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Effect of Hydrolysis Parameters with Pectinase on the Recovery Efficiency, Total Phenolic Content, DPPH of Filtrate from the Cashew Pulp Puree

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Abstract

The cashew tree is commonly grown in tropical countries. The cashew fruit includes cashew nut with a high economic value and cashew fruit pulp as a by-product from the fruit, but it contains a lot of nutritional value and healthy antioxidant compounds. The effect of factors in the hydrolysis process using pectinase on the recovery efficiency (H), total polyphenol content (TPC), and radical scavenging activities (DPPH) was investigated. Enzyme-assisted processing significantly ($p < 0.05$) improved H, TPC, DPPH. There was an overall increase of 17.1 % in H, 31.0 % in TPC, and 21.6 % in DPPH at the recommended enzyme hydrolysis conditions including pH of 4.0, a temperature of 40°C, enzyme concentration of 0.3 % (v/w), and incubation time of 90 minutes. The use of suitable conditions for the hydrolysis process using pectinase can offer an opportunity for developing foods and beverages with enhanced nutritional value and high antioxidant activity.

Keywords: *Cashew; Pectinase; Recovery efficiency; Total polyphenol content; DPPH*

Introduction

Anacardium occidentale L. (Cashew) is a native tree from Brazil. Nowadays, it is widespread in other tropical countries. It has a high economic value due to products from the nut which were much appreciated all over the world (Camila Silva Tamiello-Rosa et al., 2019). Besides, other products such as cashew nut shell oil and cashew fruit beverage have been also commonly used (Talita Lopes Honorato, 2007).

Cashew fruit is composed of two main components: cashew nut and cashew fruit pulp (Maria et al., 2006). Cashew nuts account for 10 % of the fruit weight, which is the part with the highest economic value. Cashew kernels account for 25 - 30 % of the weight of the nut, of which carbohydrates account for 22 - 33 %, fat accounts for 44 - 49 %, protein account for 15 - 28 %. In addition, there are vitamin B1, vitamin E, amino acids, total polyphenol compounds, antioxidant compounds, and many minerals that are essential for the human body. Therefore, cashew kernels are high-class nutritional foods that are favored by consumers in many countries around the world (Jônatas et al.,2019). Cashew pulp accounts for 90 % of the fruit weight which can be consumed fresh or processed into value-added products such as juices, jams, jellies, various beverages, and other food products. Furthermore, it can be used as a substrate for fermentation or other biotransformation processes (Mariana, 2012). The pectin content in cashew fruits accounts for 0.11 - 0.16 %, so it is very important to hydrolyze the pectin component to promote the extraction process and increase the content of nutrients in the filtrate.

Promoting the extraction of nutritional

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components in the filtrate by pectinase is due to the reduction of viscosity and thereby the extraction process becomes easier, which results in increasing the extraction efficiency (Demir et al, 2001). Plant cell walls contain polysaccharides such as pectin, cellulose, hemicellulose (Horvaùth–Kerbai, 2006). Besides, inside the cells of vegetables and fruits, there are also other components such as sugar, amino acids, vitamins, so pectinases are used to break down pectic substances and include two groups – de-esterification and depolymerization enzymes. Endo-polygalacturonase (endo-PG) is a depolymerizing enzyme, which is used in food, textile, and paper industries (Kashyap et al., 2001), (Kaur et al., 2004).

This study was conducted to find out the values of the factors being suitable for the hydrolysis process of cashew fruits using pectinase.

Materials and Methods

Materials

Chemicals

Foline-Ciocalteu, sodium carbonate, and acid gallic were supplied by Merck DPPH and Trolox were purchased from Sigma –Aldrich, USA.

Cashew fruits

Cashew fruits were purchased at Cau Ngang district, Tra Vinh province, Vietnam.

Pectinase

Pectinase (Pectinex Ultra SP-L) from Aspergillusaculeatus (enzyme activity of polygalacturonase: 26000 PG/ml) was purchased from Novozymes, Denmark, and stored at 4^{o}C.

