

Concentration-Dependent Investigation of the Inhibition of Bromelain Mediated Protein Hydrolysis on Egg Albumen through *Coffea arabica*

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Abstract

The implications of caffeinated beverages on human health has been widely debated. In a population that consumes an average of 250 mg of caffeine daily, investigation of health concerns is of high importance. Analysis of five variations of *Coffea arabica* concentration was conducted on the hydrolysis of egg albumen by the proteolytic enzyme bromelain, over 15 minutes.

The results suggest a statistically significant difference ($p < 0.05$) in the rate of hydrolysis as a product of the concentration of *Coffea arabica* in the experimental solution. Findings of this exploration on the sensitivity of protein hydrolysis to *C. arabica* suggest greater comprehension of the inhibitory nature of *Coffea arabica* on enzymatic digestion, which may play an important role in medical advancements to support absorption of amino acids into the bloodstream, extending to promoting healthy lifestyles. The research under exploration discusses how variations in the concentration of instant coffee affect the rate of protein digestion of egg albumin using bromelain.

Introduction

Exploration of factors that affect the rate of protein hydrolysis is of high value: the absorption of proteins into the bloodstream through hydrolysis into amino acids plays a key role in the formation of macromolecules such as new protein conformations, sources of energy and molecules bound to nitrogen such as DNA and RNA (Pagán et al., 2021). The hydrolysis process affects the size, level, and composition of free amino acids and small peptides, consequently influencing biological activity (Chen et al., 2011). If amino acids derived from food sources are not available, amino acids are acquired from existing tissues within the human body, such as muscles (Sleisenger & Kim, 1979). Proteins taken in through the oral cavity undergo mechanical breakdown and denaturation by HCl in gastric juices, enabling accessible digestion through proteolytic enzymes such as pepsin in the stomach. The shorter polypeptides formed are further broken down into constituent amino acids by other proteases in the small intestine, such as trypsin and chymotrypsin, from where the amino acids are absorbed into the bloodstream for the synthesis of new macromolecules (Li et al., 2020).

Enzymes are globular proteins that act as biological catalysts for chemical reactions; a substrate reacts with an enzyme, and this enzyme-substrate complex lowers the activation energy of a specific chemical reaction; the result of which is the initial enzyme and a product. Bromelain is a proteolytic enzyme, and therefore plays an important role in the breakdown of protein structures in the formation of monomeric amino acids (Huber et al., 1982).

The addition of an enzyme accelerates the reaction, leading to a shorter observation period. Conversely, *C. arabica* (coffee) acts as a non-competitive inhibitor of the discussed reaction, allowing for the examination of the role of coffee in impeding protein hydrolysis (Hammes, 2002). This highlights the complex interplay between enzymes, inhibitors, and substrates in biochemical reactions, shedding light on the diverse factors influencing protein breakdown and digestion in the field of human nutrition.

The hydrolysis reaction breaks down protein structures into amino acids, which is the reverse reaction to the condensation polymerization of protein structures from amino acids. The primary, secondary, tertiary, and quaternary structures of proteins enable them to perform various functions in organisms to sustain biological processes necessary for survival (Baker & Šali 2001). Protein hydrolysis differs from denaturation of proteins in that the structure is broken down into amino acids, rather than involving the loss of its three-dimensional configuration (Robinson & Robinson, 2001). The primary, secondary, tertiary, and quaternary structural features of proteins are conserved from yeast to mammals, and the energy released during the formation of these quaternary coiled-coil structures has been proposed to contribute to vesicle membrane fusion for cellular processes (Foster et al., 2000).

The aim of this exploration is to investigate the research question: How do variations in the concentration of instant coffee affect the rate of protein digestion of egg albumin in 15 minutes using bromelain? Various studies have been conducted on the role of coffee to act through non-competitive inhibition to decrease the rate of protein digestion. For example, Turesky et al. (2003) demonstrated the role of coffee influencing enzymatic activity through inhibition of key enzymes responsible for metabolic activity, which explored concentrations related to human consumption, and reflected them through animal studies. A more recent study revealed an inhibitory effect of starch hydrolysis relative to concentrations in black tea; black tea may inhibit digestive amylase activity and result in decreased levels of starch hydrolysis, which may influence research concerning human diet (Freitas et al., 2019). These studies indicate that substances containing caffeine delay digestion of macromolecules in heterotrophic organisms, which may be attributed to the quality of caffeine to act as a phosphodiesterase inhibitor, therefore demonstrating inhibitory effects on protein digestion.

