

Isolation and characterization of bromelain enzyme from pineapple and its utilization as anti-browning agent

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ABSTRACT

Bromelain is a complex mixture of proteases and non-proteases components found in pineapple (*Ananas cosmosus*). The objective of this study was to find out a suitable extraction and purification process. Then characterize the pulp and stem bromelain enzyme and compare the effectiveness of those enzyme as anti-browning agent with commercial anti-browning agents. Both the stem and pulp extracted with sodium citrate buffer had higher protein content 1.178 and 0.332 mg/mL, respectively. Enzyme activity of stem bromelain was higher in extracted with sodium citrate buffer (0.0031 U/mL) and enzyme activity of pulp bromelain was higher in distilled water extraction (0.0085 U/mL). Bromelain with ethanol precipitation had higher protein content and enzyme activity than ammonium sulfate. The results showed that ammonium sulfate precipitation was achieved higher purification fold (1.76) and activity yield 27.53%. Bromelain showed a maximum activity at pH 8.5 and at 50°C temperature for 30 min. Stem bromelain with 1% concentration had better anti-browning activity than ascorbic acid and citric acid. Although ethanol had achieved a lower purification fold but it had higher protein content and enzyme activity and it seems to be more suitable for bromelain recovery, based on process time, low ionic strength and low cost.

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1. Introduction

In recent year, enzymes production has been increased due to their numerous industrial and therapeutic applications. Proteases are the most significant of all industrial enzymes which represent about 60% of all commercial enzymes worldwide [1]. In the field of biotechnology and medicine, plant proteases have been gaining unique attention due to their exploitable properties. The most recognized plant proteases with greater commercial values are papain from *Carica papaya*, ficin from *Ficus spp.* and bromelain from pineapple plant (*Ananas cosmosus*) [2]. Pineapple (*Ananas cosmosus*) belongs to the Bromeliaceace family and is widely grown in Cuba, Hawaii, West Indies, Philippines, Malaysia, Brazil, Mexico and India. The quality of pineapple fruits is evaluated internally by broad range of constituents, especially by the high levels of sugars

and proteolytic enzymes, particularly bromelain [3]. Bromelain is a crude aqueous extract of protein digesting enzymes from stems and immature fruits of pineapple [4,5] and constitutes with an unusually complex mixture of different thiol endopeptidase, being able to break peptide bonds, separating proteins and amino acids [6]. Usually Stem bromelain (EC 3.4.22.32) and fruit bromelain (EC 3.4.22.33) are the two main enzymes extracted from stem and fruit respectively [7]. Bromelain is also present in pineapple wastes such as core, peel, crown & leaves in relatively smaller quantities as compared to those in the stem [5]. Stability of the bromelain enzyme is influenced by numerous factors such as pH, temperature and time. It is stable at pH 3.0-6.5 and the effective temperature range is 40–65°C with the optimum being 50–60°C. Previous works have been used ethanol [8], ammonium sulfate [6] and cold distilled water for isolation and purification of bromelain. Various modern technique like affinity chromatography, ion exchange, gel filtration,

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aqueous two phase extraction and extraction by using reversed micelle are also be used to extraction and purification of various enzyme from different sources [9]. But these techniques are complicated and production cost is so high [10,11]. Thus, ammonium sulfate precipitation and ethanol precipitation are frequently used due to simple equipment requirements, low energy needs and easy scale up. Ethanol precipitation is a promising technique and also could be recycled in the final process by simple distillation [12]. Bromelain has numerous applications in the food industry as well as in pharmaceutical industry. It has been used for meat tenderization, solubilization of grain proteins, stabilization of beer, baking cookies, production of protein hydrolysates, softening skins in leather and textiles [13]. Bromelain also act as an anti-browning agents [14]. Using anti-browning agents such as sulphites is a way to the prevention of enzymatic browning in fruit juices but it has health effect [15]. Therefore, the most attractive way to inhibit browning would be by natural methods [16]. Pineapple fruit proteases-bromelain is some of the proposed natural agents that have inhibitory effect on polyphenol oxidase (PPO) [14]. However, the inhibitory effect of stem bromelain as anti-browning agent was found to be minimal [14]. Therefore, in point of economic and technical aspects it is necessary to find out the suitable extraction and purification process for bromelain enzyme. The little information is available about effectiveness of extraction and purification of bromelain enzyme from stem and pulp of pineapple using ethanol and ammonium sulfate. To our best knowledge, no one compares the effectiveness of stem and pulp bromelain enzyme as an anti-browning agent in apple juice. Therefore, the purposes of the present investigation were to find out the suitable extraction and purification process of stem and pulp bromelain and compare its effectiveness as anti-browning agents with commercial anti-browning agents.

