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Extraction and Partial Purification of Bromelain from Fruit and Crown of Pineapple (*Ananas comosus*) and It's Application as a Meat Tenderizer

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Abstract
Bromelain is a protease found in pineapples having high demand in the pharmaceutical, cosmetic and food industries. Bromelain is distributed in different parts of plants. In the present study bromelain was extracted from fruit and crown of pineapple. The protein concentration was estimated by Lowry's method. In fruit bromelain concentration of protein was found to be higher as compared to crown. The activity of fruit and crown part of bromelain was determined by using Casein as a substrate. Both the enzymes had shown optimum activity at alkaline pH and they are quite stable at high temperature. The enzyme was partially purified by using ammonium sulphate precipitation method. The specific activity of fruit bromelain is more as compared to crown bromelain. The enzyme acts as a tenderizer. The physio-chemical properties like water holding capacity, cooking yield, moisture content and pH were determined.

Keywords
Pineapple, Bromelain, meat tenderization.

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INTRODUCTION
Pineapples are sweet, juicy fruits. The scientific name of Pineapple is Ananas comosus. Ananas means "excellent fruit" in an Indian language. Pineapples grow in tropical (warm) areas around the world. Bromelain is an enzyme extract derived from the stems of pineapples, although it exists in all parts of the fresh plant and fruit. The extract has a history of folk medicine use. As a culinary ingredient, it may be used as a meat tenderizer. The term "bromelain" may refer to either of two protease enzymes extracted from the plants of the family Bromeliaceae, or it may refer to a combination of those enzymes along with other compounds produced in an extract. Although tested in a variety of folk medicine and research models for its possible

efficacy against diseases, the only approved clinical application for bromelain was issued in 2012 by the European Medicines Agency for a topical medication called NexoBrid used to remove dead tissue in severe skin burns. [20]

Tenderness is an important characteristic of meat. The overwhelming demand for guaranteed tender meat has attracted players in the meat industry to provide an acceptable quality of product. In the USA, certified Angus beef was introduced in the late 1970s. Consumers have found it consistently flavorful, juicy, and tender [13].

MATERIALS & METHODS

Collection of sample

The pineapple fruit was bought from local market of Jadhav-vadi from Aurangabad, Maharashtra, India.

Crude Extraction of Bromelain

The fresh pineapple fruit and crown was washed using tap water and 0.1% hydrogen peroxide solution. The crown and fruit part of the pineapple was cut into small pieces and weighed individually. The crown and fruit pieces were washed 2-3 times with distilled water.

50 gm of crown and fruit pieces were blended with 50ml of sodium phosphate buffer (pH 8) separately. After blending the homogenate was collected and filtered by using muslin cloth. The filtrate was centrifuged at 10,000 rpm for 20 minutes at 4°C. Supernatant was collected and stored in refrigerator for further use. [3].

Estimation of Protein concentration by Lowry's method

Crude extract of crown and fruit were taken into separate labeled test tubes. 5 ml of reagent C was added, kept at room temperature for 15 min. 1 ml of Folin phenol reagent was added, in each test tube and incubated in dark condition for 30 min. Optical density was recorded on spectrophotometer at a wavelength of 600 nm. The concentration of unknown sample was calculated by using standard graph. [4].

Bromelain Enzymatic assay

100 ul (0.1 ml) protease sample was added in a test tube with 1.8 ml (1%) casein and 2ml phosphate buffer (pH 8). After the solution allowed for incubation at 37° C for 5 minutes. Thereafter, the 10% of TCA was added and centrifuged at 6,000 rpm for 5-10 minutes. 2-3 ml of supernatant collected and 5ml of reagent C was added. Solution was kept in room temperature for 10 minutes for mixed properly. Thereafter, 1ml of folin reagent was added and mixed properly and incubated in dark for 30 minutes. Absorbance was measured at 600nm. [2]

Effect of pH and temperature on enzyme activity

Optimum pH and temperature was evaluated of crude bromelain. Optimum pH was determined by using buffer having different pH range. Optimum temperature was accessed by changing the incubation temperature. [3,6]

Ammonium sulphate Precipitation

Crude extract of fruit and crown were subjected to ammonium sulfate precipitation by adding varying concentrations of ammonium sulfate with gentle stirring on the ice for 30 min to obtain 60–80% saturation and the contents were centrifuged at 10,000 rpm for 20 min at 4°C and pellet of their fractions were collected and resolubilized with phosphate buffer of pH 8. [4]