The null hypothesis for this exploration is that variations in the concentration of coffee will have no inhibitory effect on the rate of protein hydrolysis in the egg albumin by bromelain after 15 minutes; through a t test, $H_0: p > 0.05$. The alternative hypothesis for this exploration is therefore that as the concentration of coffee increases, there will be a significant difference in the rate of protein hydrolysis in the egg albumin by bromelain after 15 minutes; effectively the graph should depict a negative exponential correlation; the methodology used is derived from Hubert et al. (2005), in which the inhibitory effect on amylase activity was illustrated through an exponential regression with $H_a: p < 0.05$.

The denaturation of proteolytic enzymes in bromelain due to extreme temperatures or pH values is a well-documented phenomenon (Kimura et al. 2023). Specifically, extreme pH values can lead to denaturation due to the interaction of protons with the amino acids in the enzyme, this interaction can change the R groups on amino acids, thereby the charge and effectively, tertiary or quaternary conformation of proteolytic enzymes. This susceptibility of enzymes to denaturation under extreme conditions highlights the importance of maintaining optimal environmental conditions for their functionality.

In non-competitive inhibition, the molecule that acts as an inhibitor will bind to the allosteric site of an enzyme. This region is not the active site; however, it prevents the reaction from proceeding as the inhibitor effectively alters the shape of the enzyme to reduce the overall rate of successful collisions. The

role of coffee to act as a non-competitive inhibitor is explored, in which the rate of the digestion of egg albumen protein by bromelain will theoretically be decreased due to the change in the conformation of the enzymatic proteins in bromelain due to allosteric inhibition.

The inhibition of protein hydrolysis and its relationship to human health, particularly concerning the consumption of protein-rich foods and the development of functional structures in the body, underscores the significance of understanding the impact of coffee consumption on this process. The extensive body of research on coffee and its potential health benefits provides valuable insights into its influence on protein hydrolysis and its broader implications for human health and strength (Pavan, 2012).

Furthermore, research investigating the hydrolysis of proteins has underscored the role the discussed reaction in the release of bioactive peptides, which have been associated with beneficial effects on human health (Albenzio et al., 2017). Moreover, the development of functional structures in the human body, such as muscles, is heavily reliant on the consumption of proteins, ultimately impacting human survival and strength (Toldrá et al., 2020). Further research has indicated that coffee consumption may have implications for muscle cell signaling and differentiation, potentially influencing skeletal muscle mass and myogenic activity (Terruzzi et al., 2018). Additionally, the inhibition of protein hydrolysis has been shown to lead to increased protein content in human muscle cells, suggesting a potential link between coffee consumption and proteolysis inhibition, which could impact muscle protein turnover (Chanon et al., 2018).

Methodology

Five conditions of varying coffee concentrations were used to observe the allosteric inhibition of bromelain-mediated hydrolysis on egg albumen over 15 minutes. Three trials for each solution were conducted in conjunction with each of the solutions of varying coffee concentration. Nescafe instant coffee simulated the varying concentrations of coffee, where the variations in concentration ranged from 0% coffee solution to 1.72% coffee solution in terms of % weight / volume; the 1.72% was the greatest proportion, representative of the average caffeine content in a cup of coffee (150 mg caffeine / 250 mL distilled water). As 4.9 mL of Nescafe instant coffee contains 70 mg of caffeine per 2 g serving, therefore 1.72 g of Nescafe instant coffee was required for 100 mL of distilled water.

Therefore: % w/v = g of solute/100 mL of solution, 1.72% = 1.72 g of instant coffee / 100 mL distilled water. The variations in concentration were determined through differing dilution factors of the initial 1.72% solution to produce five conditions by intervals of 0.43%.

The inhibition by coffee of the rate of reaction in egg albumen from *Gallus gallus domesticus* hydrolyzed by bromelain from *Ananas comosus* was investigated through spectrophotometry equipment and a biuret test, as the color change is contingent upon the number of peptide bonds in which the increased vibrancy correlates to a greater proportion of peptide bonds. The variation in coffee concentration during this analysis was the independent variable, where the respective dependent variable was therefore the rate of egg albumen hydrolysis determined through the rate of absorbance per minute of light at 640 nm

by spectrophotometry equipment, this value is chosen due to the blue color from the biuret test, of which 640 nm is on the opposite side of the visible spectrum, and will therefore be more reliable in terms of measurement.

The control variables included: the laboratory experiment room and environment (e.g., temperature; this must remain constant as at differing temperature, enzymatic activity will vary), the substrate concentration, the enzyme concentration, and pH. Temperature, enzyme concentration, substrate concentration, and pH share a similar characteristic in that they have an effect on an enzyme's activity. A change in the environment temperature for example, would influence the enzymatic activity of catalase: if the environment temperature lies outside of the optimal range, the enzyme's rate of activity would decrease, at the optimal temperature, the enzyme activity would be maximized. Hence, alterations made to any of the listed factors would challenge the validity of this experiment.