Methods

Experiment Procedures

The cashew fruit pulp was cut into about 2 cm2 and added water with the fruit pulp/water ratio of 1/1, next, they were blended by HR2116 Philips blender for 1 minute. Then, pectinase was added to the puree for hydrolysis. The hydrolysis samples with the investigated variables were placed in a shaking water bath (120 rpm) throughout the incubation period to increase the interaction between enzyme and substrate better (Sreenath and Radola, 1986). After the incubation period, the enzyme was inactivated by heating at 85^{o}C for 10 minutes. The reaction mixture was filtered by the vacuum filtration method through a Whatman filter paper. The juice filtrate was determined weight, total polyphenol content (TPC), and radical scavenging activities (DPPH). Experiments were done in triplicate. The experiments were conducted by the change of the values of the variables as shown in table 1:

Determination of TPC

TPC was determined by using Foline-Ciocalteu (Darsini et al., 2013). 0.1 ml of the sample extract was mixed with 3ml of distilled water in a tube. Then, 0.5 ml of Foline-Ciocalteu reagent was added and placed in the dark. After 3 minutes, 2ml of sodium carbonate 20 % was added and mixed thoroughly. The tubes were incubated in a boiling water bath (100 oC) for exactly 1 minute Then, it was cooled and measured the absorbance at 650 nm by using a spectrophotometer (Genesys 6, Thermo spectroic, USA). The standard curve was linear between 10 and 60 ppm of acid gallic. The results were expressed as milligram (mg) of gallic acid equivalent (GAE) per 100 grams of cashew pulp.

Table 1. The design of variables in experiments

VARIABLES	EXPERIMENTS			
	1	2	3	4
PH	3.4 – 3.7 – 4.0 – 4.3 – 4.6	Base on experiment 1		
Temperature (°C)	45	35 – 40 – 45 – 50 – 55	Base on experiment 2	
Enzyme concentration (%v/w)	0.2		0 – 0.1 – 0.2 – 0.3 – 0.4 – 0.5	Base on experiment 3
Incubation time (minutes)	60			30 – 60 – 90 – 120 – 150
Fixed parameters: Mass of the test sample 100g, the ratio of the cashew pulp/water of 1/1. *Variable parameters:* The recovery efficiency of the juice filtrate, TPC, DPPH.				

Determination of DPPH Radical Scavenging Activity

DPPH radical scavenging activity was determined by the method developed by Brand-Williams et al. (Brand-Williams et al., 1995). Here, 0.3 ml of each test sample was mixed with 5.7 ml of a DPPH-methanol solution (A515nm = 1.1$\pm$ 0.02). Then, the mixture was vortexed vigorously and allowed to stand in the dark for 20 minutes. The absorbance was determined at 515nm, and the inhibition of DPPH radical scavenging activity in each concentration of sample could be calculated by the formula as shown:

$$\%inhibition = [\frac{1 - A.sample}{A.control}]x100] \quad (1)$$

The standard curve was linear between 100 and 700µM trolox by using a spectrophotometer (Genesys 6, Thermo spectroic, USA). The results were expressed in µM trolox equivalent antioxidant capacity (TEAC) per 100g of raw material basis.

The recovery efficiency of the juice filtrate was calculated by the formula:

$$H = \frac{The\ weight\ of\ the\ juice\ filtrate}{The\ weight\ of\ sample} \times 100\ (\%) \quad (2)$$

Statistical Analyses

Mean values of the parameters were compared by one-way ANOVA (analysis of variance) followed by Tukey's test (p-value < 0.05) using Statgraphics®Centurion XVIII software. Three replicates of each experiment were used for statistical analysis and the value of the variable was reported as mean $\pm$ SD.

Results and Findings

Through the experimental process, the results of the influence of parameters on the efficiency of hydrolysis are shown in Table 2.

