Experiment to Find Out Whether the Ingestion of Fruit Could Assist In Protein Breakdown in the Stomach to Aid Indigestion

Introduction

Background Information

Protein Indigestion

Intestinal indigestion of proteins is very frequent. Proteins undergo rapid decay in the stomach when not rapidly digested. According to The British Medical Journal, improper digestion can lead to indigestion symptoms, such as bloating or nausea. Proteases as asserted by López-Otín & Bond, act as multifunctional enzymes in both life and illness, thus determining the level of human health. Moreover, they also have a major role to play towards digestion of the proteins, therefore, investigating the role of proteases and the effects of its major functioning in humans is important towards improvement of human health. Idea about the investigation was developed after going through a number of incomplete interesting studies regarding the same. Enzymes are an elaborate example of proteases.

Enzymes

Enzymes are proteins folded into complex shapes that act as biological catalyst. Enzymes typically have three key characteristics. First, the basic function of an enzyme is to increase the rate of a reaction. Most cellular reactions occur about a million times faster than they would in the absence of an enzyme. Second, most enzymes act specifically with only one reactant (called a **substrate**) to produce products. The third and most remarkable characteristic is that enzymes are regulated from a state of low activity to high activity and vice versa (Ophardt 2003).

Fruit Proteases

Proteases digest various proteins. Plant proteases are most abundantly found in tropical plants such as papaya and pineapple. Cysteine protease is found in kiwi fruit, and bromelain is provided by Pineapple fruit, while papaya is a source of some of the most abundant commercially produced protease, papain (Hagar). Exact concentrations of these above proteases are hard to pinpoint, however, due to the fact that less ripe samples of the same fruit will inherently contain more protein-digesting enzymes. For the proteases to be viable, the fruits should not have been heated in any way (i.e. canned or cooked), or else the enzymes will have been permanently denatured and will not succeed in digesting the protein (Anne Marie Helmenstine).

Research Question: Out of kiwi, papaya and pineapple, which fruit will have the highest concentration of protease enzyme when measured through percent change in mass of a gelatin cube immersed in a puree of each fruit when compared to known concentrations of protease?

Hypothesis: Different fruits contain different levels of protease, therefore making it essential to compile data from different sources to determine the levels of protease in different fruits when they are ripe, given that there is no fully comprehensive article that covers the same. The kiwi fruit used in this experiment seemed harder and less ripe, and therefore it can be hypothesized that those samples will contain the highest levels of protease enzymes.

Variables:

Independent Variable:

Type of fruit puree used. The experiment will test blended kiwi, papaya, or pineapple, and compare them with known concentrations of protease enzyme (1, 3, and 5% concentration) to come up with a quantitative measurement for the enzyme content. The concentrations of the protease enzymes were arrived at, after thoughtful consideration of previous literature on the same.

Dependent Variable:
The percentage change in mass of a 25.4 millimeter-thick gelatin cube over time. Change in mass will be measured in intervals of 30 minutes for 150 minutes, so as to give enough time for action of the protease enzyme.

Controlled Variables:

Variable to be controlled	*Why it needs to be controlled*	*How it will be controlled*
Amount of fruit puree	Increased amounts of fruit puree would mean, availability of more enzymes, hence, more active sites that will be open for bonding. If this were to be inconsistent throughout trials, some trials would experience faster enzyme activity.	For each trial, the gelatin cube will be placed in 50 ml of the fruit puree.
Length of trial	The longer a trial is conducted for the more digestion of the gelatin cube will occur. Conversely, shorter trials would result in lower digestion overall. Therefore, trial time lengths need to be kept uniform in order to maintain consistency.	Each trial will last for 150 minutes with samples taken every 30 minutes.
Source of substrate (gelatin)	Different protease sources act upon various proteins differently. Therefore, it must be ensured that the same types of protein(s) are being used during each trial.	Throughout the experiment, the sources of substrate will be from gelatin and Protease.
Temperature	All enzymes have an ideal temperature in which they function most optimally. Therefore, to ensure each trial's enzymes are working equally as efficient, temperature must be kept consistent.	All trials will be conducted at room temperature (23 degrees Celsius),

		except refrigeration of gelatin.
Amount of substrate	The more substrate available to an enzyme, the faster the enzyme will act. Therefore, there must be a consistent amount of substrate during each trial.	25.4 millimeter cubes of gelatin will be used for every trial

Note: Before beginning experiment, all the materials and requirements should be assembled so as to make detailed observations without distraction.

