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Application of combined ultrasound and cellulase preparation to guava (*Psidium guajava*) mash treatment in juice processing: optimization of biocatalytic conditions by response surface methodology

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Abstract

In this study, guava mash was preliminary treated by ultrasound and then by cellulase preparation for juice processing. Response surface methodology was applied to optimize the conditions of the biocatalytic treatment. The optimal conditions of cellulolytic treatment were enzyme concentration of 0.12% (w/w) and treatment time of 57 minutes. Under these conditions, the extraction yield achieved maximum of 65.7% and this value was 21.2% higher than that in the control sample. In addition, the guava juice quality was improved. The levels of total sugars and phenolics were increased 13.5% and 16.4%, respectively in comparison with those in the control sample. Guava mash treatment by ultrasound and cellulase preparation reduced the ascorbic acid content in the obtained juice but its antioxidant activity increased 19.7% (evaluated by 2,2' -azinobis-3-ethylbenzothiazoline-6-sulfonic acid method) and 11.5% (evaluated by ferric ion reducing antioxidant power method) compared to that in the control sample.

Introduction

Extraction is one of the most important operations in fruit juice processing. Conventionally, juice extraction consists of fruit crushing and pressing (Lozano, 2006). However, juice cannot be completely extracted from fruit pulp in practice and this phenomenon noticeably reduces the economic efficiency of the production line. During the last two decades, application of hydrolase preparation to juice processing has attracted great attentions. Cellulase, hemicellulase and pectinase can effectively break down cell wall and plant tissue for improvement in juice release and that results in a higher extraction yield (Chopda and Barrett, 2001; Sun *et al*., 2007; Landbo *et al*., 2007; Wang *et al*., 2009; Kapasakalidis *et al*., 2009). Until present, pectinase has been widely used in fruit juice processing. Nevertheless, the application of cellulase preparation to guava juice extraction has not clearly been considered. It should be noted that cellulose is the main component in fruit cell wall (Jain *et al*., 2003; Ortega-Regules *et al*., 2006). Hydrolysis of cellulose could damage plant cell wall for facilitating juice extraction.

Recently, ultrasound has been used in grape mash disintegration and this method enhanced significantly the juice yield (Lieu and Le, 2010). In this study, combined ultrasound and cellulase treatment of guava mash was used in juice processing. The objective of this study was to optimize the biocatalytic conditions for maximizing the extraction yield in guava juice production.

Materials and Methods

Materials

Seedless guava (*Psidium guajava*) fruits used in this study were originated from Tien Giang province. Guava fruits were selected, washed and crushed. The obtained guava mash was kept at -18^{0}C for all experiments. Enzyme source: Cellulase preparation C1184 from *Aspergillus niger* was obtained from Sigma Aldrich Company (The United States). The enzyme activity was approximately 1400 units per one gram of the preparation. One unit would liberate 1.0 micromole of glucose equivalent from carboxymethyl cellulose at pH of 4.0, temperature of 50^{0}C and incubation time of 30 min. Chemicals: 2,2'-Azinobis-(3-ethylBenzoThiazoline-6-Sulfonic acid) (ABTS), 2,4,6-TriPyridyl-s-TriaZine (TPTZ) were purchased from Sigma Aldrich Company (The Unites States).

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Experimentation

Guava mash was pretreated with ultrasound under optimal conditions obtained from the previous investigations. The sonication conditions were as follows: weight ratio of water to guava mash of 1:1, ultrasonic frequency of 20 kHz, ultrasonic power of 3 W/g of fruit mash, sonication temperature of 60^0C, sonication time of 6 min (Nguyen *et al*., 2011). The pretreated mash was subsequently used for enzymatic treatment.

