Investigating the Effect of Concentration of Sodium Chloride on the Activity for Trypsin Hydrolysis of Albumin

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Abstract

The purpose of this paper is to present findings regarding the topic of enzyme activity and inhibitors. Since research on the enzyme trypsin is minimal, investigations were performed to understand the enzyme's activity and whether it differs from that of alpha-amylase. Results show that when the concentration of sodium chloride increases, the rate of trypsin activity decreases. Hence, salt takes the role of an inhibitor, blocking enzyme active sites and destabilizing its structure. The optimal concentration of sodium chloride is 1.0% for optimal enzyme performance apart from the control, showing that excessive salt intake could be fatal to health.

Background Introduction

Enzymes are globular proteins that speed up biochemical reactions through lowering the activation energy, or the minimum amount of energy required to break the bonds in the substrate and therefore allow a reaction to occur. Enzymes catalyze metabolic reactions in the body using a special region called the active site. During catalysis, substrate molecules are in continual random motion and there is a chance of collision between substrates and the active site on the surface of the enzyme. Collision results in binding, where the active site changes its shape to complement those of substrates, commonly known as induced fit. They are chemically attracted to each other and fit together. Enzymes will bend the substrate and put strain on its bond to reduce the energy needed for them to be converted into products. Later, the products are released from the active site, freeing it up to catalyze the reaction with more substrates. However, enzymes are usually substrate-specific and only catalyze a specific reaction involving one substrate. Thus, molecules other than the substrate do not fit or are not attracted and thus do not bind to the active site of a specific enzyme.

Trypsin is an enzyme that catalyzes the hydrolysis of proteins into individual amino acids such as arginine and lysine. To catalyze a reaction, a catalytic triad consisting of histidine-57, aspartate-102, and serine-195 on the active site of trypsin facilitates a nucleophilic substitution reaction, or a reaction in which an atom or group of atoms are replaced by an electron-rich species that has a lone pair of electrons (Polgár, 2005). The overall purpose of this reaction mechanism is to loosen the bond on the substrate and reduce the activation energy. In its active form, trypsin cleaves peptide bonds between the carboxyl group of lysine or arginine residues and the amino group of the adjacent amino acid residues (Olsen et al., 2004). Additionally, trypsin enzyme is commonly produced from pancreatic glands. The trypsin that was chosen for this investigation was extracted from purified extracts of porcine or bovine pancreas because pancreas is one of the most common sources used for industrial production and extraction of trypsin (Kortt, 1978).

Epidemiological evidence suggested that the intake of salt, especially sodium chloride, may increase the risk of diseases such as hypertension and other cardiovascular diseases (Meneton et al., 2005). In fact, one past study conducted by Chao et al demonstrated that the concentration of sodium chloride intake influenced protein digestion and protein composition in

the feces of mice (Chao et al., 2017). This shows evidence that salinity level influences the rate of protease activity. Many researchers demonstrated that high concentration of sodium chloride could disrupt the solubility and stability of enzymes by changing the conformation of the active site and denature the enzyme (Sinha & Khare, 2014). Thus, identifying the optimum concentration of sodium chloride provides insight about how dietary habits regarding salt consumption can affect the digestion of albumin protein by trypsin into smaller peptides and amino acids, which are important nutrients critical for growth and development in human bodies.

However, there have been a limited number of studies investigating the effect of sodium chloride concentration on trypsin activity. Similar studies in the field have investigated the effect of salt concentration (0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, 3.0%, 3.5%, 4.0%) on the activity of α -amylase enzyme (Audipudi et al., 2017). Referencing the previous study, while 0% was used as the control, the same range of sodium chloride concentrations from 0.0% to 4.0% with the interval as 1.0% were tested to observe the effect of the concentration of sodium chloride on the rate of trypsin activity in this investigation.

To investigate the optimum concentration of sodium chloride, the biuret reagent was used to measure the rate of hydrolysis of albumin by trypsin. While the color of the biuret solution is initially pale blue, once the solution is mixed with albumin, a component of the alkaline biuret solution called cupric ions will intertwine with the peptide bonds within albumin to create a colored complex that changes the color of the mixture to purplish-violet (Chang-Hui Shen, 2019). After trypsin is added to the mixture and starts hydrolyzing albumin, the color of the purplish-violet mixture will start to shift towards the initial pale blue color.