2. Experimental

2.1. Materials

Immature fruits of honey queen variety of pineapple and stem were collected from research field of Agro-forestry Department, Hajee Mohammad Danesh Science and Technology University, Dinajpur. Coomassie brilliant blue, casein, bovine serum albumin (BSA) and others reagents were purchased from local market. All the buffers and reagents were of analytical grade.

2.2. Methods

2.2.1. Preparation of crude extract from pineapple pulp and stem

- Extraction by distilled water

Extraction by distilled water was done by following Paulo et al. method with some modification [8]. The stem was separated by knife from pineapple fruit and washed with water to remove soil and dirt. After that pineapple fruits were peeled with a stainless steel knife to remove outer layer. Then cut into small pieces and crushed in a laboratory blender (KA-735, konka, China) with distilled water (1:1 w/w) to get approximately 115 mL of juice. The juice was filtered through a muslin cloth to remove the fibrous material. The

filtered juice was centrifuged (Biofuge 15R, USA) at 14000 rpm for 15 min at 4 °C to remove pellet. The clear supernatant obtained was used as crude extract and stored at -20°C for further uses.

- Extraction by sodium citrate buffer

This method was used for extraction of bromelain by sodium citrate buffer as described by Srinath et al. [6]. The stem and small size of peeled pineapple were dried at 55°C at several hours using a cabinet drier. 10 g of peeled pineapple and stem were blended in a blender (KA-735, konka, China) by adding 90 mL of pre-cooled sodium citrate buffer and filtered using a muslin cloth to remove fiber. After that it was centrifuged (Biofuge 15R, USA) at 5000 rpm for 15 min. The pellet was discarded and the supernatant used as crude protease was stored at -20°C for further uses.

- Extraction by citric phosphate buffer

Bromelain extraction using citric phosphate buffer was followed according to Chaiwut et al. with some modification [17]. 10 g of the dried samples were soaked in 90 mL of 0.1 M citric phosphate buffer (pH 6.5) for 10 min and then filtered. The filtrate was centrifuged (Biofuge 15R, USA) with 14000 rpm at 4°C for 15 min and supernatant was collected and finally stored at -20°C for further uses.

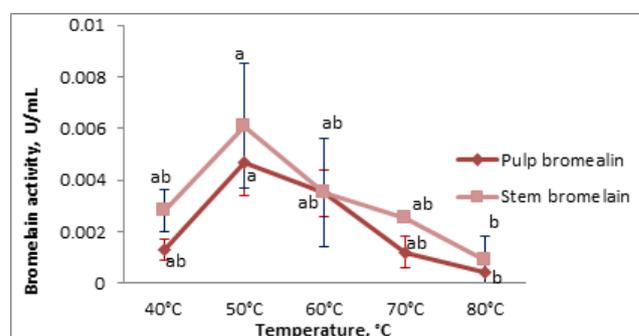


Fig. 1 - Effect of temperature on bromelain activity. ^{a-b} Means followed by different superscript in each sample are significantly different among various temperature ($p \leq 0.05$).

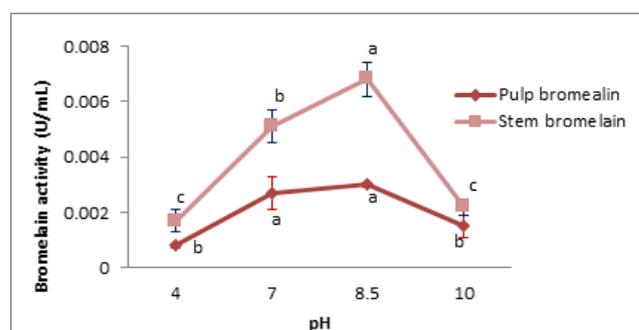


Fig. 2 - Effect of pH on bromelain activity. ^{a-c} Means followed by different superscript in each sample are significantly different among various pH ($p \leq 0.05$).

Table 1 - Protein content, enzyme activity and specific activity of pulp and stem bromelain enzyme extracted by distilled water, citric phosphate buffer and sodium citrate buffer on bromelain extraction.