Effect of concentration of cellulase preparation on extraction yield

In this experiment, the concentration of cellulase preparation was varied as follows: 0, 0.06, 0.09, 0.12, and 0.15% (w/w). Other conditions of the treatment were fixed: pH of 4.0, temperature of 50^0C and cellulolytic treatment time of 40 min. Sample of guava mash was added into 200 mL beakers and the cellulolytic treatment was carried out in a thermostatic water bath. At the end of the process, the enzyme was inactivated by heating in a water bath at 100^0C for 5 min. The mash was then filtered through a filter paper. The obtained suspension was centrifuged at 10000 x g for 20 minutes. Control sample without ultrasonic and enzymatic treatment was carried out.

Effect of cellulase treatment time on extraction yield

In this experiment, the treatment time was changed as follows: 0, 20, 40, 60 and 80 min. Other conditions of the enzymatic treatment were fixed: pH of 4.0 and temperature of 50^0C; the concentration of cellulase preparation was selected from the results of the previous experiment. The experimental procedure was similar to that in the previous experiment.

Optimization of cellulolytic treatment conditions by response surface methodology

A randomized, quadratic central composite circumscribed response surface design was used to optimize the conditions of cellulolytic treatment of the sonicated guava mash for maximizing the juice yield. The enzyme concentration (X_1) and treatment time (X_2) were used as independent factors. The central points were chosen from the results of the previous experiments. The software Modde, version 5.0 was used to generate the experimental planning and to process the data.

Evaluation of guava juice quality

In this experiment, combined of ultrasound and cellulase treatment was applied to guava juice processing. Optimal biocatalytic conditions were used. A control sample without ultrasonic and enzymatic treatment was also carried out. The obtained guava juice samples were used for analysis of total sugars, total phenolics, vitamin C and antioxidant activity.

Analytical methods

Extraction yield

The extraction yield h was calculated by the following equation:

$$h = \frac{m_2 \times C_2}{m_1 \times C_1} \times 100, \ (\%)$$

where: m1 was the weight of the initial guava fruit used in the experiment, (g); m_2 was the weight of the obtained juice after centrifugation, (g); C_1 was the dry matter content in guava fruit, (%) and C_2 was the total soluble solid content in guava juice, (%). The dry matter content in guava fruit and total soluble solid content in guava juice were determined by drying the samples at 110^0C to constant weight.

Total sugars

Total sugar content was determined by spectrophotometric method using anthone reagent (Yemm and Willis, 1954). The total sugar content in guava juice was expressed as g of sucrose equivalent (SE) per 100 g dry matter of guava fruit.

Ascorbic acid

Ascorbic acid content was determined by HPLC method (Tran, 2006). C18 column (Macherey - Nagel, ET 250/8/4 Nucleosil® 120-5, Germany) was used. The mobile phase is methanol in phosphate buffer with the volume ratio of 1:9. The results were expressed as mg of ascorbic acid per 100 g of dry matter of guava fruit.

Total phenolic

Total phenolic content (TPC) was determined by spectrophotometric method using Folin-Ciocalteu reagent (Singleton *et al*., 1999). The results were expressed as mg of gallic acid equivalent (GAE) in 100 g of dry matter of guava juice.

Antioxidant activity

The antioxidant activity (AOA) of guava juice was determined by FRAP (Benzie and Strain, 1996) and ABTS methods (Re *et al*., 1999). The results were expressed in µmol Trolox equivalent (TE) in 100 g dry matters of the initial guava mash.

Statistical analysis

Each experiment was performed in triplicate. The results were expressed as mean value ± SD. The mean values were considered significantly different when P value was less than 0.05. Analysis of variance was carried out using the software Statgraphic Centurion, version XVI.

Results and Discussion

Effect of concentration of cellulase preparation on extraction yield

Figure 1 shows the effect of cellulase concentration on the extraction yield in guava juice processing. It can be noted that the extraction yield in all treated samples was significantly higher than that of the control without ultrasonic and cellulolytic treatments. The combined ultrasound and cellulase treatment of guava mash resulted in a higher juice yield than the ultrasonic treatment. Similar results were also observed in the combined ultrasound and pectinase treatment of grape mash in grape juice processing (Lieu and Le, 2010). The extraction yield achieved maximum when the concentration of cellulase preparation was 0.09% (w/w). When the enzyme concentration was higher than 0.09% (w/w), the extraction yield did not increase significantly ($P > 0.05$). The cellulase concentration of 0.09% was therefore selected for the next experiment.

