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Effects of ultrasound on catalytic efficiency of pectinase preparation during the treatment of pineapple mash in juice processing

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Abstract: This study focused on the impacts of ultrasound on catalytic activity of pectinase preparation. Pectinex Ultra SP-L was firstly treated by ultrasound and subsequently used in pineapple mash treatment for juice processing. Change in time and enzyme concentration during the ultrasonic treatment affected significantly the pectolytic activity. Sonicated pectinase enhanced extraction yield in pineapple juice processing in comparison with unsonicated pectinase as well as improved the nutritional quality of the final product. When the pectinase solution containing 63.3 polygalacturonase units/ mL was sonicated for 60 sec with the ultrasonic power of 225 W, its catalytic efficiency reached maximum. In this case, sonicated pectinase increased the extraction yield 5.6% higher than unsonicated enzyme.

Keywords: ***Ananas comosus*, juice, pectinase, ultrasound**

Introduction

Juice represents a very important product of the total processed fruit industry. Fruit juice contributes energy, vitamins, minerals and antioxidants to human nutrition (Barrett et al., 2005). In juice processing, extraction is a critical operation during which, main nutrients from vegetal cells are released (Somogyi et al., 1996). According to conventional technology, fruit is crushed for breaking down the cell tissue and then treated with pectinase preparation for facilitating pressing operation and improvement in juice yield (Kashyap et al., 2001).

During the last decade, ultrasound has been widely used in food industry (Patist and Bates, 2008). Many studies demonstrated that application of ultrasound to extraction of plant materials enhanced considerably yield and shortened process time (Toma et al., 2001). With regards to juice processing, combined ultrasound and enzyme extraction of grape was recently reported. Simultaneous treatment of grape mash by ultrasound and pectinase preparation increased extraction yield 2.0% and reduced treatment time over 4 times in comparison with conventional enzymatic treatment (Lieu and Le, 2010).

Theoretically, pectinases are able to degrade pectins in the middle lamella between the primary walls of adjacent young plant cells (Alkorta et al., 1998) while sonication can damage cell wall and plant tissue thanks to acoustic cavitation (Toma et al., 2001). Both pectolytic and ultrasonic treatments of fruit mash could enhance juice yield (Lieu and Le, 2010). Nevertheless, the interaction between ultrasound and pectinase activity during the treatment of fruit mash is still unclear. Understanding of the actual effect of ultrasound on pectinase becomes essential for optimization of combined ultrasound and enzyme treatment procedure in juice processing.

Concerning to biocatalysts, their structure and function could be changed by the ultrasonic irradiation (Sener et al., 2005). Contradictory results have been reported. Under certain conditions, ultrasound reduced the activity of some enzymes such as β-galactosidase, malate dehydrogenase, alcohol dehydrogenase (Ozbek and Ulgen, 2000) while increased the reaction yield of invertase (Sakakibara et al., 1996; Vargas et al., 2004), alpha amylase (Barton et al., 1996), lipase (Babicz et al., 2010), and cellulase (Li et al., 2004). With regards to alkaline phosphatase, its activity was unchanged upon sonication with acoustic power of 7-40 W, frequency of 20 KHz and treatment time of 1 min (Ozbek and Ulgen, 2000). Until present, there are no studies on effect of ultrasound on pectinase activity.

This study focused on the interaction between ultrasound and catalytic activity of pectinase preparation used in fruit juice processing. Pineapple (*Ananas cosmosus*) was used as material for juice extraction. This is one of the most important fruits in tropical countries (Rohrbach et al., 2002) and pineapple juice is a well-known product due to its pleasant flavor (Rattanathanalerk et al., 2005). The objectives of this paper was to evaluate the impact of ultrasound on catalytic efficiency of pectolytic

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enzymes as well as to determine sonication conditions for pectinase preparation before being used in pineapple mash treatment for enhancement of extraction yield in juice processing.

