An Investigation into The Coagulation of Proteins in Tofu

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Introduction

Tofu, often considered to be an ideal food choice towards a healthy lifestyle, has been rooted as a staple in south-east Asian cuisine for centuries. As of recently, I have been quite interested in the process of how tofu is made and what causes it to solidify. With research and some hands-on tofu making experience, I found it was due to the use of certain substances called coagulants. However, there appear to be a large variety of coagulants ranging from salts to acids. This led me to wonder if there was a difference between different types of coagulants and if certain coagulants were better than others.

Research Question: How effective are different group two chlorine salts at coagulating soy proteins (glycinin and conglycinin)?

Background Information

Proteins are long polymer chains composed of amino acids through peptide bonds. Each amino acid monomer is formed from a carboxyl group, an amino group, and an additional functional group which dictates the properties of the molecule. Due to the identity of the last functional group and its properties, the way they interact with each other through IMF, causes the entire protein chain to form different structural shapes.

The structure of proteins can be denoted into four levels. The primary structure of a protein is the unique linear sequence of amino acids in each polypeptide chain, where even the slightest of alteration can destroy the biological function of the protein.The secondary structure of a protein is the local steric structure of the protein which is stabilised primarily by intramolecular forces. It is the result of H-bonding between nitrogen and oxygen atoms and hydrogen atoms of the same AA backbone. The tertiary structure dictates the overall 3-dimensional shape of the protein and forms due to bonding interaction in the R group. The quaternary structure dictates shapes between different proteins in solution[1].

Denaturation is the process in which there is a disruption to these bonds, causing them to lose their native shape and become biologically inactive[2]. More specifically, protein denaturation can be defined to be any non-covalent change in the structure of a protein which causes alteration in the secondary, tertiary, and quaternary structures of the protein molecule. This disruption to the protein can be applied in many ways, commonly referred to as agents[3]. Physical agents include heat, mechanical stresses, and UV radiation, whereas chemical agents include additions of acids, salts, heavy metals, organic solvents, or alterations of pH. All of these methods disrupt the intermolecular forces within the protein, mainly the hydrogen bonds which can cause structural alterations on the entire protein, causing it to denature. As the applied agents only

[1] Frazer, Douglas, et al. Nelson Biology 12. Nelson, 2012.
[2] Frazer, Douglas, et al. Nelson Biology 12. Nelson, 2012.
[3] Yang, Fang Qi, et al. 豆腐您估计理的研究. Journal of the WuXi Institute of Light Industry 12.2, 1993.

affect the intermolecular forces between different amino acids in the protein and do not break any peptide bonds, the identity of the protein is not changed[4].

Globular proteins like glycinin are proteins which have a spherical form due to intermolecular forces between different amino acids within the protein chain causing the chain to 'bunch up into a ball'. This certain type of protein most commonly exists in a liquid state under normal conditions, as each polymer chain can be treated as singular molecules which have little attractive IMF towards each other. However, when denatured, these intermolecular forces become weaker, causing bonds to break, and the monomers within the proteins start developing attraction towards monomers in other chains. As these bonds are replaced, and the protein chains become bonded to one another losing their freedom and mobility, the entire solution transitions from liquid to solid.

The main focus of this investigation will be to determine which type of salt acts as the most effective agent/catalyst in the coagulation process of soy proteins. More specifically, in attacking the salt bridges within the tertiary structure of the protein which act as the main bonds that must be broken in order for denaturation and coagulation to occur. A salt bridge in the case of proteins is an intermolecular force/bond experienced by 2 amino acids of opposing charges and within close enough range for hydrogen bonding.

Design

In this experiment, we are testing the effectiveness of each of the different salts to attack the salt bridges within the protein. More specifically, it is to determine if ionic size affects the ability of a cation with charge 2 to attack the salt bridges. The solutions will be made and heated up to determine which coagulates first and therefore, which salt had the greatest effect on the intermolecular bonds. However, through experimentation, it was determined that this method was ineffective. Under the conditions where the solution was not mixed during the heated process, the solution was heated unevenly, where the part closer to the hotplate coagulated first, resulting in inaccurate temperature, and time readings. Furthermore, when the solution was mixed during the heating process, the stirring caused the coagulated particles to become very fine, resulting in difficulty in determining

Instead, as we are comparing between settings, we can simply analyse the difference in rate between the different salts. As the salts added act as a catalyst in that they increase the rate of reaction by decreasing the activation energy, through breaking bonds. Therefore, we can simply determine which solution would take the least time to reach a certain temperature as it would require less heat energy.

