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Pineapple wastes: A potential source for bromelain extraction

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ABSTRACT

This study investigates the isolation and characterization of bromelain extract from the wastes of *Nang Lae* and *Phu Lae* pineapple cultivars (economical fruits of Chiang Rai province, Thailand). The waste portions such as the peel, core, stem and crown were 29–40%, 9–10%, 2–5% and 2–4% (w/w), respectively. The extract of crown from both cultivars gave the highest proteolytic activity and protein contents, while the extract from the stem exhibited the lowest values. SDS–PAGE showed that the major protein band in the extracts was ~28 kDa. Activity staining of the crown extracts from both cultivars confirmed that the major protein band showed caseinolytic activity on the casein substrate-gel. All of the crude extracts from both cultivars gave high caseinolytic activity (>80% relative) in a board pH range (3–9). The optimum temperatures for all crude extracts were about 50–60 °C. This study founded that there is much added value into local Thailand pineapple wastes because of bromelain extraction.

Keywords: Bromelain; Extraction; *Nang Lae*; *Phu Lae*; Pineapple; Waste utilization

1. Introduction

Thailand is the biggest exporter of cannery pineapple around the world. In 2008, ~2.5 million tons of pineapples were produced (FAO, 2008). Of that amount, 520,000 tons and 150,000 tons were exported as canned pineapple and pineapple juice, respectively. Chiang Rai province is one of the main areas for pineapple cultivating, especially in Nang Lae district, where pineapple is produced year-round (MOAC, 2008). In 2008, they produced around 15,000–18,000 tons. During pineapple processing, the crown and stem are cut off before peeling. The core is then removed for further processing. These wastes (peel, core, stem, crown and leaves) generally account for 50% (w/w) of total pineapple weight. Therefore, with increasing pineapple production, pineapple wastes are also proportionally increasing. Waste disposal represents a growing problem since it is usually prone to microbial spoilage and it causes serious environmental problems. The utilization of waste would be an innovation to handle the great deal of waste from processing.

Pineapple wastes are found to have potential uses as raw materials that can be converted into value-added products. In agricultural, waste is occasionally utilized as a fertilizer or animal feed. The peel is a rich source of cellulose, hemicelluloses and other carbohydrates. It has been used to produce paper, banknotes, and cloth (Bartholomew et al., 2003). The core waste could be used for the production of frozen pineapple juice concentrates or extracted juice for alcoholic beverages or for vinegar (Thanong, 1985). In addition, the waste from pineapple has been used as a nutrient substance in culture broth (Nigam, 1998) and cellulose production (Omojasola et al., 2008). Moreover, the pineapple wastes have also been used as substrates for the production of methane, ethanol, citric acid and antioxidant compounds (Tanaka et al., 1999; Nigam, 1999; Chau and David, 1995; Kumar et al., 2003; Imandi et al., 2008).

The utilization of pineapple wastes as a source of bioactive compounds, especially in proteolytic enzymes, is an alternative means. Bromelain and other cysteine proteases are well known enzymes present in different parts of pineapple (Ketnawa et al., 2010; Rolle, 1998; Schieber et al., 2001). Bromelain has been used commercially in the food industry, in certain cosmetics and in dietary supplements (Uhlig, 1998; Walsh, 2002). It is used for meat tenderizing, brewing, baking, as well as for the production of protein hydrolysates

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(Ketnawa and Rawdkuen, 2011; Walsh, 2002). Other applications are in tanning, for leather and textile industries, hair removal, wool, skin softening, and detergent formulations (Uhlig, 1998; Subhabrata and Mayura, 2006). Moreover, bromelain has been used as a folk medicine, a wound healer, an anti-inflammatory, and an anti-diarrhea and digestive aid (Bitange et al., 2008; Koh et al., 2006).

Because of this very wide range of applications, commercial bromelain is very expensive costing up to 2400 USD/kg. The objectives of this study were to extract bromelain from the pineapple wastes of the two cultivars, *Nang Lae* and *Phu Lae*, and to investigate some biochemical characteristics of the extracts.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), casein, L-tyrosine, glycine, sodium dodecyl sulfate (SDS) and Coomassie Brilliant Blue R-250 were purchased from Fluka, Switzerland. Bromelain from pineapple stem, acrylamide, *N*,*N*,*N*′,*N*′-methylene bisacrylamide, betamercaptoethanol (βME) were obtained from Sigma-Aldrich Co., LLC, USA. Ethylene diaminetetraacetic acid (EDTA) and trichloroacetic acid (TCA) were procured from BDH, UK. A molecular weight marker was obtained from Thermo Scientific, USA (Pierce®, Cat # 26681). All other chemicals used in the experiment were analytical grade.

