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Properties of bromelain extract from different parts of the pineapple variety Morris

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A B S T R A C T

The purpose of this study is to characterize the bromelain extract from pineapples which belong to the Morris variety. The bromelain was extracted from two different batches of Morris pineapples; one with crown and the other without crown. The weight of each part such as flesh, peel, core, stem and crown were 45–52%, 28–29%, 13–14%, 5–6% and 7% (w/w), respectively. The physiochemical properties of each part of the pineapple were determined. Results showed that the pH, TSS (°Brix), volume of extract (ml), total protein (mg/ml) and bromelain activity (CDU/ml) of the pineapple were 3–4, 1–6 °Brix, 850–6000 ml, 21–98 mg/ml and 95–251 CDU/ml, respectively. Both species showed that the peel and flesh gave the highest bromelain activity and protein contents, whereas the extract from the stem revealed the lowest values. The storage stability study showed that bromelain activities of the extract decreased with time under refrigerated condition (5–8 °C).

1. Introduction

Pineapple (Ananas Comosus) is one of the most famous and popular tropical fruit in the world. According to Food and Agriculture Organization United Nations (FAO) online database revealed that the world's pineapple production in 2015 and 2016 (25.8 million tons) increased from the 2014 (25.4 million tons) and 2013 (24.7 million tons) (FAOSTAT, 2018). The increase in pineapple production attributes to increase in waste creation because humans consume only the pineapple flesh and the unsuitable components of the fruits is removed. The fresh fruits became an important element in juice and fruit products. Some of the products are concentrated juices, squash, jams and canned fruit. It was found that only about 30% of the whole weight of the pineapple fruit is the fresh fruits whereas the rest which is 70% is considered as unsuitable components (peel, core, crown and stem). Although the unsuitable components are donating to environmental problems, extraction of bromelain enzyme from a significant part of the material has fortunately been recognized as a value added product (Chaurasiya and Umesh Hebbar, 2013; Nor et al., 2015).

Bromelain is a mixture of cysteine proteases found in the tissue of plant family Bromeliaceae obtained from pineapple. The proteolytic enzymes obtained from pineapple commercially available are stem bromelain and fruit bromelain. Nevertheless, pineapple wastes such as peel, core and crown were reported to contain bromelain as well (Nor et al., 2015; Yin et al., 2011; Ketnawa et al., 2011; Zhang et al., 2010). This enzyme is being seen as valuable because of its incredible phyto-medical effects. Bromelain exhibits antibiotic, anti-inflammatory, anti-cancer, anti-thrombotic, anticoagulative and anti-edematous properties. Besides that, bromelain is a promising postoperatively agent that decreases post-surgical discomfort and swelling. It is also known to have wound healing properties. Apart from medical perspective, various industries also use bromelain in food, textile and cosmetic industries (Manzoor et al., 2016; Soares et al., 2012).

In this study, bromelain extracted from the different parts of Morris variety is characterized and the physiochemical properties of the extracts are also determined. The storage stability of the bromelain extracts was also investigated.

2. Materials and methods

2.1. Materials

Hydrochloric Acid, di-sodium hydrogen phosphates, trichloroacetic acid (TCA), sodium acetate, L-Cysteine, hydrochloride, tetrasodium tetrahydrate Salt (EDTA), acetic acid, bromelain (from pineapples), L-tyrosine were purchased from Merck, Germany. Casein was purchased from Sigma, USA. All other chemicals used in the experiment were analytical grade. The pineapple (Ananas comosus) Malaysian species, Morris with crown and no crown were collected from a plantation in the Kg Parit Gantung, Benut, Johor, Malaysia. All of pineapple parts (core, flesh, peel, stem and crown) were washed with clean water before being chopped into small pieces. Each part was determined and reported as a percentage of the proportion of a pineapple.

