ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

How different factors affect the activity of bromelain

Tang Xuanyuan

Jurong Country Garden School, Jurong City, Zhenjiang City, Jiangsu Province, 212400, China

Abstract: *Pineapple is a common tropical fruit in our daily life. Its special flavor has become its most signal feature that made it popular and distinctive. The secret of the sour flavor is bromelain, a type of enzyme contained in fruit of pineapple, which would break down proteins into small peptides or amino acid. When putting a pineapple in your mouth, the protease breaks down the crisps of oral mucosa, resulting in a slight sense of pain. Such a special flavor made it an important ingredient in Southeast Asian dishes.To eliminate the sour of pineapple, many households use salt water to soak the slice of pineapple before eating. In Southeast Asia, local people use pineapple to make dishes, by heating pineapple with other ingredients to reduce the sour. These method indicate that change in certain factors will influence the activity of bromelain.This essay will investigate several factors that would influence the flavor of pineapple. Using a series of experiment, it was found that the proteolytic activity of bromelain is influenced by temperature, acidity, salinity and types of salt. The result also proves that heat is the most effective way to eliminate the flavor.*

Keywords: *pineapple; protease; activity*

1. Introduction

Pineapple, Ananas comosus, is a common tropical plant in the family Bromeliaceae, as shown in Figure 1. It is a terrestrial herb that grows 0.75 to 1.5 meters tall and 0.9 to 1.2 meters wide, with short and stout stems. Its tip is usually needle-like and its edge is usually marked with sharp, upward-curving spines. The leaves may be all green or have various stripes of red, yellow, or ivory in the center or along the edges. At flowering, the stem elongates and expands near the apex and produces a head of small purple or red flowers, each accompanied by a red, yellow, or green bract[1]. "Chapter 2: Morphology, Anatomy, and Taxonomy". In Bartholomew, DP; Paull, RE; Rohrbach, KG (eds.). The Pineapple: Botany, Production, and Uses. Wallingford, UK: CABI Publishing. p. 21. ISBN 978-0-85199-503-8.)

Figure 1: Picture of fruit of pineapple.

After flowering, the single fruit develops from single flower come together to form a multiple fruit. The fruit, which also named pineapple, is a conical and succulent. The skin is composed of stinging hexagonal units with hard and tough waxy surface, with a cluster of short hard leaves on the top. Under the skin, the fruit has a succulent, fibrous pulp, usually yellow or green-yellow after matured, with a harder but rather juicy core inside[2]. What makes pineapple distinctive among fruits is partially the special flavor of the pulp. The sour flavor, which is different from that of lemon, generates a stinging feeling on tongue. If people keep consuming pineapple for some time, they may find they lost part of the taste and found there tongue numb. That is because the source of sour of is different from that of lemon or other fruit, which comes from organic acid. Instead, the taste comes from bromelain, a kind of enzyme

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

that found in pineapple, which molecular formula is C39H66N2O29, as shown in Figure 2.Different from generating hydrated ions that simulate the nerve, the enzyme can catalyze breaking peptide bonds of the primary structure of proteins, causing them to hydrolyze into peptides. When the humans or animals chew the pulp, the enzyme will catalyse the decompose of the protein on their oral mucosa. Thus, they can feel sting in the mouth. When the reaction is catalyzed for certain time, it will influence the nerve, which makestongue numb[3].

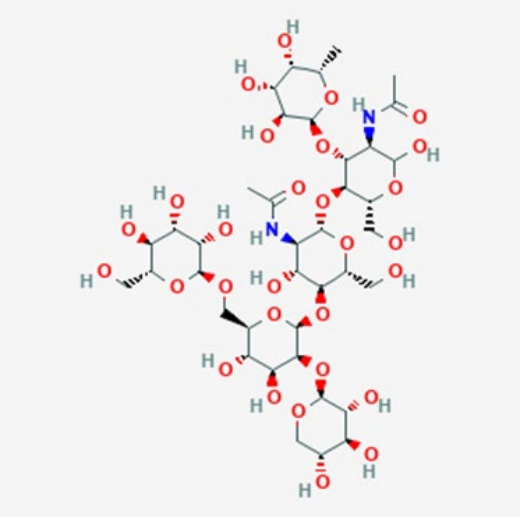

Figure 2: Molecular structure of bromelain, C39H66N2O29[4]

In daily life, to reduce the sour of pineapple, most household use salt water to immerse the slices of pineapple before consuming. It is believed that the method can eliminate sour without influence the original flavor. In other cases, like in Southeast Asia, local people use pineapple as a ingredient, heating with other food. For bakery, they often put pineapple into oven, baking under high temperature. These method has shown that high temperature and existence of metal ions can decrease the activity of bromelain[5].

2. Background information

Theory of how enzyme work:Enzyme, is a type of substance that act as organic catalyst that generate in living organisms. During the chemical reactions, the substance will not be used, but accelerate the rate of reaction. Same as other catalysts, enzyme can lower the activation energy of reaction, by correcting the orientation of collision to increase the rate of successful collision. For bromelain, its molecule has special configuration that allows certain molecules to be oriented into a proper arrangement to reduce the reaction entropy change, shown in Figure 3.