Hypothesis: I expect the control solution to take the longest to heat as there are no chemical agents used, and barium chloride to be the most effective catalyst due to its reactivity despite its large ionic size

[4] Robert, Plant proteins: Applications Biological Effects and chemistry, American chemical society Washington DC, 1986, 45.

Variables:

Independent variables: Type of dilute salt solution added to protein solution

- No salt added
- Magnesium chloride solution
- Calcium chloride solution
- Strontium chloride solution
- Barium chloride solution

The different salts used in this experiment have varying cation radii and therefore different charge densities. As the charge of the metal is remaining constant, differing radii will result in different charge densities and differing magnitudes of attractive and repulsive forces given by the cation. As such by varying the salt added, we can use the results to determine which part of the protein the salt would be attacking to weaken the bonds of. In addition to this, carrying identities of metals can also result in varied reactivities and charges which could respond differently with the proteins.

Dependant variable: Temperature at which solution coagulates

Depending on how the added salts affect the bonds and intermolecular forces within the protein solution, the required temperature to denature the proteins should also change.

Controlled variables

Controlled variable	**Reason for control**	**Method of control**
Concentration of soy solution	As the reaction must take place in a solution and it is difficult to extract the protein from the soybeans directly, the concentration of the soy solution must be controlled, as differing concentrations means different amounts of protein, and can affect results	All soy solution will be self-made from soybeans, where they will be soaked for a predetermined amount of time across all experiments to ensure there is a similar amount of water in each solution.
Concentration of salt solutions	As the salts are being added into the solution to ensure they are dissolved prior to addition and will not take additional time to dissolve in the soy solution, the concentration of these solutions needs to be maintained, as the same amount (moles) of each salt must be added in the separate trials.	The same molar concentration of 0.03M will be maintained across all salt solutions used in the experiment
Species of soybeans used	Different species of soybeans	Use same species of soybeans

	could have different protein concentrations and make-up which can affect the results of the experiment as the salts are acting on different proteins	with similar
Pressure	A difference in external pressure can affect the volatility of the solution. As the solution is being heated, a lower pressure would result in the water boiling off which will affect the concentrations of the solution.	Locating all experimental trials within the same workspace
Condition of soy solution	Soy solution left out for long periods of time can react with itself and separate	Soy solutions used in the experiment will have been produced within a 5-hour time frame prior to experimentation
Identity of anion in salt	The anion component can also potentially affect the bonds between the amino acids in the protein, and bonds in the protein to break.	A chlorine salt for each selected metal ion will be maintained

Apparatus:

- Soybeans
- 0.03M solution of MgCl
- 0.03M solution of CaCl
- 0.03M solution of SrCl
- 0.03M solution of BaCl
- Volumetric flask
- Volumetric pipet
- Powder funnel
- Mass balance
- Weighing boat
- Hot plate
- Stirring bar
- Beaker
- Large bowl
- Blender
- Funnel
- Flask
- Filter cloth

- Stirring rod
- Buret clamp
- Retort stand
- Temperature probe

Methodology:

Preparation of soy solution

1. Measure a certain mass of soybeans and place in a bowl with water, allow to soak for at least 24 hours
2. Pour out the water in the bowl, and combine with a certain mass of water to make a heterogenous mixture between the soybeans and water with a 1:8 ratio by mass between the soybeans and water
3. Blend the solution until there are no visible particles left
4. Pass the solution through the filter cloth, squeezing any excess soy solution out of the unrefined precipitate
5. Discard off the precipitate and set aside the soy solution for later use

Experimentation

1. Pour 200 ml of soy solution into a beaker
2. Place a magnetic stir base into the beaker
3. Measure out 0.01 mols of MgCl, add it to the beaker, and mix until it is completely dissolved
4. Set up a heating apparatus consisting of a hot plate, retort stand, clamp, and temperature probe according to the following diagram

Figure 1. Apparatus used to heat and measure soy solution

5. Start recording a temperature-time graph, with a reading to be taken twice a second
6. Place the temperature probe into the solution until 5 consecutive temperature readings are taken

7. Place the breaker on the hot plate that is set to 300°C while lowering the temperature probe with it and set the stirring to 750rpm
8. Stop the reading when the temperature has remained greeted tha 90°C for 10 seconds
9. Repeat the previous steps for the rest of the salt settings
10. Return equipment to their respective original positions to avoid loss or damage of lab equipment

After the conduction of the experiment, no ethical implications were present in this experimentation. No beings were injured and no horseplay was involved.

