Extraction of Bromelain Enzyme from Wastes of Pineapple and Its Application on Food Chemistry

Kalyar Min Min Htaik[1], Ohnmar Aung[2], Thwe War Aung[3]

Abstract

The increasing production of pineapple processed items, results in massive waste generations. If these wastes discharge to the environment untreated they could cause a serious environment problem. Its waste materials could be utilized for further industrial purpose. The aim of the present work is to study the activities of bromelain enzyme extracted from wastes of pineapple (peel and core) and its application in milk clotting. Bromelain is the chief protease enzyme found in pineapple plant (*Ananas comosous* (L.) Merr). Bromelain enzyme hydrolyzed casein substrate to tyrosine as a major product. The liberated tyrosine product was determined spectrophotometrically at 600 nm by using Folin-Ciocaltue's reagent. According to the results, both of pineapple wastes have protease enzyme activities and the activity of bromelain enzyme extracted from peel of pineapple is greater than that of core. The enzymic properties such as optimum pH and optimum temperature of extracted enzyme were determined by using spectroscopic method. The optimum pH and optimum temperature of bromelain from peel and core were found to be pH 7.2 and 60 °C, respectively. Application of bromelain enzyme for milk clotting was done in this research. The milk clotting time for 10 mL milk per 0.5 mL of peel extracted enzyme was 70 min and 10 mL milk per 0.5 mL of core extracted enzyme was 90 min at 60°C, respectively. The milk-clotting technique is widely used in the production of milk product such as cheese, yoghurt, etc.

Keywords: Wastes of pineapple, protease enzyme, bromelain, casein, tyrosine, milk clotting

Introduction

Pineapple Wastes

The pineapple (*Ananas comosus* (L.) Merr) is one of the most important fruits in the world and is the leading edible member of the family Bromeliaceae. This fruit juice is the third most preferred worldwide after orange and apple juices (Cabrera *et al*., 2000). Pineapple by-products are not exceptions and they consist basically of the residual pulp, peels, stem and leaves. These wastes are usually prone to microbial spoilage thus limiting further exploitation. Further, the drying, storage and shipment of these wastes is cost effective and hence efficient, inexpensive and eco-friendly utilization is becoming more and more necessary.

Their utilization has become one of the main important and challengeable aspects due to the generation of large quantities of by products including peels, seeds, leaves and core. The solid waste from pineapple canning process was estimated about 40- 50 % from fresh fruit as pineapple peels and core. Pineapple wastes are used for extraction of bromelain enzyme, production of bioethanol, phenolic antioxidants, organic acids, biogas and fiber production.

Enzyme

Enzymes are organic catalysts that speed metabolic reactions. The chemical reactions occurring in living things are controlled by enzymes (Mader, 2006). Without enzymes, most biochemical reactions would be too slow to even carry out life processes. They are biological catalysts with three characteristics. First, the basic function of an enzyme is to increase the rate of reaction. Second, most enzymes act specifically with only one reactant (substrate) to

[1,2] Lecturer, Department of Chemistry, Dagon University
[3]Lecturer, Department of Chemistry, Magway University

produce products. Third, enzymes are regulated from a state of low activity to high activity and vice versa. A living system controls its activity through enzymes (Virtual Chembook, 2019).

Protease enzyme

Protease enzymes catalyze the hydrolysis of the peptide bonds between amino acid residues of proteins. They are often referred to as proteases or proteolytic of peptidases. Proteases have been used for a long time for the benefit of humans. Proteases enzymes are produced by all life forms - plants, animals, and microorganisms (Kumar *et al*., 2010).

Bromelain enzyme

Bromelain is the name of a group of powerful protein-digesting or proteolytic enzymes that are found in pineapple plant (*Ananas comosous* (L.) Merr). Sulfhydryl proteolytic enzymes are the chief constituents of bromelain (Gautam *et al*, 2010). Bromelain is abundant in stem and fruit of pineapple plant and it can also be isolated in small amount from pineapple waste such as core, leaves, peel etc. Bromelain enzyme hydrolyzes protein to oligopeptides and amino acids. Bromelain enzyme plays a very important physiological role by interventing in metabolic reactions and protecting vegetable from the attack of infection and decreases. Bromelain has been widely used in food, medical, cosmetic, pharmaceutical and textile industries.