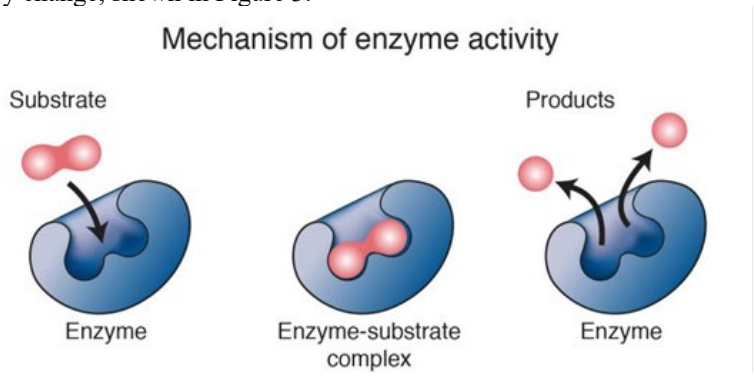

Figure 3: Mechanism of how enzyme works.

In the previous circumstance, enzyme serves as a kind catalyst by providing a proper orientation to increase the rate of successful collision. The activity of bromelain can be described as the ability of protein to provide active sites to orientating the collision. The more effective the site is, the higher activity the enzyme is. It also indicate that, to affect the activity of bromelain, it requires to deteriorate the enzyme's structure to prohibit orientating.

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

3. Variables for each experiment

The variables studied and controlled in experiments 1-4 are shown in Table 1.

Table 1: Variables for each experiment

	Experiment 1		*Experiment 2*		*Experiment 3*		*Experiment 4*	
Independent variables	*Reaction temperature (℃)*		*Acidity (pH)*		*Salinity of reaction condition (mol/dm3)*		*Types of metal ion involves in the condition*	
Dependent variable	*Absorbance of solution after reaction*	*Activity of bromelain*	*Absorbance of solution after reaction*	*Activity of bromelain*	*Absorbance of solution after reaction*	*Activity of bromelain*	*Absorbance of solution after reaction*	*Activity of bromelain*
Controlled variables	*Acidity (pH)* *Salinity (mol/dm3)* *Metal ions involved in the reaction* *Concentration of bromelain (mol/dm3)* *Time of reaction (min)*		*Reaction temperature (℃)* *Salinity (mol/dm3)* *Metal ions involved in the reaction* *Concentration of bromelain (mol/dm3)* *Time of reaction (min)*		*Acidity (pH)* *Reaction temperature (℃)* *Metal ions involved in the reaction* *Concentration of bromelain (mol/dm3)* *Time of reaction (min)*		*Acidity (pH)* *Salinity (mol/dm3)* *Reaction temperature (℃)* *Concentration of bromelain (mol/dm3)* *Time of reaction (min)*	

3.1. Reagent

Bromelain (C39H66N2O29); Casein (C81H125N22O39P); Coomassie brilliant blue G-250 (CBB); L-Tyrosine; L-cysteine; M-cysteine; Ethylenediaminetetraacetic acid (EDTA) (C10H16N2O8); Ethylenediaminetetraacetic acid disodium (EDTA-2Na) (C10H14N2Na2O8); Trichloroacetic acid (C2HCl3O2); Tris (tromethamine) (C4H11NO3); Dithiothreitol (DTT) (C4H10O2S2); Ethanol (C2H5OH); Phosphoric acid (H3PO4); Sodium hydrogen phosphate (Na2HPO4); Sodium dihydrogen phosphate (NaH2PO4); Ethanoic acid (CH3COOH); Sodium acetate (CH3COONa); CaCl2 MgCl2 ZnCl2 CuCl2 CoCl2 NiCl2 (powder); Deionized water

3.2. Instrument

Beaker(100mL; 250mL); Spectrometer; Cylinder(0-100mL,1mL; 0-25mL,0.1mL); Electronic balance(0-999.99g, 0.01g; 0-99.999g, 0.001g); Centrifuge; Centrifuge tube(15mL); Timer; Water bath; Pipette(0-25mL,0.1mL; 0-999μL, 0.1μL); Volumetric flask(1L; 100mL); Dropper; Glass rod; Glass funnel; Test tube(18mm*180mm)

3.3. Experiment procedure

3.3.1. Buffer solution

Weigh 10.00g trichloroacetic acid using electronic balance and added into beaker.Add deionized

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

water to dissolve.Put solution into volumetric flask, add deionized water to 1L

3.3.2. Preparation of protein reagent

Coomassie Bright blue G-250 (100 mg) dissolved in 50 mL 95% ethanol. The solution obtained by adding 100 mL 85% (W/V) phosphoric acid to this solution was diluted to the final volume. The final concentration of the reagent was 0.01% (W/V) Coomassie Bright Blue G-250. 4.7% (W/V) ethanol and 8.5% (W/V) phosphoric acid.

