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Short Communication

Physicochemical Properties Analysis and Bromelain Activity of Three Indexes Pineapple (*Ananas Comosus*) Peel Extract Variety Josapine

Muhammad Yazid Abd Halim[1], Siti Aimi Sarah Zainal Abidin[1,2], Noorlaila Ahmad[1], Siti Roha Ab Mutalib[1,2]*

[1]School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia

[2]Malaysia Institute of Transport, Universiti Teknologi MARA, 40450 Shah Alam, Selangor

*Corresponding author: Siti Roha Ab Mutalib, School of Industrial Technology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia; sitiroha7796@uitm.edu.my

Abstract: Industrial waste such as pineapple peels that accumulate in massive quantities contributes to environmental pollution. However, there is limited data on the physicochemical properties of pineapple peel for several ripening stages. The aim of this study was to determine the physicochemical properties and bromelain activity of three indexes of pineapple peel extract specifically for the variety Josapine. The method used for the analysis of physicochemical properties includes total soluble solid (TSS), pH, titratable acidity (TA), pulp content, sugar content and the casein digestion unit (CDU) method for bromelain activity. Significant differences at $p<0.05$ were found between TSS, pulp, pH, titratable acidity and TSS/TA ratio among an index of maturity. It was found that pineapple peel extract at maturity index 7 contained significantly the highest in titratable acidity, TSS and total sugar. From this present study, it was observed that bromelain activity in pineapple peel extract was significantly higher in maturity index 5 as compared to maturity indices 2 and 7.

Keywords: Pineapple Josapine; Pineapple Peel Extract; Maturity Index; Physicochemical Properties; Bromelain Activity

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1. Introduction

Pineapple (*Ananas comosus*) is a perennial plant from the family of Bromeliaceae (Britannica, 2021). The pineapple fruit is believed to be originated from tropical and subtropical America, where it was further expanded to grow throughout the tropical and subtropical regions of the world (Lapcharoensuk *et al*., 2019). In Malaysia, pineapple fruits are one of the major cultivated fruits, ranking ninth in terms of production in the country.

Malaysia is one of the world's major producers of pineapple other than Thailand, Philippines, Indonesia, Hawaii, Ivory Coast, Kenya, Brazil, Taiwan, Australia, India and South Africa. It was reported that in 2019, Malaysia produced 299,912 metric tonnes of pineapple (MPIB, 2022). The Malaysian Pineapple Industry Board (MPIB) reported that in 2020, Johor had the highest yearly yield of pineapple in Malaysia with 212,129.64 metric tonnes followed by Sarawak and Selangor with 47,586.83 and 15,988.58 metric tonnes, respectively. The pineapple variety Josapine is one of the popular pineapple varieties in Malaysia. This pineapple variety is a hybrid of the 'Johor' variety ('Singapore Spanish' x 'Smooth Cayenne') with the 'Sarawak' variety ('Smooth Cayenne') where it is the product of research done by the Malaysian Agriculture Research and Development Institute (MARDI). As a result, agricultural waste has been increasing massively as the production of pineapple-processed items increases, due to, the selection and elimination of components that are unsuitable for human consumption (Upadhyay *et al*., 2010). According to Lun *et al*., (2014), about 30% to 50% of the total fruit weight is discarded during canning. In canning industries, pineapple at maturity index 2 was used. The pineapple waste is the by-products of the pineapple processing industries such as residual skin, peel, pulps, stem and leaves. However, food processing waste such as pineapple waste might have the potential to be recycled into a useful product that has a higher value-added product (Hamzah *et al*., 2021). The pineapple peel waste represents about 10% of its original fruit weight resulting from the processing of pineapple into slices (Kareem *et al*., 2010). Pineapple peels have potential uses as raw materials that can be converted into value-added products. In the agricultural field, pineapple peel is utilised as fertiliser or animal feed. Pineapple peel is a rich source of cellulose, hemicelluloses and other carbohydrates. Besides, it also contains abundant bromelain. Bromelain has many applications in industries including its uses in food, medical, pharmaceutical, cosmetic and other industries. Bromelain enzyme is applied for meat tenderisation, baking cookies, beer clarification, grain protein solubilisation and protein hydrolysate production. Also, bromelain is used as an enzymatic browning inhibitor in fresh apple slices. The data for physicochemical properties is important for the isolation and purification of bromelain from pineapple peel extract and stability determination of bromelain. Knowledge of the physicochemical properties is fundamental in analysing the unit operation as it influences the next processing step towards the production of bromelain. According to Lapcharoensuk *et al*., (2019), the determination of the physicochemical properties of fruits during fruit maturation is an important measure for harvesting and post-harvesting application to maintain the good quality of the fruit for the market. Therefore, this study aims to determine the physicochemical properties analysis and bromelain activity of pineapple (*Ananas Comosus*) peel extract variety Josapine at maturity index 2, 5 and 7.

