Why Won't My Jelly Set?

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Investigating the methods for altering the functioning of an enzyme catalyst: which treatment of pineapple, amongst fresh, frozen, commercially canned, and cooked has the most enzymic effect on the pineapple, and in turn the gelatin that it is placed in? Which treatment alters the enzyme present in pineapples? And what does this mean for the consumers like us?

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ABSTRACT

The primary idea investigated in this experiment is the use of heat as a method for altering the functioning of an enzyme catalyst – in this case with the use of pineapples subjected to different treatments and gelatine. Pineapple contains an enzyme called bromelain that hydrolyses certain proteins called gelatins. In this experiment, the different samples of pineapple are added to gelatin to determine whether the treatment has altered the enzyme's activity. In general, the setting of the gelatin solution indicates that the treatment has altered or denatured the enzyme that is present in the pineapple. The non-setting of the gelatin solution indicates that the treatment has not altered or denatured the enzyme's activity, and it operates just as it had before the treatment, as a proteolytic enzyme. As the results were tabulated and graphed, it became obvious that the cooking and commercial canning of the pineapples had denatured the enzyme and prevented it from digesting proteins. On the other hand, the enzymes in the fresh and frozen pineapples were not denatured, and thus still functioned as it had before. The results, however, did not reveal as clear a difference as to which treatment between cooking and canning denatured the enzymes quicker, as results were very much similar for the two treatments. Despite this, the results proved to be accurate and reliable. The results corresponded to what was predicted in the hypothesis: The four different pineapple samples will either set or not set. The fresh and frozen pineapple samples in the gelatin solution will not set, but the commercially canned and cooked pineapple in gelatin solution will set.

AIM

To determine the effect of 4 different treatments of pineapple on the solidifying process of the gelatin that each of the different pineapple samples are placed in.

HYPOTHESIS

The four different pineapple samples, when placed into the solution of gelatin, will cause the entire solution to either set or not set at all. The fresh and frozen pineapple samples in the gelatin solution will not set, but the commercially canned and cooked pineapple in gelatin solution will set.

INTRODUCTION

Why is the subject important?

Nowadays, consumers are increasingly interested in health and nutrition, so consequently, fruits and vegetables are consumed much more than in previous years. Pineapple is a fruit that is planted all over parts of the world, particularly in tropical climates, and is generally consumed fresh. Bromelain is an enzyme that is beneficial for health and is found naturally in pineapples. It has been used for a long time as a medicinal substance by several native cultures, and has been chemically known since 1876 (Taussig and Batkin, 1988). Bromelain is the collective name for proteolytic enzymes found in various members of the family Bromeliaceae, and bromelain from the pineapple (*Ananas comosus*) is one of the most studied proteolytic enzymes by scientists all over the world.

Why does it interest me?

Personally, I became interested in the subject when, in an attempt to make a fruit jelly using a range of fruits including kiwifruits, was unsuccessful. There were also instances where I had consumed puddings and other gelatin-containing desserts and had read the packaging, in which had warned the customers against adding fresh fruits such as pineapples and kiwifruits to prevent it from not setting or becoming "runny".

What is the science behind my hypothesis?

Gelatin is derived mainly from the skins and bones of cows, pigs, and horses, which are boiled in water so that the collagen comes out. Collagen is a protein, which is to say that its molecules are made of long chains of amino acids. These are arranged in three mutually twisted chains - a sort of triple helix. (Swain, 2009)

The chemical reactions in all cells of living things operate in the presence of biological catalysts called **enzymes.** There are thousands of different enzymes in a cell catalysing thousands of different chemical reactions – so one particular enzyme catalyses only one reaction. There is an enzyme in pineapple called **bromelain** and is a proteolytic enzyme, which means it digests proteins.

The enzyme that is investigated in this experiment is one that is produced in pineapple and hydrolyses certain kinds of proteins, in this case gelatin. The gelatin used in this experiment is derived from skin, bones, and/or connective tissue of animals. These proteins, when dissolved in hot water and allowed to cool, form a semi-solid or gel state; hence the name gelatin. Hydrolyse, here, refers to breaking up the protein polymer in such a way as to prevent its forming this gel state. The hydrolysing enzyme from pineapple is denatured by heat. Denature, here, means to alter in such a manner that the enzyme is no longer able to catalyse the reaction. Enzymes can also be denatured by changes in pH, subjection to detergents or radiation, etc. (Marshman, 2013) Enzymes need specific conditions to work properly, and can be destroyed if those conditions change. Temperature is one of those conditions. (DasGupta, 2013)

MATERIALS

Chemicals

- 1 x 20g sample of gelatin powder
- 1 x 445g can of canned pineapple slices
- 1 x medium – large fresh pineapple
- Approx. 2L of tap water

Equipment

- 9 x 250-mL beakers
- 4 x stirring rods
- 1 x hot plate or stove
- 1 x 1L saucepan
- 2 x chopping boards
- 2 x colander
- 1 x electronic balance
- 1 x 1L measuring cylinder
- 1 x permanent marker
- 1 x knife for cutting pineapple
- 1 x freezer
- 1 x refrigerator
- 1 x 1L mixing bowl
- 4 x pairs of tongs
- 1 x roll of cling wrap
- 3 x 1ply Coles brand paper towel
- 1 x scissors
- 1 x fine 0.5 ballpoint pen
- 1 x metal ruler
- 1 x stopwatch / timer

The approximate set up of the experiment is shown in Figure 1.