The materials utilized in this experiment included bromelain sourced from pineapple extract, egg albumin extracted from egg whites, distilled water, Nescafe instant coffee, a timer, Biuret solution, a stir stick, test tubes, beakers (with a precision of ± 1 mL), a graduated cylinder (with a precision of ± 0.5 mL), and 0.1 mol/L NaOH. The apparatus employed consisted of a spectrophotometer (with a precision of $\pm 0.1\%$ transmittance), a digital balance (with a precision of ± 0.01 g), and a pH indicator (bromothymol blue).

The experimental procedure began by preparing an egg albumin solution (30 mL egg whites / 100 mL H₂O) and a proteolytic enzyme solution (20 mL pineapple extract / 100 mL H₂O). A coffee solution was then prepared by dissolving 1.72 g of Nescafe instant coffee in 100 mL distilled water, resulting in a 1.72% coffee condition. Varying conditions were established by diluting the 1.72% solution with distilled water (as detailed in Table 1), and each condition was labeled according to its coffee content.

To maintain a pH within the optimal range for bromelain activity (pH 7), NaOH was added to each beaker, and bromothymol blue served as the pH indicator. Subsequently, equal parts (10 mL) of bromelain, egg albumen, and the coffee solution were combined in the beakers. The Biuret test was conducted by adding 5 mL of Biuret solution to each beaker. Transmittance values at the initial time were recorded using a spectrophotometer, and a timer was set.

After a 15-minute incubation period, transmittance values at the final time were recorded by analyzing samples in the spectrophotometer. This systematic experimental design aimed to investigate the allosteric inhibition of bromelain-mediated hydrolysis reactions on egg albumen under varying coffee concentrations, providing a comprehensive understanding of the interactions involved in the enzymatic digestion process. Table 1 illustrates the dilution factors for each of the experimental samples.

Observations

Qualitative observations of the results of this experiment in conjunction with the effect of *C. arabica* on the rate of protein hydrolysis were that there was a decreased rate of protein hydrolysis relative to an increase in the concentration of Nescafé instant coffee treatment in the experimental conditions (Fig. 2).

This disruption of the protein conformations in the egg albumen lead to visible changes in the viscous liquid, specifically, suspension of the egg white in the solution (Fig. 3). It seemed that this visible suspension was most prominent in the 0.00% and 0.43% *C. arabica* solution, with suspension less apparent in greater concentrations of 1.29% and 1.72% *C. arabica* solutions. This suspension is underscored by the increased rate of protein digestion of 0.00650 Au/min in the 0.00% *C. arabica* solution, which is nearly a four times greater rate than the 1.72% *C. arabica* solution. The overall change in the rate of hydrolysis between the 0.00% and 1.72% solution is 0.00482 Au/min. The stark decrease in the rate of hydrolysis between coffee concentrations 0.00% and 0.43% of 0.00250 Au/min differs from the minimal decrease between 1.29% and 1.72% of 0.00035 Au/min, this indicates that the inhibitory property of *C. arabica* does not linearly inhibit the rate of hydrolysis, but rather may plateau in terms of inhibition efficacy.

It seems that the reactions involving conditions of lower concentrations of coffee solutions were substantially faster than the protein hydrolysis reactions in greater concentrations. This decrease in the rate of hydrolysis with greater proportions of coffee in solution illustrates a direct negative correlation between *C. arabica* levels and the rate of the digestion of egg albumin protein by bromelain proteolytic enzymes. The equation for the exponential trend line was calculated through the program excel, in which the expression produced was $y = 0.0059e^{-0.789x}$ with substantial results with an R^2 value of 0.97, this value indicates that there is a statistically significant correlation between the independent and dependent variable. Therefore, the variation in *C. arabica* has a negative influence on the rate of egg albumen hydrolysis determined through the rate of absorbance per minute.

The data (Fig. 1) illustrates this negative correlation between the independent and dependent variable to a high degree of precision. Illustrative of a stark decrease in the rate of hydrolysis as a product of the addition of *C. arabica*, where the efficacy of the inhibition plateaus on the graph, theoretically as the concentration of coffee added to the experimental reaction is increased, the slower the reaction will progress.

