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Research article

QUANTITY AND QUALITY OF BROMELAIN IN SOME INDONESIAN PINEAPPLE FRUITS

Noryawati Mulyono[1*], Elisabeth Rosmeilia[1], Jessie Gabriela Philander Moi[1], Barbara Octarina Valentine[1], and Maggy Thenawidjaja Suhartono[2]

[1]School of Biology, Faculty of Biotechnology, Atma Jaya Catholic University, Jl. Jenderal Sudirman 51, South Jakarta, Jakarta 12930, Indonesia

[2]Department of Food Science and Technology, Faculty of Agricultural Technology, Bogor Agricultural University, Indonesia

ABSTRACT: This research aimed to extract bromelain from all parts of some variant of Indonesian pineapple fruits in order to evaluate their amount and characteristics as well. Each part of pineapple (crown, flesh, core, and peel) was separately blended with sodium phosphate buffer 0.01 M pH 7.0 at ratio of 1:1 (w/v) then filtered using cheese cloth. The filtrate was centrifuged at 1250 g for 10 minutes. The supernatant was collected and then centrifuged at 10000 g for 15 minutes. All extracts from each stage (blended mixture, pulp, filtrate, supernatant 1, pellet 1, supernatant 2, and pellet 2) were collected and characterized. The concentration of protein was measured by using BSA as standard and the proteolytic activity was determined using casein 1% (w/v) as substrate under the standard conditions (37 ^{o}C, pH 8.00, 10 min). The optimum temperature (ranged 27-80°C) and pH (ranged 3-10) was also evaluated. The result showed that three stepwise extraction process (blending with buffer – filtration – centrifugation) could be applied to obtain bromelain from all parts of pineapple, but some amount of bromelain was still left in the residue. Due to its price and weight, Subang pineapple was the most potential Indonesian pineapple as the source of bromelain. The maximum protein content and bromelain activity in one of Subang pineapple were 236.29 mg and 787.92 unit, respectively, while those in Bogor and Palembang pineapples were 168.27-190.10 mg and 464.97-510.37 unit, respectively.

Keywords: Bromelain, Casein, *Ananas comosus*, Protease

INTRODUCTION

Indonesia ranked sixth on the countries with highest pineapple production volume (1540630 MT in 2011), after Thailand, Brazil, Costa Rica, Philippines, and China ([FAOSTAT] 2011). Due to the flavor, texture, and nutrition, pineapple is a favorite fruit for direct consumption of further processed as syrup, jam, cocktail, and so forth. In addition, pineapple fruit is easily spoiled. Its shelf life is only about 7 days at 21 °C, therefore, when the harvest season comes, it is often oversupplied. In contrary, the core, peel, and crown are only used for feed or become solid waste. There are three major variants of Indonesian pineapple, namely Palembang, Bogor, and Subang pineapples. The first two were Queen pineapples, with size relative small (600-880 g, including their crowns), but the last one was Smooth Cayenne with size about 1380 g, including the crown (Sobir and Duri 2008).

Bromelain is a complex mixture of proteolytic enzymes that is present in all parts of the pineapple plant, but its characteristics depend on the source (Bhattacharyya 2008). This enzyme has been widely used in food industry for tenderizing meat and chill proofing beer (Ketnawa and Rawdkuen 2011; Sheu and Chen 1986), leather tanning process and in latex manufacturing (Christner *et al.* 1992; Yapa and Lionel 1980), skin care product (Frank *et al.* 2010), and pharmaceuticals (Bhattacharyya 2008; Maurer 2001). Its activities such as anti-inflammatory, platelet aggregation, fibrinolytic, blood thinner, anticancer, antimicrobial, and so forth have been reported ([AMR] 2010). The research aimed to extract bromelain from all parts of pineapple fruits in order to evaluate their amount and characteristics as well.

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MATERIALS AND METHODS

Materials. Ripe Palembang, Bogor and Subang pineapple fruits were purchased from local market (Jakarta). The chemicals for extraction and characterization were sodium phosphate, HCl, NaOH, casein, trichloroacetic acid (TCA), L-tyrosine, sodium carbonate, Folin-Ciocalteau reagent (F-C reagent), Bovine Serum Albumin (BSA), ethanol, phosphoric acid, citric acid (Merck), and Commasie Brilliant Blue (CBB) G-250 (Bio-Basic Inc). Blender (Philip), cheesecloth, incubator (Memmert INB 200-500®), pH meter (Orion 420A), centrifuge (Sorvall® Legend RT), microcentrifuge (Sorvall Pico®), spectrophotometer visible (Genesys 20), and common glassware were used for extraction and analysis.

