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Physico-Chemical Properties of Pineapple Crown Extract Variety N36 and Bromelain Activity in Different Forms

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Abstract

The purpose of this study is to produce bromelain powder from crown extract of pineapple variety N36. The physico-chemical properties of the pineapple crown extract were initially determined. It was found that the percentage of pulp, pH, TSS, percentage of acidity, percentage of fructose and glucose of the pineapple crown extract were 2.41%, 3.94, 1.6°Brix, 0.3%, 0.83% and 0.51%, respectively. In this study, the pineapple crown extract was prepared by food processor before being filtered using muslin cloth. The bromelain in the pineapple crown extract was then purified by Preparative High Performance Liquid Chromatography (HPLC) (Agilent) using cation exchange resin column. Subsequently, the salt in the purified bromelain was removed by diafiltration process using continous diafiltrator. Finally, the desalted bromelain solution was dried using freeze dryer (Christ alpha 1-4LD Plus model). It was determined that 1.0g of bromelain powder could be produced from 200ml of desalted bromelain solution or formerly 100g pineapple crown. The bromelain activity at each step towards producing of powdered bromelain was determined. It was found that bromelain activity was significantly highest in powdered bromelain (529.77 ± 5.74 CDU/mg) at the 5% level followed by purified bromelain (501.08 ± 3.31 CDU/mg), desalted bromelain (485.78 ± 8.76 CDU/mg) and pineapple crown extract (426.49 ± 8.76 CDU/mg).

Keywords: N36, PINEAPPLE CROWN EXTRACT, BROMELAIN ACTIVITY, PHYSICO-CHEMICAL PROPERTIES, HPLC, CONTINUOUS DIAFILTRATOR, FREEZE DRYER.

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1. Introduction

The pineapple variety N36 was amongst the four main varieties of pineapples planted in Malaysia [1]. This variety is mainly used for canned product. The pineapple crown leaves are the burden of the canned pineapple industry because the need of these pineapple crown for replanting is relatively small compared to the amount of the refuse generated [2]. Hence, alternatives to its efficient utilization are necessary [3]. Fortunately, the pineapple crown contains bromelain enzyme [4]. Bromelain is proteolytic enzymes or proteases [5]. It is an aqueous extract of pineapple contains a complex mixture of proteases and non-protease components. Bromelain is used in food processing for meat tenderisation [6].

Devakate et al. [7] used ammonium sulphate to precipitate bromelain or used ion exchange chromatography to purify bromelain from fruit crude juice followed by dialysis using dialysis membrane to remove salt. Finally, the desalted bromelain solution was dried using spray dryer or freeze dryer to produce bromelain powder. In this study, the bromelain powder from the pineapple crown extract was produced through purification, desalting followed by drying processes using cation exchange chromatography, continuous diafiltrator and freeze dryer, respectively. Initially, the physico-chemical properties of the pineapple crown extract was determined. Then, the bromelain activity in the pineapple crown extract, purified bromelain, desalted bromelain and bromelain powder was determined.

2. Materials and methods

2.1. Materials

The pineapple crown of variety N36, index 2 was obtained from Peninsula Plantations Sdn Bhd at Simpang Renggam, Johor, Malaysia. Analytical grade methanol and acetonitrile and food grade acetic acid, sodium hydroxide, hydrochloric acid and sodium chloride were purchased from Merck Sdn Bhd (Petaling Jaya, Selangor, Malaysia). All other chemicals of analytical grade including standard bromelain were purchased from Sigma Technologies Sdn Bhd (Petaling Jaya, Selangor, Malaysia). Custom-made cation exchange resin and diafiltrator were purchased from IT Tech Research (M) Sdn Bhd (Subang Jaya, Malaysia) and Isetake Enterprise (Kajang, Selangor, Malaysia), respectively.

2.2. Extraction

The pineapple crowns were cut into small pieces and blended using fruit juice processor with ratio of pineapple crown to purified water 1: 1. The extract was filtered through a muslin cloth. Then, the pineapple crown extract was centrifuged at 360 x g for 10 min at 4˚C. The clear supernatant was collected and used for analysis. The bromelain activity of the pineapple crown extract was determined.

2.3. Physico-chemical properties determination

The percentage of pulp of the pineapple crown extract was determined by using the centrifugal method [8]. pH was determined at room temperature using pH meter after being standardized with pH 4 and pH 7 buffers. The TSS was determined using an Abbe refractrometer. The total titratable acidity was determined by titration method [9]. The sugar content was determined using an analytical High Performance Liquid Chromatography (HPLC), Waters model 600 instrument with a Refractive Index detector model 2414 [10]. Acetonitrile and purified water (90: 10; v/v) was used as mobile phase. Sugar in the sample was quantified by comparing peak areas of the samples with those of the sugar standards such as fructose, glucose and sucrose.