3.3.3. Casein Substrate

Measure 400 mg casein using electronic balance. Dissolve casein in 100 mL 50 mM sodium phosphate solution, which pH is 8.5.Boil for 10 minutes. Filter the solution to obtain casein stock.Dilute casein solution to 300 μg mL-1 using buffer solution.Put the solid analysis pure in the baking oven to dry for 10 minutes

3.3.4. Protein activity determination

Table 2: The amount of protein, NaCl solution and Coomassie brilliant blue used in each tube.

Number	*0*	*1*	*2*	*3*	*4*	*5*	*6*
1mg/mL Standard protein solution/μL	*0.0*	*10.0*	*20.0*	*40.0*	*60.0*	*80.0*	*100*
0.15mol/L NaCl solution/μL	*100*	*90.0*	*80.0*	*60.0*	*40.0*	*20.0*	*0.0*
Coomassie brilliant blue/mL	*5.00*	*5.00*	*5.00*	*5.00*	*5.00*	*5.00*	*5.00*

The amount is shown in Table 2,use pipette to move casein substrate of certain amount into test tube.Use buffer adjust the volume in the test tube to 0.1 mL..Add 5mL protein reagent in the test tube.Put mixed solution into colorimeter, use test tube as controlled group, and measure absorbance at 595nm.

3.3.5. Measurement of activity of bromelain

Measure 5mL casein solution using cylinder.Put solution into the tube and put tube into water bath, heat under 37℃ for 10min.Measure 0.1mL bromelain solution and dilute with EDTA-2Na and L-cysteine to 1mL.Put mixed solution into water bath, heat under 37℃ for 30 minutes.Add 5mL Trichloroacetic acid to terminate reaction.Cool down solution to room temperature.Put solution into centrifuge tube, centrifuge and centrifuge at 3500r/min for 10 minutes.Take upper layer of solution into cuvette, measure absorbance under 275nm, recorded as A.Switching the step 5 and step 1 to 4, measure absorbance, and recorded as A0. The reagent steps and dosage for the sample and control group are shown in Table 3.

Table 3: The step and amount of reagent used for sample and controlled group.

Step	*Sample*	*Controlled groups*
1	*Casein* *5mL, stored under 37±0.1℃*	*Casein* *5mL, stored under 37±0.1℃*
2	*Enzyme* *1.00mL, react for 10 minutes*	*Trichloroacetic acid* *5.00mL, react for 10 minutes*
3	*Trichloroacetic acid* *5.00mL, stand for 30min, then contrifuge*	*Enzyme* *1.00mL, stand for 30min, then contrifuge*

3.3.6. Relationship between temperature and activity of bromelain

Stored bromelain solution under 0, 25, 37, 45, 55, 65℃ in water bath and fridge separately for 2 minutes.Repeat the step mentioned.

3.3.7. Relationship between pH and activity of bromelain

Mix bromelain solution with solution of pH=5.0, 6.0, 7.0, 8.0, 9.0, 10.0 for 10 minutes , shown in table 4. After solutions are finished, measure the pH sing pH meter to ensure the acidity.

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

Table 4: The reagent and their mount used to make solution of specific pH.

Solution (pH±0.1)	*Method*
pH=5.0	*1. Move 0.57mL ethanoic acid into test tube using pipette.* *2. Measure 1.476g sodium acetate using electronic balance.* *3. Mix sodium acetate with ethanioc acid, add deionized water to 100mL.*
pH=6.0	*1. Measure 7.16g Na2HPO4 using electronic balance.* *2. Mix with 100mL deionized water.* *3. Measure 3.12g NaH2PO4.* *4. Mix with 100mL deionized water.* *5. Use pipette to move 12.3mL Na2HPO4 solution and 87.7mL NaH2PO4 solution to mix together and get 100mL solution.*
pH=7.0	*1. Use pipette to move 62mL Na2HPO4 solution and 38mL NaH2PO4 solution to mix together and get 100mL solution.*
pH=8.0	*1. Measure 1.2114g Tris using elctronic balance.* *2. Dissociate in 100mL deionized water.* *3. Measure 0.84mL HCl solution.* *4. Mix with 100mL deionized water.* *5. Use pipette to move 50mL Tris solution and 29.2mL HCl solution to mix.* *6. Add 20.8mL deionized water*
pH=9.0	*1. Use pipette to move 50mL Tris solution and 7.0mL to mix.* *2. Add 43.0mL deionized water.*
pH=10.0	*1. Measure 1.2114g Tris using elctronic balance.* *2. Dissociate in 100mL deionized water.*

3.3.8. Relationship between Metal ions and activity of bromelain

Measure certain amount of salt to get solution with concentration of 10mM, the procedure is shown in table 5, Mix the above solutions with bromelain respectively.

Table 5: The reagent and their mount used to get specific hydrated ions.