Risk Assessment

Soybeans are relatively safe and pose no harm. However, magnesium chloride and calcium chloride can both cause skin, eye, and respiratory irritation (FischerSci, 2015). Proper laboratory measures such as goggles and a lab coat will be worn to protect against these risks. Strontium and barium chloride both pose serious threats as they cause burning pain if in contact with the skin and eyes. They are also harmful if absorbed through the skin and release fumes which are harmful if inhaled (FischerSci, 2015) . In addition to the previous safety measures, a mask will also be worn in protection against toxic fumes. The hot plate and hot soy solution can also cause serious burns if mishandled in spillages or splashes. The products are relatively safe, and though this is a process used to produce tofu, nothing should be ingested within this experiment.

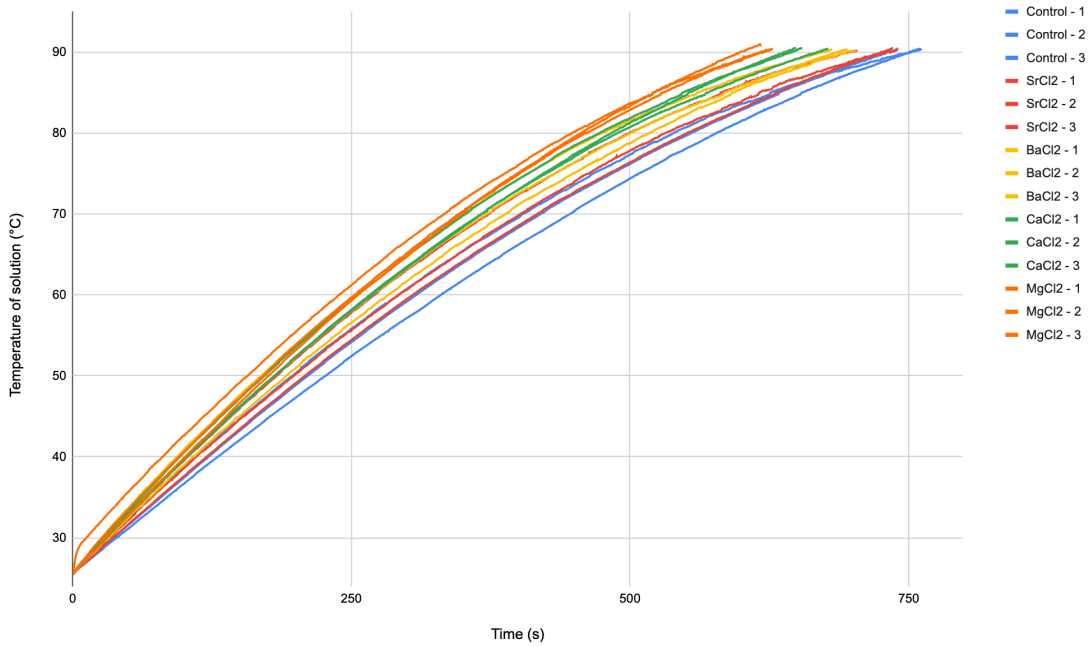
Raw Accumulated Data

Table 1. Captured time required for the solution to reach 90°C when placed on 300°C hotplate

	Time required to reach 90°C (+/- 0.5) / sec		
Type of salt	Trial 1	Trial 2	Trial 3
Control	751.5	750.5	730.5
SrCl2	726.0	694.0	730.5
BaCl2	685.0	671.0	693.0
CaCl2	667.5	639.0	644.5
MgCl2	616.5	618.0	598.5

