Extraction and Characterization of Bromelain Enzyme from *Ananas comosus* L. (Pineapple Fruit)

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Abstract

Pineapple (*Ananas comosus* L.) is one of the major fruit crops of many countries. In this research paper, the bromelain enzyme was extracted from pineapple fruit. Pineapple fruit sample was collected from Pyin Oo Lwin Township, Mandalay Region. The bromelain enzyme was extracted from pineapple fruit by using phosphate buffer solution. Enzymic properties such as optimum pH, optimum temperature, velocity at various reaction time were determined by spectroscopic method. The crude bromelain enzyme was isolated from pineapple fruit by using phosphate buffer pH 7.2. Then the partially purified bromelain enzyme was extracted by ammonium sulphate precipitation method.

Key words : Bromelain enzyme, pineapple fruit, phosphate buffer, spectrophotometer

Introduction

Nowadays, people are increasingly interested in health and nutrition, so consequently, fruits and vegetables are consumed much more than in previous years.

Pineapple (*Ananas comosus* L.Merr) is one of the major fruit crops of many countries. Furthermore, it is an herbaceous tropical plant which contains a special enzyme with high pharmacology. The enzyme complex of *A.cosmosus* called bromelain is known for its clinical application. In some developed countries, bromelain is used as a nutritional supplement to "promote digestive health" and as an anti-inflammatory medication. Moreover, proteolytic enzymes such as bromelain inhibit the action of cholera toxin, and are also chosen enzymes for food processing.

Stem bromelain (EC 3.4.22.32) and fruit bromelain (EC 3.4.22.33) are two main kinds of pineapple proteases which can be found in extracts of pineapple. As many other enzymes, the stability of this enzyme absolutely depends on intrinsic as well as extrinsic factors. Among those, temperature and pH are main factors profoundly affecting bromelain activity. The study was carried out to investigate the fruit bromelain activity.

Aim and Objectives

Aim

This research is aimed to study on the properties of fruit bromelain from pineapple.

Objectives

- To extract the bromelain from the pineapple fruit
- To isolate bromelain by ammonium sulphate method
- To study the effects of temperature and pH on the bromelain catalyzed reaction
- To study the effect of substrate (casein) concentration on the bromelain catalyzed reaction

Botanical Description

Botanical name	:	*Ananas comosus*
Family	:	Bromeliaceae
Genus	:	*Ananas*
Species	:	*A. comosus*
English name	:	Pineapple
Myanmar name	:	Na-Nut

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Figure 1. The Plant and Fruits of *Ananas comosus* L. (Na-Nut)

Materials and Methods

Sample Collection

Pineapple fruits were collected from Pyin Oo Lwin Township, Mandalay Region.

Preparation of Solutions

Preparation of Monobasic Sodium Phosphate ($NaH_2PO_4 . H_2O$) Solution (0.2 M)

Monobasic sodium phosphate (2.76 g) was dissolved in distilled water and the volume was make up to (100 mL) in the volumetric flask to give a (0.2 M) solution. It was labeled as solution A.

Preparation of Dibasic Sodium Phosphate ($Na_2HPO_4 . 12H_2O$) Solution (0.2 M)

Dibasic sodium phosphate (7.16 g) was dissolved in distilled water and the volume was made up to the mark in a (100 mL) volumetric flask to give (0.2 M) solution. It was labeled as solution B.

Preparation of pH 7.2 Sodium Phosphate Buffer Solution

Solution A (29.40 mL) was mixed with (70.60 mL) of solution B. Then the volume was made up to (200 mL) with distilled water. The pH of this solution (0.1 M) was measured by a pH meter and it was adjusted to pH 7.2, it is necessary by using (0.1 M) sodium hydroxide or (0.1 M) hydrochloric acid solution.

Procedure

Pineapple fruit was first peeled, cut into small pieces and then ground using a blender. Then (100 mL) of phosphate buffer pH 7.2 was added into the pineapple juice. Then this mixture was filtered though muslin. After that, (100 mL) of precooled acetone was added into the filtrate and stirred for 1 hour. Then, the mixture was centrifuged and the crude bromelain enzyme was obtained as precipitate.