Effect of cellulase treatment time on extraction yield

The effect of the biocatalytic time on the extraction yield in guava juice processing is presented in Figure 2. The longer the time of enzymatic treatment, the higher the juice yield. Nevertheless, the extraction yield did not statistically change ($P>0.05$) when the treatment time was higher than 40 minutes. As a result, 40 minute was the appropriate time for cellulolysis in guava juice processing. Our results were different from the findings in the study of Lieu and Le, (2010) that used ultrasound and pectinase preparation in the grape mash treatment for grape juice extraction. According to these authors, the biocatalytic time of the sonicated grape mash was only 20 minutes to reach maximum extraction yield. The cellulolytic treatment in our study needed longer time for cell wall damage for juice release. The reason may that guava fruit was rich in cellulose (Jain *et al*., 2003; Ortega-Regules *et al*., 2006) and hydrolysis of cellulose was a prolonged operation.

Optimization of cellulolytic treatment conditions by response surface methodology

In this experiment, enzyme concentration of 0.09% (w/w) and treatment time of 40 minutes was chosen as central condition of the central composite circumscribed response surface design. The results of extraction yield obtained are presented in Table 1. Multiple regression analysis was performed on the

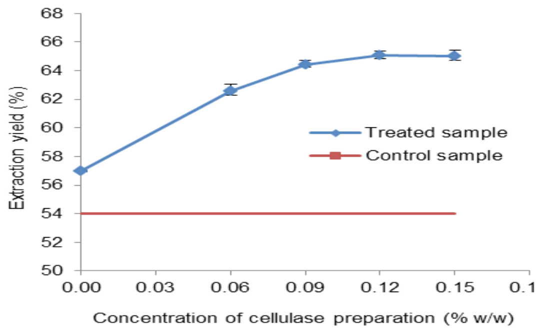

Figure 1. Effect of concentration of cellulase preparation on extraction yield in guava juice processing

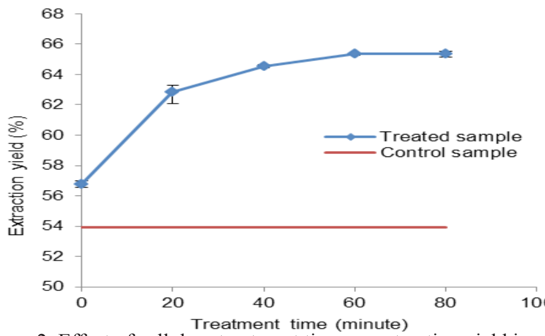

Figure 2. Effect of cellulase treatment time on extraction yield in guava juice processing

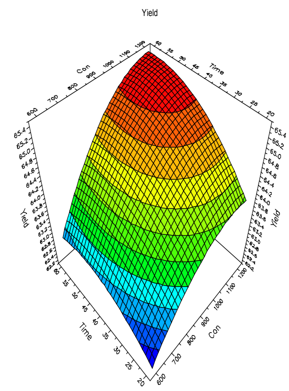

Figure 3. Response surface prediction of extraction yield in guava juice processing as a function of cellulase preparation concentration and treatment time

experimental data to establish the fitted model. The obtained prediction equation was given as follow:

$$Y = 64.67 + 0.92X_1 + 0.48X_2 - 0.44X_1^2 - 0.33X_2^2$$

Where: Y, X_1, X_2 were the extraction yield (%), concentration of cellulase preparation (%w/w) and biocatalytic time (min), respectively.