Discussion

The results from Table 1 showed that the pH values affected significantly the recovery of the filtrate, TPC, and DPPH in the filtrate from the cashew fruit pulp samples. This could be explained that pH affected the ability of substrate ionization. When the pH value was too low or too high, the active site of the enzyme was significantly affected by changes in the protein structure, resulting in the decreased activity or possibly inactivation of the enzyme (Belitz, 2009). This was clearly shown through the results of the study at pH of 4.0, H, TPC, and DPPH in the filtrate

Table 2. Effect of the parameters on the efficiency of cashew fruit pulp puree hydrolysis

The parameters of the hydrolysis	Samples	H (%)*	TPC (mgGAE/L)*	DPPH (μM TEAC)*
pH	Control	65.5 ± 1.3^{c}	665.4 ± 39.2^{d}	4,178.4 ± 110.8^{b}
	3.4	67.4 ± 2.1^{c}	727.3 ± 18.6^{c}	4,252.1 ± 279.4^{b}
	3.7	71.6 ± 1.3^{a}	768.7 ± 6.2^{b}	4,497.8 ± 255.7^{ab}
	4.0	**78.6 ± 0.8^{a}**	**820.8 ± 30.6^{a}**	**4,841.8 ± 150.1^{a}**
	4.3	77.7 ± 0.8^{b}	805.4 ± 3.7^{ab}	4,710.7 ± 147.4^{a}
	4.6	73.5 ± 1.2^{b}	703.2 ± 16.5^{cd}	4,506.0 ± 159.9^{ab}
The temperature of the hydrolysis process (°C)	Control	65.5 ± 1.3^{c}	665.4 ± 39.2^{c}	4,153.8 ± 331.8^{c}
	35	67.9 ± 1.6^{c}	758.3 ± 18.7^{b}	4,313.5 ± 145.5^{bc}
	40	**77.3 ± 1.0^{a}**	**826.3 ± 6.7^{a}**	**4,776.3 ± 232.2^{a}**
	45	77.5 ± 0.2^{a}	826.7 ± 6.7^{a}	4,809.0 ± 289.7^{a}
	50	74.2 ± 2.8^{b}	783.5 ± 2.3^{b}	4,612.5 ± 98.3^{ab}
	55	72.1 ± 1.3^{b}	690.3 ± 20.2^{c}	4,440.5 ± 265.8^{bc}
The concentration of the pectinase (%v/w)	Control	65.5 ± 1.3^{c}	677.7 ± 10.0^{d}	4,145.6 ± 138.4^{c}
	0.1	75.5 ± 0.4^{b}	720.8 ± 4.6^{c}	4,522.4 ± 262.7^{b}
	0.2	78.5 ± 1.3^{a}	830.8 ± 15.2^{b}	4,825.4 ± 86.3^{a}
	0.3	**79.0 ± 0.7^{a}**	**847.3 ± 15.7^{ab}**	**4,899.1 ± 99.3^{a}**
	0.4	79.0 ± 0.8^{a}	845.4 ± 5.6^{ab}	4,882.7 ± 161.1^{a}
	0.5	79.3 ± 0.8^{a}	849.5 ± 3.7^{a}	4,997.4 ± 110.8^{a}
The time of the hydrolysis process (min.)	Control	65.5 ± 1.3^{d}	687.7 ± 7.3^{d}	4,170.2 ± 112.6^{a}
	30	74.7 ± 0.4^{c}	741.8 ± 21.6^{c}	4,514.2 ± 88.6^{b}
	60	79.8 ± 0.8^{a}	861.2 ± 7.7^{b}	4,972.8 ± 148.1^{a}
	90	**82.6 ± 1.0^{a}**	**900.9 ± 5.3^{a}**	**5,071.1 ± 209.0^{a}**
	120	83.0 ± 0.8^{a}	904.2 ± 4.4^{a}	4,989.2 ± 75.1^{a}
	150	83.5 ± 0.9^{a}	889.1 ± 4.5^{a}	4,923.7 ± 197.1^{a}

*Mean of triplicate measurements ± SD.
^{a}highest values; $^{b, c, d}$lower values.

reached the highest values of 78.6 $\pm$ 0.8 %, 820.8 $\pm$ 30.6 mgGAE/L and 4,841.8$\neq$ M TEAC respectively while at the higher or lower pH during hydrolysis, these values were all lower and had statistically significant differences ($p < 0.05$) compared to pH 4. With the control sample (without enzyme), H, TPC, and DPPH in the filtrate only reached 65.5$\pm$ 1.3 %, 665.4$\pm$ 39.2 mgGAE/L và 4178.4$\pm$ 110.8 $\neq$ M TEAC respectively. Therefore, the pH value of 4.0 was used for the next experiment. This result is in agreement with the previous study (Mona et al., 2013) (Mona et al., 2014) reported that the immobilized pectinase properties were studied at pH 4. The trend of changes of H, TPC, DPPH according to the change of pH during hydrolysis is clearly shown in Figure 1.