Dialysis

The dialysis membrane is filled with Bromelain juice of Pineapple Fruit and Crown obtained from ammonium sulfate fractionation and sealed. The dialysis membrane is kept in a beaker containing pre-cooled phosphate buffer. The dialysis bag along with the buffer is stirred using magnetic stirrer. [18]

Application of Bromelain:

Meat Tenderization

Meat was cut into cubes of approximately 2 cm^3. The meat cubes were randomly divided into three groups. One group was treated with crown enzyme, another with fruit enzyme and one is kept as control. [9,15]

Water Holding Capacity (WHC)

1-2 gm meat pieces placed in centrifuge tube containing 2.5 ml 0.6M Nacl. Stirred with a glass rod for 1 min and kept at 4° C for 15 min. Centrifuged at 3,000 rpm for 20 min at 4° C. Collect the supernatant and measure it.

$$\text{WHC (\%)} = \frac{\text{Vol. of NaCl before centrifuge} - \text{Vol. of NaCl after centrifuge}}{\text{Vol. of NaCl before centrifuge}} \times 100$$

- **Cooking Yield**

The treated sample 1-2 gm were steamed for 1 min. and then cooled at room temperature. Cooked sample was surface dried with a filter paper and reweighed using an analytical balance. The cooking loss was calculated according to Sultana et al. (2009) and expressed in percentage (%) as the following equation:

$$\text{Cooking loss (\%)} = \frac{\text{Weight (before cooking)} - \text{Weight (after cooking)} \times 100}{\text{Beef weight before cooking}}$$

- **pH**

1-2 gm of the treated sample were homogenized with 10 ml chilled distilled water the pH value was measured with pH meter.

- **Moisture Content**

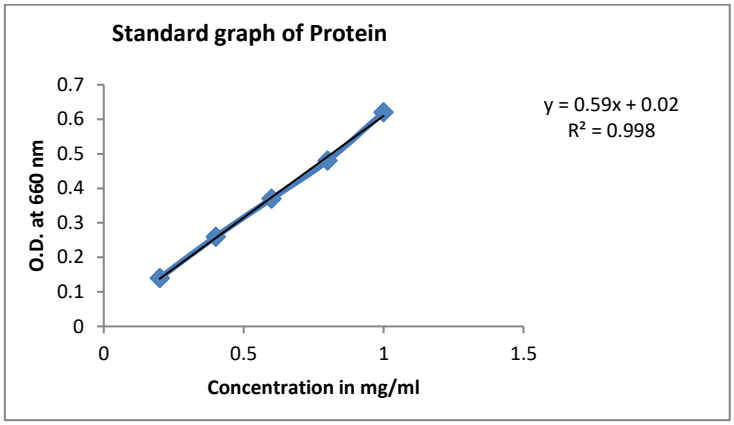
1-2 gm of treated sample were suspended in 5 ml of 0.6 M sodium chloride solution for 30 min. weighted was noted and placed on hot plate and heated at 100° C for 20 min. The weighted was determined.

Percentage of moisture content = ((Initial weight – Final weight) /Initial Weight) x 100.

RESULTS

Protein estimation by Lowry's Method:

The estimation of enzyme was done by the Lowry's method that gave the following result

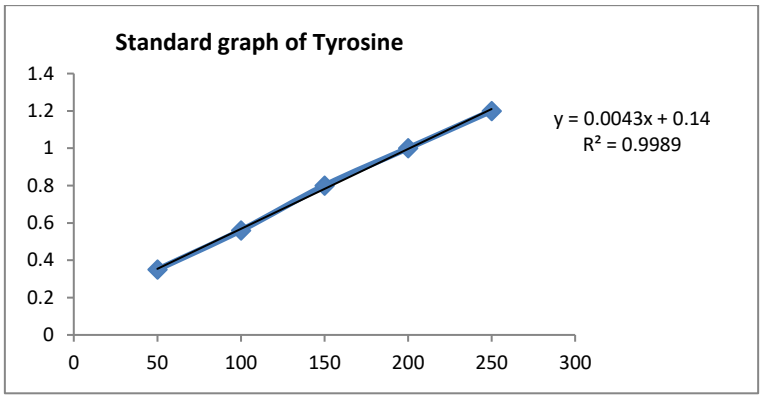


The concentration of all the unknown samples were determined using the equation y= 0.59x+0.02 which was obtained from the standard graph. The results are tabulated as below:

Sample	Protein Concentration mg/ml
Pineapple Fruit	1.11
Pineapple Crown	0.89

Enzyme assay of crude enzyme:

Qualitative assay of Bromelain enzyme gives the following result.