Materials and Methods

Materials

Enzyme source

In this study, Pectinex Ultra SP-L from *Aspergillus aculeatus* purchased from Novozymes Switzerland AG, Dittengen, Switzerland, was used. This enzyme preparation contains mainly pectinolytic enzymes such as endo-polygalacturonase (EC 3.2.1.15; C.A.S. No.9032-75-1), pectin-lyase (EC 4.2.2.10; C.A.S. No. 9033-35-6), and pectin esterase (EC 3.1.1.11; C.A.S. No. 9025-98-3)]. The activity of Pectinex Ultra SP-L is 9,500 polygalaturonase units (PGU) per mL. The catalytic temperature and pH of this enzyme preparation are 50°C and 4.5, respectively (Demir et al., 2001).

Pineapple mash

Pineapple (*Ananas comosus*, Cayen variety) used in this study was originated from a local farm in Long An, Vietnam. Pineapple was destemmed, washed and crushed in a blender (Mode: T1GN, National, Ho Chi Minh city, Vietnam). The pH of pineapple mash was then adjusted to value of 4.5.

Experimental methods

Optimization of pineapple mash treatment by pectinase preparation

A randomised, quadratic central composite circumscribed response surface design was used to optimize Pectinex Ultra SP-L concentration and treatment time for maximizing the extraction yield. The software Modde version 5.0 was used to generate the experimental planning and to process data. The sample treated by pectinase preparation under optimal conditions obtained was considered as the control for the following investigation. The experimental design is presented in Table 1. Samples of 100 g of pineapple mash were taken for each assay. The samples were placed into 250 mL flasks. Treatment temperature was adjusted to 50°C by using a thermostatic water bath (Memmert, WNB 45, Yogyakarta, Indonesia). 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 and centrifuged at 6,500 rpm for 10 min by a refrigerated centrifuge (Sartorius, Sigma 3K30, Geneva, Switzerland). The obtained supernatant was collected for further analysis.

Effect of ultrasound on catalytic efficiency of pectinase preparation during the treatment of pineapple mash

First series: Pectinex Ultra SP-L was diluted with distilled water with the dilution factor of 100 times (1 mL of diluted enzyme solution contained 95 PGU). 20 mL of diluted enzyme solution was added into a 100 mL beaker and subsequently treated by ultrasound at the power of 225 W. The treatment time was varied from 0 to 90 sec.

Second series: Pectinex Ultra SP-L was diluted with distilled water; the dilution factor was ranged from 1 to 200 times (1 mL of diluted enzyme solution contained from 9,500 to 47.5 PGU). Each diluted enzyme solution was added into a 100 mL beaker and then sonicated at acoustic power of 225 W for 60 sec.

For both series, diluted enzyme solutions were directly treated by a stick shaped ultrasonic probe (Mode: VC 750, Sonics & Materials Inc., Newtown, USA). This equipment operated at frequency of 20 kHz with the maximum ultrasonic power of 750 W.

After sonication treatment, each enzyme solution was added into a flask of sample containing 100 g of pineapple mash. The enzyme concentration in all samples was fixed at 456 PGU per 100 g of fruit mash. All samples were then kept in the period of 50 min. At the end of the pectolytic treatment, enzymes in the sample were inactivated by heating the mash at 90°C for 5 min. The following steps were similar to those in the previous investigation.

Comparison in nutritional quality of pineapple juices obtained from the mash treatment with sonicated and unsonicated pectinase preparation

Two samples of 100g of pineapple mash were used in this experiment. One sample was treated with Pectinex Ultra SP-L (control sample) while the other was treated with the same enzyme preparation which was diluted with distilled water with the dilution factor of 150 times and subsequently sonicated at ultrasonic power of 225 W for 60 sec. The pectinase concentration in the two samples was similar (456 PGU/100 g of pineapple mash). At the end of the biocatalysis, enzyme inactivation and further treatment of samples were also carried out in the same way of the previous study.

Analytical methods

Extraction yield in pineapple juice processing was calculated according to the formula below:

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

Table 1. Experimental planning and results of extraction yield for pectolytic treatment of pineapple mash

Run	X1	X2	Enzyme concentration, X1 (% v/w)	Treatment time, X2 (min)	Yield, Y (%)
1	-	-	0.02	20	74.4
2	+	-	0.06	20	76.8
3	-	+	0.02	60	76.8
4	+	+	0.06	60	77.8
5	$-\sqrt{2}$	0	0.01172	40	75.3
6	$+\sqrt{2}$	0	0.06828	40	78.5
7	0	$-\sqrt{2}$	0.04	11.72	76.2
8	0	$+\sqrt{2}$	0.04	68.28	78.6
9	0	0	0.04	40	78.4
10	0	0	0.04	40	78.5
11	0	0	0.04	40	78.4

where Y was the extraction yield (%), m_1 and w were the mass (g) and the moisture (%) of the initial pineapple mash, respectively; m_2 and C were the mass (g) and the content of soluble extract (%) of the juice obtained, respectively.