Similar to pH, The appropriate hydrolysis temperature had the effect of accelerating the enzymatic reaction. In contrast, the enzyme has decreased the activity or is inactivated at temperatures outside the enzyme's active range. It is because of the temperature effects on the structural stability of the protein that the enzyme's activity was affected. The results

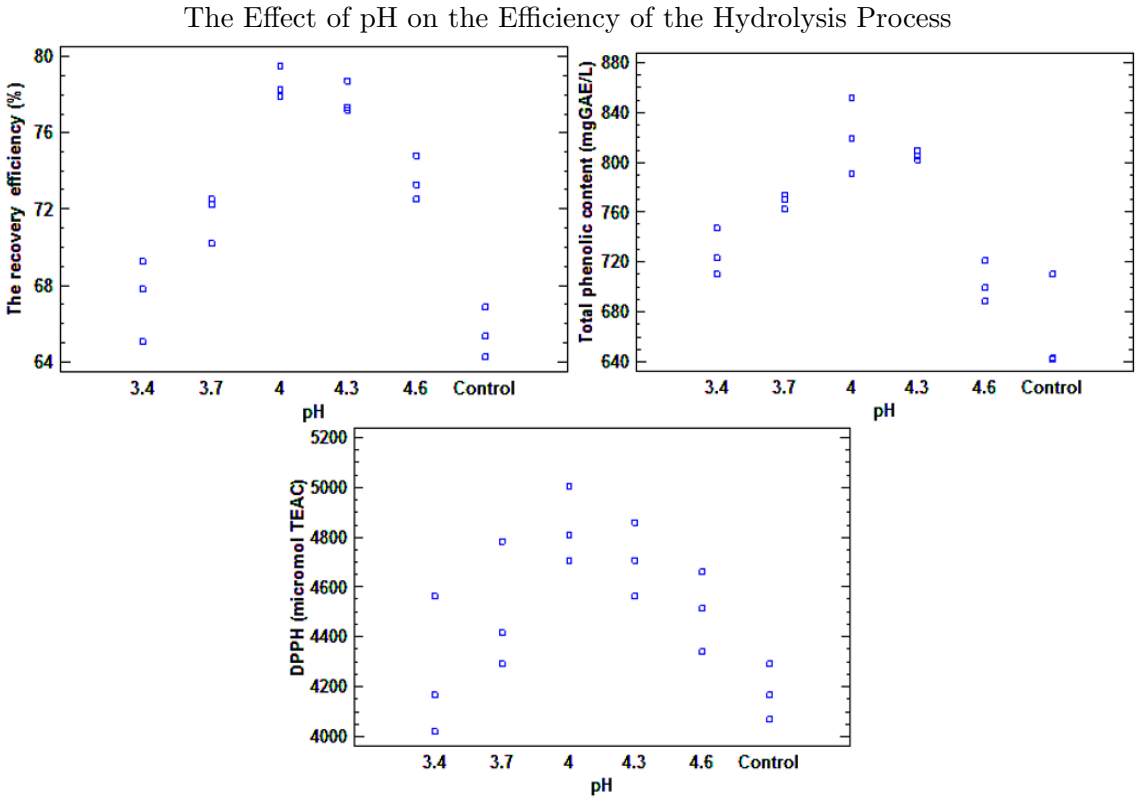

Figure 1. *The effect of the pH on H, TPC, DPPH of the hydrolysis process*

in Figure 2 clearly showed the agreement of this trend through the assessment of H, TPC, and DPPH in the filtrate from the hydrolysis process. The results in Table 2 showed that at the temperature of 40 ^{o}C, H, TPC, and DPPH values reached the highest values of 77.3$\pm$ 1.0 %, 826.3$\pm$ 6.7 mgGAE/L, 4776.3$\pm$ 232 $\neq$ M TEAC respectively. When the temperature increased to 45^{o}C, H, TPC, DPPH still maintained the highest values and there were no statistically significant differences ($p < 0.05$) compared to the achieved values at 40 ^{o}C. In contrast, when hydrolyzed at temperatures outside the range of 40$\div$ 45 ^{o}C, the values of H, TPC, and DPPH were all significantly lower. This result was similar to the previous study that reported that endo- polygalacturonase of pectinase was stable at 40 ^{o}C (Hendges, 2011).