Ion	*Method*
Na+	*1. Measure 0.585g NaCl using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*
Ca2+	*1. Measure 1.11g CaCl2 using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*
Ion	*Method*
Mg2+	*1. Measure 0.952g MgCl2 using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*
K+	*1. Measure 0.746g KCl using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*
Co2+	*1. Measure 1.30g CoCl2 using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*
Ni2+	*1. Measure 1.30g NiCl2 using elctronic balance.* *2. Add into volumetric flask of 1L.* *3. Add deionized water to 1L.*

3.3.9. Relationship between NaCl and Bromelain Activity

Mix certain amount of NaCl and mix with deionized water to get solution with salinity of 1.0%, 3.0%, 5.0%, 7.0%, 9.0%, 11.0%, shown in the table 6, Mix the solution of bromelain with solution of different salinity for 10 minutes.

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

Table 6: The reagent and their mount used to get solution of specific salinity.

Salinity	*Method*
1.0%	*1. Measure 1.00g NaCl using elctronic balance.* *2. Measure 99.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*
3.0%	*1. Measure 3.00g NaCl using elctronic balance.* *2. Measure 97.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*
5.0%	*1. Measure 5.00g NaCl using elctronic balance.* *2. Measure 95.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*
7.0%	*1. Measure 7.00g NaCl using elctronic balance.* *2. Measure 93.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*
9.0%	*1. Measure 9.00g NaCl using elctronic balance.* *2. Measure 91.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*
Salinity	*Method*
11.0%	*1. Measure 11.00g NaCl using elctronic balance.* *2. Measure 89.0mL water using cylinder.* *3. Solute the NaCl in water, stringing by glass rod.*

3.4. Safety precautions

NiCl2 may cause eye irritation with susceptible persons, may cause sensitization by skin contact, or cause skin irritation in susceptible. During experiment, ensure adequate ventilation, especially in confined areas, wear impervious gloves and glasses. Clean hand right after the experiment.CoCl2 during experiment, ensure adequate ventilation, especially in confined areas, wear impervious gloves and glasses. Clean hand right after the experiment.Water bath is dangerous when boiling water, which can cause scald when contacting the water or the outer surface of the equipment. Second, the hot water vapor can also cause scald. which may lead to scald. Wear gloves and long-sleeved shirt when operating water bath. Use crucible tongs to pick beakers from boiling water instead of using hands.If centrifuge doesn't balance before it works, it will damage the rotor. When the rotor rotates at high speed, the centrifugal tube may broke. Balance the rotor before it works, and find a proper speed and time region. Choose a proper centrifugal tube and tighten the cover.

4. Data and analysis

4.1. The concept of activity of bromelain

The concept of bromelain is defined as: a measure of the "effective concentration" of a species in a mixture, in the sense that the species' chemical potential depends on the activity of a real solution in the same way that it would depend on concentration for an ideal solution.

In this experiment, the activity of bromelain can be defined as: The amount of enzyme required to produce 1ug tyrosine after 1min of action on casein substrate. According to the definition, the unit activity of bromelain can be defined as:

$$Activity/unit = \frac{A - A_0}{A_s} \times M_S \times \frac{V_0}{t} \times \frac{k}{V}$$

A: absorbance of sample.A0: absorbance of controlled group. As: Standard tyrosine absorbance of 50 ug/mL at 275nm.Ms: Concentration of standard tyrosine solution.V0: Total volume of reaction system.V: Volume of measured sample.t: Time of reaction.k: Dilution ratio.

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

4.2. Experiment 1

4.2.1. Raw data

Table 7: The absorbance measured under each temperature.

V0 = 6.0 mL	
Temperature (℃)(±0.1℃)	*Absorbance (±0.001)*
30.0	*0.076*
37.0	*0.104*
45.0	*0.113*
50.0	*0.161*
55.0	*0.183*
60.0	*0.169*

Note. Table 7 shows the measured data under different conditions.

4.2.2. Data processing

A0=0.0544,As=0.381,Ms=50μg mL-1,V0=6.0mL,V=1.0mL,t=10min,k=10

Sample calculation:

$$Activity/unit=\frac{0.076-0.0544}{0.381}\times 50\mu g/mL\times\frac{6.0mL}{10\min}\times\frac{10}{1.0mL}=16.97$$

Uncertainty:

$$\left(\frac{0.001}{0.076-0.0544}+\frac{0.001}{0.381}+\frac{1\mu g/mL}{50\mu g/mL}+\frac{0.01\min}{10.0\min}+\frac{1mL}{6.0mL}+\frac{0.01mL}{1.00mL}\right)\times 100\%=5\%$$

The activity of bromelain at various temperatures is shown in Table 8 and Figure 4.

Table 8: The activity of bromelain under each temperature.