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2.2. Preparation of crude extract

Each pineapple part was cut into small pieces and crushed using extruder to obtain pineapple juices. After that, the resulting juice was centrifuged for 10 min at 3500 rpm to remove all suspended impurities. The obtained supernatant (crude enzyme extract) was collected, recorded and used for further experiments. The bromelain activity of each part of the pineapple extract was determined.

2.3. Physiochemical properties

The pH of each sample was measured using pH meter at room temperature (Mettler Toledo). The total soluble solid (TSS) was determined using hand refractometer (Atago) and stated as degrees Brix.

2.4. Proteolytic activity determination

The enzyme activity of all samples was determined by the casein digestion unit (CDU) using casein and L-tyrosine as a substrate and a standard, respectively. The assay was based on proteolytic hydrolysis of the casein by the enzyme. Bromelain hydrolysed the casein to release L-tyrosine. One unit of enzyme activity was defined as the amount of enzyme, releasing a product equivalent to 1 μg of tyrosine/min/ml under the standard assay conditions and expressed as CDU/ml. The absorbance was read using spectrophotometer at wavelength 275 nm.

2.5. Protein content determination

Bradford method was used to measure protein content in the crude enzyme extract using BSA as standard using spectrophotometer (Shimadzu UVmini) at 575 nm.

2.6. Storage stability testing

The stability of bromelain enzyme from different parts of the pineapple was also studied during storage under refrigerated condition (5–8 °C) by assaying the activity of extract of each part of fruits for 9 days.

3. Results and discussion

A total of 40 pineapple fruits with a total weight of 45.8 kg were collected. This batch collected belongs to the Morris species which is 20 pineapples with crown and another 20 pineapples without crown attached. The basis of choosing 20 pineapples is to produce sufficient juice from every part of the pineapple for processing in our large scale filtration systems. Both Morris species with and without crown were collected from a plantation in the Kg Parit Gantung, Benut, Johor, Malaysia. The state of Johor has especially planted Morris pineapple as a suitable variety for peat soils. We were made to understand the fruit with no crown has been sprayed with hormones to promote pollination and produce evenly fruit size in the same period. As we can see from Fig. 1a and b, pineapple with no crown have evenly fruit size compared to pineapple with no crown. From the graph below, it shows that pineapple without crown gained more weight than the pineapple with crown. The average weight of each pineapple is 1.04 kg for pineapple with crown and 1.25 kg for pineapple without crown. The weights of the pineapples collected were plotted in Fig. 1 (a) and (b). The ripening stage for Morris pineapple with and without crown is Index 5 where approximately 50% of eyes at the base is yellowish indicating the fruit is ripe. The flesh of the fruits is yellow in color, solid, smooth, sweet and fragrant.

Table 1 displays the different parts proportion of pineapple fruits variety Morris with crown and without crown. The largest waste proportion were peels (28–29%, w/w) followed by cores (12–13%, w/w), stems (5–6%, w/w) and lastly crown (5%, w/w). Whilst, the flesh

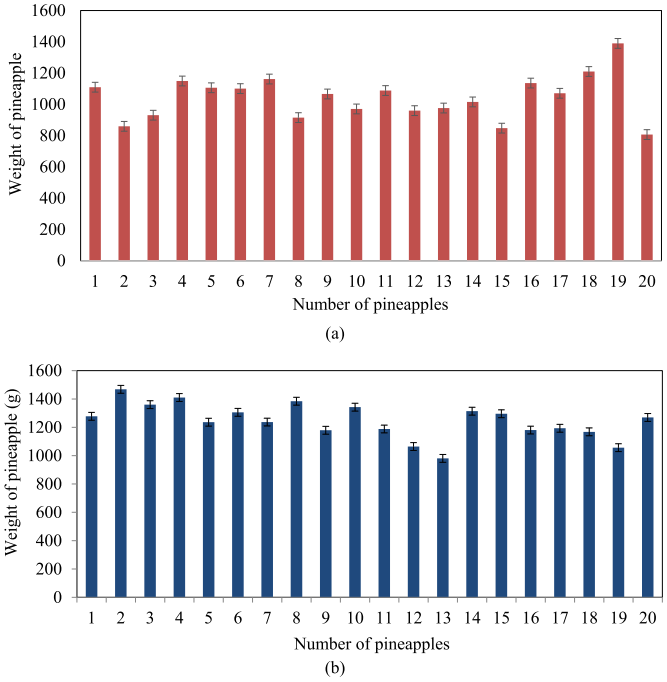

Fig. 1. The weights of every pineapples with (a) crown (b) no crown.

