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Identification of Amino Acid and Proteolytic Activity on Protein by Pineapple (*Ananas Comosus*) Enzyme Extract

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Research Article

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ABSTRACT

It is derived from the Spanish word pina meaning pine cone that was used in 1398. 300 years later it was called pineapple in order to identify the fruit individually. Scientifically the pineapple is known as *Ananas comosus* belongs to the family Bromeliaceae. It is the leading edible member of the family Bromeliaceae. Pineapple pulp contains different nutrients, and pineapple peel is known to have antioxidants helpful in warding off free radicals. Sulfhydryl proteolytic enzymes are the difconstituents of bromelain. It has been found that pineapple is very good digestive enzyme, so we have to find out that this enzyme protein having proteolytic activity or not and we have to identify that what are the different type of protein and amino-acid present in that compound because enzyme is a protein, what are the different composition of protein we have to identify. After performing all the identification test of amino acids, we found following amino acids present in the pineapple: Cysteine, methionine, histidine and tyrosine. And by performing the proteolytic activity on gelatin and albumin by the UV Visible spectroscopy we can see that the pineapple enzyme can break the proteins. If this herbal formulation prepared into drug it is beneficial for the human kind.

Keywords: Bromelain; Amino acids; UV-VIS spectroscopy; Proteolytic activity; Gelatin and albumin; Herbal formulation

INTRODUCTION

It is derived from the Spanish word pina meaning pine cone that was used in 1398. 300 years later it was called pineapple in order to identify the fruit individually. It was discovered on the island of Guadalupe in 1493 by Europeans. It was treated as a luxurious fruit because of the sort of prestige that it had. The skin of pineapple is used to make alcohol, animal food and vinegar. Pineapple is considered the third most important fruit in the world with a projected production of 27.8 million tons for 2020 [1]. The pineapple is also known as *Ananas comosus* belongs to the family Bromeliaceae. It is the leading edible member of the family Bromeliaceae, grown in several tropical and subtropical countries including, Philippines, Thailand, Indonesia, Malaysia, Kenya, India, and China. It has been used as a medicinal plant in several native cultures. Pineapples as a fruit have effective juice and a vibrant tropical flavour that balances the tastes of sweet and tart [2].

The pineapple is an herbaceous perennial, which grows to 1.0 to 1.5 m (3 ft 3 in to 4 ft 11 in) tall, although sometimes it can be taller. The plant has a short, stocky stem with tough, waxy leaves. When creating its fruit, it usually produces up to 200 flowers, although some large fruited cultivars can exceed this. Once it flowers, the individual fruits of the flowers join together to create a multiple fruit. After the first fruit is produced, side shoots (called 'suckers' by commercial growers) are produced in the leaf axils of the main stem [3]. These suckers may be removed for propagation or left to produce additional fruits on the original plant. Commercially, suckers that appear around the base are cultivated. It has 30 or more narrow, fleshy, trough-shaped leaves that are 30 to 100 cm (1 to 3+1⁄2 ft) long, surrounding a thick stem; the leaves have sharp spines along the margins [4]. In the first year of growth, the axis lengthens and thickens, bearing numerous leaves in close spirals. After 12 to 20 months, the stem grows into a spike-like inflorescence up to 15 cm (6 in) long with over 100 spirally arranged, trimerous flowers, each subtended by a bract.

The ovaries develop into berries, which coalesce into a large, compact, multiple fruit. The fruit of a pineapple is usually arranged in two interlocking helices, often with 8 in one direction and 13 in the other, each being a Fibonacci number.

The ovaries develop into berries, which coalesce into a large, compact, multiple fruit [5]. The fruit of a pineapple is usually arranged in two interlocking helices, often with 8 in one direction and 13 in the other, each being a Fibonacci number.