Procedure

Materials

- 3 packets of gelatin Premium Roche enzymes, with same dates of expiry(Each 6 grams)
- Plastic Tray
- Pot
- Stove
- Distilled water
- Refrigeration/Low Temperature
- 4 kiwis from the United States.
- ½ pineapple
- ½ papaya
- cutting board
- Knife
- A conventional Oster Blender (350 Watts)
- Assorted beakers (50ml, 600 ml)
- Measuring cylinder (100 ml) with a precision of 0.1 ml
- Protease Enzyme powder obtained from a store (Premium Roche enzymes, 3 active units per gram)
- Weighing boats
- Electric scale (0.1 gram precision)

Method

- Combine 18.5 grams of gelatin in a bowl
- Boil 700 ml of water and add into bowl
- Stir until gelatin is completely dissolved
- Pour gelatin mixture into tray and refrigerate until firm
- Cut multiple 25.4 mm-thick cubes from gelatin tray
- Peel and cut kiwis, pineapple, and papaya

- Blend each fruit and pour contents into 3 different 600ml beakers, making sure to thoroughly rinse the blender between each different fruit's blending
- Create different concentrations of cysteine protease enzyme using powder by measuring 1,3, and 5 milligrams and placing in a graduated cylinder before filling with distilled water until 100 ml line
- Pour each fruit puree and protease solution into 3 different 50 ml beakers
- Measure the mass of 8 gelatin cubes on the electric scale before placing each one into a beaker of protease source, completely submerging it
- After 30 minutes measure the mass of each cube and record the change
- With a clean paper towel, wipe and place back the cubes into each protease source and repeat in 30 minute intervals until 150 minutes are done.
- Reach trial 5 times for reputable data, rinsing and drying the same beaker after each trial
- Statistically analyze the data, using the Chi-Square test
- Use a line graph to present the findings

Safety Concerns:

- When handling boiling water during the gelatin-making process, it is imperative for it to be poured slowly and carefully. Any splashing could cause serious burns. To prevent any scalding of skin, closed toed shoes should be worn, long pants and long sleeves, and an apron. Tie hair back so vision is not obscured while handling burning water
- Make sure the knife used to dice fruit is sharp; this will ensure smooth, quick cuts. If blunt, the sawing motion required to cut through some of the tough skins of the fruits increases the risk of the knife slipping and cutting into skin. As the knife is sharp, it should be handled with care: do not run with the knife, and use a chopping board to protect bench top and prevent fruit from sliding and the knife slipping as a consequence.
- Since protease enzymes act on proteins, make sure to wear gloves when handling them as it could irritate the skin. Moreover, it's important that it doesn't enter any orifice such as the eyes, nose, or mouth so protective goggles and face masks should be worn as well.

Data collection and processing

From all the three fruit samples, mass of gelatin cubes were measured at times: 30, 60, 90,120 and 150 minutes with precision of 0.5 minutes, before each cube was completely submerged into a beaker containing cysteine protease and after 30 minutes. Each trial was repeated 5 times so as to obtain reputable data.

The times before and after submersion of the gelatin cubes was subtracted and the value converted into percentages, by dividing with the original time and multiplied by 100%. The results were then recorded in the table below.
At 120 minutes two of the gelatin cubes split during the weighing process and attempts to salvage the bits for the subsequent trials was almost likely imperfect.

Figure 1: Percent change in mass of gelatin over time for each source of protease (±0.5 percent)

	30 (± 0.5 Minutes)	60 (± 0.5 Minutes)	90 (± 0.5 Minutes)	120 (± 0.5 Minutes)	150 (± 0.5 Minutes)
Pineapple	7.88	13.89	19.9	33.79	53.69
Kiwi	8.64	15.35	22.06	37.41	59.47
Papaya	5.03	9.33	13.63	22.96	36.59
0% protease	0.12	0.24	0.36	0.6	0.96
1% protease	3.48	6.39	9.3	15.69	24.99
3% protease	5.37	10.71	16.05	26.76	42.81
5% protease	7.97	16.26	24.55	40.81	65.36