Extraction. After being washed thoroughly, all pineapple parts (flesh, core, peel, and crown) were separated. Each part was blended with sodium phosphate buffer 0.01 M pH 7.0 at ratio of 1:1 (w/v) then filtered using cheese cloth. The filtrate was centrifuged at 1250 g for 10 minutes. The supernatant was collected and then centrifuged at 10000 g for 15 minutes. All extracts from each stage (blended mixture, pulp, filtrate, supernatant 1, pellet 1, supernatant 2, and pellet 2) were collected and analyzed. The scheme of extraction process is shown in
Figure **1**1.

Activity Assay. Proteolytic activity was determined using casein 1% (w/v) as substrate ([Sigma] 1999). One unit of bromelain activity was defined as 1 µg of tyrosine released in 1 minute per mL of sample when casein was hydrolyzed under the standard conditions (37 ^{o}C, pH 8.00, 10 min).

Protein Concentration Assay. Protein content in each bromelain extract was measured by Bradford method (1976) using BSA as standard.

Characterization. Optimum temperature (ranged 27-80°C) was determined by measuring the caseinolytic activity at pH 8.00 for 10 minutes. Optimum pH (ranged 3-10) was determined by measuring caseinolytic activity at optimum temperature of each extract for 10 minutes in universal buffer with determined pH. The enzyme stability at 80°C and 90°C and their optimum pH were determined for 3 hours incubation of bromelain from selected extracts.

RESULTS AND DISCUSSION

Relevant to report of Sobir and Duri (2008), we found that Smooth Cayenne (Subang pineapple) was bigger than Queen (Bogor and Palembang pineapples) (Table 1). Moreover, the percentage of the flesh part of Queen pineapples were less than that of Smooth Cayenne.

Table 2 demonstrated that the recovery of bromelain from all parts of pineapples used in this research was less than 100%. The distribution of bromelain in the blended mixture of pineapple with buffer from all parts of pineapples was not homogenous, indicated by the higher of total enzyme unit in the filtrate than that in the blended mixture. Table 2 also showed that the waste of bromelain extraction still contained bromelain. It implied that the enzyme was trapped in the solid matrix of waste. It could be seen that applying centrifugation could improve the homogeneity of bromelain in the solution. Multiple loop homogenization might be used to release enzymes that are trapped in the tissue matrix and intracellular enzyme, resulting higher proteolytic activity and protein yield (Shian *et al.* 2005).

Table 1. Weight and percentage of each part in pineapple fruit

	Bogor		Palembang		Subang	
	Weight (g)	%	Weight (g)	%	Weight (g)	%
Flesh	240.33	39.0	290.67	44.0	504.00	48.0
Core	88.83	14.4	116.93	17.7	154.35	14.7
Peel	195.33	31.7	137.80	20.9	229.64	21.9
Crown	91.67	14.9	115.03	17.4	162.02	15.4
Total	616.16	100	660.43	100	1050.01	100

Similar to Table 2, Table 3 demonstrated that the recovery of protein from all parts of pineapples used in this research was less than 100%. The distribution of protein in the slurry from all parts of pineapples was not homogenous, as indicated by the recovery of protein amount in the filtrate was higher than that in the slurry (blended mixture).

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Figure 2 showed the estimated of bromelain and protein obtained from one pineapple fruit. Though the distribution of bromelain and protein was not very homogenous, it obviously indicated that all of parts of pineapple fruit were potential to be used as the source of bromelain and protein. As the prices of those pineapple fruit was the same (USD 50 cent for 1 fruit) and Subang pineapple was the biggest one, so Subang pineapple was the most potential sources of bromelain. However, the bromelain activity and protein content in Bogor and Palembang pineapples were still considerable. The protein content in the waste from all Indonesian pineapple was less than that from Nang Lae and Phu Lae (Thailand pineapples), which was 238-398 mg. (Ketnawa *et al*. 2012).