Pineapple is a type of fruit that is high in nutrients. One of the pineapples that are popular in is honey pineapple. This type of pineapple has a delicious and refreshing taste. The pulp of honey pineapple has a thicker pulp than pineapples in general, is more extensive than regular pineapples, and has a slightly yellowish colour to orange. Pineapple fruit contains lots of nutrients, including vitamin A, calcium, phosphorus, magnesium, iron, sodium, potassium, dextrose, sucrose (cane sugar), and the enzyme bromelain, which is a 95% mixture of cysteine proteases that can hydrolyze protein (proteolysis) and resistant to heat [6-8].

Extensive studies have been done with bromelain to explore its clinical properties. Bromelain is a chief protease enzyme found in pineapple plant. It has been known chemically since 1876 and was identified for the first time by Marcano in 1891. The investigation and isolation of bromelain has been started since 1894. It was first therapeutically supplemented in the year 1957. Sulfhydryl proteolytic enzymes are the chief constituents of bromelain. 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.

MATERIALS AND METHODS

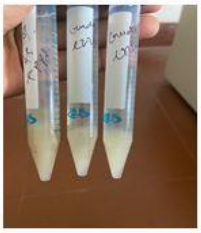
Fruit collection

To start the research first pineapple is bought from the local market of Barasat, West Bengal.

Preparation of extract: The pineapple peels were cut off into slices. Then taking the pineapple core and cut into a small slice. These small slices were given in to the mixer grinder for crushing (Figure 1 and 2). After completely crushing the liquid extract was filtered through the gauze. After filtration, 10% ammonium sulphate solution was mixed with this filtrate. Then the filtrate is put into centrifugation tubes. The centrifugation was done in centrifuge machine at 8000 rpm for 20 minutes. After the centrifugation the pineapple extract is ready for tests [9].

Figure 1. Ammonium sulphate solution was mixed with this filtrate.

Figure 2. The liquid extract was filtered through the gauze.



Identification test of amino acids

Ninhydrin test: At first few drops of the sample and add few drops of Ninhydrin reagent was taken. Then heat the solution was heated for five minutes. If the result shows blue colour, then it confirms that there are alpha amino acid present [10].

Xanthoproteic acid test: One ml solution added to sample solution. Now heated the solution with concentrated Nitric Acid. After cooling the solution added few drops of Sodium Hydroxide. If it shows orange colour then it confirms there are aromatic amino acids present [11-16].

Folin's Mc Carthy Sullivan's test: At first one ml sample taken then added few drops of 40% Sodium hydroxide. Now added few drops of glycine and sodium and nitroprusside solution. Then placed the test tube on water bath for 15 mins. Then added 6N Hydrochloride. If red colour shows it confirm that it will be methionine.

Lead sulphide test: 1 ml sample taken and add few drops of sodium hydroxide and putting marble chips in the solution. Then heated the solution up to 5 to 10 minutes. Then cooling the solution by running water. After that added few drops of 10% lead acetate solution. If black precipitate comes then it will be cysteine.

Millon's test: 1 ml sample taken and add few drops of Millon's reagent, shaked the solution. Then add few drops of concentrated nitric acid. If red color shows, then it will be tyrosine.

Histidine test: At first 2 ml sample taken and added few drops of 5% bromine and 33% acetic acid solution and place for 10 minutes. Then added 2 ml of ammonium carbonate and boil for 5 minutes. If blue colour shows, then it will be histidine [17].

TLC studies of enzyme

Thin layer chromatography is a technique used to isolate non-volatile mixtures. The experiment is conducted on a sheet of aluminum foil, plastic, or glass which is coated with a thin layer of adsorbent material. The material usually used is aluminum oxide, cellulose, or silica gel [18].

Thin layer chromatography was performed with various crude extracts such as such as hexane, chloroform, ethyl acetate and methanol of *Ananas comosus*.

For each extract,

The ratio of the solvent was 1:1 of acetone and water, methanol and water

TLC plate should be 8 cm long in which spots were kept at above 1 cm. Silica gel plate is used to separate the compounds. Based on the band that appear in TLC plate Retention Factor (Rf) value can be calculated,

Rf value=Distance travelled by the compound/Distance travelled by solvent front.