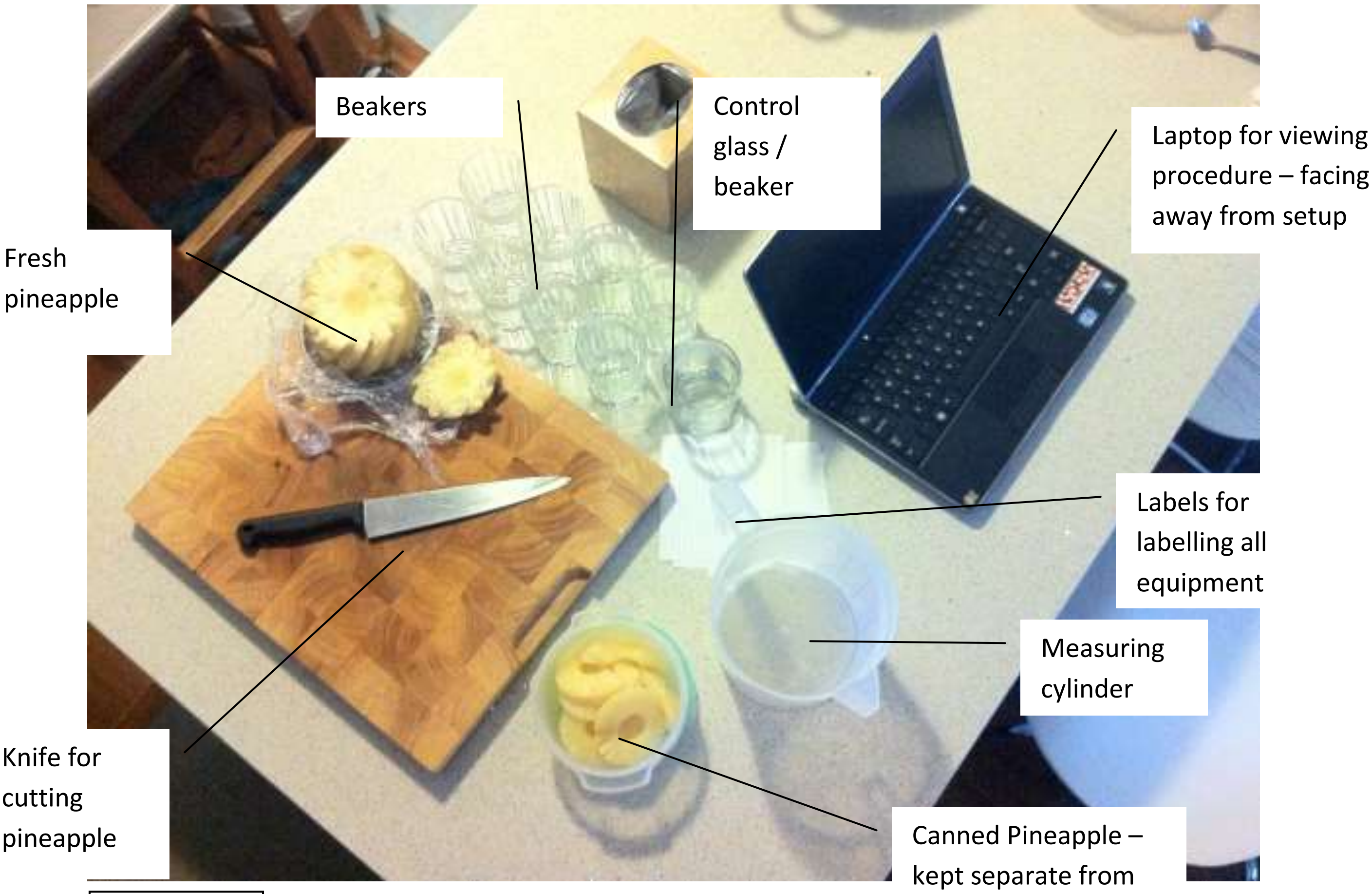
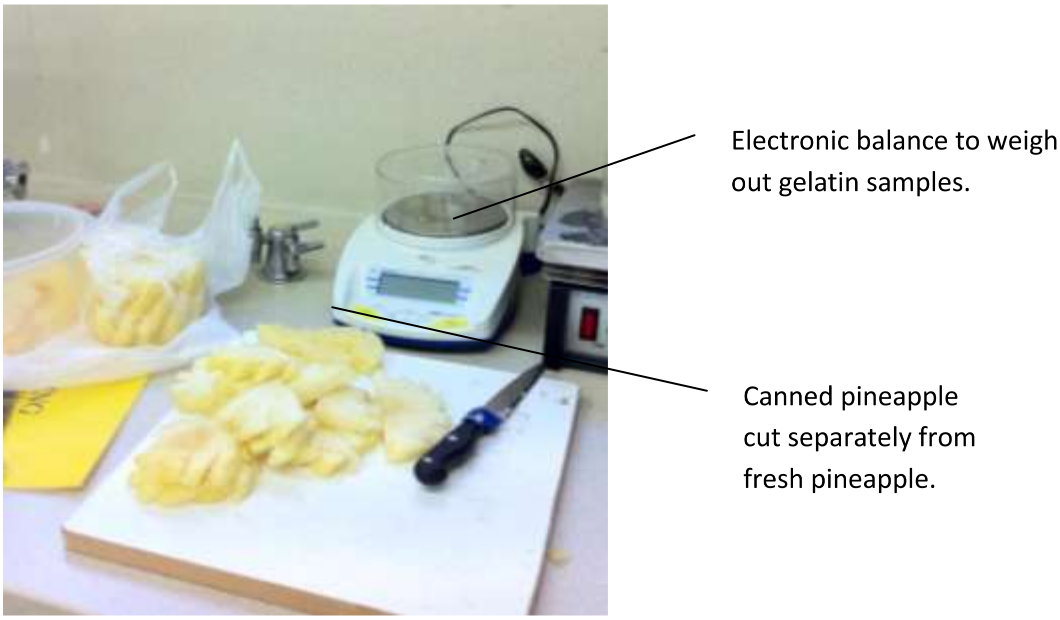