Preliminary Examination of Fruit Bromelain Activity

Two pieces of photographic film (3 × 3 cm) were used to examine the activity of bromelain enzyme. One piece of film and (50 mL) of phosphate buffer pH 7.2 were placed into a beaker and (50 mL) of enzyme solution (0.1 % in pH 7.2 phosphate buffer) and piece of film were placed into another beaker. The two beakers were heated at 50°C for 1 hr. After that the proteolytic activity of the bromelain was observed. The colour of film in the enzyme solution was disappeared.

Extraction of Partial Purified Bromelain Enzyme from Pineapple (*Ananas comosus* L.) Fruit

Procedure

Pineapples (*Ananas comosus* L.)were washed and then peeled. Pineapple slices were cut into small pieces and then ground using a juice extractor. Pineapple juice (100 g) was thoroughly mixed with (200 mL) of phosphate buffer of pH 7.2 and the mixture was filtered through two layer muslin. The filtrate was collected and left overnight at 4°C. After that, it was filtered and the filtrate was used for extraction of enzyme.

The crude extract containing the enzyme was used for the isolation of bromelain. The filtrate was brought to 20 % saturation (22.68 g) by slow addition of solid ammonium sulphate. The filtrate was collected by centrifugation at 3000 rpm for 20 min. The ammonium sulphate (49.40 g) was then added into the supernatant to achieve 60 % saturation. The partially purified bromelain was then collected by centrifugation at 6000 rpm for 30 min.

Determination of Maximum Absorption Wavelength of Standard Tyrosine Solution

Procedure

Standard tyrosine solution (1.10 mM) was placed in a quartz cell and the absorbance was measured between the wavelengths of 250 nm to 350 nm using UV-visible spectrophotometer. Trichloroacetic acid 5 % solution was used as a blank. A plot of absorbance against wavelength was drawn.

Construction of Calibration Curve for Standard Tyrosine Solutions

Procedure

For construction of calibration curve, six different concentrations of standard tyrosine solution, (0.11, 0.22, 0.44, 0.66, 0.88 and 1.10 mM) were used. The absorbance values of these standard solutions were measured at 273.9 nm by a UV-visible spectrophotometer and 5 % trichloroacetic acid solution was used as a blank solution. The calibration curve was obtained by plotting the concentration of tyrosine solution Vs absorbance.

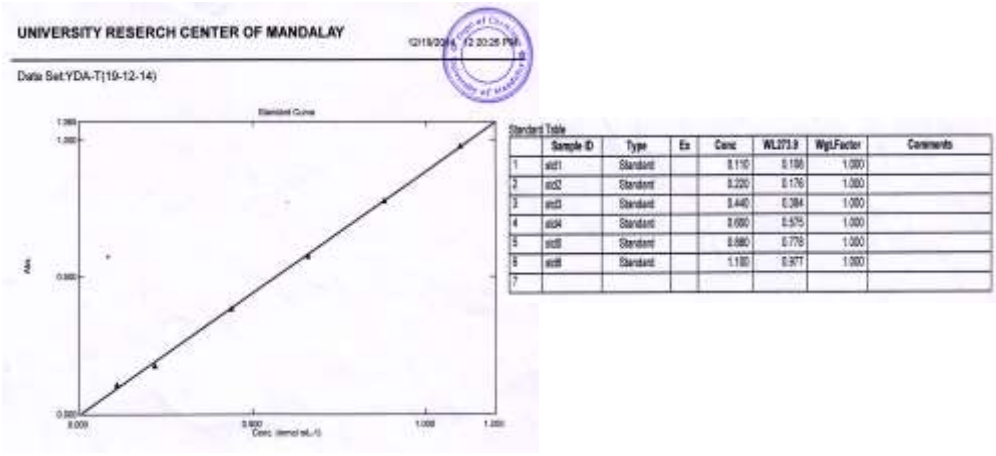

Figure 4. Construction of Calibration Curve for Standard Tyrosine solutions

Results and Discussion

Extraction of Bromelain from Pineapple Fruit

The bromelain enzyme was extracted from (*Ananas comosus* L.) by using acetone and phosphate buffer solution. It was partially purified by solid ammonium sulphate precipitation method. Ammonium sulphate precipitation method was chosen for salt fractionations because of its high solubility in water, lack of toxicity, cheapness, no harmful effect on enzyme activity. Various organic solvents such as acetone, alcohol and diethyl ether and salts of ammonium sulphate can be used to isolate partial purification of enzyme.