V0 = 6.0 mL	
Temperature (℃)(±0.1℃)	*Activity (±5%)*
30.0	*16.97*
37.0	*39.02*
45.0	*46.11*
50.0	*83.90*
55.0	*101.23*
60.0	*90.20*

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

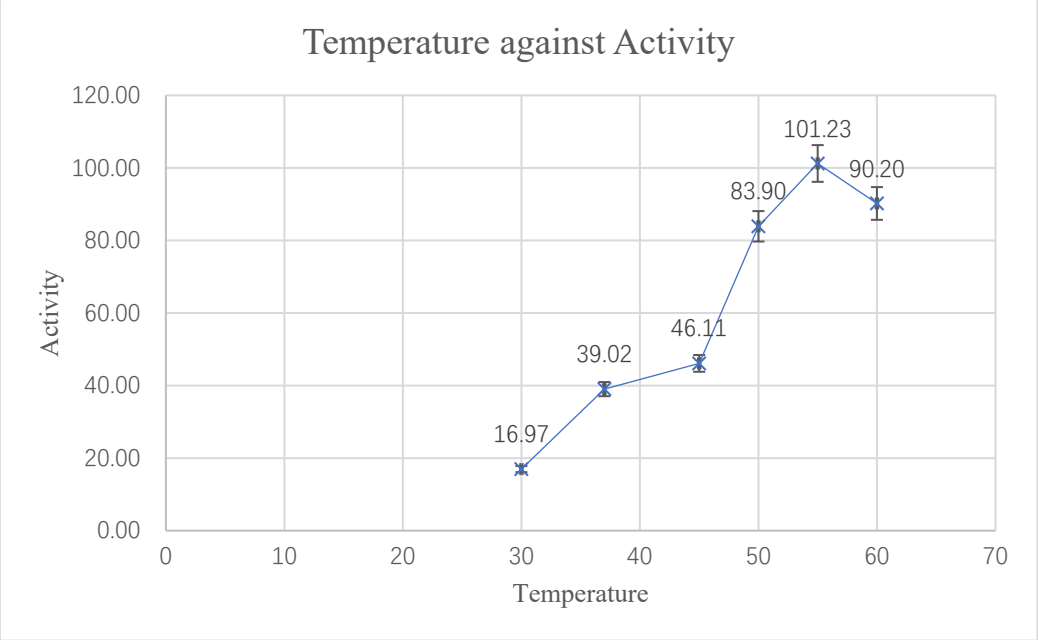

Figure 4: The activity of bromelain under each temperature.

4.2.3. Analysis

From the data and figure, we can deduce that the activity of bromelain reach its maximum at temperature around 55℃. Below this temperature, as temperature increase, the rate of reaction increase. This is because, as the temperature increase, the kinetic energy of molecule increase. Thus, the chance of effective collision increase. Above 55℃, the structure of the enzyme begins to break apart. That is because at higher temperature, the atom gains more energy, intermolecular bonds in the bromelain break down. Thus, the structure of enzyme is changed, then the molecule can't allocate in a proper orientation to decompose. As a result, the rate of reaction decrease.

4.3. Experiment 2

4.3.1. Raw data

Table 9: The absorbance measured under each pH.

V0 = 6.0mL	
pH (±0.1)	*Absorbance (±5%)*
5.0	*0.066*
6.0	*0.087*
7.0	*0.114*
8.0	*0.101*
9.0	*0.090*
10.0	*0.078*

Note.Table 9shows the measured data under different conditions.

4.3.2. Data processing

A0=0.0544,As=0.381,Ms=50μg mL-1,V0=6.0mL,V=1.0mL,t=10min,k=10

Sample calculation:

$$Activity/unit=\frac{0.066-0.0544}{0.381}\times 50\mu g/mL\times\frac{6.0mL}{10\min}\times\frac{10}{1.0mL}=8.85$$

Uncertainty:

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

$$\left(\frac{0.001}{0.066-0.0544}+\frac{0.001}{0.381}+\frac{1\mu g/mL}{50\mu g/mL}+\frac{0.01\min}{10.0\min}+\frac{1mL}{6.0mL}+\frac{0.01mL}{1.00mL}\right)\times 100\%=5\%$$

The activity of bromelain under each pH is shown in Table 10 and Figure 5.

Table 10: The activity of bromelain under each pH.

V0 = 6.0mL	
pH (±0.1)	*Absorbance (±5%)*
5.0	*8.85*
6.0	*25.58*
7.0	*46.99*
8.0	*36.38*
9.0	*27.83*
10.0	*18.90*

