

Lab #12

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Denaturation of Proteins and Enzymes

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ABSTRACT

For this Lab, our group wanted to examine some of the visual aspects of proteins being denatured. Enzymes are a distinct type of protein which typically catalyze reactions. They are the type of protein we are denaturing for this lab. Four experiments were conducted by the lab groups to see the results of enzymes denaturing as well as enzymes that experienced no reaction. We saw enzyme denaturing due to pH change, the presence of organic compounds or heavy metal, and heat for enzymes in foods.

INTRODUCTION

The purpose of this experiment was to see some of the chemical changes that occur when an enzyme is denatured. This was accomplished through the process of four experiments. The pineapple and gelatin experiment, where enzymes are denatured with heat, the pudding experiment where amylase in saliva denatures enzymes in pudding cups, the milk and lemon juice experiment, where lemon juice changes the acidity of milk and heavy cream, and the egg albumin experiment, which denatures proteins using, heavy metal, heat, acid, base, and alcohol.

Denaturing of proteins is a process by which proteins lose their secondary, tertiary, or quaternary structure resulting in different chemical properties. While some of the experiments used controls, for most, a chemical change can be expected from denaturing various foods that contain enzymes.

MATERIALS AND METHODS

The two experiments that our group conducted were the pudding saliva, and egg albumin one. For these experiments we used:

packaged puddings (2)

spoons (2)

spreading map

A hot plate

6 large test tubes with egg albumin

10% HNO_3

10% NaOH

95% ethyl alcohol solution

10% AgNO_3

EXPERIMENTAL PROCEDURE

For the pudding and saliva experiment, the procedure was fairly straightforward.

Step 1: Separate the contents of a pudding cup into two beakers evenly.

Step 2: Put some pudding from one beaker into your mouth before spitting it back into the beaker.

Step 3: Stir both cups for 2 minutes

Step 4: Examine results, specifically observe the difference in thickness between the puddings.

For the egg albumin experiment:

Step 1: Place 2 mL of egg albumin solution into each of the 6 large test tubes.

Step 2: Place one test tube in a hot water bath

Step 3: Add 2 mL of 10% HNO_3 to one test tube.

Step 4: Add 2 mL of 10% NaOH .

Step 5: Add 4 mL of a 95% ethyl alcohol solution.

Step 6: Add 2 mL 1% AgNO_3 .

Step 7: Examine and compare results for different methods of denaturing.

RESULTS

Observations for part A:

	Observations
Fresh Pineapple	Darker yellow, gelatinous (should be more liquidly, would be if it was fresh, enzyme in pineapple that would break down the protein isn't nearly as strong due to not being fresh)
Canned Pineapple	Light yellow gelatin, maintains gelatinous texture
Control	Clear gelatin, keeps together well

Observations for part B:

	Observations
Pudding without spit	The pudding kept together in a glob when put onto the spreading mat
Pudding with spit	The pudding dispersed gradually, forming a small puddle similar to how water would.

Observations for part C:

	Observations	PH
Milk	Looks like milk	6
Heavy Cream	Similar to milk, some very small bubbles	5
Lemon Juice	Slightly transparent and yellow	3
Milk with Lemon Juice	Looks yellow and chunky, very thick	3
Heavy Cream with Lemon Juice	Some curding on the surface, very thick and slightly yellow but opaquer than the milk with lemon	3

Observations for part D:

	Observations
Heat	Hardening and becoming a similar substance to cooked egg white
HNO₃ (acid)	Much cloudier than control
NaOH (base)	Completely clear
Ethanol	A bit cloudier, more dense
AgNO₃ (Heavy Metal)	Very opaque / cloudy
Control	Maintained liquid consistency and mostly transparent color

DISCUSSION

For part A, the gelatin with fresh pineapple was darker in coloration than the canned pineapple. There would have been more significant differences in the structural integrity of the two pineapples if we had had access to real fresh pineapple in lab. The lack of real fresh pineapple certainly skewed our results slightly for this experiment but we were still able to detect some denaturing of enzymes in pineapple from the breaking down of the gelatinous texture.

For part B, the pudding without spit seemed to have an easier time staying together when placed upon the spreading mat. The pudding with spit seemed to behave more similarly to watery and spread out on the mat. This is likely due to the amylase enzyme present in saliva breaking down the structures in the pudding. Were fresh pineapple used in part A of the experiment, we would have seen similar results there as well, with the enzymes in the pineapple breaking down the gelatin and become more liquidy.

In part C, the addition of lemon juice to milk and heavy cream caused them to become more thick and lowered the PH to be more similar to that of lemon juice (representing an increase in acidity). We can see the PHs of milk and heavy cream reduce from 6 and 5 to 3. The thickness of both dairy products changes as well, with both becoming more thick and displaying signs of curding.

Finally, for part D, egg albumin, which is often used as a stabilizer similar to gelatin, is combined with various reactants. Heat causes the albumin to harden, somewhat similar to the texture of a cooked egg. adding HNO_3 increased the cloudiness of the mixture, whereas NaOH turned it completely transparent. Ethanol thickened the albumin and AgNO_3 turned the mixture the most opaque. Most of the chemical changes within this part of the experiment are revealed through changes in color.

CONCLUSION

Within these experiments we witnessed chemical changes due to proteins being denatured within foods. Results showed successful denaturing in the form of chemical changes for the different

foods. We can see enzymes breaking down the structure of gelatin and turning it into a more liquidy substance, as well as coloration differences for egg albumin.