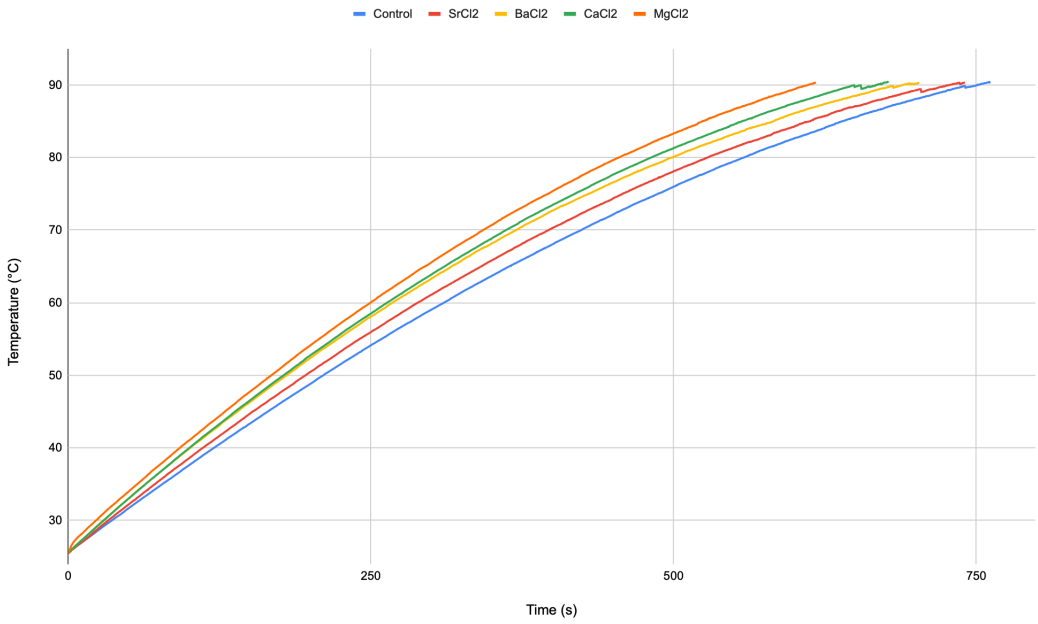
*Note 90°C is chosen as the solution will likely start boiling at higher temperatures

Figure 1. Temperature vs. Time graph of solutions of 200ml of soy solution and 0.01mol of varying salts subjected to 300°C with stirring



Processed Data

Figure 2. Temperature vs. Time graph of solutions of 200ml of soy solution and 0.01mol of varying salts subjected to 300°C with stirring (Average of 3 trials)



Finding average time required to reach 90°C

The arithmetic mean formula will be applied to find the average and it given by:

$$A = \frac{1}{n}\sum_{i=0}^{n} a_i$$

Where:

- A = arithmetic mean
- n = count of terms within the dataset
- a = value of data

Table 2. Average amounts of time required for each solution to reach 90°C

Setting	**Time needed to reach 90°C**
Control	$t = \frac{(751.5 \pm 0.5) + (750.5 \pm 0.5) + (730.5 \pm 0.5)}{3}$ $t = \frac{2232.5 \pm 1.5}{3}$ $t = 744.2\,s \pm 0.5$
SrCl2	$716.8\,s \pm 0.5$
BaCl2	$683.0\,s \pm 0.5$
CaCl2	$650.3\,s \pm 0.5$
MgCl2	$611.0\,s \pm 0.5$

The heat absorbed by each solution is composed of two components, the heat being supplied to increase the average kinetic energy or temperature of the solution, and that towards breaking the bonds. As the temperature of the hotplate is maintained constant throughout the trials, the amount of heat being transferred to the beaker within any given time interval would also be the same. Therefore, if we isolate a single point in time, and use the trial where no salt was added as a control, we can determine how effective each salt was in breaking the bonds, and how much energy was saved by the salt.

Using the lowest average time required to reach 90°C, we take the average temperatures for each setting as follows.

Table. 3 Average temperatures for each solution at 611 seconds of heating

Setting	**Temperature at 611 seconds / °C**
Control	$T = \frac{(84.00 \pm 0.01) + (82.13 \pm 0.01) + (83.53 \pm 0.01)}{3}$ $T = \frac{249.66 \pm 0.03}{3}$ $T = 83.23\,°C \pm 0.01$
SrCl2	$84.92\,°C \pm 0.01$

BaCl2	$86.71\,°C \pm 0.01$
CaCl2	$88.04\,°C \pm 0.01$
MgCl2	$90.00\,°C \pm 0.01$

The following formula can then be applied to solve for the heat absorbed by each solution:
$Q = mc\Delta T$
Where:

- Q = heat energy
- m = mass of substance
- c = specific heat capacity
- ΔT = Difference in temperature

*We can assume that the density and the specific heat capacity of the solution to be equal to that of water as it is very dilute
*As the difference of two temperatures is the same in celsius as in kelvins, we do not need to convert units

Table. 3 Average temperatures for each solution at 611 seconds of heating

Setting	**Heat energy consumed in 611 seconds / J**
Control	$Q = (200 \pm 5\%)(4.186)((83.23 \pm 0.01) - (25.50 \pm 0.01))$ $Q = (200 \pm 5\%)(4.186)(57.73 \pm 3.46 \bullet 10^{-4}\%)$ $Q = 48331 \pm 5.000346\%\,J$ $Q = 48.3kJ \pm 2.4$
SrCl2	$Q = 49.7kJ \pm 2.5$
BaCl2	$Q = 51.2kJ \pm 2.6$
CaCl2	$Q = 52.4kJ \pm 2.6$
MgCl2	$Q = 54.0kJ \pm 2.7$

With the heat absorbed by each of the different settings, the difference will tell us the amount of energy that was used to heat the solution instead of breaking the bonds.