A two-tailed t-test was used to determine the statistical significance ($p \leq 0.05$) of the role of *C. arabica* on the inhibition of the hydrolysis of egg albumin by bromelain over a 15 minute period. This t-test for one independent mean was chosen to assess if each set was numerically greater or less than another. Although the data demonstrates a direct correlation between the independent and dependent variable with a relatively high R^2 value of 0.97, a t-test further supports this correlation, an ANOVA test was not possible because of the limited number of trials conducted; therefore, a t-test was the best option to determine statistical significance. The H_0 null hypothesis, that alterations to the concentration of *C. arabica* will have no inhibitory effect on the rate of protein hydrolysis in the egg albumin by bromelain after 15 minutes was rejected through the t-test, with $H_0: p > 0.05$.

Discussion

The primary objective of this investigation was to examine the impact of varying concentrations of instant coffee on the rate of protein digestion facilitated by bromelain-mediated hydrolysis of egg albumin. The statistical analysis revealed a t-value of 5.271 and a corresponding p-value of 0.000013, indicating a high level of statistical significance ($p < 0.05$). Consequently, the null hypothesis, which posited that changes in the concentration of *C. arabica* would not exert an inhibitory influence on the rate of protein hydrolysis in egg albumin by bromelain after 15 minutes, is rejected. Conversely, the alternative hypothesis, which suggested that an increase in the concentration of *C. arabica* would lead to a significant difference in the rate of protein hydrolysis in egg albumin by bromelain after 15 minutes, is accepted. The graphical representation of the data exhibited a negative exponential correlation, as evidenced by the general expression $y = 0.0059e^{-0.789x}$ with a notably high and statistically significant R^2 value of 0.97.

The outcomes of this experiment therefore establish a relationship between the concentration of *C. arabica* and a respective decrease in the rate of hydrolysis of the egg albumin by the proteolytic enzyme bromelain. The statistical significance of the results was assessed through a two-tailed t-test, which delineate a substantial variance in the concentration of *C. arabica* and the reaction rate over a 15-minute period, with a significance level of $p < 0.05$. This indicates that an elevated concentration of *C. arabica* in the experimental solution effectively diminishes the rate of hydrolysis of the egg albumin protein.

Protein hydrolysis is achieved through enzymatic digestion, that effectively break down proteins into shorter polypeptides, that are later broken down into individual amino acids for absorption into the bloodstream (Freeman et al., 1979). This process of enzymatic digestion by proteases is essential for biological processes that support healthy functioning of tissues within the body.

Caffeine, a naturally occurring alkaloid, has been found to act as an inhibitor for certain enzymes, particularly those involved in protein hydrolysis (Nawrot et al., 2003). As the concentration of caffeine increases, there is a noticeable decrease in the rate of protein hydrolysis, suggesting a potential interaction with the active sites of protease enzymes. This inhibition can occur through competitive or non-competitive mechanisms, with competitive inhibition involving the inhibitor (caffeine) competing with the substrate (protein) for binding to the enzyme's active site, while non-competitive inhibition involves the inhibitor binding to a different site on the enzyme, leading to a conformational change that reduces the enzyme's activity (Szabó et al., 2019).

The concentration-dependent effect of caffeine on protein hydrolysis suggests a non-linear relationship, with an increasing concentration of caffeine leading to a more pronounced inhibitory effect on protein hydrolysis. This observation may be attributed to the saturation of binding sites on the enzyme or other complex interactions between caffeine and the enzyme. Moreover, this non-linear relationship between caffeine concentration and its impact on protein hydrolysis may be indicative of the complex pharmacokinetic interactions of caffeine with enzymes, affecting their activity and leading to pharmacokinetic interactions with medications and other compounds (Romero-Martínez et al., 2021; Rasmussen et al., 1998). Additionally, the inhibitory effect of caffeine on specific enzymes has been

explored in different biological systems, highlighting its potential as an enzyme inhibitor in various contexts (Liu et al., 2022; Selby & Sancar, 1990). Furthermore, the non-linear relationship between caffeine concentration and protein hydrolysis may be influenced by the diverse physiological effects of caffeine. Caffeine has been shown to modulate various physiological processes, including its impact on vision, cell cycle, and neurotransmitter agents, which may contribute to its non-linear effect on protein hydrolysis (Nguyen et al., 2017; Traganos et al., 1991; Delbari et al., 2022).

The methodology of this investigation may be subject to potential limitations, including the absence of control over external temperature, which was not manipulated to achieve the optimal conditions for the hydrolysis reaction. This lack of control may have implications for the analysis of the inhibitory properties of *C. arabica*, as the setting may not be ideal. A potential modification to address this limitation could involve conducting the analysis in a closed system at 50 °C, a temperature significantly different from the approximate 20 °C surrounding the investigation. Another limitation pertains to the insufficient quantity of biuret solution, which is essential for producing the characteristic blue-purple color indicative of the presence of peptide bonds. This limitation may have impacted the observations and analysis of the experiment. Furthermore, challenges were encountered in relation to the duration of the experiment, particularly concerning the accurate measurement of initial transmittance values. These challenges may have led to deviations from the ideal initial value, which is crucial for monitoring the progression of the reaction. A potential modification to mitigate this limitation could involve conducting each analysis individually to ensure meticulous organization and planning.