Thus, the paler-more bluish the reaction mixture, it means that less peptide bonds are present and that most proteins are hydrolyzed, indicating greater rate of trypsin activity. Meanwhile, the more purplish the reaction mixture, it means that more peptide bonds are present and that only a miniscule of proteins are hydrolyzed, proving slower rate of trypsin activity. According to Beer Lambert's law, a higher absorbance value corresponds to a higher concentration of peptide bonds or proteins present in the reaction mixture, which means slower rate of protein hydrolysis and slower rate of trypsin activity. Thus, the mathematical relationship between absorbance value and enzyme activity is best described as inversely proportional. Hence, the absorbance value for the color of the reaction mixtures could be measured with a spectrophotometer to examine the rate of trypsin activity.

Research Question

How does the concentration of sodium chloride affect the rate of trypsin activity, measured as the absorbance value for the color of the reaction mixture solution using a spectrophotometer?

Hypothesis

Concentration of sodium chloride: If the concentration of sodium chloride increases, then the rate of trypsin activity will decrease possibly due to its blockage of enzyme active sites and disturbance of the stability of trypsin.

Variables

Table 1. Set up of the different variables

Variable	Name of the variable	How to set/measure/control
Independent variable (IV)	Concentration of sodium chloride (0%, 1.0%, 2.0%, 3.0%, 4.0%)	Solutions with different concentrations of sodium chloride were prepared by preparing a standard 5.0% sodium chloride solution and adding different volumes of the solution into the reaction beaker to achieve the desired concentration of sodium chloride.
Dependent variable (DV)	Absorbance value for the color of the reaction mixture (AU)	Trypsin is added to the reaction mixture, then a timer is turned on immediately and after 9 minutes and 30 seconds, 1 cm^3 of reaction mixture will be harvested and mixed with 1 cm^3 of biuret solution to be added to each cuvette. Then the cuvette is placed in a calibrated spectrophotometer and absorbance value is recorded.
Controlled variables	1. Final concentration of trypsin in mixture after dilution	Final concentration of trypsin in reaction mixture was kept constant at 1.0% because changes in the enzyme concentration affect the rate of successful collisions between trypsin and albumin.
	2. Final concentration of albumin in mixture after dilution	Final concentration of albumin in reaction mixture was kept constant at 1.0% because changes in the substrate concentration affect the rate of successful collisions between trypsin and albumin.
	3. Volume of biuret solution	Volume of biuret solution was kept constant at 1.0 cm^3 because changing the volume of biuret solution affects the absorbance value for the color of the reaction solution.
	4. Time to harvest reaction mixture and added to cuvette	The time to harvest reaction mixture was kept constant at 9 minutes and 30 seconds because changing the harvest time allows more or less time for the reactants to react and makes it difficult to isolate the effect of different sodium chloride concentration.

	5. Volume of harvested reaction solution	Volume of harvested reaction solution was kept constant at 1.0 cm^3 because changing its volume affects the absorbance value for the color of the reaction solution.

Materials

Table 2. A list of materials and apparatuses

5.00 g Albumin	5.00 g Trypsin Powder	2.50 g Sodium Chloride
600 cm^3 Distilled Water	100 cm^3 Biuret Reagent	Seven 100 cm^3 Beakers ($\pm$5 cm^3)
One 100 cm^3 Graduated Cylinder ($\pm$1 cm^3)	Glass Rod	Electronic Balance ($\pm$0.01 g)
Electronic Stopwatch ($\pm$0.01 s)	Five Spectrophotometer Cuvettes	One Automatic Volume-Adjustable Lab-Use Pipette
One Spectrophotometer	Spoon	Weighing Papers

Procedures

Preparation of 10.0% trypsin solution:

1. An electronic balance was used to measure 5.00 g of trypsin, and a 100 cm^3 graduated cylinder was used to measure 50 cm^3 of distilled water.
2. Trypsin and distilled water were poured into a 100 cm^3 beaker and mixed using a glass rod until trypsin was dissolved completely.

Preparation of 10.0% albumin solution:

1. An electronic balance was used to measure 5.00 g of albumin, and a 100 cm^3 graduated cylinder was used to measure 50 cm^3 of distilled water.
2. Albumin and distilled water were poured into a 100 cm^3 beaker and mixed using a glass rod until albumin was dissolved completely.

