Thesis Title	Separation, characterization and application of bromelain from pineapple wastes in Chiang Rai
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ABSTRACT

This study investigated the extraction, isolation, characterization and application of bromelain extract from wastes of *Nang Lae* and *Phu Lae* pineapple cultivars. 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. The peel was considered as the source with the highest potential for bromelain extraction. The best extractant for bromelain extraction was sodium phosphate buffer pH 7.0 containing cysteine and EDTA (PB-CE) due to the highest bromelain activity obtained (867 and 1,032 units for *Nang Lae* and *Phu Lae* cultvivar, respectively). TCA-soluble peptides content of all the treated muscles (beef, chicken and squid) with bromelain extract increased when the amount of bromelain extract was increased ($P<0.05$). Reduction in myosin heavy chains (MHC) and actin (AC) was observed in the entire muscle types when bromelain extract was applied.

Bromelain from pineapple peel (*Nang Lae* and *Phu Lae* cultivars) was predominantly partitioned to the polyethylene glycol (PEG) rich phase in aqueous two-phase system (ATPS). For *Nang Lae* cultivar, the highest enzyme activity recovery (113.54%) with purification fold of 2.23 was observed in the top phase of 15% PEG2000-14% $MgSO_4$. The bromelain extract showed

the highest activity at pH 7.0 and 55°C. Its activity decreased continuously when concentration of NaCl was increased (up to 1.5%, w/v) ($P<0.05$). The β, α_1, α_2 of giant catfish skin collagen extensively degraded into lower MW proteins when treated with 0.02 units of the bromelain extract. For *Phu Lae* cultivar, the best ATPS condition for bromelain partitioning was 18% PEG6000-17% $MgSO_4$, which increased the purity by 3.44-fold and the activity recovery to 205.78%. The obtained bromelain showed the highest relative activity at pH 8.0. The highest activity of bromelain was found at 60°C and then decreased 70% after 5 min of incubation at 90°C. SDS-PAGE and activity staining showed that bromelain from crude extract of both cultivars had the MW approximately 28 kDa.

According to the activity recovery, TCA soluble peptides content and hydrolysis of muscle proteins, *Phu Lae* was selected for application study. The tenderizing effect of bromelain extract powder (0, 3, 7, and 20%, w/w) obtained from the top phase of ATPS comprising of 18% PEG 6000–17% $MgSO_4$ on muscle foods (beef, chicken and squid) was investigated. Lower of pH and moisture content were observed in the samples treated with bromelain extract, but the TCA-soluble peptides content significantly increased ($P<0.05$). Reduction of meat firmness, toughness, water holding capacity and cooking yields were observed when the concentration of bromelain extract was increased ($P<0.05$). Electrophoretic patterns also revealed extensive proteolysis of the treated samples. At the microstructural level, tissue fibers were broken; cell membranes were much more degraded and the generation of numerous gaps was clearly observed when 20% (w/w) of bromelain extract was added. From the results, the bromelain extract derived from two-phase extraction of pineapple peel could be used as an effective meat tenderizer. Results from this study suggests that local pineapple wastes are rich sources of bromelain and application of bromelain as meat tenderizer helps add value to such sources.

Keywords: Bromelain / *Nang Lae* / *Phu Lae* / Pineapple wastes / Aqueous two phase system / Meat tenderization