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Research Article

Application of cellulase preparation to guava mash treatment in juice processing: optimization of treatment conditions by RSM

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Abstract

The objective of this study was to use cellulase preparation in guava mash treatment for improvement in juice yield and quality. Firstly, the effect of enzyme concentration and biocatalytic time on the juice yield was investigated. Response surface methodology was then employed to select enzyme dosage and treatment time for maximizing juice yield. The results showed that optimal concentration of cellulase preparation and treatment time were 0.13% (w/w) and 93 minutes, respectively. Under these conditions, the extraction yield, total sugars and phenolics level in the obtained juice increased 22.3%, 20.0% and 11.5%, respectively in comparison with those in the control sample. Although cellulolytic treatment decreased the content of ascorbic acid in guava juice, the antioxidant activity of the product was increased 21.8% in comparison with that in the control sample.

Keywords: *Psidium guajava*, beverage, optimization, response surface methodology, cellulolytic treatment, Vietnam.

Introduction

Consumption of fruit juice has increased in recent years in Asian countries due to different health benefits [1]. Among tropical and subtropical fruits, guava is widely popular because of its typical fragrance [2]. In addition, guava is rich in different nutrients such as sugars, organic acids, vitamins and phenolic compounds [3]. As a result, guava juice is a highly nutritional beverage for human consumption.

Juice extraction is one of the most important processes in fruit juice production. Lately, various hydrolase preparations have been used in fruit mash treatment for increase in juice yield [1, 3].

Until present, the application of cellulase preparation to guava mash treatment has not been considered.

The aim of this study was to investigate the effect of concentration of cellulase preparation and biocatalytic time on the juice yield and to optimize the conditions of cellulase treatment for maximizing extraction yield in guava juice processing.

Materials and Methods

Materials

Enzyme source

Cellulase C – 1184 (Sigma-Aldrich, USA) originating from *Aspergillus niger* was used in this study. The activity of this preparation was 0.3 cellulase activity unit/mg. The optimal temperature and pH were 50^0C and 4.0, respectively.

Guava mash

Seedless guava (*Psidium guajava* L.) fruit were purchased from a local market in Ben Tre, Vietnam. The fruit were de-stemmed, washed and crushed in a juice extractor (Panasonic, MJ – 170, Malaysia). The obtained guava mash was diluted with water with the weight ratio of 1:1 and adjusted to pH of 4.0. Each 50 gram sample was placed into 250 mL beaker. These samples were used for the cellulase treatment.

Chemicals

Chemicals used in this study were purchased from Merck KGaA (Germany) and Sigma-Aldrich Co. LLC (USA).

Experimentation

Effect of concentration of cellulase preparation on extraction yield

Samples were treated with 0.1, 0.12, 0.14, 0.16 and 0.18%w/w cellulase preparation. The treatment time was 60 min. The temperature of enzymatic treatment was maintained at 50^0C by using a thermostatic water bath. At the end of the process, enzymes in the sample were inactivated by heating the mash at 90^0C for 5 min in a water bath. The mash was then filtered through a filter paper. The obtained suspension was centrifuged at 10,000 g for 20 min by a refrigerated centrifuge (Sartorius, Sigma 3K30, Geneva, Switzerland) and the supernatant was collected for further analysis.

Effect of cellulase treatment time on extraction yield

Cellulase preparation was added into samples using the concentration chosen from the previous experiment. The cellulolytic time was varied: 0, 40, 60, 80, 100, 120 min. At the end of the biocatalytic treatment, the mash was filtered and centrifuged in the same way of the previous section.

Optimization of cellulase treatment conditions by response surface methodology

The quadratic central composite circumscribed response surface design was used to optimize the conditions of cellulolytic treatment of the guava mash for maximizing the extraction yield. The software Modde version 5.0 was used to generate the experimental planning and to process data. The enzyme concentration (X_1) and biocatalytic time (X_2) were the input variables while the extraction yield was the output variable (Y). The central points were chosen from the results of the previous experiments. The complete design consisted of 12 experimental points including 4 factorial points, 4 axial points and 4 centre points.

Comparison of extraction yield and guava juice quality from cellulase treatment sample and control sample without enzymatic treatment

The extraction yield and some physicochemical characteristics of the obtained guava juice from the cellulase treatment sample were evaluated. Control sample without enzymatic treatment was also carried out and compared.