proportion of pineapple Morris without crown and pineapple Morris with crown were about 52% (w/w) and 45% (w/w), respectively. This results exhibited that the waste proportion is about 50% (w/w) of the total weight of the pineapple. Pineapple Morris without crown has more consumable portions than pineapple Morris with crown due to larger size and the findings are in agreement with other researchers (Ketnawa et al., 2012).

The characteristics of each part of pineapple extract are tabulated in Table 2. The characteristics include pH, TSS, volume of extract, protein content and bromelain activity. The pH of the crude extracts from Morris with crown and without crown was around 3.8–4.2. The stem portion showed the highest value of pH (4.20) whereas core of both species gave the lowest value (3.89–3.92). Ripe pineapple fruit contains organic acid such as citric and maleic acid (Bartolomé et al., 1995; Ketnawa et al., 2012). The flesh and the core almost have the same pH (3.89–3.93) due to existence of citric and maleic acid in the extract. High acidity of citric and maleic acid causes the pH value to be lower.

The total soluble solid of Morris (with crown) and Morris (without crown) were reported to be 1.9–6.1°Brix and 1.7–7.5°Brix, respectively. This TSS values might be different from other species due to different cultivars and plantation area as also agreed by previous researchers (Ketnawa et al., 2012).

Table 2 also depicts that the flesh and peel of both species have the highest protein content while core, crown and stem exhibit lower protein content. The lowest protein content of both species is stem, 29.3 mg/ml for Morris with crown while 21.7 mg/ml for Morris without crown. Like the protein content, highest bromelain activity was found in flesh and peel portion whereas stem is found to have the lowest bromelain activity for both species. The differences in protein content and bromelain activity in each portion was most probably due to the different types of enzymes contained in the pineapple, such as enzyme from the fruit and stem: ananin and comosain (Hale et al., 2005).

The stability of the enzyme during storage under refrigerated condition (5–8 °C) was studied for 9 days. The change in bromelain activity was analyzed for Morris pineapple without crown (Fig. 2) and with crown (Fig. 3). Bromelain activity of all the portions decreased slightly throughout the storage period. This results indicate that in order to achieve higher specific activity of bromelain the extract should be used as soon as possible or better to use fresh extract (Chaurasiya and Umesh

Table 1
Proportion of pineapple fruits variety Morris with crown and without crown.

Cultivar proportion	Morris (with crown)		Morris (without crown)	
	Weight (g)	% (w/w)	Weight (g)	% (w/w)
Flesh	7282.13 ± 3.32	45.10 ± 0.81	9205.45 ± 2.41	52.52 ± 1.43
Peel	4739.00 ± 1.47	29.35 ± 0.33	4913.58 ± 5.61	28.06 ± 3.25
Core	2071.22 ± 2.99	12.83 ± 1.42	2401.08 ± 4.84	13.73 ± 1.70
Stem	1104.71 ± 6.40	6.84 ± 4.12	999.44 ± 2.38	5.69 ± 2.02
Crown	950.44 ± 4.06	5.89 ± 2.36	--	--
Total	16147.50 ± 6.22	100.00 ± 7.50	17519.55 ± 2.91	100.00 ± 88.15

Mean ± SD from triplicate determinations.

Table 2
Characteristics of each part of pineapple extract.