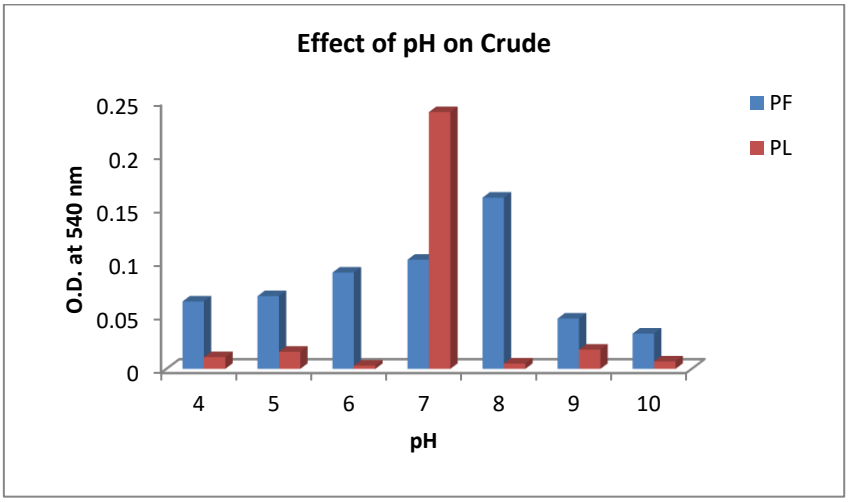


The concentration of all the unknown samples were determined using the equation y= 0.004x+0.14 which was obtained from the standard graph. The results are tabulated as below

Crude Enzyme	Bromelain Activity U/ml	Specific Activity U/mg
Pineapple Fruit	24	21.6
Pineapple Crown	18	20.22

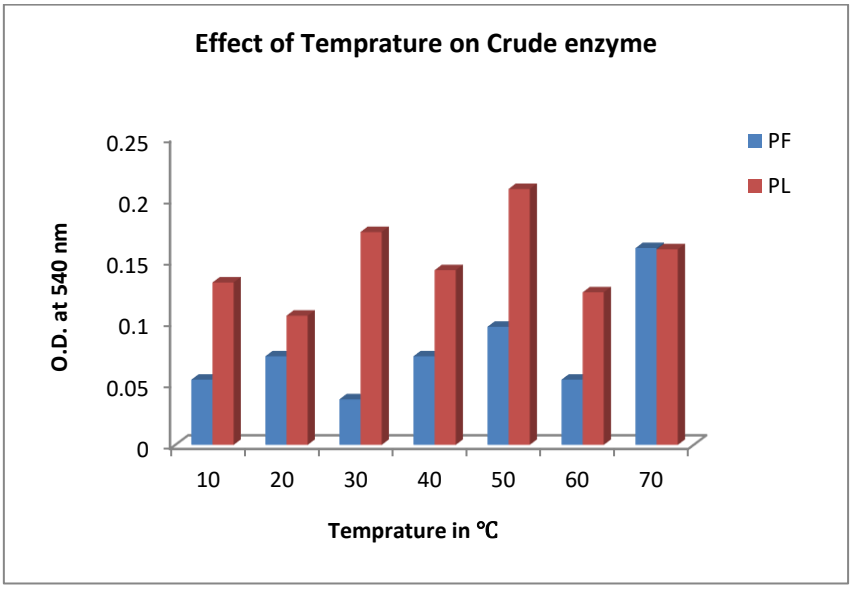
Effect of pH

The effect of pH on the enzyme activity of the Fruit and Crown enzyme was determined. At pH 8 and 7, the fruit and crown enzyme shown maximum activity which is about 5 and 25 U/ml respectively.



Effect of Temperature:

The effect of temperature on the enzyme activity of the Fruit and Crown enzyme was determined. At 70°C and 50°C, the fruit and crown enzyme shown maximum activity which is about 5 and 15 U/ml respectively.



Ammonium Sulphate Precipitation:

The enzyme was purified by using Ammonium Sulphate Precipitation method and determined its activity given as follows.