Preparation of 5.0% sodium chloride solution:

1. An electronic balance was used to measure 2.50 g of sodium chloride, and a 100 cm^3 graduated cylinder was used to measure 50 cm^3 of distilled water.
2. Sodium chloride and distilled water were poured into a 100 cm^3 beaker and mixed using a glass rod until sodium chloride was dissolved completely.

Investigating the effect of the concentration of sodium chloride on the rate of trypsin activity:

Table 3. Combination of solutions added to reaction beaker for each interval of IV

Final concentration of sodium chloride in mixture after dilution (%)	Volume of 5.0% sodium chloride solution (cm^3)	Volume of distilled water (cm^3)	Volume of 10.0% albumin solution (cm^3)	Volume of 10.0% trypsin solution (cm^3)
4.0	8.0	0.0	1.0 (1.0%)*	1.0 (1.0%)*
3.0	6.0	2.0	1.0 (1.0%)*	1.0 (1.0%)*
2.0	4.0	4.0	1.0 (1.0%)*	1.0 (1.0%)*
1.0	2.0	6.0	1.0 (1.0%)*	1.0 (1.0%)*
0.0	0.0	8.0	1.0 (1.0%)*	1.0 (1.0%)*

*Final concentration of albumin or trypsin in mixture after dilution (%)

1. Turn on the spectrophotometer and set the mode to absorbance, then set corresponding wavelength to 540 nm.
2. Calibrate the spectrophotometer to 0 AU with a blank cuvette.
3. Using the pipette and referencing Table 3, from left to right, extract the correct amount of solution from respective beakers and pour into the 100 cm^3 reaction beaker. Hence, pour trypsin enzyme last.
4. Immediately turn on an electronic stopwatch to keep track of time. Do not swirl or touch the reaction beaker.
5. At $t = 9.5\,minutes$, 1.0 cm^3 of reaction mixture and 1.0 cm^3 of biuret solution were extracted and added to each of 4 cuvettes corresponding to a total of 4 trials.
6. The 4 cuvettes were immediately placed into the spectrophotometer and their absorbance values were recorded on a paper at $t = 10\,minutes$.
7. Steps 2-6 were repeated for each tested concentration of sodium chloride (0%, 1.0%, 2.0%, 3.0%, 4.0%).

Safety Considerations

Table 4. Safety Considerations

Source	Risk	Justification	Action Plan
Trypsin powder	May cause breathing difficulties and serious eye damages; can be hazardous to health (CLEAPSS).	Wear gloves and goggles to avoid direct contact with any part of the skin; avoid inhaling the powder or vapor (CLEAPSS).	Rinse the eye with tap water; wipe spilled powder with a cloth (CLEAPSS).
Biuret reagent	May corrode electronics or lab equipment; is irritant to the skin and may be hazardous to health (CLEAPSS).		Rinse the eye or skin with tap water; mop spilled solution with cloth; dilute solution with distilled water when disposing (CLEAPSS).

Sodium chloride solution	Consuming sodium chloride at high concentration can cause high blood pressure and heart disease (CLEAPSS).	Wear eye protection and avoid raising dust (CLEAPSS).	Do no more than rinse and spit with drinking water. Do not induce vomiting (CLEAPSS).
Usage of glassware	May damage the skin when the glassware is broken (Edulab).	Handle all glassware gently; dispose of broken glassware (Edulab).	Seek medical attention when the damage is severe (CLEAPSS).

Ethical Considerations:

- Only use trypsin powder from the laboratory rather than extracting it from wildlife.

Environmental Considerations:

- Dilute all solutions with water before disposing them into the sink.