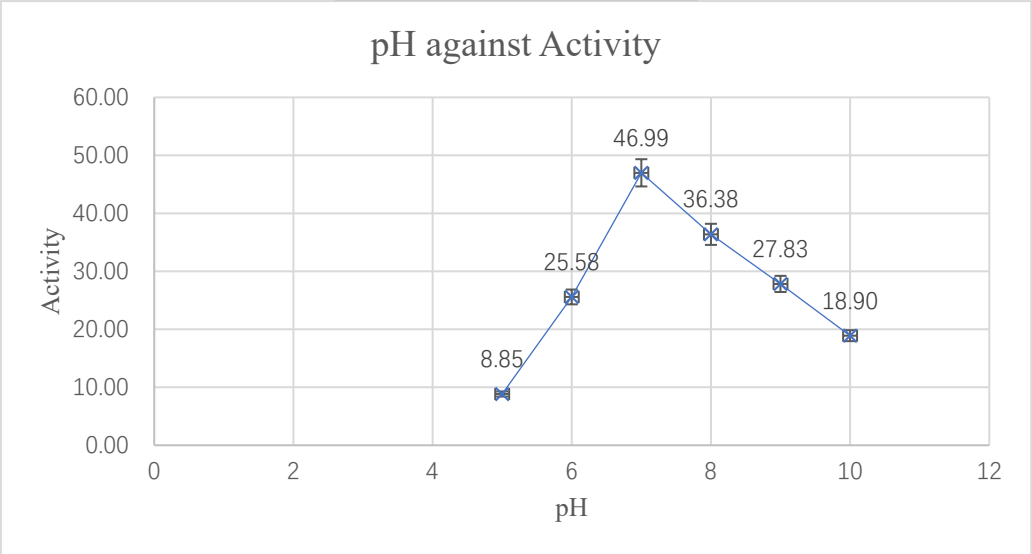

Figure 5: The activity of bromelain under each pH.

4.3.3. Analysis

From the table and figure, we can deduce that, the bromelain has highest activity when pH is around 7. Below this point, as pH increase, activity increase, while above this value, as pH increase, activity decrease. That is because, free hydrated hydrogen ion has empty orbitals, which will attract electrons of the bromelain, which thus change its configuration. When hydrogen ions is at certain concentration, the configuration is at highest efficiency.

4.4. Experiment 3

4.4.1. Raw Data

Table 11: The absorbance measured under each salinity.

V0 = 7.0mL	
Salinity	*Absorbance (±0.001)*
1.0%	*0.100*
3.0%	*0.090*
5.0%	*0.086*
7.0%	*0.081*
9.0%	*0.071*
11.0%	*0.068*

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

Note.Table11 shows the measured data under different conditions.

4.4.2. Data processing

A0=0.0544,As=0.381,Ms=50μg mL-1,V0=6.0mL,V=1.0mL,t=10min,k=10

Sample calculation:

$$Activity/unit=\frac{0.100-0.0544}{0.381}\times 50\mu g/mL\times\frac{7.0mL}{10\,\min}\times\frac{10}{1.0mL}=42.23$$

Uncertainty:

$$\left(\frac{0.001}{0.100-0.0544}+\frac{0.001}{0.381}+\frac{1\mu g/mL}{50\mu g/mL}+\frac{0.01\,\min}{10.0\,\min}+\frac{1mL}{7.0mL}+\frac{0.01mL}{1.00mL}\right)\times 100\%=5\%$$

The activity of bromelain under each salinity is shown in Table 12 and Figure 6.

Table 12: The activity of bromelain under each salinity.

V0 = 7.0mL	
Salinity	*Activity (±8%)*
1.0%	*42.23*
3.0%	*32.20*
5.0%	*28.99*
7.0%	*24.39*
9.0%	*15.21*
11.0%	*12.34*

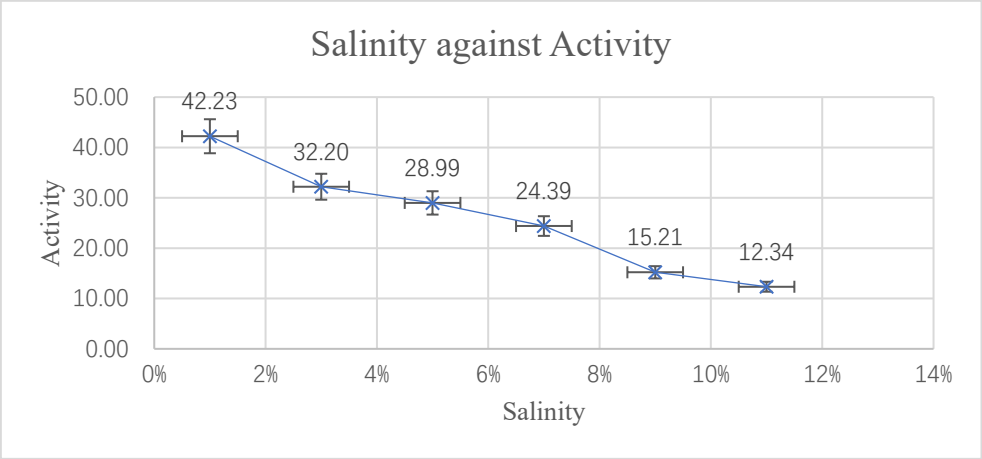

Figure 6: The activity of bromelain under each salinity.

4.4.3. Analysis

From the data and figure, we can deduce that the activity of bromelain decrease as the concentration of Na+ increase. The reason is similar to how pH influence the activity, the difference is that sodium ion has greater effective nuclear charge, so the electrostatic attraction is larger, so the protein configuration is distorted more. As a result, the activity decrease sharply.

4.5. Experiment 4

4.5.1. Raw data

Table 13: The absorbance measured under existence of different ions.