Materials and Methods

Extraction of bromelain from wastes of pineapple (peel and core)

In this research, bromelain enzyme was extracted from pineapple wastes such as peel and core by using phosphate buffer solution (pH 7.0). Pineapple was purchased from Sanpya Market, Thingangyun Township, Yangon Region. Peel of pineapple (50 g) was weighed and placed in a blender. Then (100 mL) of phosphate buffer (pH 7.0) solution was added and the mixture was blended. It was allowed to response for 20 minutes and then filtered through a cheese cloth. The solution was centrifuged at (3000 rpm) for 30 min. Supernatant containing crude bromelain enzyme was stored at 4 °C. Using the same procedure, the extraction of bromelain enzyme from core of pineapple was also extracted.

Determination of the maximum absorption of standard tyrosine solution

The wavelength of maximum absorption should be chosen for determination of a substance when using an ultraviolet-visible spectrophotomer (Burstone, 1962). In the determination of wavelength of maximum absorption of standard tyrosine solution, the absorption spectrum of tyrosine was recorded in the range from 400 to 800 nm.

Construction of calibration curve for standard tyrosine solution

After deciding upon the conditions for the analysis, it is necessary to prepare a calibration curve from a series of standard solution. In this research, a calibration curve was constructed by standard tyrosine solutions (55.18, 27.16, 13.79, 3.49 and 1.72 mM) and their absorbances were measured at 600 nm**.**

Enzyme assay

Enzyme solution (0.5 mL) was added to 1 mL of 1 % casein solution. They were mixed well. After 20 min incubation time, 1 mL of 5 % trichloroacetic acid solution was added to the reaction mixture in order to terminate the enzyme reaction.

After 20 min, 1 mL of supernatant was added into a test tube. A 5 mL of 0.5 M sodium carbonate solution and 1 mL of 1 M sodium hydroxide solution were added to the test tube. After 10 min, a 0.5 mL of Folin-Ciocalteu reagent was added to the test tube and mixed. The mixture was allowed to stand for 30 min and it was measured for absorbance at 600 nm by using a UV-visible spectrophotometer (Mukhtar and Ikram, 2008). A blank solution was prepared by carrying out the procedure as described above except that 0.5 mL of phosphate buffer was used instead of 0.5 mL of enzyme solution.

Determination of optimum pH and temperature of crude bromelain enzyme

The optimum pH of the enzyme was determined by using the enzyme solution of different pH value ranging from pH 5.2 to 8.0. For the determination of the optimum temperature, the enzyme reaction was conducted at temperatures between 30 °C and 80 °C.

Determination of milk clotting time for bromelain enzyme

Each 10 mL of milk was placed in three beakers. This is labelled (A, B and C). A 0.5 mL of peel extracted enzyme was added in beaker B and 0.5 mL of core extracted enzyme was added in beaker C. The three beakers are placed in an incubator controlled, at a temperature of 60 ˚C. After 70 min and 90 min, it was found that the milk in beaker (B) and (C) were clotted.

Results and Discussion

Crude bromelain enzyme from pineapple waste

In this research, crude bromelain enzyme extracted from peel and core of pineapple were shown in Figure 1 and 2.

Figure (1). Bromelain enzyme extracted from peel of pineapple

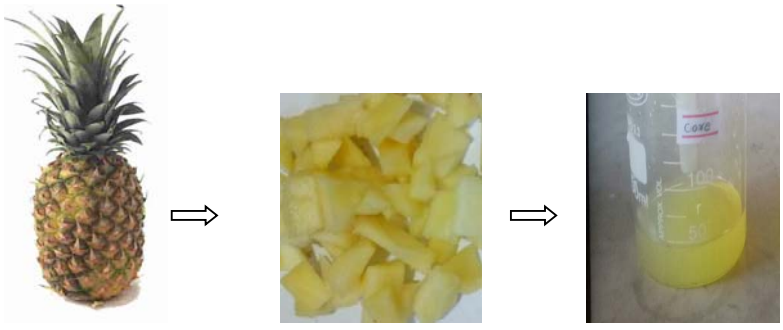

Figure (2). Bromelain enzyme extracted from core of pineapple

Wavelength of maximum absorption of standard tyrosine

For quantitative analysis of a compound by UV-visible spectroscopy, it is firstly necessary to know the wavelength of maximum absorption (λ_{max}). In the present work, casein was used as the substrate in all the experimental studies of enzyme-catalyzed reactions. The protein, casein, is decomposed by protease to give degraded stages of protein products which includes among them, tyrosine.