Table 2. Percentage of bromelain recovery during extraction based on the enzyme unit

	Bogor	Palembang	Subang
Flesh:			
Filtration	19.07-114.98	48.36-89.98	92.96-106.27
1st centrifugation	90.50-96.60	84.19-84.58	73.77-74.37
2nd centrifugation	94.91-98.38	89.83-103.08	69.24-85.70
Core:			
Filtration	88.86-94.22	77.28-93.82	89.77-96.19
1st centrifugation	77.36-94.50	79.52-91.76	76.39-88.77
2nd centrifugation	58.94-97.38	74.50-93.89	68.08-87.30
Peel:			
Filtration	80.64-195.92	89.66-103.31	90.91-97.37
1st centrifugation	60.33-93.27	55.10-85.24	50.52-61.84
2nd centrifugation	84.07-93.83	43.47-89.34	78.24-82.56
Crown:			
Filtration	96.44-146.70	82.95-112.33	78.58-96.91
1st centrifugation	76.96-95.56	80.95-85.62	83.16-93.28
2nd centrifugation	99.27-113.33	71.92-98.13	82.05-98.85

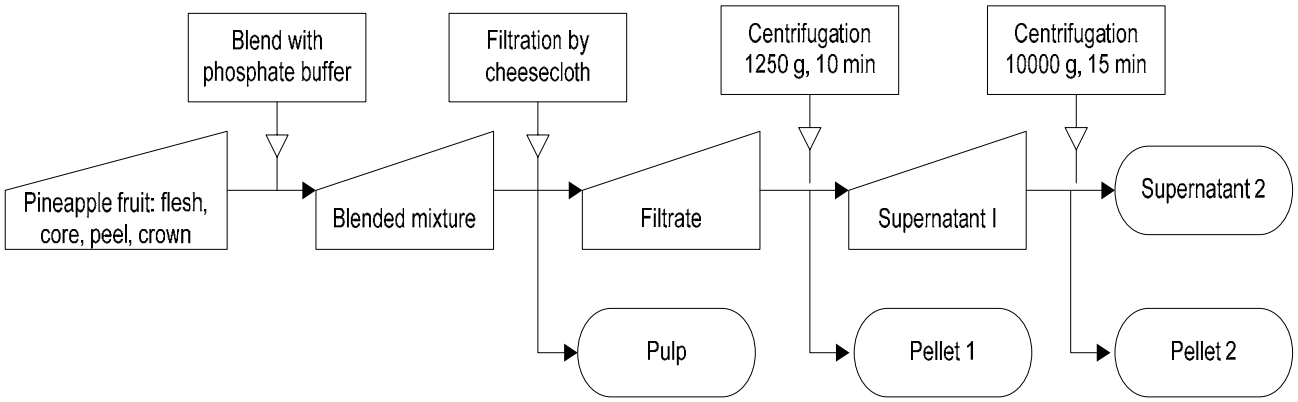

Figure 1 Bromelain extraction process

From the data of protein concentration and enzyme unit in 100 g of each part of pineapple fruits shown in Table 4, it could be seen that the bromelain with the highest caseinolytic activity was present in the flesh part of all tested pineapple. However, the pineapple waste, especially the crown of Bogor and Subang pineapples contained the highest protein concentration among other parts. It indicated that there was some non enzyme protein in the crown of Bogor and Subang pineapple. In addition, this part was potential as high protein sources for feed. The protein content in flesh part of those three variants of Indonesian pineapples was less than that in Malaysian pineapples, which was 500 mg per 100 g edible part (Zulkipeli 2007).

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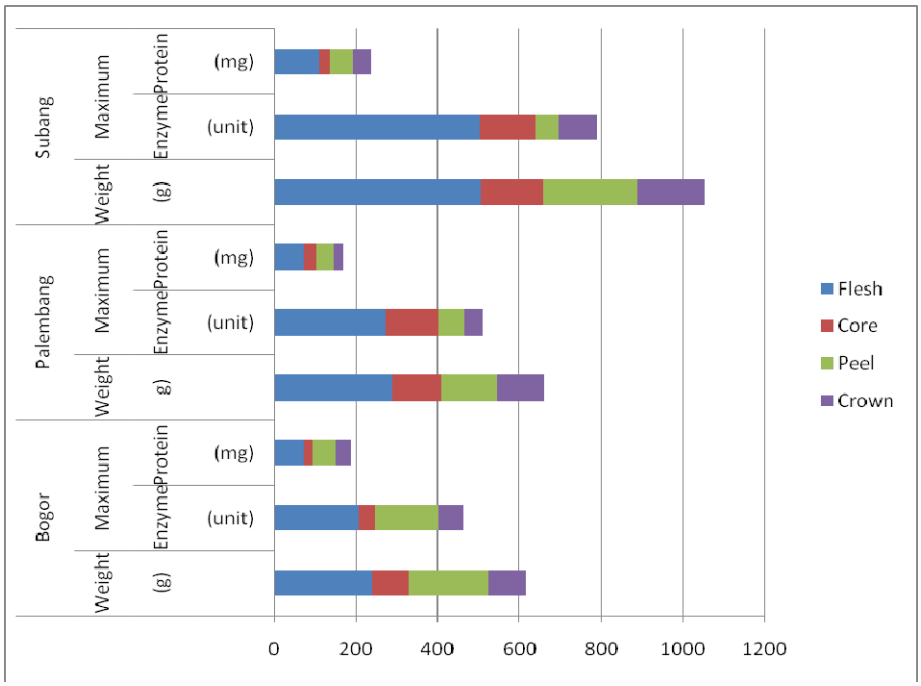