V0 = 7.0mL

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

Metal Ion	*Absorbance (±0.001)*
Na+	*0.106*
Mg2+	*0.178*
K+	*0.094*
Ca2+	*0.188*
Co2+	*0.140*
Ni2+	*0.115*

Note.Table13 shows the measured data under different conditions.

4.5.2. Data processing

A0=0.0544,As=0.381,Ms=50μg mL-1,V0=6.0mL,V=1.0mL,t=10min,k=10.

Sample calculation:

$$Activity/unit = \frac{0.106-0.0544}{0.381} \times 50\mu g/mL \times \frac{7.0mL}{10\,\min} \times \frac{10}{1.0mL} = 47.53$$

Uncertainty:

$$\left(\frac{0.001}{0.106-0.0544}+\frac{0.001}{0.381}+\frac{0.5}{1000}+\frac{1\mu g/mL}{50\mu g/mL}+\frac{0.1\,\min}{10.0\,\min}+\frac{1mL}{7.0mL}+\frac{0.01mL}{1.00mL}\right)\times 100\% = 21\%$$

The activity of bromelain under existence of different ions is shown in Table 14 and Figure 7.

Table 14: The activity of bromelain under existence of different ions.

V0 = 7.0mL	
Metal Ion	*Activity (±20%)*
Na+	*47.53*
Mg2+	*113.46*
K+	*36.43*
Ca2+	*122.77*
Co2+	*78.38*
Ni2+	*55.61*

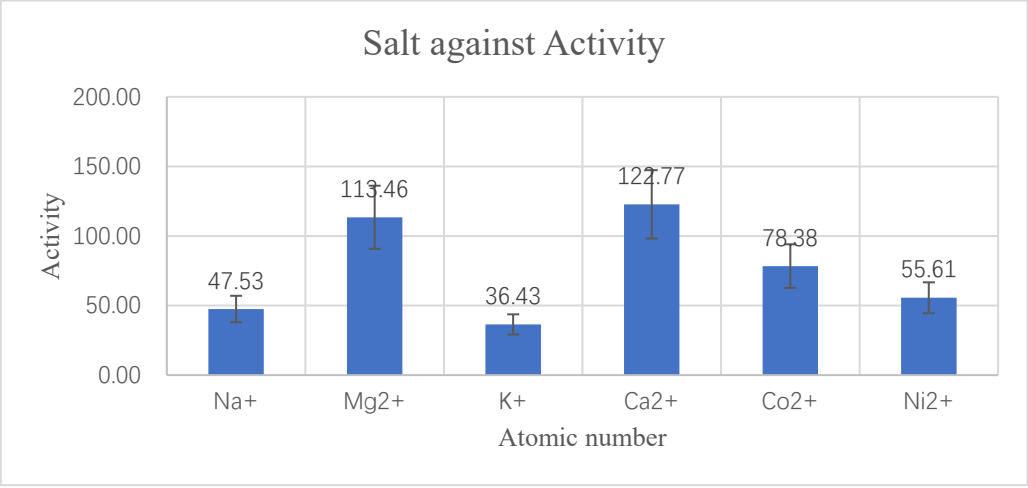

Figure 7: The activity of bromelain under existence of different ions.

4.5.3. Analysis

To explain the phenomenon, it should use the concept of volume charge density, a measure of the quantity of charge per unit volume at any point in a volume. The following table 15 shows the charge density of ions used in the experiment.

Table 15: The activity of bromelain under existence of different ions.

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

Metal Ion	*Charge density(Cm-3)*
Na+	*24*
Mg2+	*120*
Metal Ion	*Charge density(Cm-3)*
K+	*11*
Ca2+	*52*
Co+	*108*
Ni2+	*134*

When the charge density is higher, the charge per volume is larger, leading to greater polarization between atoms. As the polarization increase, the secondary structure of enzyme, which change the helix of protein.

Deducing from Table 14 and Table 15, the ion with lower charge decrease the activity more efficient than that with higher charge. A possible reason is that the change in helix may improve the structure of enzyme, making it works more efficiently. Since the high concentration of heavy meta ions would cause denaturation of protein, the measurement only shows the situation of low concentration of solution. The result implied that, when the concentration of metal ion is small, the ions might accelerate the reaction.

The problem throughout this group is that the uncertainty of activity reach 20%, which cause the range of certain activity to overlap. So, it is hard to justify whether the electric density is directly correlated to the variables investigated.

5. Evaluation

5.1. Errors and solutions

During the experiment, there are various source leading to random errors.

First, the whole experiment only measured the activity of bromelain under limited conditions in a small range. The data collected cannot give a precise answer. Also, the trend predicted is extrapolation, which means there may be unexpected changes.

Second, when dropping the solution of different ions into solution, it turned out that heavy metals, Co and Mn, would lead to deposit of protein. A possible explanation is that heavy metal would break the bond of protein leading to metamorphism.

Third, the whole process take consider of the possible result when two factor changes simultaneously. A possible changes in pressure or other factors may influence the result as well.