Data Collection and Processing

Qualitative Data

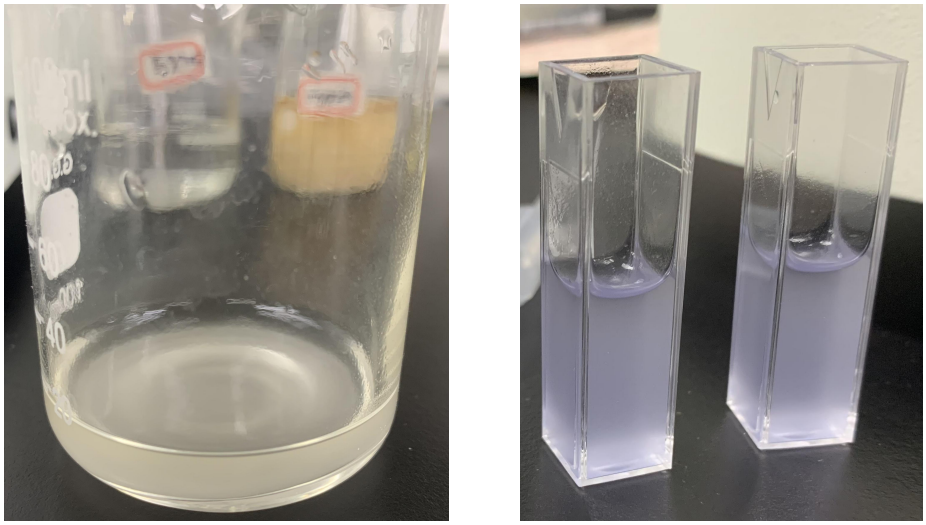

Figure 1. Observation of the reaction process and color change

The picture on the left shows the reaction mixture consisting of sodium chloride, distilled water, albumin, and trypsin solution. The combination of these compounds in a solution yields a uniformly colored yellow-translucent solution.

After trypsin hydrolyzes albumin for 9.5 minutes during the reaction process, 1 cm^3 of the reaction mixture and 1 cm^3 of biuret solution were being transferred into the cuvette. The picture on the right shows the color of the resultant solution in the cuvette, a uniform intermediate of

purplish-violet and pale-blue. Because the color shifted from purplish-violet to pale-blue, this is evidence for protein hydrolysis and lessening of the number of peptide bonds in existence.

Raw Quantitative Data

The raw data regarding the absorbance value of the color for the reaction mixture after reacting for 10 minutes were measured with a spectrophotometer and recorded on a paper.

Table 5. Absorbance value of reaction mixture per tested concentration of sodium chloride

Sodium chloride concentration/%	Absorbance value of reaction mixture/AU (±0.001 AU)			
	Trial 1	Trial 2	Trial 3	Trial 4
0.0	1.311	1.340	1.314	1.343
1.0	1.381	1.390	1.357	1.382
2.0	1.416	1.408	1.409	1.382
3.0	1.476	1.466	1.467	1.457
4.0	1.526	1.514	1.516	1.510

Sample Calculations

Mean:

$$Mean = \frac{Sum\ of\ the\ absorbance\ values\ for\ reaction\ mixture\ from\ all\ trials}{Number\ of\ trials}$$

Sample calculation using Trial 1 to 4 for concentration of sodium chloride at 0.0%:

$$Mean = \frac{1.311+1.340+1.314+1.343}{4} = 1.327$$

Standard deviation:

$$Standard\ deviation = \sqrt{\frac{\sum(Absorbance\ value\ for\ reaction\ mixture - Mean)^2}{Number\ of\ trials}}$$

Sample calculation using Trial 1 to 4 for concentration of sodium chloride at 0.0%:

$$Standard\ deviation = \sqrt{\frac{(1.311-1.327)^2+(1.340-1.327)^2+(1.314-1.327)^2+(1.343-1.327)^2}{4}} \approx 0.014$$

Analysis: The effect of the concentration of sodium chloride on the rate of trypsin activity

Processed Data: Concentration of Sodium Chloride and Trypsin Activity

Table 6. Mean and standard deviation for absorbance values per tested concentration of sodium chloride

Concentration of sodium chloride/%	Absorbance value of reaction mixture/AU (±0.001 AU)	
	Mean of 4 trials/AU	Standard deviation/AU
0.0	1.327	0.014
1.0	1.378	0.012
2.0	1.404	0.013
3.0	1.466	0.007
4.0	1.516	0.006