Figure 1



RISK ASSESSMENT

Mandatory precautions: Covered shoes, safety glasses, hair exceeding shoulder length tied back. (PTO for Risk Assessment)

METHOD

Preparation of Paper Towel Strips for Solidity Measurements

1. Before conduction of the experiment: the 1ply paper towel was placed vertically onto the table and, using the metal rules, 4 centimetre intervals were measured and marked out onto the paper towel. (See Fig. 2)
2. Using scissors, each of these intervals were cut out into longs strips. They were folded vertically in half, so that the width now became 2 centimetres.
3. Using a fine 0.5 ballpoint pen (so that the ink did not chromatograph), a defined mark was made, 1 centimetre from the bottom of each strip.

Figure 2

4cm being measured out on the paper towel, marked, and cut out into long strips.

4. Steps 1 – 3 were repeated until there were 84 strips altogether, and was set aside.
5. 7 strips were labelled “Fresh Pineapple 1”.
6. Step 5 was repeated for “Fresh pineapple 2”, “Fresh pineapple 3”, “Frozen pineapple 1”, “Frozen pineapple 2”, “Frozen pineapple 3”, “Canned pineapple 1”, “Canned pineapple 2”, “Canned pineapple 3”, and “Cooked pineapple 1”, “Cooked pineapple 2”, and “Cooked pineapple 3”.

Figure 3

Fresh pineapple cut and wrapped in cling wrap to be frozen.

Preparation of the Fresh and Canned Pineapples

7. The medium – large fresh pineapple was peeled and cut horizontally into three equal portions.
8. Each portion of the pineapple was cut horizontally into slices of width approximately 1 – 1.5 cm. Approximately 12 pieces were cut out of each slice. Portions were kept separate.
9. One portion was placed into the freezer and left for a minimum of 2 hours, or until frozen. (See Fig. 3) The second portion was left aside.
10. Canned pineapple slices were drained using a colander.

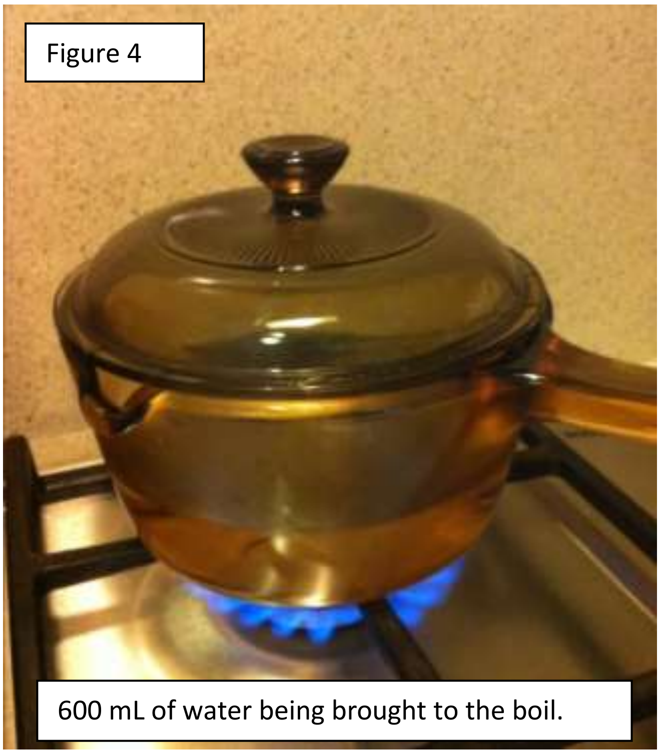


Figure 4

600 mL of water being brought to the boil.

11. Canned pineapple slices were doubled up to match the approximate width of fresh pineapple. Each of these portions of the drained canned pineapple slices were cut into pieces that correspond to the size of the fresh pineapple pieces.