2. Materials and Methods

2.1 Preparation of Samples

Pineapple variety Josapine with different maturity indices of 2, 5 and 7 used in this study were purchased from Pasar Moden Seksyen 6, Shah Alam, Selangor, Malaysia. The pineapple peel from maturity index 2 was chosen because in industrial practices, pineapples are harvested at maturity index 2 when it is suitable for canning, while maturity index 5 represents the middle ripening stage of pineapple and maturity index 7 represents the fully ripe stages of pineapple. Pineapple peels were crushed using a fruit crush processor with a ratio of pineapple peel to purified water 1:1. The extract was filtered through a muslin cloth into a cool iced water bath. The filtered extract was then stored in a freezer before being used for analysis.

2.2 Physicochemical Analysis

2.2.1 Total soluble solids (TSS)

The total soluble solid (TSS) of the pineapple peel extracts of different maturity was determined using an Abbe digital refractometer. Firstly, a drop of the extract solution was placed on the glass prism. Next, the reading of the TSS percentage was obtained directly from the digital refractometer. Analyses were done in triplicate.

2.2.2 pH

The pH of the pineapple peel extracts of each maturity indexes was determined using a pH meter at room temperature. The pH meter was dipped into pineapple peel extract solution and the reading was taken directly from the device.

2.2.3 Titratable acidity (TA)

The titratable acidity (TA) of the pineapple peel extracts was determined by using a method as outlined by AOAC 962.12. Firstly, 10 mL of pineapple peel extract was transferred into a 250 mL conical flask. A 250 mL of distilled water was added to dilute the pineapple peel extract. Next, about 1 mL of phenolphthalein indicator was added to the conical flask. The sample in the conical flask was titrated with 0.1 M NaOH solution until a faint pink end point was observed. After that, the total acidity of the sample was calculated using Equation (1) and expressed as the concentration of citric acid.

$$\% \text{ Acid (as anhydrous citric acid)} = \text{Volume of 0.1 N NaOH (ml)} \times 0.64 / 10 \quad (1)$$

2.2.4 Pulp content

The pulp content of pineapple peel extracts was centrifuged at 1500 rpm for 7 mins. Then, the pulp was weighed and expressed in the percentage using Equation (2) as followed:

$$\% \text{ Pulp} = (\text{weigh of pulp/weigh of extract}) \times 100 \quad (2)$$

2.2.5 Sugar analysis

Pineapple waste was analysed for glucose, fructose and sucrose using a High-Performance Liquid Chromatography (Waters, USA) model 600 instrument with a Refractive Index detector model 2414. The sugar in pineapple waste was extracted into purified water and then filtered through a 0.45 µm membrane filter. The chromatographic conditions are as follows:

Column: Carbohydrate High Performance 4µm (4.6 mm x 250 mm cartridge)

Column temperature: Room temperature (22°C)

Mobile phase: Acetonitrile: distilled water (90:10)