Analytical methods

Extraction yield

The extraction yield was defined as the mass (g) of total soluble solids in guava juice after centrifugation from 100g total solids in guava mash. Extraction yield was calculated according to the following equation:

$$Y = \frac{M_1 \times C}{M \times (100 - w)} \times 100$$

Where Y was the extraction yield (g/100g dry matters) of sample, M and w were the mass (g) and the moisture (%) of the initial guava mash, respectively; M_1 and C were the mass (g) and the total soluble solids content (%) of the obtained guava juice after centrifugation, respectively.

Total sugars

Total sugar content was determined by spectrophotometry using anthrone reagent [4]. The total sugar content was defined as the mass (g) of sucrose equivalent calculated from 100g dry matter of the initial guava mash.

Vitamin C

Vitamin C content was determined by HPLC method with C-18 column (Macherey – Nagel, ET 250/8/4 Nucleosil® 120-5, Germany). The mobile phase was methanol in phosphate buffer pH 2.8 with the ratio of 1:9 (v/v), the flow-rate was 1 mL/min and the detection wavelength was 245 nm [5]. Vitamin C content was defined as the mass (mg) of vitamin C calculated from 100 g dry matter of the initial guava mash.

Total phenolics

Total phenolic content of guava juice was quantified by spectrophotometric method using Folin–Ciocalteu reagent [6]. Total phenolic content was defined as the mass (mg) of total phenolics calculated from 100 g dry matter of the initial guava mash.

Antioxidant activity

Antioxidant activity of guava juice was determined by spectrophotometric method using 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) reagent [7]. Antioxidant activity was defined as µmol Trolox equivalent calculated from 1 g dry matter 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 that the extraction yield in guava juice processing increased 17.5% when the cellulase concentration increased from 0 to 0.12% (w/w). Cellulase hydrolyzed cellulose in the cell wall and plant tissue. This phenomenon released the intracellular substances and improved the extraction yield. Therefore, the higher the concentration of cellulase preparation, the higher the extraction yield. Similar observations were previously reported by Kaur [8], who used pectinase in the guava mash treatment in juice production. However, when the concentration of cellulase preparation was higher than 0.12% (w/w), the extraction yield remained stable ($p < 0.05$). It was explained by the limit of intracellular substances in the guava fruit.

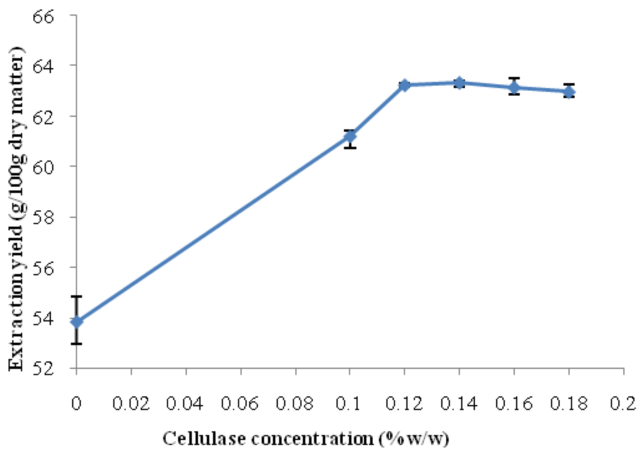

Figure 1. Effect of cellulase concentration on extraction yield in guava juice processing.

The concentration of cellulase preparation of 0.12% (w/w) was therefore selected and used in the next experiment.

Effect of cellulase treatment time on extraction yield

Figure 2 presents the influence of cellulase treatment time on extraction yield. The extraction yield increased significantly when the enzyme treatment time was prolonged from 0 to 80min. The extraction yield reached maximum with the treatment time of 80min. This value increased 20.8%, compared with that of the control sample. When the treatment time was longer than 80min, increase in juice yield became statistically insignificant.

Similar treatment times were also reached in other work on guava juice processing, Chopda [9], reported that the biocatalytic time of Pectinex Ultra SP – L treatment of guava mash was 90 min.

Optimization of enzymatic treatment conditions by response surface methodology (RSM)
The experimental data for extraction yield under different treatment conditions are presented in Table 1. Table 2 presents the estimated coefficients of each variable. The R^2 value of this model was determined to be 0.944, which proved that the regression model was significant. The results showed that linear coefficients (X_1, X_2) and pure quadratic coefficients (X_{12}, X_{22}) were significant, but the interaction coefficient ($X_1 \times X_2$) was not (P = 0.4468). The influence of enzyme concentration and catalytic time of the cellulolytic treatment on the extraction yield were calculated and expressed in quadratic model by the following equation:

$$Y = 62.984 + 0.621X_1 + 0.940X_2 - 0.435X_1^2 - 0.566X_2^2$$

Where Y, X_1 and X_2 were the extraction yield of cellulase treatment of guava mash (g/100g dry matters of the initial guava mash), the concentration of cellulase preparation (%w/w) and the treatment time (min), respectively.