Table. 4 Average temperatures for each solution at 611 seconds of heating

Setting	**Energy of bonds broken by salt / J**
Control	$E = (48.3kJ \pm 2.4) - (48.3kJ \pm 2.4)$ $E = 0kJ \pm 4.8$ *Though there is an uncertainty of 4.8, the energy of the bonds broken by the agent remains zero as no agent was added

SrCl2	$E = 1.4kJ \pm 4.9$
BaCl2	$E = 2.9kJ \pm 5.0$
CaCl2	$E = 4.1kJ \pm 5.0$
MgCl2	$E = 5.7kJ \pm 5.1$

Results

Qualitative results:

There appeared to be little to no coagulation in the trial where there was no coagulant added. The amount of tofu produced remained relatively consistent throughout the other trials.

Quantitative results:

Energy of salt bridges broken by $SrCl_2$: 1.4 ± 4.9 kJ
Energy of salt bridges broken by $BaCl_2$: 2.9 ± 5.0 kJ
Energy of salt bridges broken by $CaCl_2$: 4.1 ± 5.0 kJ
Energy of salt bridges broken by $MgCl_2$: 5.7 ± 5.1 kJ

Discussion

The results obtained from the experiment show that magnesium chloride was the most effective catalyst in the experiment. In other words, it acted as the best chemical agent in attacking the salt bridges between the amino acids and allowed for the protein to denature more easily. This is likely due to $MgCl_2$ having the highest charge density between the salts and therefore being more prone to attacking the charged salt bridges. This varied from the hypothesis that strontium chloride would have been the most effective agent due to it being the most reactive. Additionally, due to the smaller size of the magnesium ion, it could have potentially affected its steric (spacital) capabilities to reach and attack areas within the structure of the globular protein.

It should also be noted that the reason that there was no tofu produced in the solution without coagulant added was likely due to the boiling temperature of the solution being lower than that of the coagulation temperature.

Evaluation

Table 5. Lit of experimental errors with respective effects on result and potential corrections

Error	**Type of Error**	**Effect on result**	**Correction**
Large uncertainty value	Systematic	Though each result of energy absorbed by solution was fine, the differences calculated or the chemical energy provided by the salts was highly inaccurate, with a range that could result in a negative result. Negative energy	Utilisation of measurement devices with higher precision such as transfer pipet or graduated cylinder instead of the beaker for more accurate

		does not make sense here as it would signify that breaking bonds is an exothermic process which is not the true.	readings or use a large amount of soy solution to maximize the difference between different settings
Assumed no heat was lost	Systematic	Due to the rate of cooling being different at different temperatures, the readings for time and temperature could have been skewed which would result in inaccurate.	Use an insulated container such as a styrofoam cup, with a cover on top to prevent heat loss. As the container can no longer be heated using a hot plate, an alternative electrical heating device which can be placed directly into the liquid can be used.
Assumed specific heat capacity and density to be that of water	Systematic	Resulting in inaccurate heat energy results as the specific heat capacity of the proteins and salts and the mass of the entire solution could have been set off by a certain margin.	Determine the values for mass and specific heat capacities of proteins and salts and add them to the equation.
Inability to obtain all liquid form soy solution production	Random	As not all of the liquid portion of the soy solution was pressed out of the filter, the ration could be set off by a certain margin. In addition to this, pouring errors where certain amounts of liquid is left between transfer could also effect final results	Use a weighted object press the soy solution to ensure that the maximum amount of liquid was extracted.

Conclusion

The results of the experiment is that magnesium chloride was the most effective coagulant out of all of the coagulants. This makes sense, as $MgCl_2$ is one of the most popular coagulants used to make tofu and is the most popular salt coagulant. A large part of this is due to how effective it is in coagulating the protein, however, some of it is also due to its affordability. Research can be continued, to compare other types of coagulants, particularly acid coagulants, and also how certain coagulants can potentially work in tandem with each other for better results. Alternatively, additional investigation could also be done to find which specific amino acid pairs or groups the coagulants attack.