Flow rate: 1.3 ml/min

Injection volume: 20 µL

Duration of analysis: 15 mins

2.3 Bromelain Activity

Bromelain activity in the sample was determined using the casein digestion unit (CDU) method (Enzyme Development Corporation, 2015). Firstly, 5 mL of the casein substrate was transferred into a test tube. Then, the test tube was immersed in a water bath at 37^{o}C for 10 mins. Then, 5 mL of trichloroacetic acid (TCA) stopping reagent and 1 mL of enzyme solution were added into the blank test tube, while only about 1 mL of enzyme solution was added to the sample test tube. The test tubes were vortexed and placed into the water bath at 37^{o}C for 10 mins. Then, 5 mL of the TCA stopping reagent was added into the blank test tube and sample test tube and were vortexed, and, placed into the water bath at 37^{o}C for 30 mins. The blank test tube and sample test tube were removed from the water bath and left to cool to room temperature. Then, the blank and sample were filtered using filter paper. The filtrate was collected in a beaker and the absorbance was measured at 280 nm using a UV-Visible spectrophotometer. The bromelain activity was calculated using the formula shown in Equation (3).

$$CDU/mg = [(Et – Eb)/Es] \times 50 \times (11/10) \times DF \quad (3)$$

Where;

DF: Dilution Factor

Et = Absorbance of enzyme sample tube

Eb = Absorbance of blank sample tube

Es = Absorbance of standard Tyrosine

2.4 Data Analysis

All experiments were performed in triplicates. The data obtained were analysed using software, Statistical Package for the Social Sciences (SPSS). The analysis of variance (ANOVA) tests were performed.

3. Results and Discussions

Table 1 shows the physicochemical properties and bromelain activity of the pineapple peel extracts. From these results obtained, the maturity index of the pineapple has a great influence on the physicochemical properties and bromelain activity of pineapple peel extracts. From these results, it was found that the pH of pineapple peel extracts decreased as the maturity index increased. The pH values of pineapple peel extracts were in the range 3.80-3.93 which indicated that the extracts were acidic. According to (USFDA, 2003), the pH of pineapple should be in the range of pH 3.2 to pH 4. pH is an internal ripeness indicator and an important factor in the fruit processing industry (Alam *et al*., 2021).

Table 1. Physicochemical properties and bromelain activity of pineapple peel extract variety Josapine of maturity indices 2, 5 and 7.

Assay	Maturity Index		
	2	5	7
pH	3.93 ± 0.04[a]	3.80 ± 0.00[a]	3.83 ± 0.06[a]
Titratable Acidity (%)	0.15±0.00[b]	0.23±0.00[a]	0.24±0.00[a]
Pulp (%)	3.88±0.40[a]	2.55±0.18[b]	2.56±0.09[b]
Total soluble solid (oBrix)	6.60 ± 0.00[c]	7.56 ± 0.05[b]	8.30 ± 0.00[a]
TSS/TA ratio	44±0.000[a]	32.86±0.000[c]	34.58±0.000[b]
Sugar			
Glucose (%)	1.17±0.17[a]	1.30±0.20[a]	1.28±0.20[a]
Sucrose (%)	Not Detected	Not Detected	9.10±4.32[a]
Fructose (%)	1.36±0.15[b]	1.71±0.21[b]	4.25±0.32[a]

Assay	Maturity Index		
	2	5	7
Total sugar (%)	2.53[b]	3.01[b]	15.91[a]
Bromelain Activity (CDU/mg)	723.30 ± 66.90[b]	1118.00 ± 118.08[a]	360.35 ± 47.02[c]

Titratable acidity indicates the sourness of the fruit. In this present study, titratable acidity increases along with the maturation of the fruit. This is due to the low respiration activity of the pineapple fruit. Usually, the organic acids in fruit decrease as fruit maturation increases, where the organic acids are converted to simple sugars during the respiration activity of fruit. Acidity in pineapple is reported as citric acid. A maximum of 1% acidity was established by CODEX and FAO/WHO in international trade to guarantee consumer acceptance of pineapple (Coppens d'Eeckenbrugge & Leal, 2003). Therefore, the titratable acidity value from this study can be accepted because the values were in the range of 0.15% to 0.24%. Titratable acidity was significantly higher in index 7 of pineapple maturity.