Preparing the Solution

12. In a 1L glass beaker, 600mL of water was boiled on the hot plate. (See Fig. 4)
13. A 15-g sample of gelatin was weighed out and placed in the 1L mixing bowl. (See Fig. 5)
14. 600mL of boiling water was carefully added to the gelatin sample and was stirred until completely dissolved. (See Fig. 5)
15. With stirring, 300mL of cold water was added to the solution.
16. Another 500mL of water was heated in the 1L saucepan on the stove.

Figure 5

15g sample of gelatin weighed and 600 mL of water being added to the gelatin.

Labelling

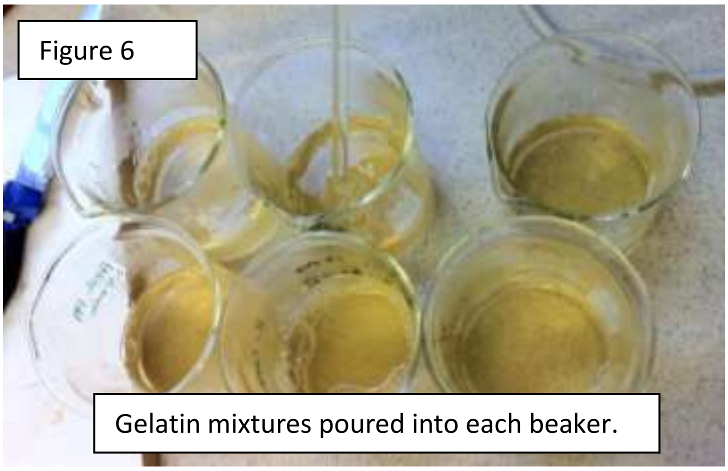
17. Tongs were labelled as follows:
    - Fresh pineapple
    - Canned pineapple
    - Frozen pineapple
    - Cooked pineapple
18. Twelve beakers were labelled as follows:
    - Fresh pineapple 1

- Fresh pineapple 2
- Fresh pineapple 3
- Canned pineapple 1
- Canned pineapple 2
- Canned pineapple 3
- Frozen pineapple 1
- Frozen pineapple 2
- Frozen pineapple 3
- Cooked pineapple 1
- Cooked pineapple 2
- Cooked pineapple 3

Experiment Set Up

19. 60mL of gelatin solution was measured in the measuring cylinder and poured into a 250mL beaker. Mix well. (See Fig. 6)
20. Step 19 was repeated for all twelve beakers.
21. The second portion of fresh pineapple (that had been left aside) was placed into the 500mL of water being heated and boiled.

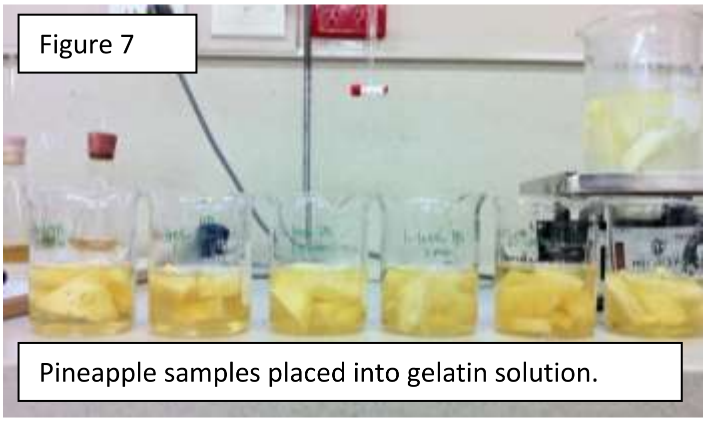
Figure 6



Gelatin mixtures poured into each beaker.

22. The pair of tongs labelled "Fresh pineapple" was used to place approximately 4 pieces of fresh pineapple pieces into the beakers labelled "Fresh pineapple 1", "Fresh pineapple 2", "Fresh pineapple 3"
23. Enough pineapple pieces were placed in the beaker for the pineapple to be fully submerged in solution but the level of solution to reach 200mL on the beaker. (Approx. 4 pieces), (See Fig. 7)
24. The pair of tongs labelled "Cooked Pineapple" was used to take the boiled pineapple out of the water. The pineapple was drained using a different colander to the one that had been previously used. Place the drained, boiled pineapple into the beakers labelled "Cooked pineapple 1", "Cooked pineapple 2" and "Cooked pineapple 3". Step 23 was repeated.
25. The pair of tongs labelled "Canned Pineapple" was used to place the canned pineapple into the beakers labelled "Canned pineapple 1", Canned pineapple 2" and "Canned pineapple 3".

Figure 7



Pineapple samples placed into gelatin solution.

Step 23 was repeated.