When the enzyme concentration was low, the amount of substrate in the mixture was high, so the rate of enzyme-substrate complex formation was low, resulting in fewer hydrolysis products. As a result, the H, TPC, DPPH of the filtrate were low. On the contrary, as increasing enzyme concentration, the values only tended to increase linearly in a certain concentration range, then that gradually stabilized under the same survey conditions of pH, temperature, and hydrolysis time. This could be explained by the limited solubility of the substrate or by competitive inhibitors from the resulting product (Owen, 1996) as clearly shown in Figure 3.

The research results in Figure 3 showed that H, TPC, DPPH of the filtrate increased continuously as changing enzyme concentration from 0 % to 0.3 % w/w. At the 0.3 %, H, TPC, DPPH reached the highest value of 79.0$\pm$0.7 %, 847.3$\pm$15.7 mgGAE/L và 4899.1$\pm$99.3 $\neq$M TEAC respectively. This trend continued to be maintained when the enzyme concentration continued to increase to 0.4 and 0.5 %. Therefore, we chose the enzyme concentration of 0.3 % for the next experiment to avoid wasteful use of the

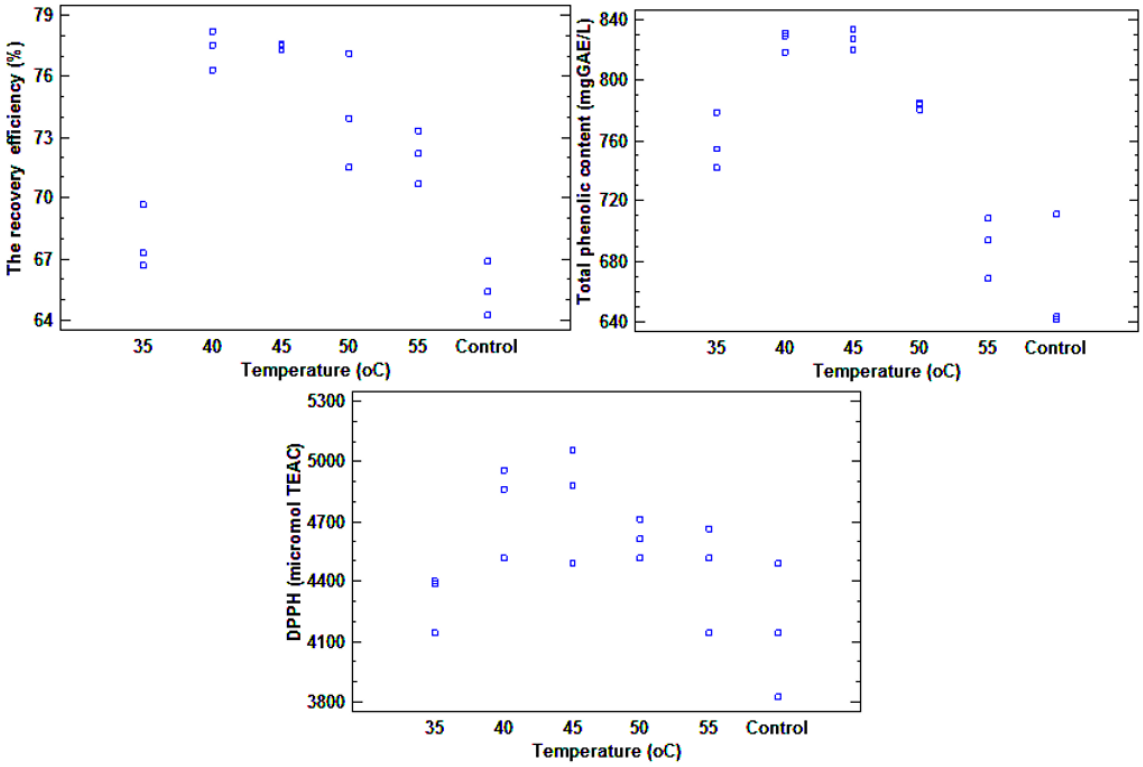

Figure 2. *The effect of the incubation temperature on H, TPC, DPPH of the hydrolysis process*