It was found that the percentage of pulp content in pineapple peel extracts was significantly higher in maturity index 2 followed by maturity index 5 and 7. The percentage of pulp was in the range of 2.55–3.88%. These findings were in agreement with studied done by Nadzirah *et al*., (2013) who found that pulp content was higher in the lower stage of maturity.

TSS in the pineapple peel extracts was significantly increased as the maturity index increased which was similar to studies performed by Nadzirah *et al*. (2013), and Siti Rashima *et al*. (2019), where the range of 6.60–8.30 °Brix was obtained. In addition, it was found that the peel colour of the pineapple had linear relationships with TSS as indicated by the increasing trend in TSS and the pineapple peel's colour during the storage period (Nadzirah *et al*., 2013). The increase in TSS was due to the conversion of starch into simple sugars during the fruit ripening process. TSS is an important quality factor as it indicates the sweetness of fruit. According to Moneruzzaman *et al*. (2008) and Md Anowar *et al*. (2014), during the maturation and ripening of fruits, there are changes in their TSS. The TSS increases from the mature green stage to the yellow ripe stage.

Increases in TSS and TA can cause a decrease in TSS/TA ratio and can contribute to sour tastes (Lu *et al*., 2014). These results showed that the TSS/TA ratio for pineapple peel extracts decreased with an increase in the maturity index. In our study, the result was in agreement with Wijesinghe and Sarananda (2002), who found that pineapples at 40% yellow stage have significantly lower TSS/TA ratio compared to pineapples at the 20% yellow stage. This could be due to the onset of senescence. Therefore, pineapples should be harvested at index 2 of the maturity stage as at this stage, pineapples taste better and are better in quality. It is generally recognised that the TSS/TA ratio is the most reliable parameter index for evaluating pineapple fruit quality. To obtain high-quality pineapple fruit, those cultivars with

TSS/TA ratio from 20 to 40 were recommended (Lu *et al*., 2014). Wijesinghe and Sarananda (2002) further reported that balance values between TSS and TA gave a better taste of pineapple.

In pineapple fruit, glucose, sucrose and fructose play an important role in terms of flavour. From the result obtained, it was found that glucose, sucrose and fructose were detected in pineapple peel extract index 7. However, only glucose and fructose were detected in pineapple peel extracts index 2 and 5. The percentage of glucose in pineapple peel extracts indices 2, 5 and 7 were 1.17%, 1.30% and 1.28%, respectively. Fructose content in maturity index 7 was significantly higher at 4.25% compared to index 5 (1.71%) and index 2 (1.36%). This means that the pineapple peel extract with maturity index 7 was the sweetest among the three indices. Meanwhile, pineapple peel extract maturity index 7 also contained the highest total sugar (15.91%) followed by maturity index 5 (3.01%) and maturity index 2 (2.58%). These results were in agreement with the explanation from Ersoy *et al*. (2007) and Wijesinghe and Sarananda (2002) that sugar content and its quantity changes in fruits may depend upon the fruit maturity stages.

Bromelain activity in different maturity of pineapple peel extract was also determined. It was found that bromelain activity was significantly higher in maturity index 5 (1118.0 CDU/mg) followed by maturity indices 2 (723.30 CDU/mg) and 7 (360.35 CDU/mg). From this present study, it was observed that bromelain activity in the pineapple peel extracts was significantly higher in maturity index 5 as compared to maturity indices 2 and 7. These results were in agreement with previous studies done by Chaurasiya and Hebbar (2013) and Rifani (2005) which found that as the ripening progress, bromelain activity will decrease. From this finding, it was found that bromelain enzymes were more stable because the pineapple peel extracts were in an acidic condition. Besides, bromelain activity is less affected due to the low pulp content in pineapple peel extract. Furthermore, pulp content is related to the cloudiness of pineapple peel extract. Cloudiness will influence the rheological behaviour of pineapple peel extract which is important for the condition of isolation and purification of bromelain.

5. Conclusions

The physicochemical properties of pineapple waste extract were affected by different indexes of maturity. Index 7 of the maturity was the highest in titratable acidity, TSS and total sugar. Bromelain activity in pineapple peel extract was found more stable as the extract was acidic.

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