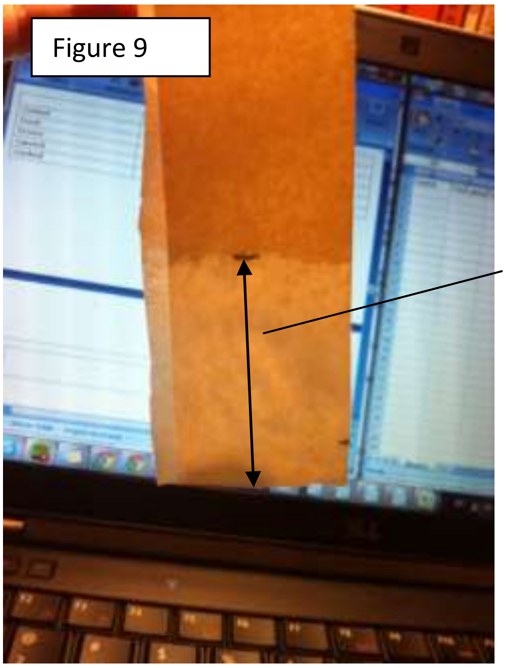
26. When the 2 hours (minimum) had passed, or when the pineapple in the freezer had frozen, the pair of tongs labelled "Frozen Pineapple" was used to place the frozen pineapple into the beakers labelled "Frozen pineapple 1", "Frozen pineapple 2" and "Frozen pineapple 3". Step 23 was repeated.
27. All beakers were sealed with cling wrap. (See Fig. 8)

Figure 8

Beakers being sealed with cling wrap – prepared to be put into refrigerator to speed up the gelling process.

28. A strip of the paper towels that had been set aside was submerged into the solutions (corresponding to the label on the paper towel) until the base of the meniscus of the solution reached the 1cm mark that had been drawn on. (See Fig. 9)
29. The paper towel was kept submerged in the solution for 5 seconds before taken out and hung on a line, being careful that the spreading of the solution up the paper towel was not disturbed in any way. (See Fig. 9)
30. All the samples were placed into the refrigerator and the timer was started immediately.
31. When the stopwatch / timer reached the 30 minute mark, steps 28 – 29 were repeated.
32. Steps 28 – 29 were repeated for each beaker of solution. A metal ruler was used to measure the length of the wet part of the paper towel from the highest point of the spread to the bottom of the paper towel. All results were recorded in centimetres.
33. Step 31 – 32 was repeated every 30 minutes until the 3 hour mark. (7 tests altogether on each solution.)
34. Samples were removed from the refrigerator.
35. Samples were observed and all qualitative results were recorded in a table.

Figure 9



This length was measured – from the highest point of the spread to the base of the paper towel.

RESULTS

Qualitative Data

Type of Pineapple	Set	Did not set
Fresh		✓
Frozen		✓
Canned	✓	
Cooked	✓	

Quantitative Data

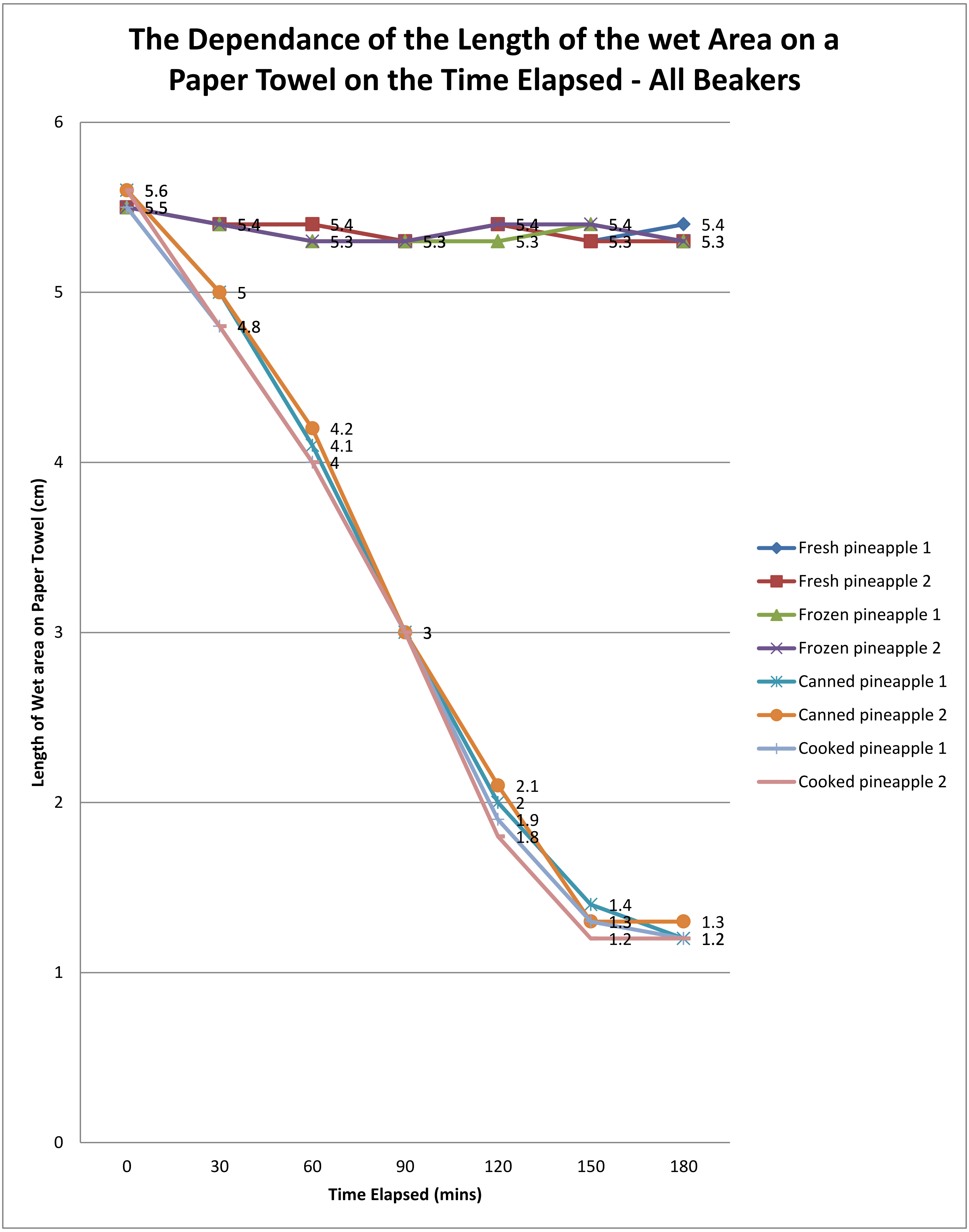
Raw data for the length of the wet area measured from the highest point of spread to the base of the paper towel.