Samples	Extraction methods	Protein content (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)
Pulp	Distilled water	0.151 ± 0.004 ^b	0.0085 ± 0.0002 ^a	0.056 ± 0.0002 ^a
	Citric phosphate buffer	0.197 ± 0.001 ^b	0.0036 ± 0.0002 ^b	0.018 ± 0.0020 ^b
	Sodium citrate buffer	0.332 ± 0.008 ^a	0.0040 ± 0.0005 ^b	0.012 ± 0.0020 ^b
Stem	Distilled water	0.182 ± 0.004 ^b	0.0020 ± 0.0002 ^b	0.011 ± 0.0010 ^a
	Citric phosphate buffer	0.212 ± 0.005 ^b	0.0025 ± 0.0005 ^{a-b}	0.012 ± 0.0030 ^a
	Sodium citrate buffer	1.178 ± 0.080 ^a	0.0031 ± 0.0006 ^a	0.003 ± 0.0003 ^a

^{a-b} Means followed by different superscript in each column are significantly different among distilled water, citric phosphate buffer and sodium citrate buffer extraction ($p \leq 0.05$).

Table 2 - Protein content, enzyme activity, specific activity, purification fold and yield of pulp bromelain enzyme purified by ammonium sulfate and ethanol precipitation.

Samples	Extraction methods	Protein content (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)	Purification fold	Yield (%)
Pulp	Distilled water	^B 0.076 ± 0.003 ^b	^{NS} 0.0046 ± 0.0006 ^a	^{NS} 0.061 ± 0.0090 ^a	^{NS} 1.09 ± 0.173 ^a	^{NS} 7.48 ± 0.39 ^a
	Citric phosphate buffer	^{NS} 0.136 ± 0.010 ^b	^{NS} 0.0023 ± 0.0007 ^a	^{NS} 0.018 ± 0.0060 ^b	^{NS} 0.95 ± 0.267 ^a	^{NS} 11.14 ± 0.87 ^a
	Sodium citrate buffer	^{NS} 0.272 ± 0.020 ^a	^{NS} 0.0027 ± 0.0006 ^a	^{NS} 0.010 ± 0.0014 ^b	^{NS} 0.84 ± 0.005 ^a	^{NS} 10.01 ± 1.07 ^a
Stem	Distilled water	^A 0.136 ± 0.003 ^b	0.0048 ± 0.0001 ^a	0.036 ± 0.0021 ^a	0.65 ± 0.063 ^a	7.88 ± 0.18 ^a
	Citric phosphate buffer	0.166 ± 0.009 ^b	0.0028 ± 0.0003 ^a	0.017 ± 0.0007 ^b	0.91 ± 0.115 ^a	11.25 ± 3.51 ^a
	Sodium citrate buffer	0.302 ± 0.006 ^a	0.0028 ± 0.0010 ^a	0.010 ± 0.0035 ^b	0.83 ± 0.418 ^a	12.34 ± 8.75 ^a

^{a-b} Means followed by different superscript in each column are significantly different among distilled water, citric phosphate buffer and sodium citrate buffer extraction ($p \leq 0.05$).

^{A-B} Means followed by different superscript in each column are significantly different among extraction methods, between ammonium sulfate and ethanol ($p \leq 0.05$).

^{NS} means non-significant.

Table 3 - Protein content, enzyme activity, specific activity, purification fold and yield of stem bromelain enzyme purified by ammonium sulfate and ethanol precipitation.

Purification methods	Extraction methods	Protein content (mg/mL)	Enzyme activity (U/mL)	Specific activity (U/mg)	Purification fold	Yield (%)
Ammonium Sulfate	Distilled water	^B 0.121 ± 0.004 ^b	^{NS} 0.0017 ± 0.0009 ^a	^{NS} 0.014 ± 0.0084 ^a	^{NS} 1.26 ± 0.631 ^a	^{NS} 14.93 ± 5.40 ^a
	Citric phosphate buffer	^{NS} 0.166 ± 0.004 ^b	^{NS} 0.0024 ± 0.0006 ^a	^{NS} 0.015 ± 0.0035 ^a	^{NS} 1.31 ± 0.598 ^a	^{NS} 27.53 ± 5.26 ^a
	Sodium citrate buffer	^B 0.499 ± 0.018 ^a	^{NS} 0.0025 ± 0.0002 ^a	^{NS} 0.005 ± 0.0007 ^a	^{NS} 1.76 ± 0.463 ^a	^{NS} 14.31 ± 3.91 ^a
Ethanol	Distilled water	^A 0.151 ± 0.003 ^b	0.0019 ± 0.0002 ^a	0.012 ± 0.0014 ^a	1.13 ± 0.272 ^a	13.95 ± 6.46 ^a
	Citric phosphate buffer	0.182 ± 0.010 ^b	0.0025 ± 0.0007 ^a	0.014 ± 0.0035 ^a	1.22 ± 0.578 ^a	14.14 ± 6.53 ^a
	Sodium citrate buffer	^A 0.982 ± 0.006 ^a	0.0030 ± 0.0002 ^a	0.003 ± 0.0000 ^a	1.16 ± 0.127 ^a	13.08 ± 2.19 ^a