Figure 5. The Bromelain Enzyme Extracted from Pineapple (*Ananas comosus* L.)Fruit

Table 1. Yield Percent of Bromelain Enzyme Extracted from (*Ananas comosus* L.)

Pineapple used (g)	Enzyme powder (g)	Yield %
400	6.00	1.50
400	6.36	1.59
400	6.24	1.56
	Mean	1.55

The yield percent of bromelain enzyme was found to be 1.55 %.

Determination of Maximum Absorption Wavelength of Tyrosine

For UV-visible spectrophotometric determination, the wavelength of maximum absorption (λ_{max}) must be firstly determined. In the presence research, the absorption spectrum of tyrosine was recorded in the range from 250 nm to 350 nm. The wavelength of maximum absorption was found to be 273.9 nm, Figure (6) in accordance with literature value of 274.2 nm (Fasman, 1976).

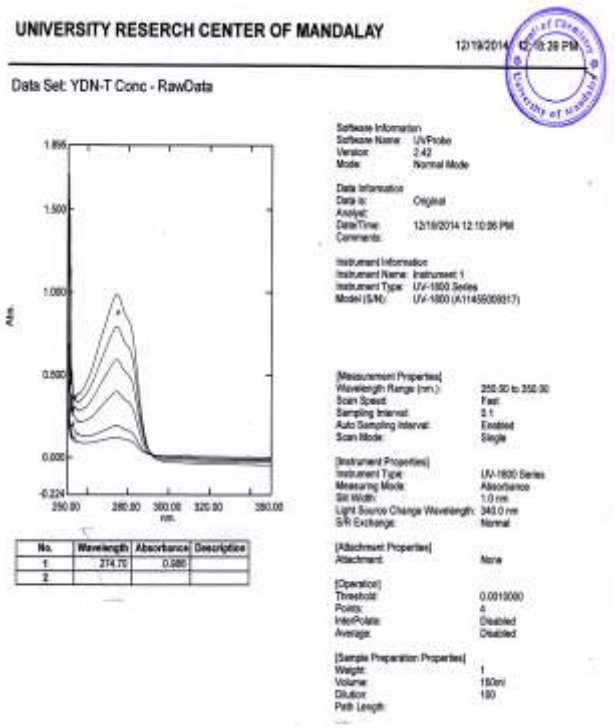

Figure 6. Wavelength of Maximum Absorption of Tyrosine

Construction of Calibration Curve for Standard Tyrosine Solution

For quantitative analysis of a compound by spectrophotometric method, the wavelength of maximum absorption (λ_{max}) must be known. In the present research, casein, (milk protein) is decomposed by bromelain to produce degraded protein products which include among them tyrosine. To determine the activity of bromelain, the concentration of the product tyrosine must be known. The calibration curve of standard tyrosine solution was constructed by measuring the absorbance value of different concentrations of standard tyrosine (0.11 mM, 0.22 mM, 0.44 mM, 0.66 mM, 0.88 mM and 1.10 mM) at 273.9 nm. The results were shown in Table (2) and Figure (7).