Sample	Protein concentration mg/ml	Bromelain activity U/ml		Specific activity U/mg
60% Pellet of Pineapple Fruit.	0.49	18.5	40	37.75
60% Pellet of Pineapple Crown	0.30	8.00	40	26.66
80% Pellet of Pineapple Fruit	0.17	6.5		38.23
80% Pellet of Pineapple Crown	0.14	5.5		39.28

Application of Bromelain

Meat Tenderization

- **Assessment of pH:**

pH was determined by using pH meter. Reduction in pH was observed in both Bromelain treated sample as compared to control which is in range 4.94-5.50.

- **Assessment of Moisture content:**

Moisture content is a measure of total water contained in a food. Moisture content was increased in treated sample as compare to control. The moisture contain of control was 37.16% whereas, Pineapple fruit and Pineapple crown treated meat sample 69.69% & 58.14% respectively.

- **Assessment of Water-holding capacity:**

WHC is defined as the ability to hold its own and added water during the application of forces like centrifugation, pressing, heating. There was not any difference observed in both the control and treated sample of meat.

- **Assessment of cooking yield:**

The cooking loss was calculated according to Sultana et al. (2009) and expressed in percentage (%). The cooking yield of treated meat sample was found to be decreased as compared to untreated sample. The cooking yield of control was 49.21%. The cooking yield of meat sample treated with Pineapple Fruit Bromelain was 48.21% and Pineapple Crown Bromelain 46.96%.

DISCUSSION

Crude bromelain is a mixture of cystein protease that catalyses the hydrolytic cleavage of the internal peptide bonds of protein substrate. Bromelain was extracted and purified from crown leaf and fruit Pineapple. The purification process carried out by ammonium sulphate precipitation followed by dialysis.

In present study Pineapple fruit and crown bromelain were identified for its meat tenderization activity.

During our study we found that pineapple fruit bromelain having the more protein content than the crown bromelain.

A various other parameter like pH and temperature were analyzed. Fruit bromelain shown maximum activity at pH 8 whereas, crown bromelain shown maximum activity at pH 7. Fruit enzyme show maximum activity at temperature 70°C and crown enzyme shown maximum activity at 50°C. Our results resemble with the results reported by C. Ramalingam, R. Sirnath and N. Nasimun Islam in 2012.

In case of crude Pineapple fruit and crown leaf extract shown protease, activity which is about 24 and 18 U/ml respectively. By using ammonium sulphate precipitation method, the crude enzyme was purified and its activity and specific activity was determined. The specific activity of purified fruit and crown bromelain is about37.75 U/mg and 39.28 U/mg respectively.

The physio-chemical and quality characteristics of meat like water-holding capacity, cooking yield, pH, moisture content was determined of the Bromelain enzyme (BE) treated meats.

The pH value in meat product is highly important because it has major influence on other physio-chemical and quality characteristics like water-holding capacity, cooking yield, pH, moisture content. The pH on treated sample was reduced as compared to untreated sample. Similar results were reported by. Janhvi Manohar et. al., 2016.

Rawdkuen and Benjakul (2012) reported that enzymes increased the collagen solubility and promoted the structural alterations through the action on collagens crosslink.

Heating process would accelerate the meat tenderization process. This is because, during heating of meat, the sarcoplasmic, myofibrillar and connective proteins undergo denaturation (Kołczak et al., 2008) The cooking yield of treated meat sample was found to be decreased as compared to untreated sample. The cooking yield of control was 49.21%. The cooking yield of meat sample treated with Pineapple fruit bromelain was 48.21% and Pineapple crown bromelain 46.96%.

The decrease of WHC was due to the action of bromelain in the denaturation of myofibrillar proteins which play a role in water retention (Murphy and Marks, 2000). Besides, the decrease of WHC was due to the myofibrillar shrinkage as well as the movement of water from the myofilament space into the extracellular space (Ketnawa and Rawdkuen, 2011). The decrease in the moisture content of bromelain– treated beef was in agreement with Sultana et al. 2009. The majority of water in meat is held within the structure of muscle and muscle cells. Therefore, the decrease of moisture content was caused by the destruction of the structure of muscle cells due to the denaturation of myofibrillar proteins by the action of heat and meat tenderizer (Huang et al., 2011).

CONCLUSION

- The present study as shown that the protein concentration was found to be higher in Pineapple fruit as compared to crown.
- The fruit and crown bromelain are active at alkaline pH and quite stable at high temperature.
- This study shows how the tough meat can be easily get softened by the use of natural product, pineapple without compromising quality of the meat.
- Bromelain has an economic importance as well, as the enzyme is obtained from waste part of pineapple like crown.
- It can be used as a better alternative to chemical tenderizer.

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