^{a-b} Means followed by different superscript in each column are significantly different among distilled water, citric phosphate buffer and sodium citrate buffer extraction ($p \leq 0.05$).

^{A-B} Means followed by different superscript in each column are significantly different among extraction methods, between ammonium sulfate and ethanol ($p \leq 0.05$).

^{NS} means non-significant.

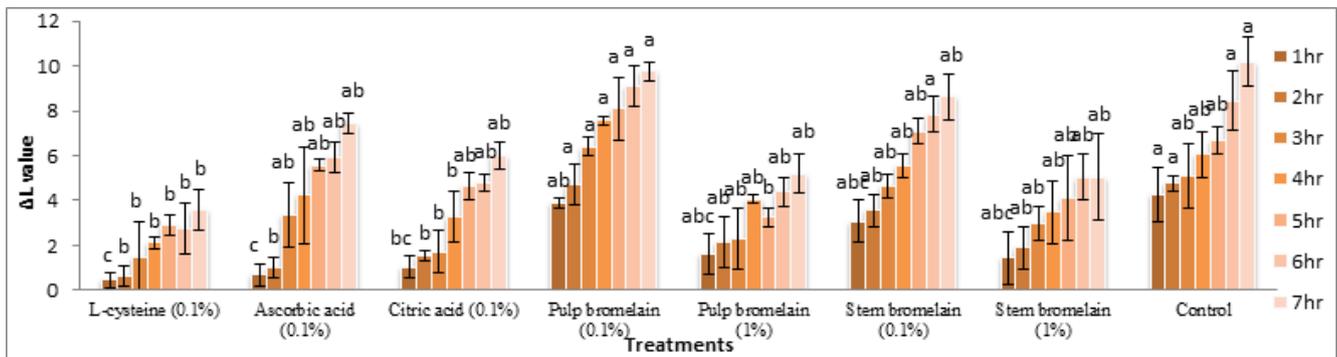


Fig. 3 - Browning of apple juice at different time interval treated with commercial anti-browning agents and bromelain enzyme. ^{a-c} Means followed by different superscript in each hour are significantly different among various anti-browning agents ($p \leq 0.05$).

2.2.2. Purification of crude protease

Crude protease which was collected from distilled water, sodium citrate buffer and citric phosphate buffer extraction, was taken for purification by using ammonium sulfate precipitation and ethanol precipitation.

- Ammonium sulfate precipitation

Ammonium sulfate precipitation was performed by the method as described by Srinath et al. with some modification [6]. 15 ml of crude stem and pulp were mixed along with 9 g of ammonium sulfate to get 60% saturation under constant stirring using a magnetic stirrer for 10 min. The salt enriched solution was then subjected to centrifugation (Biofuge15R, USA) at 14000 rpm for 15 min and the precipitate was collected. After that, precipitate was dissolved with distilled water, sodium citrate buffer and citric phosphate buffer, respectively, and then the solution was poured into dialysis tube and sealed. Then the dialysis tube was soaked in distilled water and kept overnight at 4°C. Distilled water was changed subsequently over the period of 12 h.

- Ethanol precipitation

Bromelain precipitation was performed according to methodology as described by England and Seifter with some modification [18]. 15 ml of crude stem and pulp were taken and 98% cold ethanol was added drop wise until concentration of 70% (w/w) was reached. The solution was then centrifuged (Biofuge 15R, USA) at 14000 rpm for 15 min at 4°C and the resulting pellet was suspended in 1.0 mL of phosphate buffer (0.03 M, pH 7.0) and stored at -8°C for further uses.