Figure 2. Maximum bromelain activity and protein content (mg) in one pineapple

Table 3. Percentage of protein recovery during extraction based on the protein concentration

	Bogor	Palembang	Subang
Flesh:			
Filtration	87.30-96.87	72.35-96.60	95.44-104.88
1st centrifugation	80.36-91.78	76.35-83.00	73.97-75.79
2nd centrifugation	83.21-96.61	93.71-93.86	68.70-86.35
Core:			
Filtration	97.79-112.87	93.06-97.28	96.96-115.56
1st centrifugation	85.84-95.67	56.01-85.68	83.32-89.91
2nd centrifugation	66.02-95.23	89.54-99.92	55.26-87.85
Peel:			
Filtration	98.07-106.02	95.68-98.98	93.59-100.41
1st centrifugation	81.03-92.87	82.71-94.46	80.32-83.02
2nd centrifugation	65.68-93.93	88.13-88.20	72.65-92.48
Crown:			
Filtration	87.47-98.28	62.52-98.94	83.79-91.51
1st centrifugation	92.72-104.79	85.10-97.29	92.48-92.66
2nd centrifugation	69.81-97.49	92.89-103.25	86.46-92.66

Table 4. Protein concentration and enzyme unit in 100 g of each part of pineapple

	Bogor		Palembang		Subang	
Part	Protein (mg)	Enzyme (unit)	Protein (mg)	Enzyme (unit)	Protein (mg)	Enzyme (unit)
Flesh	20.124	82.1	19.24	81.008	11.13	50.773
Core	14.933	20.821	15.481	65.61	7.952	46.235
Peel	15.25	39.779	22.5	11.215	14.729	9.937
Crown	29.232	60.478	16.416	22.781	21.306	38.733

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Our results showed that bromelain from all parts of pineapples had optimum temperature at 70-80 ^{o}C, except that from core, which showed more thermophilic. Other exceptional was shown by bromelain from Subang pineapple peel, which had lower optimum temperature. All of bromelain obtained in this research showed that they favored neutral to basic condition, especially for bromelain from the peel of Queen pineapples (Bogor and Palembang pineapples) (Table 5). Our result was relevant to previous report that fruit bromelain had its maximum activity at pH 8, 70 ^{o}C (Suh *et al*. 1992).

With the same variant of pineapple, Martowibowo *et al.* (2009) reported that the optimum temperature and pH of bromelain from the crown of Subang pineapple was 60 ^{o}C and 7.0, respectively, eventhough the enzyme preparation was quite different. They used potassium phosphate 0.01 M as extracting buffer and they precipitated the bromelain using acetone and ammonium sulphate. Bromelain from other pineapple, such as Perola variety from Brazil, had their maximum activity at 70 ^{o}C and pH 8.0 (Silvestre *et al*. (2012). Other research reported that the bromelain from pineapple fruit, but the variant of pineapple was not reported. while Kamunang and Kamu (2011) reported that the optimum temperature and pH was 65 ^{o}C and pH 6.5. Bromelain from the fruit and core parts of pineapple from some other researchers showed their lower optimum temperature (Yamada *et al.* 1976; Ratnawati 2001).

Table 5. Optimum temperature and pH of each part of pineapple

	Optimum temperature			Optimum pH		
	Bogor	Palembang	Subang	Bogor	Palembang	Subang
Flesh	≥80	70	70	7-8	7	8
Core	≥80	≥80	≥80	8	8	8
Peel	70	70	50-60	≥10	≥10	7
Crown	70	60-80	60-70	8	8	7

Table 6. Residual activity of bromelain from each part of Indonesian pineapples

	Bogor	Palembang	Subang
Flesh	100.0	100.0	100.0
Core	89.0	88.5	97.6
Peel	83.6	81.1	100.0
Crown	94.4	87.9	100.0

Table 6 reported the residual activity of bromelain after being incubated at 80 ^{o}C and their optimum pH for 3 hours. In general, bromelain from Subang pineapple was more stable than that from Queen pineapples. Therefore, from Table 5 and Table 6, it can be concluded that bromelain from these Indonesian pineapples, especially Subang pineapple, was potential to be applied in many industries in which pasteurization, blanching or other mild treatments were involved.

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