5.2. Real life application

From the previous study, we can found that, to eliminate the sour, the ways is to use high temperature or low temperature and pH, with high concentration of NaCl.

To prove this result, I conducted the following experiments as shown in Table 16.

Cut a pineapple into slice of 2cm thick, then doing following work.

Table 16: Experiment

Group	*Method*
A	*Measure 60mL water, as controlled group.* *Put slice into solution for 10 min, under room temperature.*
B	*Measure 10mL vinegar, mix with 50mL water, to mimic acid condition.* *Put slice into solution for 10 min, under room temperature.*
C	*Measure 15g NaHCO3, mix with 60mL water, to mimic alkaline condition.* *Put slice into solution for 10 min, under room temperature.*
Group	*Method*
D	*Heat water to 80℃, mimic high temperature.* *Put slice into solution for 10 min, under room temperature.*
E	*Heat water to 55℃, mimic temperature of highest activity.* *Put slice into solution for 10 min, under room temperature.*
F	*Measure 10g salt, mix with 60mL water, to mimic high salinity.* *Put slice into solution for 10 min, under room temperature.*

To make the result more reliable, I invited 10 people as volunteer to test the sour of pineapple. After tasting each groups, volunteer is required to drink water to eliminate the leaving bromelain. After all

ISSN 2706-6819 Vol.4, Issue 10: 30-42, DOI: 10.25236/IJFM.2022.041006

process, the volunteers are asked to give a ranking of the sour of each groups. None volunteer were told how each groups are operated.

After collecting the reflection, the result is that F<D<E<C≈A≤B. Generally, the result follows the conclusion of experiment. But, there are some outliers that doesn't fit in the conclusion. First, even though B is reacted in acidic condition, the sour increased, meaning that the activity didn't affected. A probably explanation is that the sour of vinegar influenced the original flavor, which made the pineapple sour.

Another outlier is that E is less sour than controlled group. According to previous experiment, bromelain reach its maximum activity at 55℃, so the sour would be the greatest theoretically. But the result shows that the activity decreased. A convincing explanation is that: as temperature increase, the kinetic energy of molecule increase. Thus, more bromelain in the pulp dissolve in the water, which made the concentration of bromelain decrease.

In conclusion, we can deduce that the application prove the result of the experiment.

6. Conclusion

This essay studied the factors that influence the activity of bromelain. By using casein as substrate, the passage examine the absorbance of reacted solution and use proper formula to find out the activity of bromelain under certain conditions.

From the data, it is shown that, the activity of bromelain reach its maximum at temperature around 55℃. Below this temperature, as temperature increase, the rate of reaction increase. It has highest activity when pH is around 7. Below this point, as pH increase, activity increase, while above this value, as pH increase, activity decrease. The activity of bromelain decrease as the concentration of Na+ increase. Also, the ions with high electric density would decrease the activity most.

From the result, it can also conclude that, high temperature is more effective and feasible to eliminate the activity of bromelain. That is because the activity of bromelain reach its peak at 55℃and the activity is sensitive to the change in temperature. It is fairly easy to change its activity through changing temperature, both in laboratory and daily life. It is also impossible for consumers to use heavy metal ions in daily food consumption.

From the experiment, the bromelain reach its maximum activity at 55℃, pH=7, with no metal ions in the water. It can be conclude that our daily method, like sinking the slices into water or cook under high temperature are all effective way to eliminate the sour. The real life application proved the result of the experiment, which helped giving a conclusion that could help guide the consumption of fresh pineapple in daily life.

Further, to make the result more precise and comprehensive, it is necessary to take more groups of experiment, both expanding the range of variables and decrease the interval between factors. During the process, the measurement accuracy should be improved, in order to decrease random error. Also, to make the result more convinced, an experiment of examining whether anions in water will affect activity should be done.

References

[1] Coppens d'Eeckenbrugge, G; Leal, F. (2003). "Chapter 2: Morphology, Anatomy, and Taxonomy". In Bartholomew, DP; Paull, RE; Rohrbach, KG (eds.). The Pineapple: Botany, Production, and Uses. Wallingford, UK: CABI Publishing. p. 21. ISBN 978-0-85199-503-8.

[2] Anwei Chen, Wenliang Wang, Tongcheng Xu, "Research progress of factors affecting bromelain activity", Food and Nutrition in China, No. 10, 2009.

[3] Di Di, Yongxing Zhou, Xinye Zhang, "Investigation on the conditions affecting bromelain activity", BULLETIN OF BIOLOGY, Volume 55, Number 11, 2020.

[4] National Center for Biotechnology Information (2022). PubChem Compound Summary for CID44263865, Bromelain. Retrieved January 31, 2022 from https:// pubchem.ncbi.nlm.nih.gov/ compound/Bromelain.

[5] GALLAGHER, JACQUELINE, et al. "Hydrolysis of Casein: A Comparative Study of Two Proteases and Their Peptide Maps." International Journal of Food Science & Technology, vol. 29, no. 3, 6 July 2007, pp. 279–285, 10.1111/j.1365-2621.1994.tb02069.x