2.2.3. Determination of protein content

Protein content in the samples was measured by spectrophotometer according to Bradford with little modification [19].

2.2.4. Enzyme activity assay

Enzyme activity was determined according to the Liggieri et al. with slight modification [20]. Purified enzyme (0.1 mL) was mixed with 1.1 mL of 1% casein containing 0.1 M Tris-HCl buffer (pH 8.5).

Then incubated at 42°C for 2 min and 1.8 mL of 5% trichloroacetic acid (TCA) was then added to stop the reaction. After that, centrifuged (MF-300, Korea) at 3000 rpm for 30 min and the absorbance of the supernatant were measured at 280 nm. The enzyme unit (Ucas) was defined as the amount of enzyme (g) that produces an increase of one absorbance unit per minute in the assay conditions [21].

2.2.5. pH stability of bromelain enzyme

The pH stability of bromelain enzyme was estimated by using azo-casein as a substrate.

2.2.6. Thermal stability of bromelain enzyme

The effect of temperature on bromelain enzyme was evaluated by azo-casein assay. The bromelain was subjected to various temperatures (40 to 80° C).

2.2.7. Application of bromelain enzyme on apple juice as natural anti-browning agents compare to commercial anti-browning agents

Fresh apples were purchased from local market and were washed, peeled, de-stoned and juiced using kitchen blender (KA-735, konka, China). 10 mL of juice samples were transferred into beakers containing anti-browning agents (L-cysteine, citric acid, ascorbic acid, pulp bromelain and stem bromelain) and stirred with a vortex for 10 s [22]. The concentration of the commercial anti-browning agents was 0.1%, while concentrations of 0.1% and 1% were chosen for stem and pulp bromelain enzyme, respectively [6]. Without any anti-browning agent was used as control. The degree of browning was calculated by following formula [23]:

$$\Delta L = (L^* \text{ value at initial} - L^* \text{ value at given time})$$

Where, L^* values were measure using a spectrophotometer (Minolta Camera, Tokyo, Japan).

2.2.8. Statistical Analysis

Each experiment was done in triplicate. The results were expressed as mean \pm standard deviation and were analyzed by R (version 2.13.1). Significant differences between the means were determined by Duncan's Multiple Range test. $P \leq 0.05$ was considered as a level of significance.

3. Results and discussion

3.1. Protein content, enzyme activity and specific activity of crude extract

Table 1 shows the effects of distilled water, citric phosphate buffer and sodium citrate buffer on protein content, enzyme activity and specific activity of bromelain enzyme from crude extract of pineapple stem and pulp. It was observed that the protein content of crude extract of pulp and stem ranged from 0.151–0.332 mg/mL and 0.182–1.178 mg/mL, respectively. Protein content was significantly highest in sodium citrate buffer extract whereas in distilled water extraction recorded lowest protein content for both samples. This variation might be difference in protease composition between pulp and stem. These results were lower than pineapple pulp (0.871 mg/mL) extracted by sodium citrate buffer and higher than that of stem and bark of ripe pineapple (0.23 mg/mL) extracted with deionized water [6,8]. This variation may be due to difference in source material used to extract enzyme. The enzyme activity of crude extracts was found 0.0040–0.0085 U/mL for pulp whereas for stem it was 0.0020–0.0031 U/mL. Significantly maximum enzyme activity was observed at sodium citrate buffer (0.0031 U/mL) for stem, while maximum enzyme activity was found to be at distilled water (0.0085 U/ml) for pulp. These results opposed to Paulo et al. and Martins et al. who got enzyme activity of crude extract 2.86 U/mL and 16.25 U/mL, respectively [8,24]. The highest specific activity of bromelain enzyme was found in stem (0.012 U/mg) and pulp (0.056 U/mg) extracted with citric phosphate buffer and distilled water, respectively. This might be due to the lower enzyme activity and higher protein concentration.