The potential benefits of caffeine's inhibitory nature have been extensively discussed in the current literature. For instance, caffeine has been associated with a reduced risk of neurodegenerative diseases, such as Alzheimer's and Parkinson's, which are characterized by the accumulation of specific proteins, including beta-amyloid in Alzheimer's and alpha-synuclein in Parkinson's. The inhibitory effect of caffeine on protein hydrolysis may influence the clearance or breakdown of these proteins, potentially reducing their accumulation and the associated neurodegenerative effects (Zheng et al. (2023)Zheng et al., 2015; Yang et al., 2020; Mitani et al., 2017; Ko, 2023).

A study by Eskelinen et al. (2010) found an association between midlife coffee and tea drinking and a decreased risk of dementia in later life, suggesting a potential link between caffeine consumption and cognitive health (Eskelinen & Kivipelto, 2010). This observation aligns with the potential neuroprotective effects of caffeine, as evidenced by its association with improved cognitive performance and reduced amyloid positivity in older adults (Zheng et al., 2023).

Furthermore, the potential benefits of caffeine consumption on cognitive function and overall nutrient adequacy have been highlighted in various studies, suggesting a positive association between caffeine intake and cognitive performance (Beydoun et al., 2014; Jarvis, 1993). These findings underscore the multifaceted impact of caffeine on various physiological processes, including cognition, lipid metabolism, and neuroprotective functions.

Conclusions

C. arabica plays a significant role in the inhibition of the rate of hydrolysis of egg albumen by bromelain. Over 15 minutes the rate of hydrolysis expressed through absorbance per minute demonstrated statistical significance in the greater rate of reaction observed in lower concentrations of *C. arabica*, compared to conditions containing higher concentrations. These results are substantiated by those of Turesky et al. (2003) and Freitas et al. (2019) who demonstrated the role of coffee on enzymatic activity through inhibition of key enzymes responsible for metabolic activity in addition to the inhibitory effect of coffee on starch hydrolysis relative to concentrations in black tea, respectively. These studies illustrated how coffee likely plays a role on the rate of digestion of macromolecules in heterotrophic organisms, which may be attributed to the quality of caffeine to act as a phosphodiesterase inhibitor, therefore demonstrating inhibitory effects on protein digestion.

An understanding of the critical role that coffee plays in the inhibition of protein hydrolysis into its constituent amino acids can help researchers to understand the health implications associated with the consumption of caffeine. As discussed, the overall impact of caffeine on the inhibition of protein hydrolysis concerns its influence on the absorption of amino acids or by altering protein conformation, potentially affecting protein synthesis or overall nutritional uptake. This, in turn, may have implications for muscle health and neurological processes. Consequently, the role of caffeine in inhibiting enzymes involved in drug and nutrient metabolism implies physiological consequences for individuals and their absorption of these essential components. Advancements in scientific research to expand current understanding of the diverse effects of caffeine may expand to potential therapeutic implications for neurodegenerative diseases and other health conditions. Such advancements would underscore the multifaceted nature of caffeine and its far-reaching impact on human health and well-being.

Declarations

Author Contribution

C.L. wrote the manuscript text and collected the data embedded

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Tables

Table 1 *Dilution factors for each coffee concentration condition.*

Concentration of Coffee (%)	0.0	0.43	0.86	1.29	1.72
Coffee solution ($\pm$ 0.1 mL)	0.0	25.0	50.0	75.0	100.0
Water solution ($\pm$ 0.1 mL)	100.0	75.0	50.0	25.0	0.0

Table 2 *Absorbance data calculated from collected transmittance values*

	Absorbance (± 0.001 Au)				
Coffee (%)	0.00	0.43	0.86	1.29	1.72
Initial	0.16749	0.44370	0.64207	0.97881	1.2007
	0.17199	0.44612	0.63827	0.98197	1.2147
	0.19247	0.47366	0.63451	0.96257	1.2218
Average	0.17731	0.45449	0.63828	0.97478	1.2124
Final	0.069560	0.38510	0.61979	0.94692	1.1871
	0.080922	0.39147	0.58838	0.94310	1.1938
	0.088842	0.40671	0.58670	0.94310	1.1805
Average	0.079775	0.39443	0.59829	0.94437	1.1871
Difference	0.09793	0.05860	0.02228	0.03189	0.01357
	0.09106	0.05464	0.04989	0.03987	0.02085
	0.1036	0.06695	0.04781	0.01948	0.04139
Average	0.09754	0.06006	0.03999	0.03041	0.02527