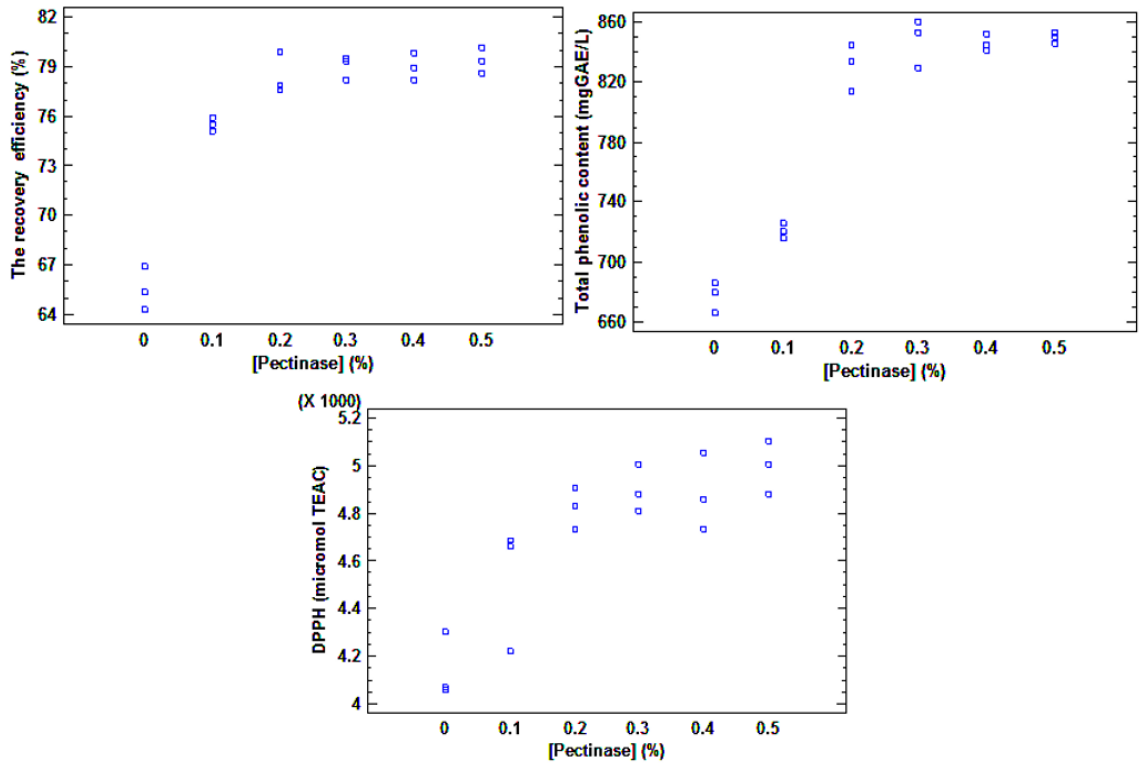

Figure 3. *The effect of the enzyme concentration on the H, TPC, DPPH of the pulp hydrolysis*