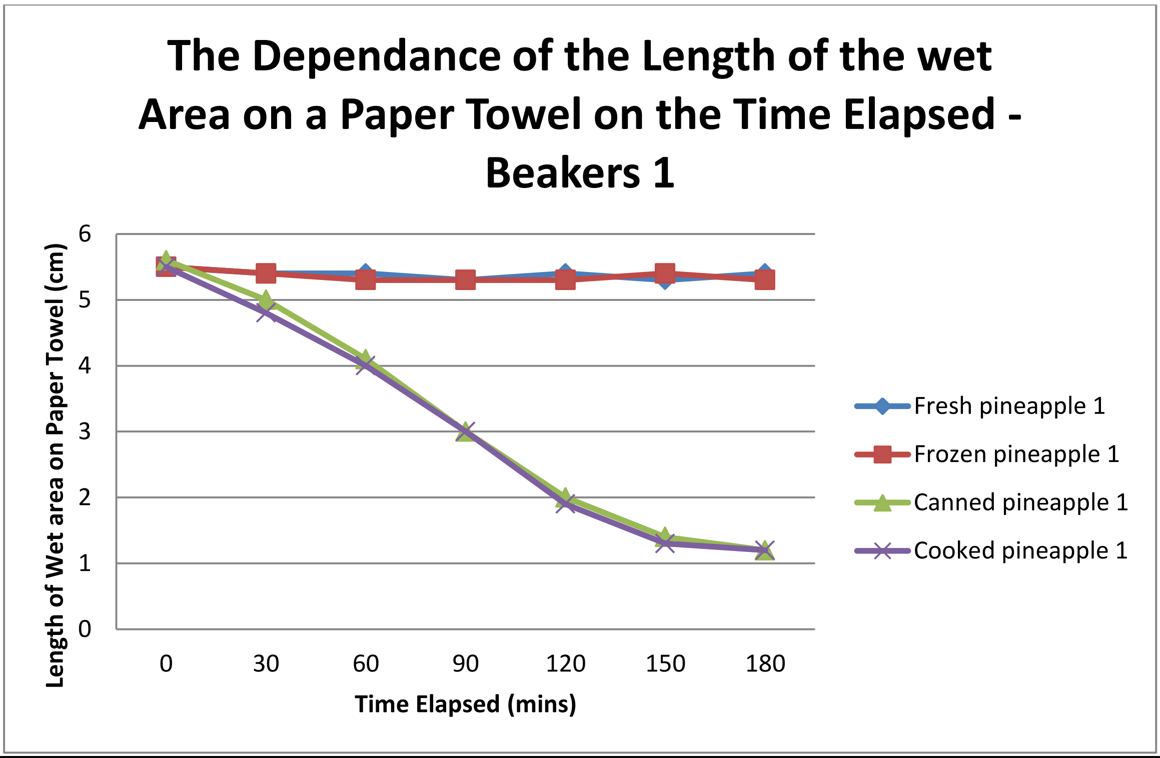
Time Elapsed(min)	Fresh pineapple 1 (cm)	Fresh pineapple 2 (cm)	Frozen pineapple 1 (cm)	Frozen pineapple 2 (cm)	Canned pineapple 1 (cm)	Canned pineapple 2 (cm)	Cooked pineapple 1 (cm)	Cooked pineapple 2 (cm)
0	5.5	5.5	5.5	5.5	5.6	5.6	5.5	5.6
30	5.4	5.4	5.4	5.4	5.0	5.0	4.8	4.8
60	5.4	5.4	5.3	5.3	4.1	4.2	4	4
90	5.3	5.3	5.3	5.3	3.0	3.0	3.0	3.0
120	5.4	5.4	5.3	5.4	2.0	2.1	1.9	1.8
150	5.3	5.3	5.4	5.4	1.4	1.3	1.3	1.2
180	5.4	5.3	5.3	5.3	1.2	1.3	1.2	1.2