Tyrosine is an amino acid relatively less readily soluble in water whose absorbance may be easily measured in the visible region using Folin-Ciocalteu reagent (Mukhtar and Ikram, 2008). Therefore, tyrosine was used as a standard in this work. The wavelength of maximum absorption of tyrosine was measured by spectrophotometer. It was recorded in the range from 400 to 800 nm and the wavelength of maximum absorption of the standard tyrosine solution was found to be 600 nm (Figure 3).

Standard calibration curve for standard tyrosine using uv-visible spectroscopy

The calibration curve for standard tyrosine was drown and the standard comparison method was used for the determination of results. It was found that the nature of the plot of absorbance *vs.* concentration of tyrosine (Figure 4) was straight line passing through the origin. It was showing that beer's law was obeyed.

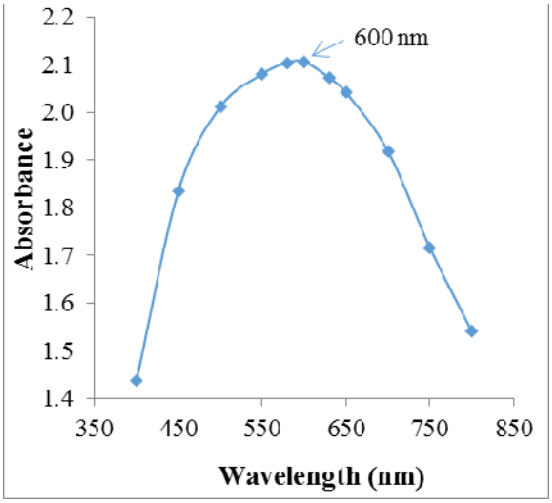

Figure (3). Wavelength of maximum absorption of standard tyrosine solution

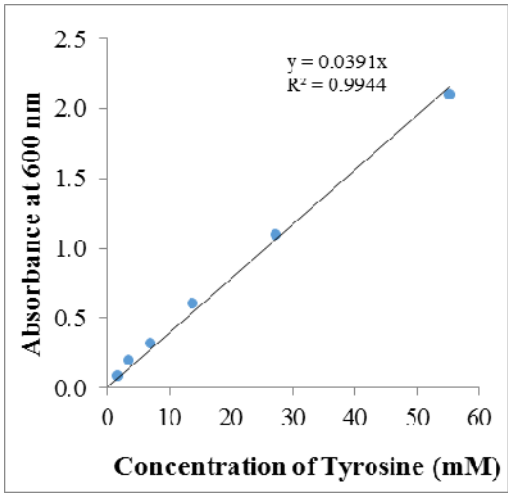

Figure (4). Plot of absorbance as a function of concentration of standard tyrosine

Investigation of proteolytic activity for liquid crude bromelain enzyme

The enzyme activity was determined by calculating the micro mole of tyrosine liberated per milliliter per minute of enzyme solution. It was observed that both of pineapple wastes have protease enzyme activity and the activities of crude bromelain enzyme extracted from peel and core were 8.269 and 7.346 µmol min^{-1} mL^{-1}, respectively. The comparison of the activities of protease enzyme (Bromelain) from pineapple peel and core are shown in Table 1 and Figure 5. From the above experimental data, it was found that the activity of crude bromelain enzyme extracted from peel of pineapple is greater than that of core.

Table (1). Comparison of the Activities of Bromelain Enzyme from Pineapple Wastes (Peel and Core)

No.	Pineapple Wastes	Absorbance at 600 nm	Enzyme Activity ($\mu mol\ min^{-1}\ mL^{-1}$)
1	Peel	0.421	8.269
2	Core	0.374	7.346