TLC for saponins: To detect the saponins in the extract we take chloroform, glacial acetic acid, methanol and water as a standard ratio of 64:32:12:8. We take the ratio of solvent as 16:8:3:2. After preparing the solvent we make the TLC chamber. And then spotted in TLC plate through capillary tube.

TLC for flavonoids: To detect the saponins in the extract we take ethyl acetate: Formic acid: Glacial acetic acid: Water as a standard ratio of 25:2.75:2.75:6.5. After preparing the solvent we make the TLC chamber. And then spotted in TLC plate through capillary tube.

Estimation of proteolytic activity on gelatin under UV-VIS spectroscopy.

Gelatin solution preparation

100 mg of gelatin is dissolved in 100 ml water *i.e.,* 0.1% solution. From 0.1% stock solution 1 ml was taken and dissolved in 100 ml of distilled water which is 0.01% solution.

Measurement of fresh gelatin absorbance

In fresh gelatin we have made three concentrations which are 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml and placed in an UV spectrometer to see the absorbance and we found that 1 mg/ml there is no absorbance but we have seen 0.764 and 0.581 reading in 0.1 mg/ml and 0.01 mg/ml respectively.

Absorbance for gelatin+protein precipitate

In gelatin+protein ppt we have made three concentrations which are 1 mg/ml, 0.1 mg/ml and 0.01 mg/ml and placed in an UV spectrometer to see the absorbance and we found that in 1 mg/ml and 0.1 mg/ml there is no absorbance and in 0.01 mg/ml we found 0.079 absorbance.

Albumin solution

We have made 5% dilute albumin solution tested under UV spectrometer to find out the absorbance.

Pineapple Enzyme+Albumin solution

Test I

We have taken albumin and mixed with 9 ml enzyme and placed under UV spectrometer with 307 and 263 nm and got the absorbance 1.500 and 2.672 respectively. We have diluted the solution 10 times and find the absorbance of 0.756 at 307 nm.

Test II

We have taken 1 ml albumin and mixed with raw enzyme and placed under UV spectrometer with 307 and 263 nm and got the absorbance 1.904 and 3.200 respectively (Tables 1-7 and Figures 3-5). We have diluted the solution 10 times and find the absorbance of 0.823 at 307 nm.

RESULTS AND DISCUSSION

Table 1. Results for Identification test of amino acids.

Name of the tests	Result	Amino acids
Ninhydrin test	(-) Ve	-
Xanthoproteic acid test	(-) Ve	-
Lead sulphide test	(+) Ve	Cysteine
Folin's Mc Carthy Sullivan's test	(+) Ve	Methionine
Histidine test	(+) Ve	Histidine
Millon's test	(+) Ve	Tyrosine

Table 2. Result of TLC studies of enzyme.

Visible light		
Solvent Name	Solvent ratio	Rf value
Acetone: Water	1: 1	0.65
Methanol: Water	1: 1	0.454

Table 3. Result of TLC studies for saponins.

Solvent name	Solvent ratio	Rf Values			
		Long UV		Visible UV	
		Crude Enzyme	Centrifuged enzyme	Crude enzyme	Centrifuged enzyme
Chloroform: Glacial acetic acid: Methanol: Water	64:32:12:8	0.85	0.75	0.66	0.7

Table 4. Result of TLC studies for flavonoids.

Solvent name	Solvent ratio	Rf Values			
		Long UV		Visible UV	
		Crude enzyme	Centrifuged enzyme	Crude enzyme	Centrifuged enzyme
Ethyl acetate: Formic acid: Glacial acetic acid: Water	25:2.75:2.75:6.5	0.82	0.69	0.78	0.78

Table 5. Absorbance results for fresh gelatin.

Concentration	Absorbance
1 mg/ml	No result
0.1 mg/ml	0.764
0.01 mg/ml	0.581

Figure 3. Concentration *vs.* absorbance chart of fresh gelatin.