Sugars were measured by spectrophotometric method, using 3,5-dinitrosalicylic acid reagent (Nielsen, 2003). Total acids were expressed in equivalent of citric acid content (g/L), determined by method of titration, using 0.1N NaOH solution to a pH endpoint of 8.1 (Cliff et al., 2007). Total phenolic content was quantified by spectrophotometric method, using Folin-Ciocalteu reagent (Luque-Rodrıguez et al., 2007). Vitamin C was determined by method of titration with iodine solution (Suntornsuk et al., 2002).

Statistical analysis

All experiments were performed in triplicate. Means were compared by Multiple range tests with $p < 0.05$. Analysis of variance was realized using the software Statgraphics plus, version 3.2.

Results and Discussions

Optimization of pineapple mash treatment by pectinase preparation

Table 1 shows extraction yield in the pectolytic treatment of pineapple mash. After fitting the experimental data (Table 2), the results showed that linear coefficients (X_1, X_2) and pure quadratic coefficients (X_1^2, X_2^2) were significant, but the interaction coefficient (X_1 x X_2) was not (p=0.203183). The statistical significance of the quadratic model equation was evaluated by the analysis of variance (ANOVA) in Table 3. The influence of enzyme concentration and catalytic time of the pectolytic treatment on the extraction yield were calculated and expressed in quadratic model by the following equation:

$$Y = 78.43 + 0.99X_1 + 0.85X_2 – 0.94X_1^2 – 0.69X_2^2$$

where Y, X_1 and X_2 were the extraction yield of enzymatic treatment of pineapple mash (%), the enzyme concentration (%v/w) and the treatment time (min), respectively.

Considering the model equation, both factors affected the extraction yield, but the effect of enzyme concentration was higher. Surface response graph, obtained by using the fitted model above, is presented in Figure 1. Optimal enzyme concentration and treatment time were 0.048% v/w and 50 min,

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

Factor[a]	Estimated coefficient	Standard error	P
Intercept	78.4335	0.276138	1.02652E-011
X_1	0.99075	0.169112	0.00205361
X_2	0.849328	0.169112	0.00402725
X_1 x X_1	-0.941928	0.201309	0.00543841
X_2 x X_2	-0.691852	0.201309	0.0185

X_1: enzyme concentration (%v/w), X_2: treatment time (min).
P: Indicates significance of linear regressions.
[a] Significant factors at 95% of confidence level.

Table 3. Analysis of variance for the model representing the extraction yield (Y)

Source	Degrees of freedom	Sum of squares	Mean square	F-value	P-value	Standard deviation
Total	11	65656.8	5968.8			
Constant	1	65635.5	65635.5			
Total Corrected	10	21.3203	2.13203			1.46051
Regression	5	20.1765	4.03531	17.6402	0.003	2.00881
Residual	5	1.14378	0.228756			0.478285
Lack of Fit	3	1.13711	0.379038	113.761	0.009	0.615661
Pure Error	2	0.00666637	0.00333319			0.0577338
N = 11	Q2 =	0.620	Cond. no. =	3.6208		
DF = 5	R2 =	0.946	Y-miss =	0		
	R2 Adj. =	0.893	RSD =	0.4783		

Table 4. Effect of enzyme concentration during sonication treatment on extraction yield in pineapple juice processing

Enzyme concentration (PGU/ml)	Yield (%)	Yield increase * (%)
9500 (Control sample: non-sonicated enzyme preparation)	78.8[a]	0.0[a]
9500	80.4[b]	1.6[b]
95	82.7[c]	3.9[c]
76	83.5[d]	4.7[d]
63.3	84.4[e]	5.6[e]
54.2	83.4[d]	4.6[d]
47.5	82.1[f]	3.3[f]