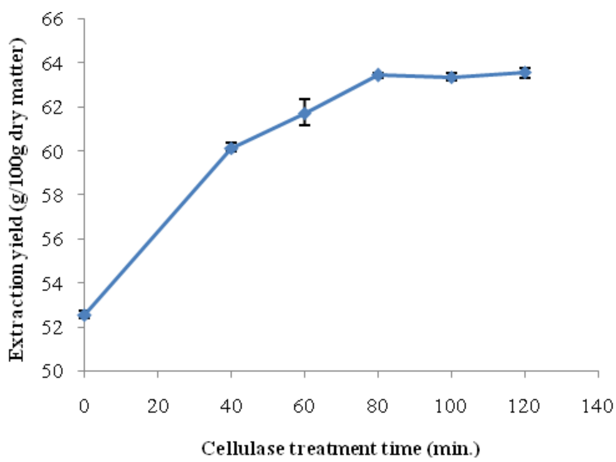

Figure 2. Effect of cellulase treatment time on extraction yield in guava juice processing.

Table 1: Experimental planning and results of extraction yield for cellulase treatment.

STT	X_1	X_2	*Enzyme conc. (%w/w)*	*Treatment time (min.)*	*Extraction yield (g/100g dry matter)*
1	0	$-\sqrt{2}$	0.12	51.7	59.1
2	0	$-\sqrt{2}$	0.12	51.7	59.6
3	-1	-1	0.1	60.0	59.9
4	-1	-1	0.1	60.0	59.8
5	1	-1	0.14	60.0	61.6
6	1	-1	0.14	60.0	61.4
7	$-\sqrt{2}$	0	0.092	80.0	60.5
8	$-\sqrt{2}$	0	0.092	80.0	61.0
9	0	0	0.12	80.0	63.3
10	0	0	0.12	80.0	63.1

STT	*X_1*	*X_2*	*Enzyme conc. (%w/w)*	*Treatment time (min.)*	*Extraction yield (g/100g dry matter)*
11	0	0	0.12	80.0	62.8
12	0	0	0.12	80.0	63.2
13	0	0	0.12	80.0	62.9
14	0	0	0.12	80.0	62.8
15	0	0	0.12	80.0	63.0
16	0	0	0.12	80.0	62.8
17	$\sqrt{2}$	0	0.148	80.0	62.8
18	$\sqrt{2}$	0	0.148	80.0	63.0
19	-1	1	0.1	100.0	61.6
20	-1	1	0.1	100.0	61.7
21	1	1	0.14	100.0	62.8
22	1	1	0.14	100.0	63.0
23	0	$\sqrt{2}$	0.12	108.3	63.3
24	0	$\sqrt{2}$	0.12	108.3	63.6

Table 2: Estimated coefficients of the fitted model for extraction yield (Y).

Extraction yield	**Coeff. SC**	**Std. Err.**	**P**	**Conf. int(±)**
Constant	62.9844	0.128873	0.00E+00	0.270752
X_1	0.620741	0.0760069	1.82E-07	0.159685
X_2	0.940447	0.0760069	3.08E-10	0.159685
$X_1 \times X_1$	-0.435297	0.071236	8.99E-06	0.149661
$X_2 \times X_2$	-0.565823	0.0707859	2.48E-07	0.148716
$X_1 \times X_2$	-0.0694	0.0892283	0.4468	0.187462
N = 24	Q^2 =	0.846	Cond. no. =	3.644
DF = 18	R^2 =	0.944	Y-miss =	0
	R^2 Adj. =	0.929	RSD =	0.3645
			Conf. lev. =	0.95

Where X_1: enzyme concentration (ppm); X_2: treatment time (min); P: significance of linear regressions.

The statistical significance of the quadratic model equation was evaluated by the analysis of variance (Table 3).

Table 3: Analysis of variance of the regression model in experiments.