3.2. Ammonium sulfate and ethanol precipitation for bromelain purification

The protein content of pulp and stem bromelain ranged from 0.076–0.272 mg/mL (Table 2) and 0.121–0.499 mg/mL (Table 3) in ammonium sulfate precipitation, respectively and 0.136–0.302 mg/mL (Table 2) and 0.151–0.982 mg/mL (Table 3) in ethanol precipitation, respectively. Significantly highest protein content was observed at ethanol precipitation than ammonium sulfate precipitation. It might be due to low ionic strength of ethanol than ammonium sulfate. These values were higher than Martins et al. who reported 0.084 mg/mL protein in stem, bark and leaves after 30–70% ethanol precipitation and consistent with Paulo et al., who observed that 0.20 mg/mL protein remained in stem, bark of pineapple after 70% ethanol precipitation [8,24]. The highest enzyme activity was found to be 0.0046 U/mL in pulp bromelain precipitated by ammonium sulfate and in ethanol it was 0.0048 U/mL extracted with distilled water. On the other hand, stem bromelain purified by ammonium sulfate and ethanol had highest enzyme activity in sodium citrate extraction. These results contrasted with Paulo et al. and Martins et al., who reported that enzyme activity after 70% ethanol precipitation was 2.77 U/mL and 15.96 U/mL, respectively [8,24]. The specific activity was observed significantly higher in pulp (0.061 U/mg) and stem bromelain (0.015 U/mg) extracted by distilled water in ammonium sulfate precipitation (Table 2 and Table 3). A relatively much higher specific

activity (189.5 U/mg and 13.85 U/mg) in the stem of the pineapple [8,24].

In case of ammonium sulfate precipitation, the purification fold and yield was ranged from 0.84–1.09 and 7.48–11.14% for pulp bromelain, respectively in Table 2. The quantification results were consistent with the findings of Paulo et al. who found purification fold of 4.4 and 44% yield after bromelain precipitate with ammonium sulfate [8]. Although highest purification fold was found in pulp extracted with distilled water (1.09) but activity yield was higher in pulp extracted with citric phosphate buffer (11.14 %) in Table 2. The highest purification fold was determined in stem extracted with sodium citrate buffer (1.76) and activity yield was highest in stem extracted with citric phosphate buffer (27.53%) in Table 3. These results were lower than fruit bromelain (2.97 fold) precipitated by ammonium sulfate obtained [25].

In case of ethanol precipitation, the purification fold was ranged from 0.65–0.91 and 1.13–1.22 in pulp and stem bromelain, respectively. The highest yield was 12.34% in pulp extracted with sodium citrate buffer and in case of stem, extracted with citric phosphate buffer was higher yield (14.14 %). The quantification results of this study were similar with the findings of Rabelo et al. who found the purification fold 1.25 purified with aqueous-two phase system [26]. There was no significant difference between ammonium sulfate and ethanol precipitation in terms of purification fold and yield.

3.3. Temperature and pH effect on bromelain

The enzyme activity increased with increasing temperature until it reached 50°C, where it began to decline rapidly (Fig. 1) for both stem and pulp. Thus the enzyme is inactivated at high temperature due to the partial unfolding of its molecule [27]. This result is compatible with those reported by Srinath et al. where maximum enzyme activity was found at 50°C [6]. Liang et al. reported that the optimum temperature for the activity of bromelain was found to be at about 55°C [28]. The result is contrasted with the ones described by Ketnawa et al., Valles et al. and Koh et al. [29–31]. At those studies, the maximum activity was reached at 60, 63 and 60°C, respectively. As the temperature increases, more molecules have kinetic energy to undergo the reaction. After the temperature is raised above the optimum temperature, a biochemical threshold, the systems energy is so high that peptide bonds and disulfide bonds are disrupted, therefore inactivating the enzymes. The stability of bromelain incubated with various pH buffers is shown in Fig. 2. Both samples of bromelain were able to retain most of the activity at pH 8.5. The enzyme activity slightly reduced in the acidic area, while it was dramatically lost at pH above 8.5. This result is similar to Liang et al., who reported that the bromelain has a wider pH range for optimum activity at 6.8–9.0 [28]. High enzyme activity was observed in pH ranging from 6.5 to 8.0, and maximum activity was near pH 7.0 [29]. Under the very acidic and alkaline pH, the charge repulsion associates with a decrease in electrostatic bonds [32].