According to analysis of variance (data not shown), the regression model was significant ($P < 0.05$) and was fitted with data. It can be mentioned that both cellulase concentration and treatment time had significant effect on the extraction yield in guava juice production. But the cellulase concentration had more significant effect on the juice yield than the

Table 1. Experimental planning and results of extraction yield for combined ultrasonic and cellulolytic treatment of guava mash

Run	Enzyme concentration % (w/w)	Time (minute)	Extraction yield (%)	Run	Enzyme concentration % (w/w)	Time (minute)	Extraction yield (%)
1	0.090	11.7	62.8	13	0.090	40.0	64.8
2	0.090	11.7	62.7	14	0.090	40.0	64.9
3	0.060	20.0	61.9	15	0.090	40.0	64.5
4	0.060	20.0	62.3	16	0.090	40.0	64.4
5	0.120	20.0	63.9	17	0.132	40.0	65.0
6	0.120	20.0	64.4	18	0.132	40.0	64.5
7	0.048	40.0	62.3	19	0.060	60.0	62.8
8	0.048	40.0	61.7	20	0.060	60.0	62.3
9	0.090	40.0	65.0	21	0.120	60.0	65.5
10	0.090	40.0	64.5	22	0.120	60.0	65.4
11	0.090	40.0	65.2	23	0.090	68.3	64.8
12	0.090	40.0	64.1	24	0.090	68.3	64.7

Table 2. Some physico-chemical characteristics of guava juice from different treatment methods

Method	Total sugars (g SE/100g)	Vitamin C (mg/100g)	TPC (mg GAE/100g)	AOA (FRAP) (µ TE/100g)	AOA (ABTS) (µ TE/100g)
C	46.6 ± 0.3^a	477.0 ± 1.5^a	1446.2 ± 14.8^a	107.6 ± 1.2^a	88.3 ± 1.5^a
UT	48.5 ± 0.1^b	345.9 ± 10.4^b	1558.7 ± 19.7^b	111.8 ± 0.8^b	96.3 ± 0.3^b
CUCT	52.9 ± 0.1^c	277.2 ± 3.2^c	1684.0 ± 14.0^c	119.9 ± 0.4^c	105.6 ± 1.7^c

Values are significantly different ($P < 0.05$) from other values within a column unless they have at least one similar superscript letter.
C: Control sample without enzyme and ultrasound treatment, UT: ultrasonic treatment, CUCT: combined ultrasound and cellulase treatment

biocatalytic time.

Figure 3 shows the impact of both cellulase concentration and treatment time on juice yield. Optimal conditions of cellulase treatment of the sonicated guava mash were enzyme concentration of 0.12% (w/w) and treatment time of 57 minutes. The predicted maximal extraction yield was 65.5% and this value was 21.2% higher than that in the control sample. We carried out the cellulase treatment under the optimal conditions obtained; the extraction yield was 65.7 ± 0.3 %.

Evaluation of guava juice quality

Table 2 indicates that the content of total sugars and total phenolics in the guava juice obtained from the combined ultrasound and cellulase treatment increased 13.5% and 16.4%, respectively in comparison of those in the control sample.

Sonication of guava mash promoted cavitation that could damage plant cell wall (Mason *et al*., 1996; Mason and Lorimer, 2002; Esclapez *et al*., 2011). In addition, hydrolysis of cellulase in plant cell wall also reduced the material size. These phenomena facilitated the release of extractive compounds such as sugars and phenolics from the fruit cells.

On the contrary, the vitamin C level in the treated sample was lower than that in the control sample. We supposed that the cellulase treatment at 50^0C for 40 minutes was the main reason for vitamin C degradation. This component is very sensitive to oxygen and high temperature (Zheng *et al*., 2011). However, the antioxidant activity of the treated sample was 11.5% and 19.7% higher than that in the control sample according to FRAP method and ABTS method, respectively. Consequently, it can be concluded that the combined ultrasound and cellulase treatment of guava mash improved juice quality than the conventional extraction method.

Conclusion

Combined ultrasound and cellulase treatment of guava mash improved both juice yield and quality. The application of ultrasound and cellulase preparation was very potential in the production of guava juice processing.

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