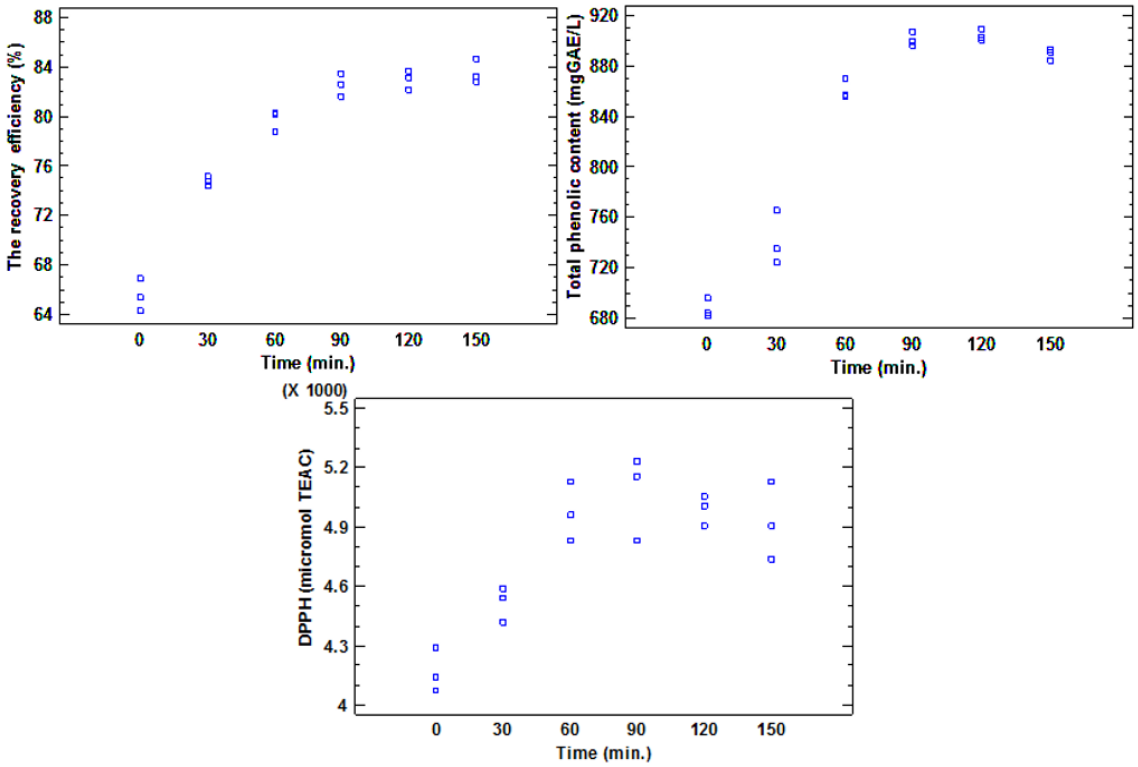

Figure 4. *The effect of the incubation time on H, TPC, DPPH of the hydrolysis process*

enzyme.

To enhance the efficiency of the hydrolysis process, the incubation time was also an important variable to consider for increasing the hydrolysis efficiency as shown in Figure 4. This was because the hydrolysis reaction required a certain time for the interaction of the enzyme [E] and substrate [S] to form a complex [ES]. This complex continued to need time to convert to product [P] and released [E] again. However, in the case of prolonged hydrolysis time, the substrate concentration gradually decreases leading to decreasing the reaction rate (Belitz, 2009).

The results in Table 2 showed a significant effect ($p < 0.05$) of the incubation time on H, TPC, DPPH of the filtrate. As gradually increasing the incubation time to 90 minutes, H, TPC, DPPH in the filtrate gradually increased and reached the highest values at 82.6±1 %, 900.9±5.3 mgGAE/L, and 5071.1±209 ≠M TEAC respectively. That increased 17.1 %, 31.0 % và 21.6 % respectively compared to the condition without the use of the enzyme. When the incubation time was extended to 120 and 150 minutes, the observed values remained at the highest level and there was no statistically significant difference from the values obtained at the incubation time of 90 minutes. The results of this study are consistent with the previous report of Anurag Singh et al., 2012 when hydrolyzing bael fruit with pectinase, the recovery yield was obtained of 84.5 % (Singh, 2012).

Conclusions

The hydrolysis of puree from cashew fruit using pectinase enzyme was significantly affected by factors including pH, temperature, enzyme concentration, and incubation time. The suitable conditions for the hydrolysis process were found at a pH of 4.0, a temperature of 40 oC, an enzyme concentration of 0.3 % (v/w), and an

incubation time of 90 minutes. The obtained filtrate can be used to process foods with a high biological activity that promises to increase the use and economic value of the by-product of cashew fruits.