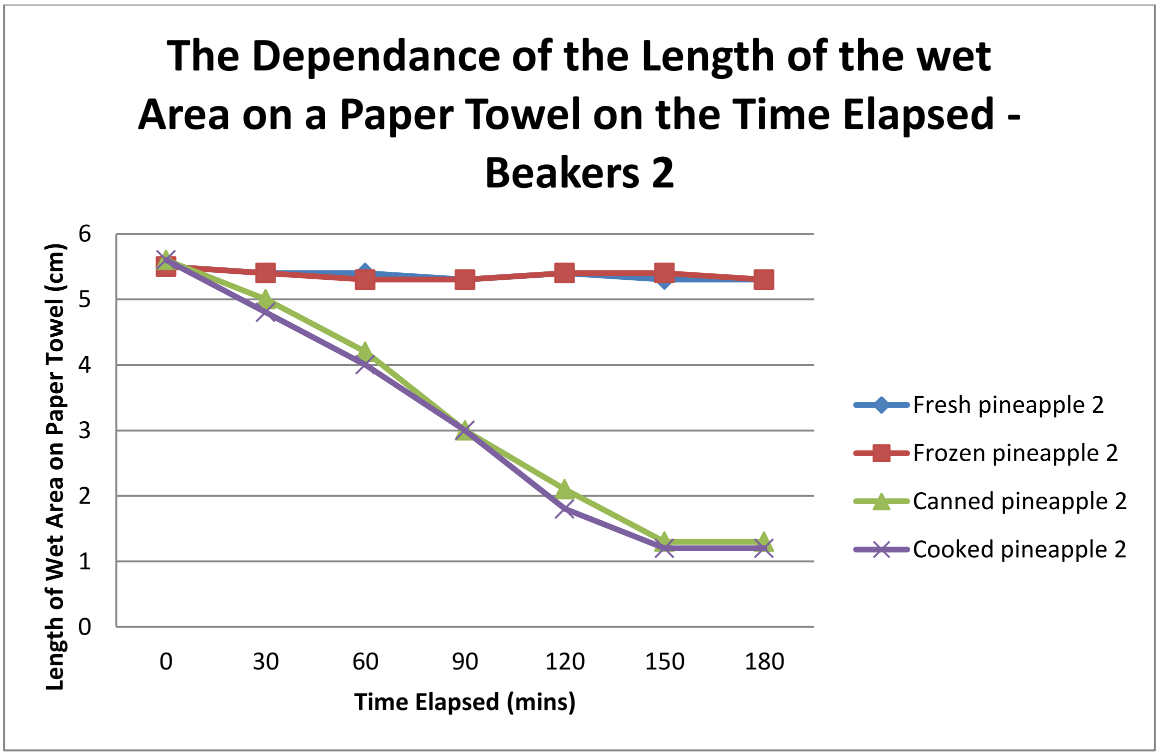
Processed Data - Average calculations of the two trials.

Time Elapsed (min)	Control (cm)	Fresh Pineapple (cm)	Frozen Pineapple (cm)	Canned Pineapple (cm)	Cooked Pineapple (cm)
0	5.6	5.5	5.5	5.6	5.55
30	5.4	5.4	5.4	5	4.8
60	5.4	5.4	5.3	4.15	4
90	5.3	5.3	5.3	3	3
120	5.3	5.4	5.35	2.05	1.85
150	5.3	5.3	5.4	1.35	1.25
180	5.4	5.35	5.3	1.23	1.2
Average	5.4	5.4	5. 4	3.2	3.1

Graphed Data*







*The first graph combines the results from both trials. The second and third graphs are separate graphs of the results of the two separate trials.

DISCUSSION

The majority of the data presented within the results supports the hypothesis that the four different pineapple samples will either set or not set. The fresh and frozen pineapple samples in the gelatin solution did not set, but the commercially canned and cooked pineapple in gelatin solution did set.

Patterns and Trends

It is obvious even at the early stage of the second testing of the solidity of the solutions, which was done at the 30 minute mark, that there is a change in the state of the canned and cooked pineapple samples in the gelatin solution, whilst the results of the fresh and frozen ones stayed similar compared to the first testing, which was done before samples were placed into the refrigerator. At the first testing, it is worthy to note that *all four different samples had very similar results when testing its solidity level – meaning that they had all begun with the same state.*

There are several trends and patterns that can be seen. The first is the difference between the development of the canned and cooked to the fresh and frozen. The results of the canned samples and the cooked samples were very similar, as emphasised when graphed. Similarly, the fresh and frozen samples also provided results that were quite alike, and the control beaker also proved similar results. This suggests that heat as a treatment to denature the enzyme in pineapple, bromelain, is an effective treatment; whilst cooling and freezing were not the case.

Enzymes (Bromelain)

'DasGupta, 2013' states, "The chemical reactions in all cells of living things operate in the presence of biological catalysts called **enzymes.** There are thousands of different enzymes in a cell catalysing thousands of different chemical reactions – so one particular enzyme catalyses only one reaction. The enzyme in pineapple is called **bromelain** and is a proteolytic enzyme, which means it digests proteins." This was particularly note-worthy in the experiment because the idea being tested is the effect of this enzyme on gelatin, of which the structural composition is as follows.