The chromatography was run using a Carbohydrate High Performance 4µm (4.6mm x 250 mm cartridge) column at 18-22 °C, flow rate of 1.3 ml/min. Injection volume was 20 µl.

2.4. Production of bromelain powder and bromelain activity determination

Production of bromelain powder and bromelain activity determination was conducted according to the method by Devakate et al. [7] with slight modifications. Purification of the pineapple crown extract was carried out by Preparative HPLC using cation exchange resin column of 21.2 mm internal diameter and 250 mm length. The eluents used were acetate buffer (25mM, pH 4.0) and 1M NaCl solution. Removal of salt in the purified bromelain samples was carried out by continuous diafiltrator with hollow fiber membrane using purified water for exchange. Finally, the desalted bromelain sample was subjected to freeze dryer (Christ alpha 1-4LD Plus model) to produce powdered form bromelain. The bromelain activity of the bromelain powder at each step towards producing of powdered bromelain was determined using casein digestion unit (CDU) method.

2.5. Statistical Analysis

All data were expressed as mean ± standard deviation. Data were analyzed using one-way ANOVA using SPSS 15.0. Duncan's multiple-range test was used to determine the difference between means. A significant difference was considered at the level of $p < 0.05$.

3. Results and discussion

Table 1 shows that there was 2.41% of pulp in the pineapple crown extract. Therefore, the extract had to be filtered using membrane filter of 0.45µm before injected to Preparative HPLC. pH of the pineapple crown extract was 3.94 which indicate that bromelain in the pineapple crown extract was stable. This is based on Gautam et al. [11] who reported that bromelain activity is stable at pH 3.0 to 6.5. The TSS in the pineapple crown extract was 1.6˚Brix. In this study, it was found that glucose and fructose was the largest contributor to the total soluble solids which was in agreement with the finding by Anthon et al. [12] who studied on tomatoes. There was no sucrose in the pineapple crown extract. Therefore bromelain activity will not be affected because according to Ngampanya and Phongtongpasuk, [13] sucrose concentration could affect on protein content, crude bromelain activity and specific activity of induced shoots of pineapple var. 'Pattavia' grown in typical liquid culture system. They found that at higher concentration of sucrose more than 45 g/l, enzyme would be less accumulated in the cells.

Table 1. Physico-chemical properties of pineapple crown extract

Physico-chemical properties	Mean values
% pulp	2.41 ± 0.08
pH	3.94 ± 0.00
Total soluble solid	1.6 ± 0.0
% acid	0.30 ± 0.00
% fructose	0.83 ± 0.04
% glucose	0.51 ± 0.01

Values are expressed as mean ± standard deviation.

The bromelain activity in the pineapple crown extract, purified bromelain, desalted bromelain and bromelain powder is shown in Table 2. It was found that the bromelain activity was significantly highest in bromelain powder at the 5% level followed by those in purified bromelain, desalted bromelain and pineapple crown extract. After purification process, bromelain activity increased. This is because only bromelain was purified from the pineapple crown extract. Gautam et al. [11] reported that the presence of impurities is accompanied by a decrease in enzymatic activity.

Desalting of the purified bromelain sample was performed using continuous diafiltrator with hollow fiber membrane whereby the sample flow through the intracapillary space whereas the dialysis buffer is pumped in the extracapillary space. It was found that there was slight decreased in bromelain activity in desalted bromelain sample. The loss may have been the result from partial denaturation of the enzyme due to desalting [14]. Maurer [15] reported that bromelain rapidly deteriorates through self-digestion in aqueous solution.

After freeze dried, bromelain activity increased. This result is in agreement with Devakate et al. [7] who found that freeze drying of pineapple juice had produced bromelain powder with higher enzyme activity because of lower drying temperature. Furthermore, freeze drying reduces the risk of protein denaturation [16]. It was found that, after freeze dried, about 1.0g of bromelain powder was produced from 200ml of desalted bromelain solution or formerly 100g of pineapple crown leaves.

Table 2. Bromelain activity (CDU/mg) in samples

Samples	Bromelain activity (CDU/mg)
Pineapple crown extract	$426.49^{d} \pm 8.76$
Purified bromelain	$501.08^{b} \pm 3.31$
Desalted bromelain	$485.78^{c} \pm 8.76$
Bromelain powder	$529.77^{a} \pm 5.74$

Values are expressed as mean ± standard deviation.

4. Conclusion

From this study, it can be concluded that the pineapple crown extract was acidic and had no sucrose content which had stabilized bromelain activity. Hence, the bromelain activity not affected. It was found that the bromelain activity in the powdered form bromelain was higher compared to those in the extract, purified and desalted forms. Therefore it is recommended to produce bromelain in the powder form.

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