DOI: 10.19184/ICL.v4i1.5885

The Effect of Concentrated Seawater Salinity on Soybean Protein Coagulation in Tofu Production

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Abstract: Seawater, which has a salinity of 35 ‰, contains essential ions such as chloride, sodium, sulfate, magnesium, and calcium. These ions play a crucial role in the coagulation of proteins. Salt-based coagulants are among the oldest and most commonly used in tofu production. Additionally, several metal cations exhibit similar coagulating effects on soybean proteins. Tofu can be produced by adding salt coagulants, like calcium sulfate (commonly known as tofu stone) and seawater extract. The seawater extract was obtained from seawater through evaporation in three distinct ponds, with varying evaporation times that can lead to differences in salinity and density. In this experiment, we used coagulants from these three ponds, labeled A, B, and C. Coagulant C, derived from the pond with the longest evaporation time, has the highest salinity of 310 ‰ and a density of 1.220 g/cm³. The mass of the tofu produced shows a consistent pattern among coagulants A, B, and C: an initial increase followed by a decrease, which is influenced by the salting-out and salting-in processes. When used at a volume of 15 mL, Coagulant C yielded the highest mass at 179.426 grams and the lowest water content at 71.152%, demonstrating its effectiveness in protein coagulation.

Keywords: coagulant, salinity, seawater, tofu.

INTRODUCTION

Water salinity, which measures the amount of dissolved salts, affects water quality and its suitability for various uses. As a physical property, salinity plays a crucial role in determining overall water quality [1,2]. The salinity refers to the total ion concentration in water. Different types of water have different salinities: freshwater has a salinity of 0.5 ‰, brackish water ranges from 0.5 to 30 ‰, seawater is between 30 and 40 ‰, and hypersaline waters have a salinity of 40 to 80 ‰ [3].

Seawater, typically with a salinity of 35 ‰, contains various significant ions such as chloride, sodium, sulfate, magnesium, and calcium [4]. These ions are crucial in protein coagulation, a process that involves the random interaction of protein molecules, leading to the formation of aggregates and solid proteins, occurs due to the cross-linking of protein molecules in soymilk with salt's cation [5,6,7].

Salt coagulants are the oldest and the most widely used tofu coagulants, while several metal cations have similar coagulating effects on soybean proteins [5]. Coagulation is the most important step in the tofu-making process [1]. Tofu production relies on protein coagulation, and two main types of coagulants are used: salt coagulants and acid coagulants. Salt coagulants, like calcium sulfate (gypsum) and magnesium chloride (nigari), cause protein aggregation through ionic interactions. Acid coagulants, such as acetic acid (vinegar) and citric acid (lemon juice), denature proteins and cause them to precipitate due to their acidity [8]. Salt coagulation, also known as salting out, works by increasing the ionic strength of a protein solution, causing proteins to precipitate or aggregate out of solution due to reduced solubility. This is different from "salting in," where increased ionic strength actually enhances protein solubility [9]. Calcium sulfate ($CaSO_4$), or tofu stone, is a frequently used salt coagulant. The choice of coagulant significantly affects the quality of tofu, influencing factors such as protein content, water content, pH, taste, aroma, and texture. Tofu made with salt coagulants tends to have a higher protein content than those made with acid coagulants, resulting in better quality [10,11].

Salt coagulants for protein coagulation can be derived from seawater extracts. Nugrahani (2014) studied seawater extract from evaporating seawater using a water bath with 10, 12.5, 25, and 50 times concentration variations [12]. The best tofu was produced with a seawater coagulant concentrated 50 times, resulting in a dense and non-brittle texture. Generally, better tofu texture correlates with higher protein content [11]. Seawater extract as coagulant might be obtained from seawater evaporation under the sunlight to evaporate seawater in shallow ponds, leaving salt deposits. This process, known as evaporation, is used to obtain salt from seawater [13]. Increasing the salt concentration during tofu coagulation can enhance the tofu's mass, texture, and protein content, particularly as the coagulation process progresses [1].

The type and concentration of salt coagulants play a crucial role in determining the final properties of the tofu curd [1]. This concentration can be achieved through the traditional salt-making process, which involves evaporating seawater in solar ponds divided into three stages: reservoir ponds, concentration ponds, and crystallization ponds [13].

Research on the saltwater in concentration ponds was necessary to evaluate its potential as a coagulant for soybean extract protein during tofu production. This study focused on the salinity and density of the water in salt ponds, as salinity influences density and indicates evaporation, leading to higher salt concentration in the water. The effectiveness of coagulants in coagulating soybean extract protein was assessed by measuring changes in protein levels throughout the tofu-making process, employing the Semi micro Kjeldahl method (SNI 01-2891-1992) [14].

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METHODS

Tools and Instrumentation

The tools used in this study encompass a hand refractometer REF103/113/103bp, a set of destruction tools, a Kjeldahl apparatus, a pycnometer, a Maspion MT-1206 blender, an OHAUS Analytical balance, magnetic stirrers, filters, tofu molds measuring (10x10x20) cm, electric heaters, thermometers.