* Yield increase was calculated by the difference between the yield of the control sample and the yield of the sample tested.
Various small letters in each column represent statistically significant difference at the level of p=0.05

Table 5. Physicochemical characteristics of pineapple juice

Characteristics	Sample treated by sonicated Pectinex Ultra SP-L	Sample treated by unsonicated Pectinex Ultra SP-L
Sugars (g/L)	63.6[b]	59.4[a]
Total acidity (g of citric acid/L)	73.4[b]	0.68[a]
Total phenolics (g/L)	0.98[b]	0.89[a]
Vitamin C (mg/L)	253[b]	241[a]

Various small letters in each row represent statistically significant difference at the level of p=0.05

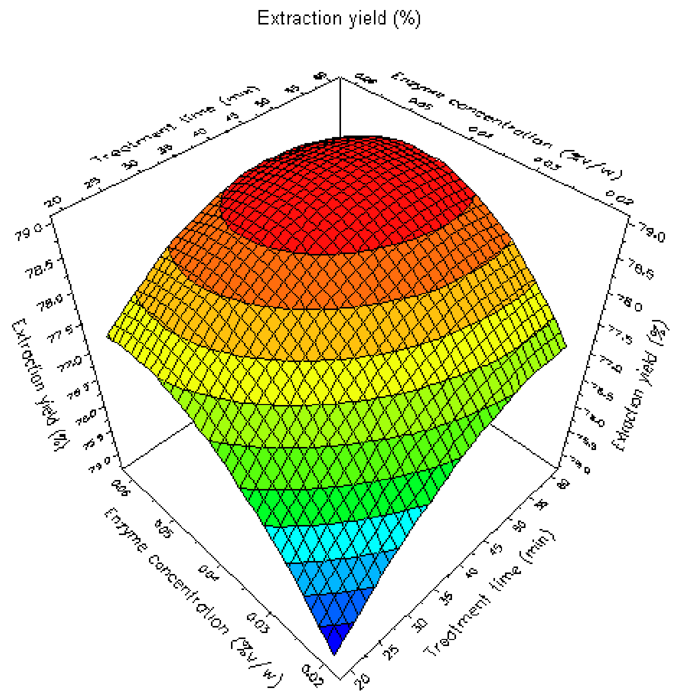

Figure 1. Response surface plot showing the effect of enzyme concentration and treatment time on extraction yield in pineapple juice processing

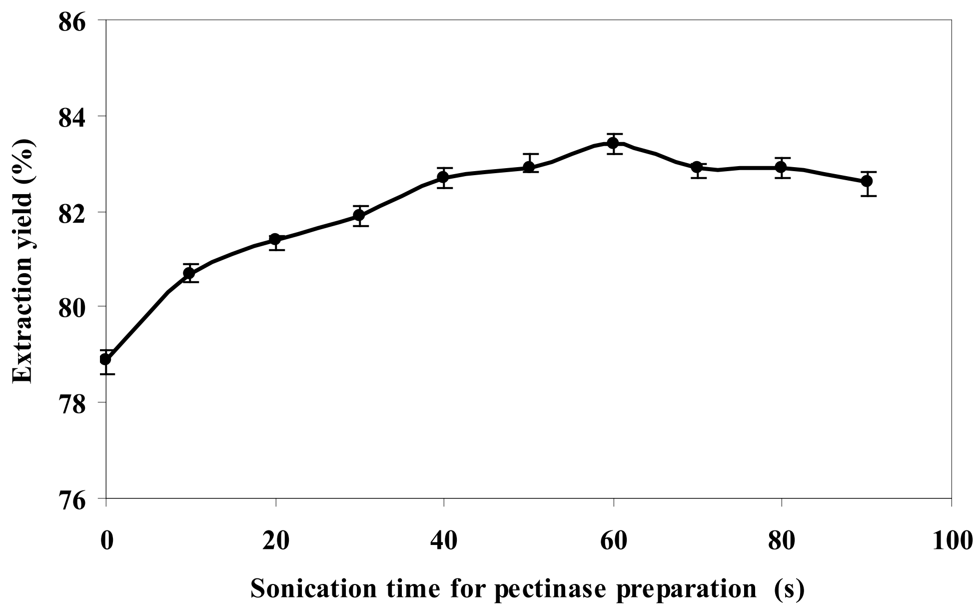

Figure 2. Effect of sonication time for pectinase preparation before being used in pineapple mash treatment on extraction yield (Ultrasonic power was 225 W for 20 mL of Pectinex Ultra SP-L solution; pectinase preparation contained 95 PGU/mL).

