Protein denaturation takes place when the attractive forces holding a protein's secondary, tertiary and/or quaternary structures are disturbed or broken. Proteins can be denatured in a number of ways including heat, detergents, mechanical action and reducing agents. Proteins can also be denatured in an acidic environment. When acid is added to milk, the attractive forces holding milk proteins together will break, causing the milk proteins to lose their structure and interact with other unfolded proteins around them. As the proteins clump together, it will form curds on top of the milk. The curds are nonfunctional proteins. Adding pineapple juice to milk will also cause the proteins to clump together. This is not due to the acidity of pineapple juice, however. It is due to the presence of a mixture of protease enzymes in pineapple, called Bromelain. Proteases are enzymes that break down, or hydrolyze, protein molecules by breaking peptide bonds and releasing free amino acids.

I did a protein-denaturation experiment (with a few little helpers) using milk, lemon juice and pineapple juice. Prior to conducting the experiment, I thought that the protein in the warm milk and lemon juice would denature more than the protein cold milk and lemon juice. I also thought that the Bromelain enzyme itself would denature when added to hot milk, causing the protein in the hot milk and pineapple mixture to be less curdled than the protein in the cold milk and pineapple juice mixture.



## Results:

Cold milk + lemon juice	Warm milk + lemon juice	Warm milk + pineapple	Cold milk + pineapple juice
		juice	
Not curdle but protein	More curdled than cold	Very curdled, milk protein	Less curdled than hot milk
very slight separation.	milk and lemon mixture in	almost completely	+ lemon, but more than
	large clumps.	separated from liquid.	cold milk + lemon.
		Not as clumpy as the hot	More of a liquid than the
		milk and lemon juice	warm milk + lemon juice
		mixture.	mixture.

The warm milk denatured much more quickly and thoroughly when lemon juice was added compared to the cold milk mixture. The warm milk mixture was very separated, with large clumps of the milk protein separated from the liquid. As stated above, I thought that the Bromelain enzyme would denature when added to hot milk, reducing the enzyme activity, and causing the protein in the hot milk and pineapple mixture to be less curdled than the protein in the cold milk and pineapple mixture, but that did not happen. I now know that Bromelain is very stable in high temperatures up to around 140 degrees Fahrenheit. The milk was heated to around 120 degrees Fahrenheit.