Proportion	pH	TSS (°Brix)	Volume of extract (ml)	Protein content (mg/ml)	Bromelain activity (CDU/ml)
Morris (with crown)					
Flesh	3.92 ± 0.01	6.10 ± 2.71	4500.33 ± 2.08	92.63 ± 3.71	219.46 ± 0.46
Peel	4.04 ± 0.02	5.73 ± 3.71	4000.47 ± 0.87	83.33 ± 0.80	229.64 ± 0.54
Core	3.89 ± 0.01	3.47 ± 0.84	1399.85 ± 1.61	45.00 ± 5.01	186.22 ± 0.47
Crown	4.09 ± 0.02	3.60 ± 1.87	1000.2 ± 1.21	36.32 ± 0.69	154.31 ± 0.20
Stem	4.20 ± 0.03	1.97 ± 0.55	850.17 ± 1.70	29.30 ± 1.06	131.42 ± 0.20
Morris (without crown)					
Flesh	3.93 ± 0.05	7.50 ± 0.82	6002.33 ± 1.53	99.72 ± 1.91	251.87 ± 2.91
Peel	4.09 ± 0.05	6.47 ± 0.32	4001.33 ± 2.08	98.08 ± 0.94	246.83 ± 2.20
Core	3.92 ± 0.03	4.07 ± 0.15	1499.67 ± 0.58	54.12 ± 1.29	218.75 ± 3.62
Stem	4.20 ± 0.04	1.77 ± 0.21	999.63 ± 0.55	21.73 ± 1.86	95.22 ± 3.86

Mean ± SD from triplicate determinations.

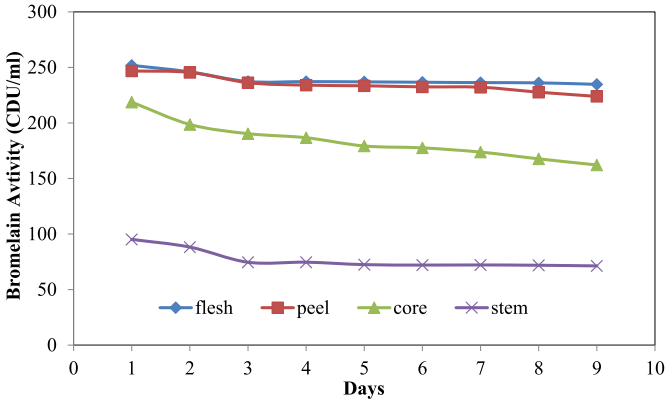

Fig. 2. Effect of storage on bromelain activity (without crown).

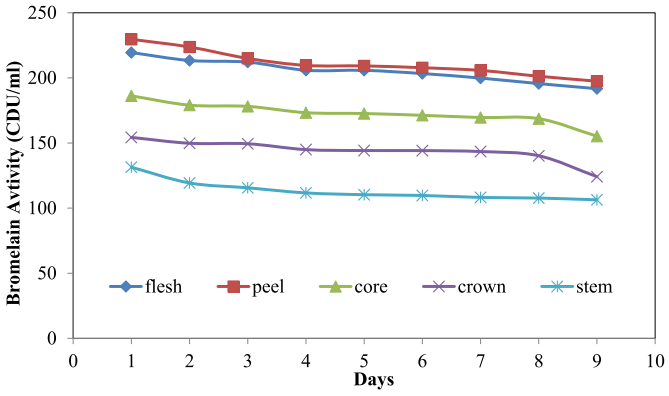

Fig. 3. Effect of storage on bromelain activity (with crown).

Hebbar, 2013).

4. Conlcusion

The flesh of the pineapple is the largest portion (w/w) for both Morris with crown and no crown followed by peel, core, stem and crown. The pH of the extract is in the range from 3 to 4 for both species. The flesh and peel exhibit the highest bromelain activity. However, peel is the major waste proportion and seems to have the utmost potential for bromelain extraction. The bromelain extracted from the peel exhibits high bromelain activity of 246.83 CDU/ml (without crown) and 229.64 CDU/ml (with crown); only slightly lower than the flesh. Thus results revealed that pineapple waste such as peels which contribute to almost 30% of pineapple waste can be a good source of bromelain.

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