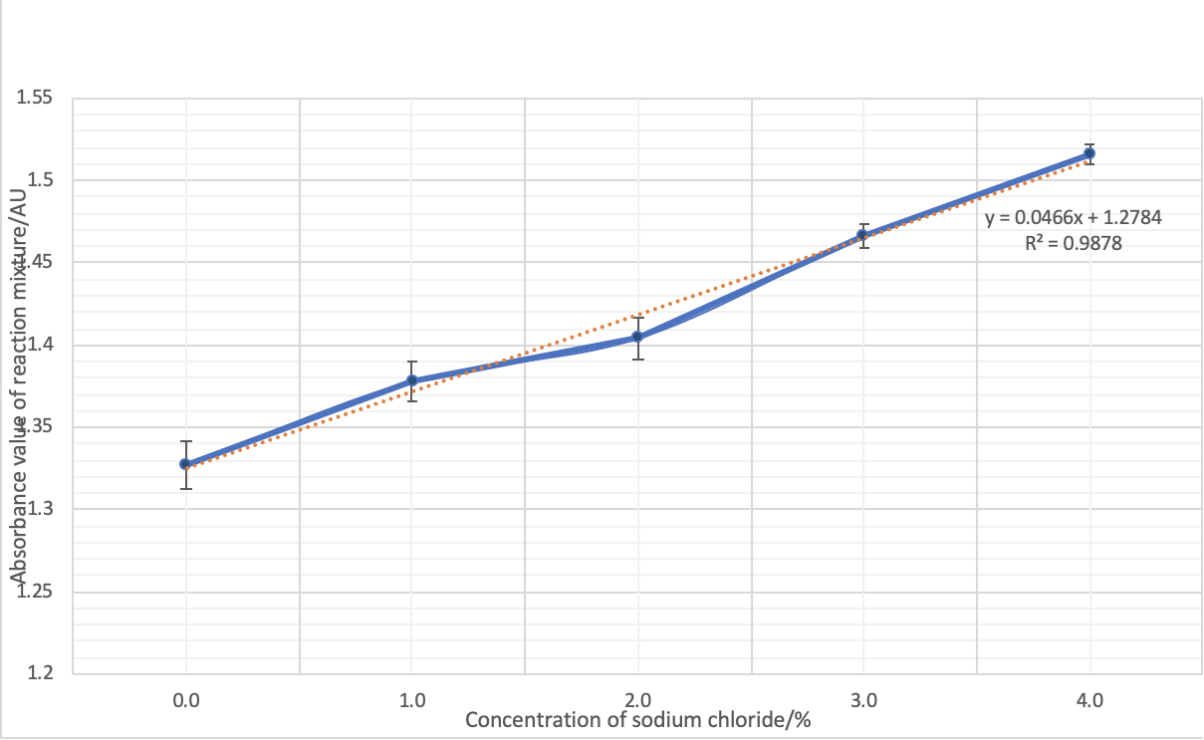

Figure 2. Absorbance value for reaction mixture per tested concentration of sodium chloride

Data Analysis: Concentration of Sodium Chloride Trypsin Activity

The processed data points, each with an error bar represented by its standard deviation from the mean, are connected with a smooth curve. From this graph, it can be observed that the higher the absorbance value of reaction mixture, the greater the amount of peptide bonds and proteins present in the mixture, the less efficient the trypsin activity is.

Overall, the graph demonstrates a positive correlation between the concentration of sodium chloride and the absorbance value of reaction mixture. As the concentration of sodium chloride increases, the absorbance value of the reaction mixture increases. This positive correlation and the increasing trend can be explained by the significant disturbance of the stability of trypsin caused by the increased concentration of sodium chloride. Loosening of the bonds within trypsin results in less successful collisions between trypsin and albumin, decreasing the rate of trypsin activity. Hence, in this specific experiment, the optimal concentration of sodium chloride is 1.0% because aside from the control (0%), the absorbance value of reaction mixture is the lowest when trypsin is hydrolyzing albumin at 1.0% of sodium chloride concentration, meaning that less amount of peptide bonds and proteins are present, so trypsin activity is highest and most efficient.

However, the rate of trypsin activity is higher than expected when the sodium chloride concentration is at 2.0%. If there were no errors during the experiment, it can also be hypothesized that sodium chloride, at 2.0% concentration, may serve as a cofactor that helps trypsin hydrolyze albumin more efficiently. On the other hand, there may have been human error, which will be more thoroughly discussed in the error analysis section during evaluation.

Statistical Analysis: Concentration of Sodium Chloride and Trypsin Activity

Meanwhile, a linear regression line is constructed on Figure 2 using Microsoft Excel. For Figure 2, the equation of the linear regression line is $y = 0.0466x + 1.2784$. The slope 0.0466 indicates that for every additional increase in the concentration of sodium chloride by 1%, the absorbance value for reaction mixture increases by 0.0466 AU. Moreover, the value of R^2 for this linear regression line is 0.9878, suggesting that the data points obtained during the experiment fit the regression model well.