Table 3 *Absorbance data calculated from collected transmittance values*

	Rate of Hydrolysis (Au/min)				
Coffee (%)	0.00	0.43	0.86	1.29	1.72
Rate of Hydrolysis	0.00653	0.00391	0.00149	0.00213	0.000905
(Au/min)	0.00607	0.00364	0.00333	0.00266	0.00139
	0.00691	0.00446	0.00319	0.00130	0.00276
Average	0.00650	0.00400	0.00267	0.00203	0.00168
Standard Deviation	0.00192	0.00115	0.000893	0.000770	0.000962

Table 4 *Descriptive statistics on the inhibition of the rate of hydrolysis as a product of variations in the concentration of C. arabica*

<i>C. arabica</i> (%)	Rate of Hydrolysis Mean (Au/min)	Uncertainty (%)	Standard Deviation	N
0.00	0.00650	19.1	0.00192	3
0.43	0.00400	0.0725	0.00115	3
0.86	0.00267	2.93	0.000893	3
1.29	0.00203	0.0289	0.000770	3
1.72	0.00168	1.46	0.000962	3

Table 5 *Table of equations and sample calculations for this exploration*

Equation	Sample Calculation
Absorbance = $\log_{10} \frac{100}{T[\%]}$	Absorbance for 0.86 % coffee concentration initial time trial 1. $= \log_{10} \left(\frac{100}{22.8} \right)$ $= 0.64207 \text{ Au}$
Element Concentration Mean = $\frac{\sum \text{Rate of Hydrolysis}}{\# \text{ trials}}$	Mean for the 0.86 % coffee concentrations rate of hydrolysis results. $= \frac{0.00149 + 0.00333 + 0.00319}{3}$ $= 0.00267 \text{ Au/min}$
Standard Deviation (SD) = $\sqrt{\frac{\sum (x_i - \mu)^2}{(n-1)}}$	SD for the 0.86 % coffee concentrations rate of hydrolysis results. $= \sqrt{\frac{(0.00149 - 0.00267)^2 + (0.00333 - 0.00267)^2 + (0.00319 - 0.00267)^2}{(3-1)}}$ $= 0.000893 \text{ Au/min}$
Systematic uncertainty = $\frac{\text{digital balance uncertainty}}{\text{mass of Nescafe coffee}} \times 100\% + \frac{\text{beaker uncertainty}}{\text{volume of water}} \times 100\%$	Systematic uncertainty for 0.86 % coffee concentration. $= \frac{0.01 \text{ g}}{0.86 \text{ g}} \times 100\% + \frac{0.5 \text{ mL}}{100 \text{ mL}} \times 100\%$ $= 1.16\% + 0.5\%$ $= 1.7\%$ $0.86 \times 1.66\% = 0.0143$ Systematic uncertainty = 0.86 ± 0.014
Random uncertainty = $\frac{\text{max.value} - \text{min.value}}{2}$	Random uncertainty for 0.86 % coffee concentration initial time trials. $= \frac{0.64207 - 0.63451}{2}$ $= 0.00378$ Using the mean of the initial time trials $\frac{0.00378}{0.63828} \times 100\% = 0.59\%$