Table 2. Relationship between Absorption and Wavelength of Tyrosine Solution

No.	Concentration (mM)	Absorbance
1.	0.11	0.108
2.	0.22	0.176
3.	0.44	0.384
4.	0.66	0.575
5.	0.88	0.778
6.	1.10	0.976

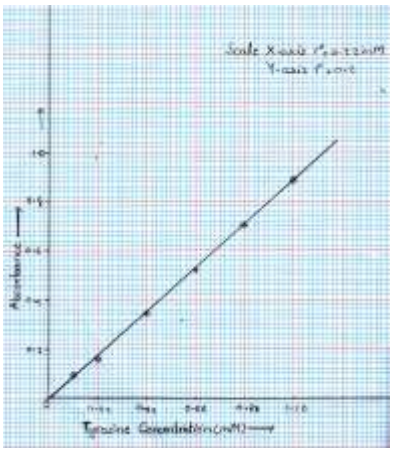

Figure 7. Plot of Absorbance as a Function of Concentration of Standard Tyrosine Solution

Determination of Optimum pH for Bromelain Catalyzed Reaction

Enzymes are very sensitive to change in pH. Most enzymes active only within a fairly limited pH range and they have an optimum pH at which their activity is greatest. In this research work, phosphate buffers of pH values ranging from 6.6 to 7.8 were used to determine the activity of the bromelain enzyme Table (3).

$$\text{Enzyme activity} = \frac{\text{Amount of product released from the reaction}}{\text{reaction time (min)} \times \text{used volume enzyme (mL)}}$$

Table 3. Relationship between Activity and pH for Bromelain Catalyzed Reaction

No.	pH	Absorbance	Concentration (mM)	Enzyme activity $\times 10^{-3}$ mmole min^{-1} mL^{-1}
1.	6.6	0.124	0.136	0.453
2.	6.8	0.135	0.149	0.497
3.	7.0	0.164	0.203	0.609
4.	7.2	0.197	0.201	0.670
5.	7.4	0.161	0.184	0.553
6.	7.6	0.140	0.163	0.489
7.	7.8	0.110	0.120	0.360

The optimum pH of fruit bromelain in phosphate buffer solution is found at 7.2.

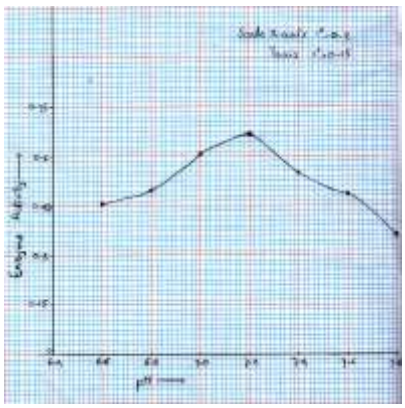

Figure 8. Plot of Activity and pH for Bromelain Enzyme Catalyzed Reaction

Determination of Optimum Temperature for Bromelain Enzyme Catalyzed Reaction

All chemical reactions are increased as the temperature is increased, including enzyme catalyzed reactions. For each enzyme, there is a certain temperature called the optimum temperature at which the enzyme activity is maximum. In this research work, the temperature was measured from 20°C to 80°C while the substrate (1 % casein) was prepared. The results were shown in Table (4).

Table 4. Relationship between Activity and Temperature for Bromelain Catalyzed Reaction

No.	Temperature (°C)	Absorbance	Concentration (mM)	Enzyme activity $\times 10^{-3}$ mmole min^{-1} mL^{-1}
1.	20	0.261	0.286	0.953
2.	30	0.321	0.352	1.173
3.	40	0.420	0.461	1.536
4.	50	0.451	0.505	1.683
5.	60	0.383	0.441	1.470
6.	70	0.299	0.332	1.106
7.	80	0.123	0.130	0.433

The optimum temperature for the activity of bromelain was found at 50°C.