2.2. Raw material

The pineapple (*Ananus comosus* L.) from *Nang Lae* and *Phu Lae* cultivars (Fig. 1A) were collected from a plantation in the Nang Lae district of Chiang Rai province, Thailand. The fruits were washed, air dried and then manually peeled. The different wastes (peel, core, crown, and stem as in Fig. 1B) were separated and then stored at 4 °C for the experiments. Each waste portion was determined and reported as a percentage of the proportion of a pineapple.

2.3. Preparation of crude extract

Each pineapple waste was chopped into small pieces before being blended (Philips HR-2011 Blender, China) with cold distilled water at a 1:1 ratio for 3 min. The resulting blend was filtered through a cheese cloth and then centrifuged at 10,000 × *g* at 4 °C for 20 min. The obtained supernatant (crude enzyme extract) was collected, recorded and used for pH measurement by using a pH meter (Eutech Instruments pH 510, Singapore). The total soluble solids were also measured by using a hand refractometer (Atago N1-E, Japan) and it was reported as degrees Brix.

2.4. Proteolytic activity determination

The proteolytic activity of the crude enzyme extracts was determined by the Murachi method (1976), using casein and L-tyrosine as a substrate and a standard, respectively. The extract (1.0 ml) was mixed with 1.0 ml of a reaction cocktail (contained 1% (w/v) of casein, 0.03 M cysteine, 0.006 M EDTA in 0.05 M phosphate, and a buffer pH 7.0). The reaction was carried out at 37 °C and was stopped by the addition of 3 ml of 5% (w/v) TCA. The reaction mixture was then centrifuged at 8000 × *g* for 10 min. The obtained supernatant was measured and showed an absorbance of 275 nm indicated by the soluble peptides. One unit of protease activity was defined as the amount of enzyme, releasing a product equivalent to 1 μg of tyrosine min^{-1} ml^{-1} under the standard assay conditions.

2.5. Protein content determination

Protein present in the crude enzyme extract was measured according to the Bradford method (1976) using BSA as a standard.

2.6. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE)

2.6.1. Protein pattern

SDS–PAGE was carried out by the method of Laemmli (1970) using 15% separating and 4% stacking gels. The samples were mixed with the sample buffer (0.5 M Tris–HCl, pH 6.8, 0.5% bromophenol blue, 10% glycerol, and 2% SDS) with and without βME at a ratio of 1:1 for reducing and non-reducing condition, respectively. The mixture was then boiled for 3 min. Four micrograms of protein were loaded in each well and then subjected to separate at 15 mA/gel by using Mini Protean Tetra Cell units (Bio-Rad Laboratories, Inc, Richmond, CA, USA). After separation, the protein was stained with Coomassie Brilliant Blue R-250 and destained with a methanol–acetic acid solution. A broad-range molecular weight standard marker containing myosin (215 kDa), phosphorylase B (120 kDa), bovine serum albumin (84 kDa), ovalbumin (60 kDa), carbonic anhydrase (39.2 kDa), trypsin inhibitor (28 kDa), and lysozyme (18.3 kDa) was used.

2.6.2. Activity staining

The bromelain activity in the protein band separated on the SDS–PAGE was verified by using activity staining according to the method of Garcia Carreno et al. (1993) with slight modification. Each well was loaded with 2 μg of the protein. After electrophoresis, the gel was immersed in 50 ml of 2% (w/v) casein in 0.05 M sodium phosphate buffer (pH 7.0), containing 0.03 M cysteine and 0.006 M EDTA, with a constant agitation at 4 °C for 45 min. The gel was then incubated at 37 °C for 30 min and then rinsed with distilled water, fixed, stained and destained as mentioned above. The bromelain activity was observed by developing clear zones against a dark background. The apparent molecular weight (MW) of the bromelain was estimated by comparing the reference distance (Rf) with those of molecular weight standard protein markers.

2.7. pH profile assay

The pH profile was determined by assaying the proteolytic activity in different pHs (3–10). Glycine (pH 3), sodium acetate (pH 4–5), sodium phosphate buffer (pH 6–7), Tris–HCl buffer (pH 8–10) were used. The residual proteolytic activity was measured and expressed as the relative proteolytic activity.

2.8. Temperature profile assay

Proteolytic activity of crude extract was performed at different temperatures (40, 50, 60, 70, 80, 90, and 100 °C) for 10 min. The assay was measured as mentioned above by using casein as a substrate. The caseinolytic activity was expressed as the relative proteolytic activity, compared with that of the control.