Figures

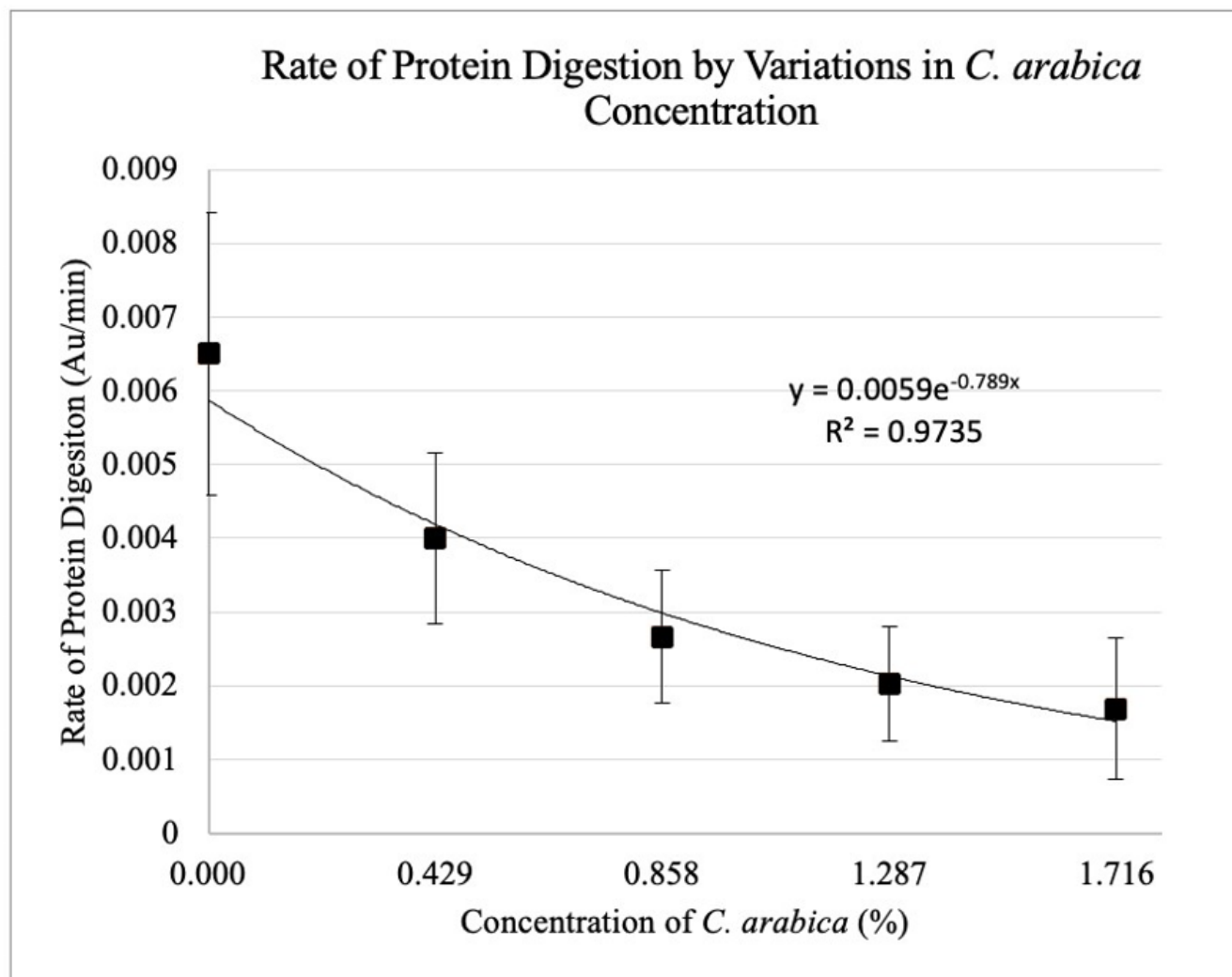


Figure 1

The inhibitory effect of *C. arabica* (coffee) on enzymatic activity of bromelain from *Ananas comosus* on egg albumen. The measurement on bromelain activity was performed as described in the presence of various concentrations of coffee. ($R = 0.97$; $y = 0.0059e^{0.789x}$). Bars indicate ± 1 standard deviation.

