treatment of sonicated fruit mash was 5.6% higher than that in the control sample. However, the combination of ultrasound and pectinase treatment did not promote high acidity level in the obtained juice as the conventional enzymatic process. It was probably due to the loss of ascorbic acid and organic acids by ultrasound (Ashokkumar *et al.*, 2008). Two mechanisms have been proposed for sonodegradation of organic compounds. The first mechanism is pyrolysis within cavitation bubbles which is likely to be the major reaction path for the degradation of polar compounds. The second mechanism is the generation of hydroxyl radicals in the cavitation bubbles which subsequently oxidise the polar organic compounds (Tiwari *et al.*, 2009).

## Conclusion

The pectolytic treatment of sonicated barbados

cherry mash enhanced extraction yield, decreased enzyme concentration, shortened pectolytic time and improved some physicochemical characteristics of the obtained juice in comparison with the pectolytic treatment of non-sonicated fruit mash. However, the combined ultrasound and pectinase treatment did not promote high vitamin C level in the barbados cherry juice as much as the conventional pectolytic treatment. The enzymatic treatment of sonicated fruit mash is a potential method in fruit juice extraction for improvement in juice yield and quality.

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