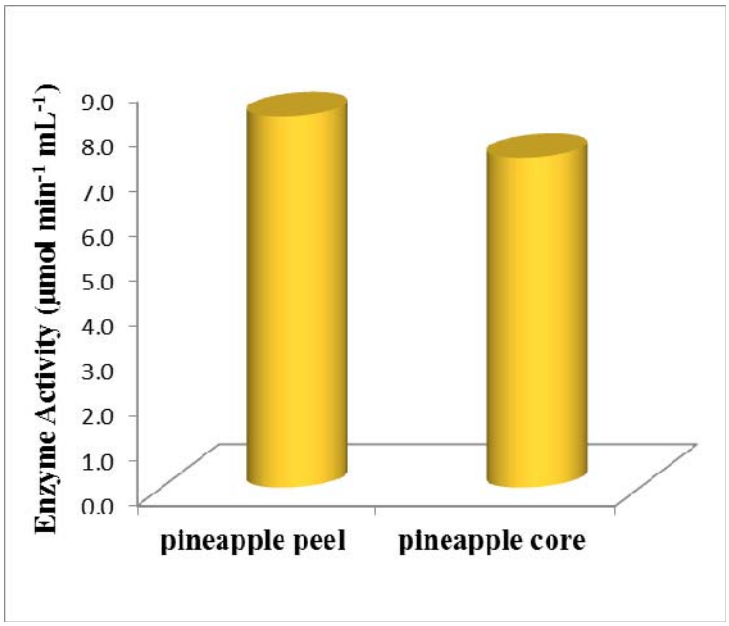

Figure (5). Protease enzyme activities of the bromelain from pineapple wastes (peel and core)

Optimum pH for bromelain-catalyzed reaction of casein

One of the most striking features of enzyme activity is its very marked dependence on the pH of the mixture in which the reaction is going on (Moss, 1968). The pH at which the enzyme catalyses the reaction at the maximum rate is called the optimum pH. Each enzyme has a characteristic optimum pH at which the activity is maximum, on each side of this optimum, the rate of enzyme-catalyzed reaction is lower (Boyer, 1993).

Phosphate buffer of pH values ranging from 5.6 t0 8.0 were used to determine the activities of crude bromelain enzyme extracted from peel and core of pineapple. The nature of the activities as pH curve of the enzyme (Figure 6 and 7) was obviously found to be unsymmetrical and optimum pH was obtained at pH 7.2 with casein substrate. This value is in accord with the literature value (Wilkison, 1961).

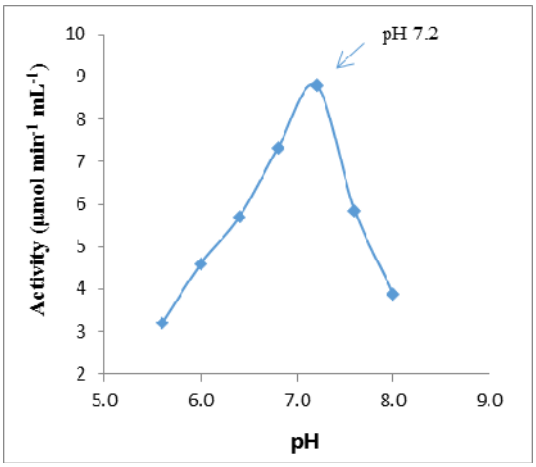

Figure (6). Plot of crude bromelain activity of peel as a function of pH of the solution

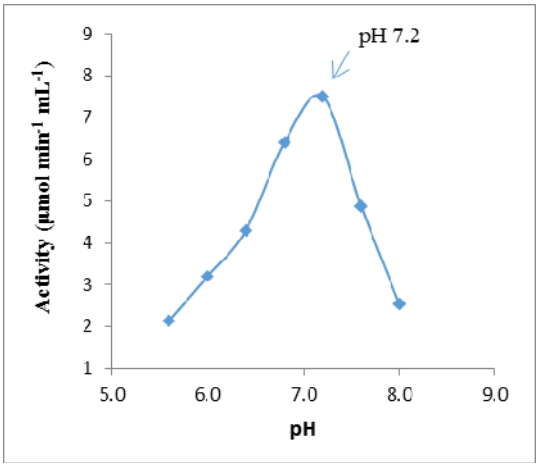

Figure (7). Plot of crude bromelain activity of core as a function of pH of the solution

Optimum temperature for bromelain-catalyzed reaction of casein

The rate of an enzyme catalyzed reaction increases as the temperature is raised. The reaction rate increases with temperature to a maximum level (optimum temperature), then declines with further increases of temperature. Because most enzymes are rapidly become denatured at temperature above optimum (Bennett, 1989).

In the present work, the optimum temperature for crude bromelain enzyme was found to be 60 °C in phosphate buffer solution (Figure 8 and 9). The functional temperature was increased from 30 to 60 °C and decreased from 60 to 80 °C for both peel and core of pineapple. The substrate medium (1% casein solution) and the optimum pH 7.2 were fixed.