Gelatin

Gelatin is derived mainly from the skins and bones of cows, pigs, and horses, which are boiled in water so that the collagen comes out. Collagen is a protein, which is to say that its molecules are made of long chains of amino acids. These are arranged in three mutually twisted chains, like a triple helix. 'The Boston Globe, 2013' states, "When dissolved in liquid, the long chains of amino acids form a tangled network reminiscent of a big plate of spaghetti, with water molecules stuck to the spaghetti in layers. The huge surface area of all the strands means they can hold a lot of water, and because the strands are so tangled, you can pick it all up as a lump."

The Effect of Bromelain on Gelatin

The enzyme that is investigated in this experiment, bromelain, is one that is produced in pineapple and hydrolyses certain kinds of proteins called gelatins. The gelatin used in this experiment is derived from skin, bones, and/or connective tissue of animals. These proteins, when dissolved in hot water and allowed to cool, form a semi-solid or gel state; hence the name gelatin. Hydrolyse, here, refers to breaking up the protein polymer in such a way as to prevent its forming this gel state.

Denatured Enzymes

The hydrolysing enzyme from pineapple is denatured by heat. Denature, here, means to alter in such a manner that the enzyme is no longer able to catalyse the reaction. Enzymes can also be denatured by changes in pH, subjection to detergents or radiation, etc. (Marshman, 2013.)

'DasGupta, 2013' also states, "The rate of an enzyme-catalysed reaction depends on a number of factors, such as the concentration of the substrate, the acidity and temperature of the environment, and the presence of other chemicals. At higher temperatures, enzyme reactions occur more rapidly, but only up to a point. Because enzymes are proteins, excessive amounts of heat can change their structures, rendering them inactive. An enzyme altered by heat is said to be *denatured.*"

The Effect of Denatured Enzymes on Gelatin

Pineapple contains a chemical called **bromelain**, which contains two enzymes capable of digesting proteins, which are called **proteases**. Gelatins get their structure from links formed between chains of collagen, which is a protein. When you add pineapple to any form of gelatin, you break the links as fast as they form, so the gelatin never sets up. On the other hand, when the enzymes are **denatured**, they are effectively unable to function properly, and thus would not be able to break down any chains formed in gelatin. Denatured enzymes therefore do not affect the gelatin in any way, thus allowing the gelatin to properly set up.

The enzyme, bromelain, in pineapple disrupts the gelling process when both fresh and frozen. Freezing the fruit does not affect or denature the enzymes in any way. However, as mentioned earlier, if the fruit is heated, then the enzymes are permanently inactivated, making the fruit perfectly fine for making gelatin-containing deserts. (Helmenstine, 2013) This is why the gelatin solution did not set when the frozen and fresh pineapple were placed into it, but managed to set when the canned and cooked pineapple were placed into it.

What does this mean for consumers?

It is also noteworthy that a substantial amount of heat is applied to the pineapples during the canning process. The enzymes in bromelain are inactivated once they have been heated to about 70° Celsius. (Marshman, 2013). This heating step is mandatory during the process of canning many different fruits that have enzymes that break down gelatin too. There are certain fruits that break gelatin down because they contain the enzymes called proteases which break the chemical bonds that try to form between chains of protein as gelatin tries to gel. These include the tested chemical, bromelain, in pineapple, actinidin in kiwi, ficain in figs, papain in papaya and pawpaw, and others in mango, guava and ginger root. All these fruit will have effectively the same effect on gelatin according to their different treatments. This means that consumers will have to be aware of these particular fruits when mixing them with gelatin-containing substances and foods, to prevent an inadequate outcome.

Improvements, Validity and Reliability of Experiment

There were no particular outliers in this experiment, as the gelatin solution had either set, or not set.

Repetition – having two samples of each tested treatment of pineapple in the experiment had allowed determination of whether the results are reliable. It is evident, when looking at the two separately graphed results of the two separate trials that the results are extremely alike, therefore showing that the results are reliable. The data displayed was consistent and therefore appears to be reliable. Both trials conducted provided very similar results, and this shows that results are reliable, in both trials. The data obviously shows the two different paths that the gelatin solutions took – either setting or not setting, and this was shown when data was tabulated and graphed. The data presented within the results supports the hypothesis and relatively accurately shows the development and process of solidifying in the canned and cooked samples, whilst also showing the frozen and fresh samples remaining stably in the same state when compared to the first testing. (Hendrickson, 2011)

The method is valid because there was much care taken in the handling of the pineapple samples: different tongs and chopping boards, and other instruments were used to deal with the different types of pineapple samples. Besides incorporating suitable equipment and controlling variables to the most accurate level possible, measuring procedures were designed so that it did not affect the results and development of results *and* experiment at all, so that the most accurate form of data was extracted from it.

There are several areas of possible improvement within the experiment and method. One of these would be, if given more time, to repeat the entire experiment over, so that comparisons may be made between the results of both trials to assess reliability of results.

CONCLUSION

The enzymes in bromelain are inactivated once they have been heated to about 70° Celsius. (Marshman, 2013) When being cooked and during the commercial canning process, exposure to this amount of heat is mandatory. While fresh and frozen pineapple prevented the gelatin-solution from solidifying because this enzyme that “broke down” the gelatin was not denatured, the enzymes in canned and cooked pineapple *were* denatured, and therefore still allowed the gelatin-solution to solidify.

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