respectively. Under optimal conditions, the model predicted a maximum response of 78.9%. Similar level of extraction yield was also observed in a study on grape juice processing (Lieu and Le, 2010). The obtained optimal conditions in this experiment were used to conduct the control sample in the next investigation.

Effect of ultrasound on catalytic efficiency of pectinase preparation during the treatment of pineapple mash

Figure 2 presents the extraction yield in pineapple mash treatment by pectinase preparation previously sonicated with different times. Sonication of pectinase preparation before the pectolytic treatment of pineapple mash improved significantly juice extraction. The maximum extraction yield reached 83.4% when the sonication time was 60 sec. The yield obtained was 4.5% higher than that in the control sample. It can be deduced that sonication with a power of 225 W for 60 sec had a positive effect on catalytic activity of Pectinex Ultra SP-L. In fact, ultrasound causes acoustic cavitation which includes the formation, growth, and violent collapse of small bubbles or voids in liquids as a result of pressure fluctuation (Suslick, 1988). According to Vargas et al., (2004), ultrasonic energy may act on the tridimensional structure of the enzyme and leads to an increase in enzyme activity.

Some researchers reported that ultrasound augmented yield of biochemical reactions but in their studies, substrate solution and enzyme preparation were mixed and subsequently treated by ultrasound. Thus, enhancement of reaction yield was probably due to increase in enzyme activity as a result of modification of enzyme conformation and/or improvement in transport of the enzyme macromolecules toward the substrate surface (Barton et al., 1996; Khanal et al., 2007). Based on our results, it can be suggested that ultrasound impacted on physicochemical properties of pectinase preparation and that led to an increase in catalytic activity. However, for detailed explanation of this phenomenon, further research on enzyme structure during the sonication should be carried out.

In addition, enzyme concentration in Pectinex Ultra SP-L solution during the ultrasonic treatment had certain effect on pectinase activity. Table 4 demonstrates that reduction in enzyme concentration from 9500 to 63.3 PGU/mL during the sonication augmented the extraction yield in pineapple mash treatment by pectinase preparation. However, ultrasonic treatment of Pectinex Ultra SP-L with lower pectinase concentration decreased slightly the extraction yield obtained. It can be noted that application of sonicated Pectinex Ultra SP-L with all dilution factors tested to pineapple mash treatment increased obviously the extraction yield in juice production.

In conclusion, sonication of Pectinex Ultra SP-L solution with enzyme concentration of 63.3 PGU/mL and subsequent use in pineapple mash treatment enhanced the extraction yield 5.6% in comparison with application of the same pectolytic prearation without ultrasonic treatment.

Comparison in nutritional quality of pineapple juices obtained from the mash treatment with sonicated and unsonicated pectinase preparation

Table 5 shows some physicochemical characteristics of pineapple juices obtained from the mash treatment by sonicated and unsonicated Pectinex Ultra SP-L. The level of sugars, organic acids, phenolics and vitamin C in the sample treated by sonicated pectinase preparation was significantly higher than that in the control sample. Increase in catalytic activity of the sonicated pectolytic enzyme during pineapple mash treatment was therefore confirmed. In other words, ultrasound had a positive effect on catalytic efficiency of Pectinex Ultra SP-L used in pineapple juice production.

Conclusion

Under certain conditions, ultrasonic energy did not degrade or denature the enzyme. Ultrasonic treatment could improve the activity of enzyme preparation. Sonication time and pectinase concentration during the ultrasonic treatment affected the catalytic efficiency of the biocatalyst obtained. Sonicated pectinase preparation enhanced the extraction yield in pineapple juice processing in comparison with unsonicated pectinase as well as increased nutritional quality of the obtained juice.

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