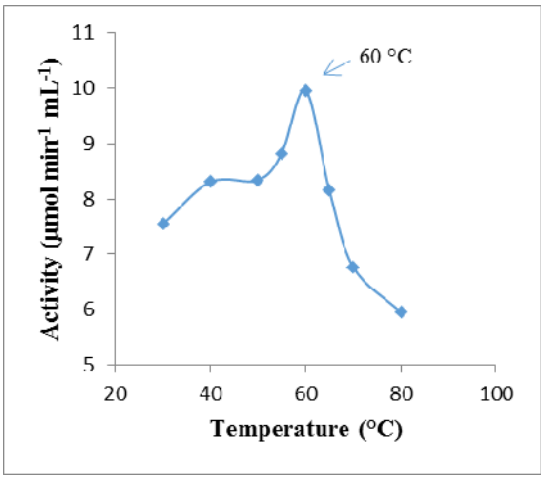

Figure (8). Plot of crude bromelain activity of peel as a function of temperature of the solution

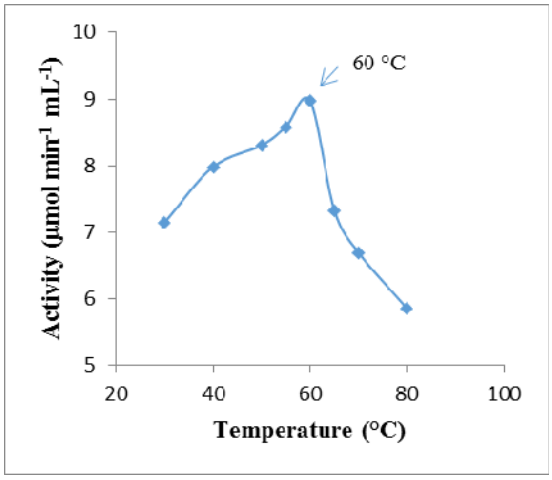

Figure (9). Plot of crude bromelain activity of core as a function of temperature of the solution

Application of bromelain enzyme

The most widely used unit system for bromelain is the milk-clotting unit (MCU). The milk clot assay is a very accurate and yet simple test procedure which measures the amount of time required to form clotted milk in the presence of the proteolytic enzyme under specified and controlled conditions, i.e., temperature etc. Figure 3.11 shows the application of bromelain enzyme for milk clotting. The beaker (A) is milk without bromelain enzyme and beaker (B) and (C) are the milk with bromelain enzyme. After 70 min, milk-clot was observed in beaker (B) and after 90 min, milk-clot was observed in beaker C. The milk-clotting technique is widely used in the production of milk product such as cheese, yoghurt, etc.

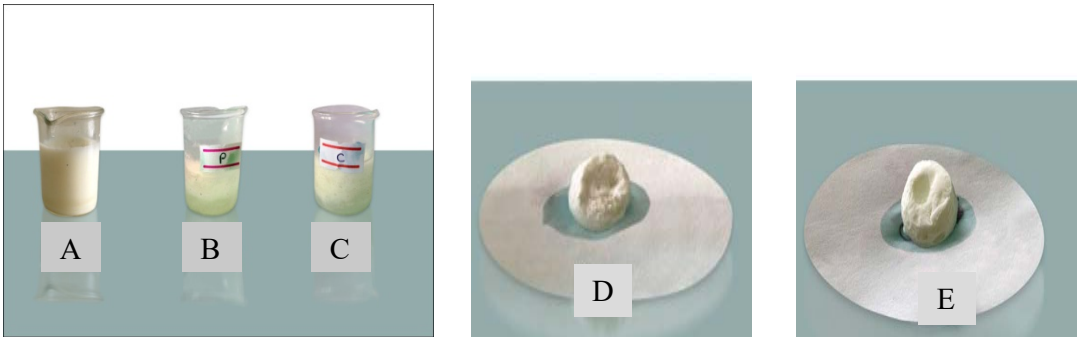

Figure (10). Application of bromelain enzyme for milk clotting
(A) Milk without bromelain enzyme
(B) Milk with peel extracted bromelain enzyme
(C) Milk with core extracted bromelain enzyme
(D) Milk clot by peel extracted bromelain enzyme
(E) Milk clot by core extracted bromelain enzyme

Conclusion

Pineapple (peel and core) are the major wastes of pineapple juice industry. From this study, it is concluded that core extracted bromelain shows slightly lower proteolytic activity than peel extracted bromelain enzyme.

The optimum pH and temperature of bromelain enzyme (peel and core) were found to be pH 6.0 and 60˚C, respectively. Bromelain enzyme obtained from wastes of pineapple was able to clock milk. The milk clotting time for peel extracted enzyme was 70 min and core extracted enzyme was 90 min at 60°C. So that peel can be major sources for the bromelain production and can be used for food industry.

Acknowledgements

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