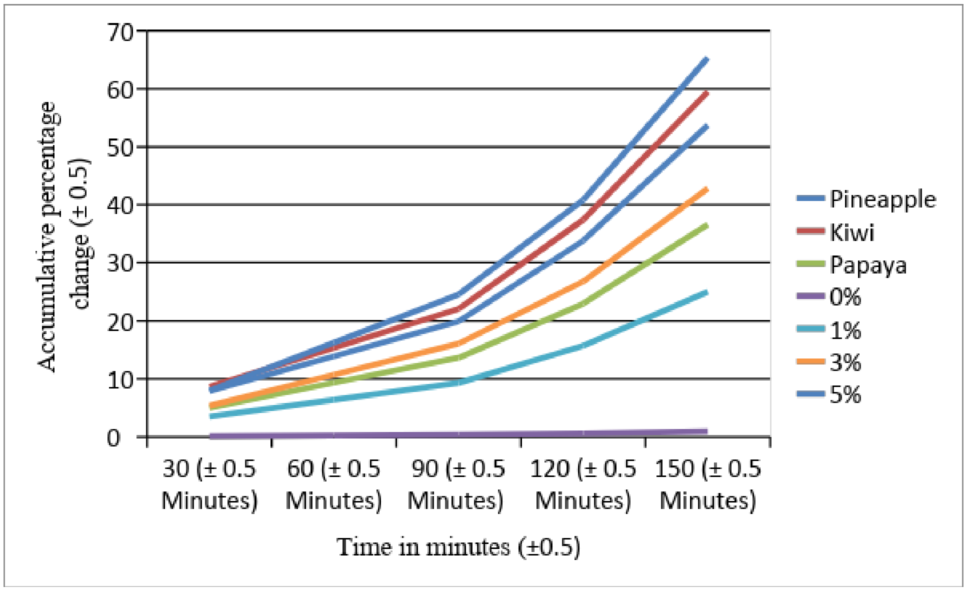
The values from the table above were used to plot the following graph of Percent change in mass of gelatin against time for each source of protease.

Error bars were calculated using upper and lower ranges

Sample calculations are as follows:

Lower value: 6.25
Upper value: 7.55
Mean: 7.11
Upper range: 7.55-7.11= 0.44
Lower range: 7.11-6.25= 0.86

Figure 2: <u>Accumulative Percent Change in Mass of Gelatin over Time</u>



<u>Analysis:</u>

As can be seen from the figures above, it would seem that pineapple and kiwi display the stronger protease enzymes. Not only are the percent changes in mass of each gelatin cube greater overall compared to that of papaya fruit, both kiwi and pineapple show more sustained enzymatic activity. Although the two fruits have dips and peaks between the 30 minute intervals, they are shown to oscillate around a given value whereas the papaya graph shows a consistent downwards trend.

<u>Conclusion and Discussion</u>

In conclusion, should one be suffering from any symptoms of indigestion brought by the over/rapid consumption of protein, a good home remedy could be to perhaps consume some kiwi or pineapple fruit. Evidently, this agrees in part with the original hypothesis which states that kiwi would contain the highest levels of protease enzyme, added to the fact that acids in the stomach only start action on the food an hour after it is consumed.

The two fruits clearly feature high amounts of the enzyme bromelain which will aid in the breakdown of large polypeptides causing indigestion symptoms in an individual. In the trials conducted, pineapple fruit consistently caused mass reductions in the gelatin cubes of 6% or greater. Similarly, gelatin digestion by protease enzyme present in kiwi fruit did not attain below 6.7% between 30-minute sampling intervals. Should neither of those fruit be available, however, papaya fruit could also be considered as a slightly less effective substitute. As stated in the data analysis, not only was papaya fruit’s overall digestion of the gelatin cube lower (peaked at 5%

mass change), the level of enzymatic activity steadily dropped with each 30-minute trial taken. This could potentially suggest that the enzymes in the papaya are not viable after a certain period of time; however, change in pH would not have been the cause.

When compared to known concentrations of protease enzymes, the possible concentrations of each fruit's enzymes can be interpolated. As can be seen in figure two, the papaya's graph bears striking similarity to the 3% protease solution, suggesting that the papain content in a papaya could be very close to 3%. Moreover, both the kiwi and pineapple showed similar trends when compared to the 5% solution, suggesting that these two samples of fruit contain around 5% concentration of the enzyme bromelain.

Possible scientific explanations for the above results could be through the ripeness of each fruit sample. As stated in the hypothesis, the kiwi fruit was predicted to have the most protease enzymes due to the fact that it was quite hard to the touch, suggesting un-ripeness. Similarly, the pineapple flesh was not tender when cutting and could suggest higher levels of enzyme than normal. Moreover, the papaya was extremely mushy and practically dematerialized once it was cut, since it was not fresh. This could suggest over-ripeness, and could be a contributing factor to why it housed more inactive enzymes.

Evaluation

The error bars for each trial were quite small throughout most trials, suggesting that the data was, overall, quite accurate. There were, however, some examples of anomalous data points throughout. The first example can be seen with the 0% protease concentration. At the 90-minute sample, the digestion was actually negative, suggesting that the cube had increased in mass. This piece of data addresses a potential advantage of the osmotic power of gelatin. Because each cube was soaked in a liquid, when they were taken out to be measured there would still be droplets of residual solution or fruit juice still attached to the cube. While throughout the data collection process, the cubes were cleaned with paper towels to remove liquid, this is an imperfect process and could have led to inflated measurements due to residual liquids on the cube.