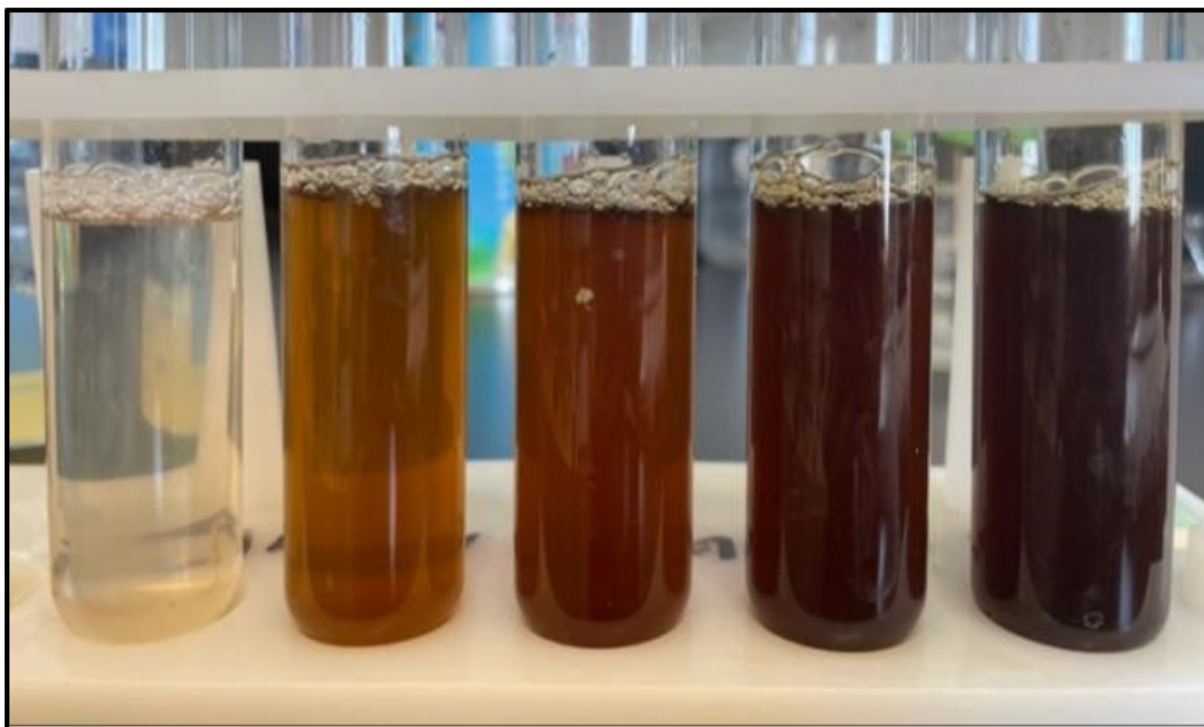


Figure 2

Initial photograph of the experimental setup, with variations in *C. arabica* concentration (%) increasing from left to right (0.00, 0.43, 0.86, 1.29, 1.72).

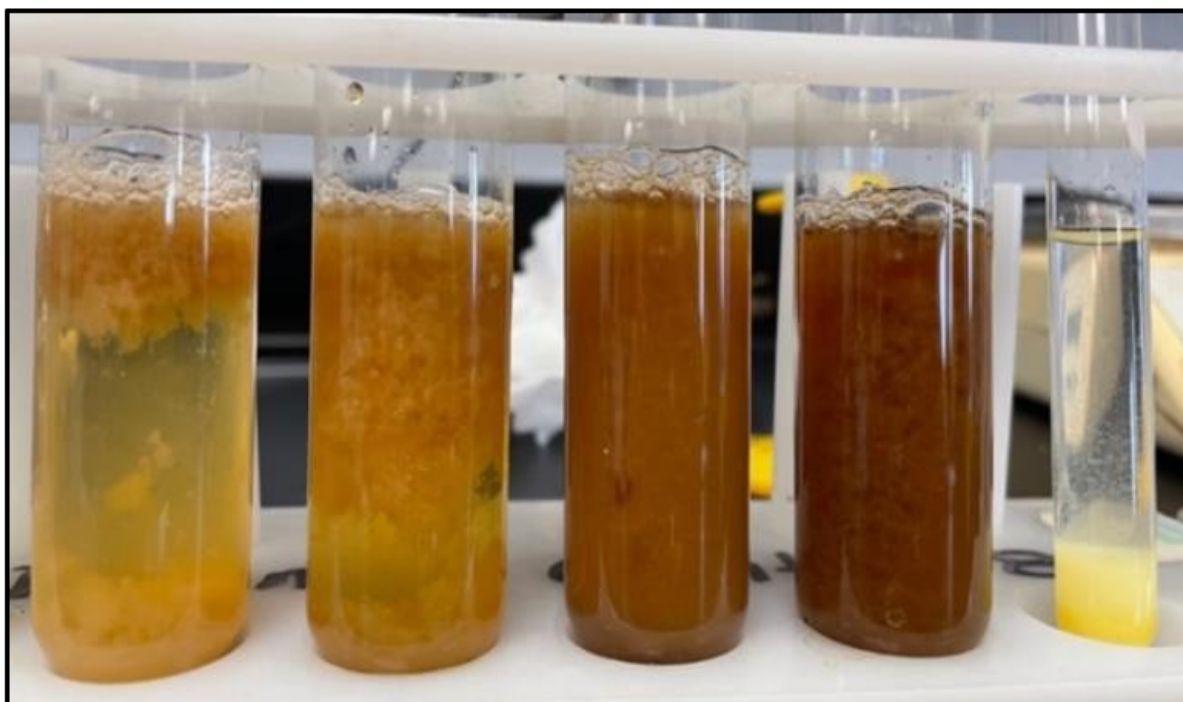


Figure 3

Final photograph of the experimental setup after 15 minutes, with variations in *C. arabica* concentration (%). Lowest concentration (0.00 %) is pictured on the far right, where left to right progression is from concentration conditions (%) of 0.43, 0.86, 1.29, 1.72, 0.00. Illustration of the visible suspension of egg albumin by the proteolytic enzyme bromelain.