3.4. Comparing bromelain as anti-browning agent on apple juice with commercial anti-browning agents

From Fig. 3, we see that bromelain is a weak anti browning agent when compared with other. Amongst the anti-browning agents L-

cysteine was significantly most effective followed by citric acid, ascorbic acid, pulp and stem bromelain. These results are compatible with those reported by Tochi et al. who said that L-cysteine is a more effective browning inhibitor as compared to either ascorbic acid or stem bromelain [14]. The bromelain extracted from pineapple stem was significantly more capable for preventing browning than the bromelain extracted from the pulp. This can be explained by the fact that protein content was more in stem part than pulp part. Lower concentration 0.1% of stem bromelain was not so potent anti-browning agent whereas higher concentration 1% stem bromelain proved to be better anti-browning agent than ascorbic acid and citric acid. Similar result was found by Srinath et al., who said that bromelain extracted from the fruit pulp had better anti-browning agent than ascorbic acid and acetic acid with a concentration of 1% [6]. Because, the effectiveness of ascorbic acid as anti-browning agent is temporary [33] and it can be easily oxidized by endogenous enzyme, as well as decomposed by auto oxidation [34].

4. Conclusion

In the present study, the bromelain was extracted from pineapple stem and pulp with distilled water, citric phosphate buffer and sodium citrate buffer and purified by ammonium sulfate and ethanol. The highest protein content was in bromelain extracted with sodium citrate buffer and lowest was in bromelain extracted with distilled water. Enzyme activity was higher in stem bromelain extracted with sodium citrate buffer whereas enzyme activity was higher in pulp bromelain extracted with distilled water. Bromelain with ethanol precipitation had higher protein content and enzyme activity but lower purification fold and yield than ammonium sulfate precipitation. Bromelain showed a maximum activity at pH 8.5 and at 50°C temperature for 30 min. As observed stem bromelain at a concentration of 1% had the potential to be a better agent than ascorbic acid and citric acid. Although ethanol had achieved a lower purification fold but it had higher protein content and enzyme activity and it seems to be more suitable for bromelain recovery, based on process time, low ionic strength and low cost.

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REFERENCES

- [1] D. Leary, M. Vierros, G. Hamon, S. Arico, C. Monagle, Marine genetic resources: a review of scientific and commercial interest. *Marine Policy*, 33 (2009) 183-94.
- [2] V.K. Dubey, M. Pande, B.K. Singh, M.V. Jagannadham, Papain-like proteases: applications of their inhibitors. *African Journal of Biotechnology*, 6 (2007) 1077-1086.
- [3] R.V. Devakate, V.V. Patil, S.S. Waje, B.N. Thorat, Purification and drying of bromelain. *Separation and Purification Technology* 64 (2009) 259-264.
- [4] L.P. Hale, P.K. Greer, C.T. Trinh, C.L. James, Proteinase activity and stability of natural bromelain preparations. *International Immunopharmacology* 5 (2005a) 783-793.
- [5] H.U. Hebbar, B. Sumana, K.S.M.S. Raghavarao, Use of reverse micellar systems for the extraction and purification of bromelain from pineapple waste. *Bioresource Technology* 99 (2008) 4896-4902.
- [6] R. Srinath, C. Ramalingam, N.N. Islam, Isolation and characterization of Bromelain from pineapple (*Ananas Comosus*) and comparing its anti-browning activity on apple juice with commercial antibrowning agents. *Food Science* 45 (2012)7822-7826.
- [7] B.R. Babu, N.K. Rastogi, K.S.M.S. Raghavarao, Liquid-liquid extraction of bromelain and polyphenol oxidase using aqueous two-phase system. *Chemical Engineering and Processing* 47 (2008) 83-89.
- [8] A.G.S. Paulo, F.M.V. Antônio, T.S.C. Maria, P.J. Adalberto, G.C. Maria, Purification of bromelain from pineapple wastes by ethanol precipitation. *Separation and Purification Technology* 98 (2012) 389-395.
- [9] M.A. Desai, *Downstream Processing of Proteins: Methods and Protocols*, first edition, Humana Press, New Jersey 2000.
- [10] B. Hans-Jorg, P. Stephan, *Industrial Scale Natural Products Extraction*, first edition. Wiley-VCH, Weinheim (2011).
- [11] R. Hutti-Kaul, B. Mattiasson, *Isolation and purification of proteins*, first edition. Marcel Dekker, New York (2003).
- [12] S. Golunski, V. Astolfi, N. Carniel, D. Oliveira, M. Di Luccio, M.A. Mazutti, H. Treichel, Ethanol precipitation and ultrafiltration of inulinases from *Kluyveromyces marxianus*. *Separation and Purification Technology* 78 (2011) 261-265.
- [13] G. Walsh, *Protein biochemistry and biotechnology* (1st edition). New York: John Wiley and Sons (2002).
- [14] B.N. Tochi, Z. Wang, S.Y. Xu, W. Zhang, Effect of stem bromelain on the browning of apple juice. *American Journal of Food Technology* 4 (2009) 146-153.
- [15] Anon, Sulphites banned. *Food Ingredients Process, International*. 11 (1991) 11-11.
- [16] E. Laurila, R. Ahvenainen, Minimal processing of fresh fruits and vegetables. In: *fruits and vegetable processing-improving quality*, Wim, J. (Editors). CRC Press, Washington. DC (2002) 1-22.
- [17] P. Chaiwut, S. Nitsawang, L. Shank, P. Kanasawud, A comparative study on properties and proteolytic components of papaya peel and latex proteases. *Chiang Mai Journal of Science* 34 (2007) 109-118.
- [18] S. Englard, S. Seifter, *Precipitation techniques.in: Guide to protein purification*, Editors. Deutscher, M.P., Academic Press, San Diego, USA (1990).
- [19] M.M. Bradford, A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry* 72 (1976) 248-254.
- [20] C. Liggieri, W. Obregón, T. Sebastián, N. Priolo, Biochemical analysis of a papain-like protease isolated from the latex of *Asclepias curassavica* L. *Acta Biochimica Biophysica Sinica* 41 (2009) 154-162.
- [21] H.M. Manjunath, G.J. Chandrashekhar, R.Vijay, M. Mahesh, Comparative Analysis of Papain from Different varieties of Papaya Plant Latex. *International Journal of Agricultural and Food Science*, 4 (2014) 123-127.
- [22] H. Ozoglu, A. Bayindirli, Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents. *Food Control*, 13 (2002) 213-221.
- [23] C.M. Ley, A. Tsiami, Q. Ni, N. Robinson, A review of the use of bromelain in cardiovascular diseases. *Chinese Journal of Integrative Medicine* 9 (2011) 702-710.
- [24] B.C. Martins, R. Rescolino, D.F. Coelho, B. Zanchetta, E.B. Tambourgi, E. Silveir, Characterization of Bromelain from