Another example of anomalous data can be seen with the pineapple fruit at 120 minutes in where it spikes to 8.11 % change in mass. Because of their delicate texture and the enzymes breakdown for 120 minutes, the structural integrity of the gelatin was compromised and bits broke off as they were weighed. While it was attempted to salvage all the pieces in the trials to come, again, it was most likely imperfect.
Moreover, the data gathered in this experiment was for the explicit purpose of exploring whether the ingestion of fruit could assist in protein breakdown in the stomach to aid indigestion; however, the experimental design did not do a great job in simulating the environment of a stomach. Stomachs contain low pH acids that could potentially disrupt the function of papain, protease and bromelain.

Suggestions for Improvement

The above anomalous data points essentially came because the method for measuring the activity of the enzyme was open for a few errors. Therefore, another way to measure the activity could have been to place gelatin in a petri dish, dig a well and the source of protease poured into the well. Changes in measurement should be recorded from on an electronic scale as the enzymes works its way through the gelatin. Here, photos could have been taken with the petri dish and evaluated in programs such as imagej to ensure quality results. This would remove the possibility of residual water affecting mass measurements and any loss of mass due to gelatin breakage.

Handling of gelatin cubes should in future be done with care to avoid inconsistencies in results. It would also be very important to increase the number of trials in future so as to provide more elaborate data for statistical analysis

Moreover, further investigation could be done to see how these proteases behave in low pH environments like those found in the stomachs of humans. This extension would contribute to the data found and strengthen claims about whether fruit can really be a viable remedy to protein-related indigestion.

<u>Works Cited</u>

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Appendix (Raw Data)

The tables below shows the raw data obtained during the experiments for each of the fruits.

Figure 3: pineapple

Pineapple	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	8.87	8.3	7.92	7.36	6.83	6.34
Trial 2	7.31	6.88	6.48	6.02	5.62	5.22
Trail 3	9.05	8.32	7.89	7.42	6.76	6.41
Trail 4	10.5	9.72	9.01	8.39	7.77	7.36
Trail 5	9.25	8.45	7.99	7.54	6.99	6.51

Figure 4: kiwi

Kiwi	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	10.5	9.8	9.16	8.5	7.87	7.29
Trial 2	9.99	9.1	8.45	7.89	7.45	6.88
Trail 3	7.45	6.83	6.4	5.87	5.61	5.18
Trail 4	8.84	8.1	7.6	7.05	6.44	6.21
Trail 5	7.89	7.33	6.96	6.39	6.05	5.56

Figure 5: Papaya

Papaya	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	7.42	7.16	7.02	6.8	6.52	6.33
Trial 2	8.4	7.91	7.41	7.21	7.01	6.94
Trail 3	10.34	9.82	9.51	9.33	9.01	8.92
Trail 4	9.11	8.61	8.26	7.99	7.81	7.69
Trail 5	7.94	7.63	7.26	7.01	6.84	6.75

Figure 6: 0% protease solution.

0%	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	10.12	10.15	10.11	10.12	9.99	10.13
Trial 2	9.53	9.53	9.58	9.54	9.59	9.59
Trail 3	8.34	8.29	8.38	8.34	8.34	8.37
Trail 4	9.24	9.23	9.19	9.27	9.28	9.24
Trail 5	10.14	10.12	9.98	10.11	10.14	10.13

Figure 7: 1% protease solution.

1%	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	7.42	7.26	7.12	6.9	6.62	6.33
Trial 2	8.3	8.01	7.81	7.61	7.31	7.12
Trail 3	10.34	9.92	9.71	9.53	9.31	9.1
Trail 4	9.11	8.71	8.36	8.09	7.91	7.69
Trail 5	7.84	7.63	7.36	7.11	6.94	6.75

Figure 8: 3% protease solution.

3%	0 Minutes	30 Minutes	60 Minutes	90 Minutes	120 Minutes	150 Minutes
Trial 1	9.21	8.7	8.22	7.79	7.46	7.11
Trial 2	10.22	9.71	9.21	8.77	8.31	7.93
Trail 3	7.88	7.51	7.14	6.78	6.44	6.11
Trail 4	9.3	8.84	8.39	7.97	7.66	7.23
Trail 5	11.3	10.7	10.2	9.71	9.19	8.74

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