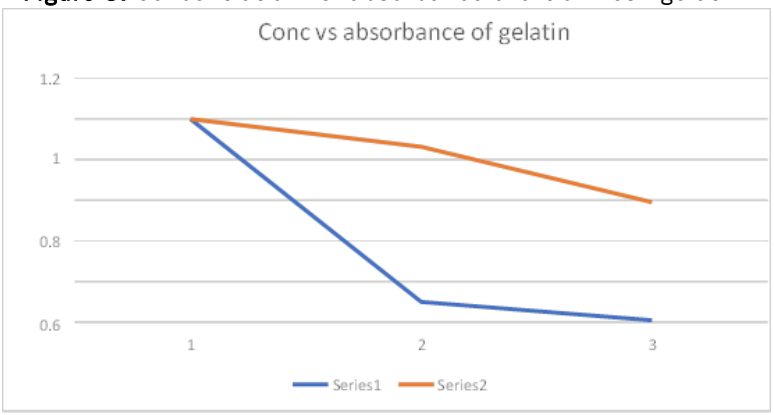


Table 6. Absorbance results for gelatin+protein precipitate.

Concentration	Absorbance
1 mg/ml	No result
0.1 mg/ml	0.578
0.01 mg/ml	0.079

Figure 4. Concentration *vs*. Absorption chart of gelatin and pineapple enzyme.

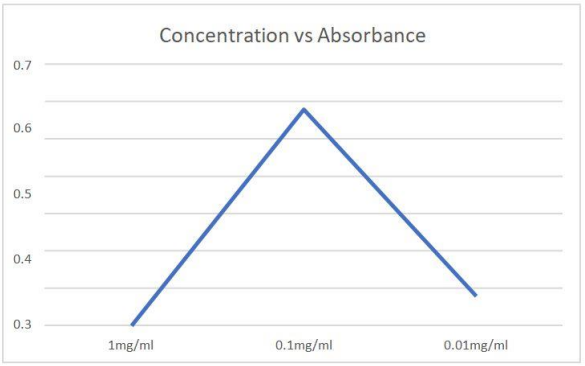
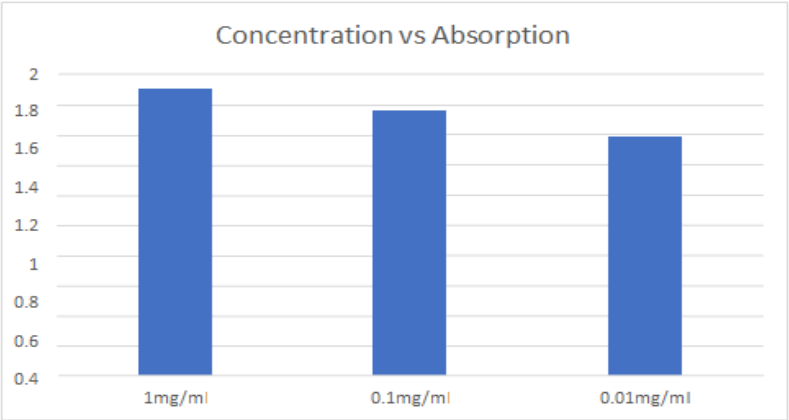


Table 7. Absorbance results of albumin and pineapple enzyme.

Λmax	Concentration	Absorbance
307 nm	1 mg/ml	1.904
307 nm	0.1 mg/ml	1.762
307 nm	0.01 mg/ml	0.586

Figure 5. Concentration *vs*. absorption of albumin and pineapple enzyme.



CONCLUSION

After performing all the identification test of amino acids, we found following amino acids present in the pineapple: Cysteine, methionine, histidine and tyrosine.

By performing the proteolytic activity, we can see that the pineapple enzyme can break the proteins. In cases of different people having digestion problem after having animal food. Some of the person can have the problem of having synthetic drug with different side effects. So the people can use the herbal products which are having low side effects and great bioavailability.

If this herbal formulation prepared into drug beneficial for the human kind. There is different formulation of pineapple having different activity and here in this research we are having proven proteolytic activity of pineapple extract.

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