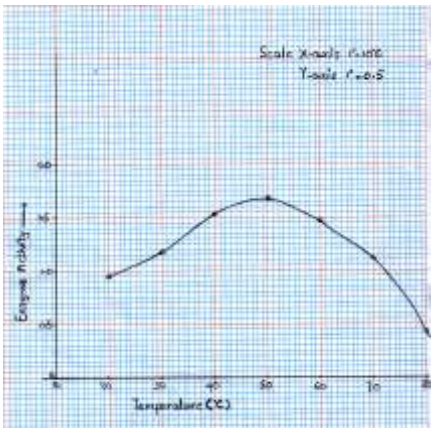

Figure 9. Plot of Activity and Temperature for Bromelain Catalyzed Reaction

Determination of Concentration of Tyrosine Liberated at Various Reaction Time

The bromelain enzyme breaks down casein into degraded protein product which include among them tyrosine. The amount of Tyrosine librated during the various reaction times of 10, 15, 20, 25, 30, 35 and 40 min were determined by UV-visible spectrophotometric method. The results were shown in Table (5). The velocity of enzyme catalyzed reaction can be calculated by the following equation.

$$\text{Velocity, } V = \frac{\text{concentration}, (\Delta C)}{\text{Time}, (\Delta T)}$$

Table 5. Relationship between Velocity and Reaction Time for Bromelain Catalyzed Reaction

No.	Time (min)	Absorbance	Tyrosine concentration (mM)	Velocity $10^{-5} \times$ mM min^{-1}
1.	10	0.132	0.142	–
2.	15	0.174	0.206	1.28
3.	20	0.241	0.274	1.36
4.	25	0.303	0.345	1.42
5.	30	0.380	0.428	1.66
6.	35	0.361	0.418	0.20
7.	40	0.360	0.416	0.04

The rate of bromelain enzyme catalyzed reaction on casein increases linearly with incubation time up to about 30 min and velocity falls at longer incubation.

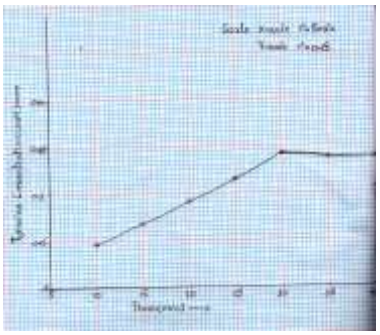

Figure 10. Plot of Concentration of Tyrosine Liberated from Bromelain Catalyzed Reaction as a Function of Reaction Time

Changes in pH also alter an enzyme's shape. Different enzymes work best at different pH values. The optimum pH for an enzyme depends on where it normally works. For example, intestinal enzymes have an optimum pH of about 7.5. Figure 8 shows the optimum pH of Bromelain enzyme was found to be 7.2. As the temperature increases, so does the rate of reaction. But very high temperature denature enzymes. The Figure (9) shows the typical change in an enzyme's activity with increasing temperature. The optimum temperature for Bromelain enzyme was found at 50°C. Then as the temperature continues to rise, the rate of reaction falls rapidly as heat energy denatures the enzyme. Moreover to investigate the optimum reaction period of the enzyme solution, reaction was carried out using 0.1 M sodium phosphate buffer pH 7.2 at 50°C in a water bath at different time intervals and the enzyme activity was then measured. Figure (10) shows the optimum reaction time of Bromelain enzyme from pineapple plays an important role in digestive health. Therefore everybody should eat the pineapple for digestive health.

Conclusion

In this research, pineapple (*Ananas comosus* L.) fruits were collected from Pyin Oo Lwin Township, Mandalay Region. The crude bromelain enzyme was isolated from pineapple fruit by using phosphate buffer pH 7.2. Then the partially purified bromelain enzyme was extracted by ammonium sulphate precipitation method. In this research work, casein, (milk protein) as a substrate and phosphate buffer solution were used for its stability. After partial purification, 1.55 % of the bromelain enzyme extract was obtained.

Enzyme properties such as optimum pH, optimum temperature and effect of reaction time were determined by UV-visible spectrophotometric method. The optimum pH of

bromelain enzyme solution was found to be pH 7.2 in phosphate buffer and optimum temperature for bromelain enzyme solution was found at 50°C. The concentration of tyrosine liberated during 30 min reaction time increased gradually. Then the tyrosine concentration becomes nearly constant after 30 min reaction time. Therefore, the reaction time for 30 min was chosen as an effective reaction time of the bromelain enzyme catalyzed reaction. If I have sufficient time in order to obtain complete purified enzyme and its application in medicinal field should be done.

Acknowledgement

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Online Materials

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