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References Cited

Belitz, H.-D, Grosch, W., Schieberle, P. (2009.). *Food Chemistry (4th ed.)*. Berlin: Springer. DOI 10.1007/978-3-540-69934-7

Brand-Williams, W., Cuvelier, M. E., Berset, C. (1995). Use of a free radical method to evaluate antio-xidant activity. *Food Science and Technology, 28*, 25-30. https://doi.org/10.1016/S0023-6438(95)80008-5

CarvalhoSilva, J., Françab, P. R. L., Convertic, A., SouzaPorto, T. (2019). Pectin hydrolysis in cashew apple juice by *Aspergillus aculeatus* URM4953 polygalacturonase covalently-immobilized on calcium alginate beads: A kinetic and thermodynamic study. International *Journal of Biological Macromolecules, 126*, 820-827. https://doi.org/10.1016/j.ijbiomac.2018.12.236

Darsini, D. T. P., Maheshu, V., Vishnupriya, M., Nishaa, S., Sasikumar, J. M. (2013). Antioxidant potential and amino acid analysis of underutilized tropical fruit *Limonia acidissima* L. *Free Radicals and Antioxidants, 3*, 62-69. https://doi.org/10.1016/j.fra.2013.08.001

Demir, N., Acar, J., Sarioglu, K., Mutlu, M. (2001). The use of commercial pectinase in fruit juice industry. *Journal of Food Engineering, 47*(4), 275-280. https://doi.org/10.1016/S0260-8774(00)00127-8

Esawy, M. A., Gamal, A. A., Kamel, Z., Ismail, A. M. S., Abdel-Fattah, A. F. (2014). Evaluation of free and immobilized *Aspergillus niger* NRC1ami pectinase applicable in industrial processes. *Carbohydrate Polymers, 92*, 1463–1469.

Fennema, O. R. (1996). *Food Chemistry (3rd ed.)*. New York: CRC Press.

Hendges, D. H, Montanari, Q., Malvessi, E. and Moura da Silveira, M. (2011). Production and characterization of endo-polygalacturonase from *Aspergillus niger* in solid-state fermentation in double-surface bioreactor. *Brazilian Archives of Biology and Technology, 54*, 253-258. DOI:10.1590/S1516-89132011000200005

Honorato, T., Rabelo, M. C., Goncalves, L. R. B., Pinto, G. (2007). Fermentation of cashew apple juice to produce high added value products. *World Journal of Microbiology and Biotechnology, 23*(10), 1409-1415. https://doi.org/10.1007/s11274-007-9381-z

Kashyap, D. R., Vohra, P. K., Chopra, S., Tewari, R. (2001). Applications of pectinases in the commercial sector. *Bioresource Technology., 77*, 215-227. DOI:10.1016/S0960-8524(00)00118-8

Kaur, G., Kumar, S., Satyanarayana, T. (2004). Production, characterization and application of a thermostable polugalacturonase of a thermophilic mould *Sporotrichum thermophile* Aipinis. *Bioresource Technology, 94*, 239-243. doi: 10.1016/j.biortech.2003.05.003.

Rocha, M. V. A. P., Oliveira, A. H. S., Goncalves, L. R B. (2006). Natural cashew apple juice as fermentation medium for biosurfactant production by *Acinetobacter calcoaceticus*. *World Journal of Microbiology and Biotechnology, 22*(12), 1295-1299. DOI:10.1007/s11274-006-9175-8

Silveira, M. S., Fontes, C. P. M. L., Guilherme, A. D. A., Fernandes, F. A. N., Rodrigues, S. (2012). Cashew apple juice as substrate for lactic acid production. *Food and Bioprocess Technology, 5*(3), 947-953.

Singh, A., Kumar, S. and Sharma, H. K. (2012). Effect of enzymatic hydrolysis on the juice yield from bael fruit (*Aegle marmelos Correa*) Pulp. *American Journal of Food Technology, 7*(2), 62-72.

Sreenath, H. K. and Radola, B. J. (1986). The effect of removing cellulase(s) from a commercial pectinase on maceration and liquefaction of carrots. *Journal of Biotechnology, 4*, 269-282.

Tamiello-Rosa, C. S., Cantu-Jungles, T. M., Lacomini, M., Tamiello-Rosa, C. S., Cantu-Jungles, T. M., Lacomini, M. (2019). Pectins from cashew apple fruit (*Anacardium occidentale*): Extraction and chemical characterization. *Carbohydrate Research, 483*, 1-6.

Zhang, B., Gao, Y., Zhang, L., Zhou, Y. (2020). The plant cell wall: Biosynthesis, construction, and functions. Journal of Integrative Plant Biology, 63(1), 251-272. https://doi.org/10.1111/jipb.13055