Moreover, to determine whether the differences between the data points are statistically significant, the error bars can be observed. Since the error bars of the data points for 0% and 4.0% do not overlap, the difference between the two data points will most likely be statistically significant. However, the error bars of the data points for 1.0% and 2.0% do overlap, so the difference between those data points may not be statistically significant. Hence, it is necessary to clear this ambiguity by performing a student's t test.

Microsoft Excel was used to conduct a student's t test to determine whether the perceived difference for the data points of 0% and 4.0% is significant. The p-value is calculated as approximately 7.87×10^{-7}. Since a smaller p-value that is less than 0.05, or the threshold of significance, indicates that the data points are statistically significant, it can be concluded that the difference between those two data points are statistically significant, and that concentration of sodium chloride has an effect on the rate of trypsin activity.

Furthermore, when a student's t test is performed for the data points for 1.0% and 2.0%, the p-value is approximately 0.0438. This p-value is also less than 0.05, indicating that the data points are statistically significant, it can be concluded that the difference between those two data points are also statistically significant, and 1.0% and 2.0% concentration of sodium chloride have significantly different effects on rate of trypsin activity. According to a statistics test, it may be concluded that 2.0% of sodium chloride is a significant cofactor which helps trypsin hydrolyze albumin more efficiently. But because the p-value of 0.0438 is close to the threshold value of 0.05, if we want to be even more confident, then 2.0% should be attributed to human error.

Conclusion

When the concentration of sodium chloride increases, the absorbance value of reaction mixture increases, demonstrating higher presence of peptide bonds and slower rate of albumin hydrolysis by trypsin, showing decrease in the rate of trypsin activity.

Evaluation

Overall, for trypsin extracted from purified extracts of porcine or bovine pancreas, the optimal concentration of sodium chloride is 1.0%. Additionally, lower rates of trypsin activity in higher concentrations of sodium chloride highlight the diminishing activity of the essential enzyme trypsin when the intake of salt increases. This also raises awareness about how excess intake of salt from food consumption may negatively affect the digestion of albumin that is found in protein-rich food and egg-whites for people around the world.

Nevertheless, there are instances where the data points do not align with the regression line and some error bars almost overlap, meaning that we are not completely confident about the statistical significance of the result. Since these may have been caused by limitations of this investigation, it would be necessary to conduct an error analysis.

Error Analysis and Improvements

During the preparation of trypsin, albumin, and sodium chloride solutions, there may have been human error while reading the volume in a graduated cylinder. This error may also be one potential reason as to why the rate of trypsin activity is higher than expected when the sodium chloride concentration is at 2.0%. Nevertheless, to reduce this error, the number of trials were increased from 3 trials per tested concentration of sodium chloride into 4 trials.

Moreover, I cannot ensure that all the reaction mixture and biuret solution were mixed together on time to be put into a cuvette and be measured and recorded by a spectrophotometer in exactly 10 minutes. This error may also explain why the rate of trypsin activity is higher than expected at 2.0% because I hypothesize I was not quick enough to extract the mixture and allowed more time for the compounds to react, making it seem like trypsin hydrolyzed more peptide bonds and have higher rate of activity. I might need to allocate myself 45 seconds instead of 30 seconds to complete all these short and repetitive procedures, so I should record the raw data at 10 minutes and 15 seconds on a paper.

Although there are some errors that occurred during the investigation, the data points obtained still demonstrate one clear trend. Increasing the concentration of sodium chloride decreases the rate of trypsin activity. In addition to identifying the optimum concentration of sodium chloride of trypsin extracted from purified extracts of porcine or bovine pancreas, various extensions can be applied to enhance this investigation.

Extensions

In the future, the experiment can be conducted by using different types of trypsin. There are three major types of trypsin, including trypsin-1, trypsin-2, and trypsin-3 that are expressed in different regions of the body. It will be interesting to identify the optimal concentration of sodium chloride for different types of trypsin to investigate their real-world application. It will also be interesting to test the effect of the concentration of different potential inhibitors on trypsin activity. Since past studies have demonstrated that heavy metals, such as Zn and Cu, inhibit enzyme activity, the effects of heavy metals and salinity can be compared as well (Lukowski & Dec, 2018). Lastly, since the interval of each tested concentration of sodium chloride is 1.0% in the current investigation, in the future, smaller intervals can be used, such as 0.2% or 0.5%, to improve the precision of the results.

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