Extraction yield	**DF**	**SS**	**MS (variance)**	**F**	**p**	**SD**
Total Corrected	23	43.0156	1.87024			1.36757
Regression	5	40.6239	8.12479	61.1474	0	2.8504
Residual	18	2.3917	0.132872			0.36452
Lack of Fit	3	1.75795	0.585982	13.8694	0	0.7655
Pure Error	15	0.63375	0.04225			0.20555

DF: degrees of freedom; SS: sum of squares; MS: mean square; F: F-value, P: P-value; SD:

Surface response graph, obtained by using the fitted model above, is presented in Figure 3. According to the model, the optimal conditions for maximizing extraction yield were determined as follows: concentration of cellulase preparation of 0.13%w/w and cellulase treatment time of 93min.

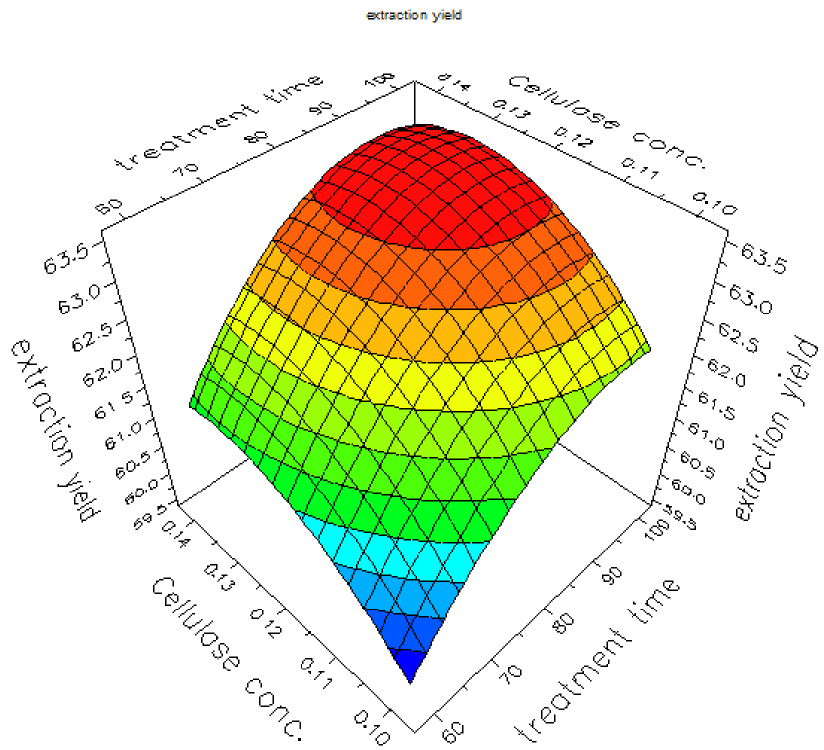

Figure 3. Fitted surface for extraction yield of cellulase treatment of guava mash as a function of enzyme concentration and treatment time.

Comparison of extraction yield and guava juice quality from cellulase treatment sample and control sample without enzymatic treatment

Table 4 shows extraction yield and some physicochemical characteristics of guava juices obtained from the cellulolytic treatment sample and control sample. The extraction yield in the optimally treated sample was 22.3% higher than that in the control sample. Sugars were the main components of the extract. Their level increased 20.0% as compared to that in the control sample.

Table 4: Extraction yield and some physicochemical characteristics of guava juice.

Sample	Extraction yield (g/100g DR)	Total sugars (g/100g DR)	Vitamin C (mg/100g DR)	Total phenolics (mg/100g DR)	Antioxidant activity (µM TE/g DR)
Control	52.06 ± 0.20	37.16 ± 0.23	314.24 ± 12.85	1472.19 ± 12.41	102.89 ± 0.81
Cellulase treatment	63.67 ± 0.40	44.59 ± 0.15	265.82 ± 11.80	1641.49 ± 0.50	125.27 ± 1.78

DR: dry matter of the initial guava mash; TE: trolox equivalent; cellulase treatment with enzyme concentration of 0.13% (w/w) and treatment time of 93 min.

Although both phenolics and vitamin C have antioxidant activity, the use of cellulase preparation in guava mash treatment affected differently their contents in the juice obtained. The phenolic content in the treated sample increased 11.5% while the vitamin C content decreased 15.4% in comparison with those in the control sample. According to Tiwari [10], vitamin C was more sensitive to high temperature than phenolics. In this experiment, the prolonged cellulase treatment time of 93 min at a relatively high temperature (50^0C) could reduce vitamin C level in the juice. However, the antioxidant activity of guava juice obtained from enzyme treatment was raised 21.8% in comparison with that in the control sample.

Conclusion

Use of cellulase preparation successfully enhanced extraction yield and quality of guava juice in juice production. The application of cellulase preparation to fruit mash treatment offers good promise in fruit juice processing.

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