- Ananas Comosus Agroindustrial Residues Purified by Ethanol Fractional Precipitation. The Italian Association of Chemical Engineering (2014) 781-786.
- [25] R.V. Devakate, V.V. Patil, S.S. Waje, B.N. Thorat, Purification and drying of bromelain. Separation and Purification Technology 64 (2009) 259-264.
- [26] A.P.B. Rabelo, E.B. Tambourgi, J. A. Pessoa, Bromelain partitioning in two-phase aqueous systems containing PEO-PPO-PEO block copolymers. Journal of Chromatography B 807(2004) 61-68.
- [27] S. Klomkiao, S. Benjakul, W. Visessanguan, H. Kishimura, B.K. Simpson, Purification and characterisation of trypsins from the spleen of skipjack tuna (*Katsuwonus pelamis*). Food Chemistry, 100 (2007) 1580-1589.
- [28] H.H. Liang, H.H. Huang, K.C. Kwok, Properties of tea-polyphenol complexed bromelain. Food Research International 32 (1999) 545-551.
- [29] S. Ketnawa, P. Chaiwut, S. Rawdkuen, Pineapple wastes: A potential source for bromelain extraction. Food and Bioproducts Processing 90 (2012)385-391.
- [30] D. Valles, S. Furtado, A.M. Cantera, Characterization of new proteolytic enzymes from ripe fruits of *Bromelia antiochana* Bertol. (Bromeliaceae). Enzyme and Microbial Technology 40 (2007) 409-413.
- [31] J. Koh, S. M. Kang, S. J. Kim, M. K. Cha, Y. J. Kwon, Effect of pineapple protease on the characteristics of protein fibers. Fibers and Polymer 7 (2006) 180-185.
- [32] S. Benjakul, W. Visessanguan, K. Leelapongwattana, Purification and characterization of heat-stable alkaline proteinase from bigeye snapper (*Priacanthus macracanthus*) muscle. Comparative Biochemistry and Physiology 13 (2003) 579-591.
- [33] P. Komthong, N. Igura, M. Shimoda, Effect of ascorbic acid on the odours of cloudy apple juice. Food Chemistry, 100 (2007) 1342-1349.
- [34] H. Ozoglu, A. Bayindirli, Inhibition of enzymic browning in cloudy apple juice with selected antibrowning agents. Food Control, 13 (2002) 213-221.

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