Chemicals and Sample

Chemicals used were Na_2SO_4, $CuSO_4$, H_2SO_4, NaOH, boric acids, indicator BCG (brom cresol green), methyl red, HCl, filter paper, and oxalic acids.

The sample used was salt pond water from Randu Tatah Village, Paiton District of Probolinggo Regency. Approximately 3 liters of water samples were collected from three different ponds (A, B, and C). Sampling was conducted at three points within each pond: the inlet, the middle, and the outlet, with about 1.0 liter taken from each point. The water was collected using a dipper submerged nearly to the bottom of the pond, as the maximum water height in each pond is only 10 cm, to ensure that the samples represent the pond rather than just the surface water. The samples were collected in opaque bottles, filtered and homogenized [15]. The sampled ponds were designated as below (Figure 1).

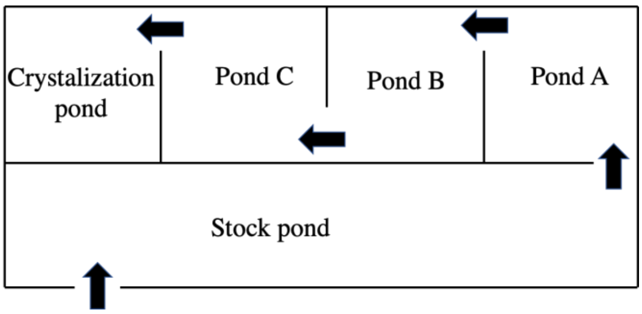

Figure 1. Seawater concentration pond (salt pond)

Salinity Measurement

Water salinity measurement uses a hand refractometer, that use the light refraction to determine the concentration of dissolved salts in a water sample [16]. Before measuring samples, the hand refractometer was calibrated with distilled water by applying a few drops of distilled water onto the prism until it completely covered the glass surface. Then, adjust the calibration screw until the blue field aligns with the zero scale. For the measurements, water from the concentration pool in the salt pond was collected using a dropper, and then a drop was placed onto the prism of the hand refractometer until it covered the glass surface. Finally, the salinity value is observed through the rear lens of the hand refractometer.

Density Measurement

Measurement of water density in three concentration ponds at salt production sites using a pycnometer [17]. The pycnometer was thoroughly cleaned with distilled water and then dried before used. The pycnometer was then filled with the concentrated sweater sample from different ponds and weighed. The density can then be calculated using the equation provided.

$$\boldsymbol{Sampel\ density} = \frac{(\boldsymbol{pycnometer\ mass+sample})-\boldsymbol{pycnometer\ mass}}{\boldsymbol{volume}} \ldots (1)$$

Tofu Production

An amount of 1,250 grams of soybeans were washed, drained, and soaked in 1,500 mL water for approximately 6 to 8 hours. Once soaked, the soybeans were ready for grinding, and an additional 7,500 mL of water were added. After grinding, the soybean pulp was filtered through a cloth, separating the dregs from the soybean extract. These two components were collected in different containers and weighed. The volume of the soybean extract was measured, and 500 mL were taken for each batch of tofu. The soybean extract was heated to a temperature of 70 to 80 °C, after which a coagulant (water from the evaporation pond in the salt pond) was added with volume ranging from 1 to 70 mL, with increments of 5 mL. The mixture was then stirred and allowed to coagulate. While still hot, the coagulated mixture was poured into a mold lined with a filter cloth and pressed firmly. Once processed, the resulting tofu was weighed.

Water Content Analysis in Tofu

Two grams of tofu was weighed in a known weight porcelain cup, placed in the oven at a temperature of 105 °C for 1 hour. After 1 hour, the sample then placed into a desiccator, and weighed after reaching room temperature. The process was repeated until the constant weight was obtained.

Protein Analysis

The semi micro Kjeldahl method (SNI 01-2891-1992) was used to determine the crude protein content of soybean extract, salt pond water, and tofu wastewater. This method, which involves three steps: destruction, distillation, and titration, was a reliable approach for ensuring the accuracy of our results. In the destruction step, 0.3 grams of the sample was placed in a destruction flask with a catalyst consisting of 1.78 grams of Na_2SO_4, 0.22 grams of $CuSO_4$, and 3 mL of concentrated H_2SO_4. The mixture was heated until a clear green solution was obtained. The clear solution containing $(NH_4)_2SO_4$ was then cooled to room temperature. A blank sample was prepared using the same procedure without adding the sample.

The cooled clear solution was added with 25 mL distilled water to dissolve the sample. Subsequently, the sample and the blank solutions were treated with 40% NaOH until a base was reached, which was indicated by a color change in the litmus paper from pink to blue. The solution was then distilled using a Kjeldahl apparatus, a meticulous process contributing to our results' accuracy.

The resulting distillate was collected in an Erlenmeyer flask contained 10 mL of 4% boric acid and the BCG-MR indicator. The distillation process lasted approximately 30 minutes and was terminated when the sample solution became cloudy. Serum was observed in the Kjeldahl flask, while the distillate in the Erlenmeyer flask changed to blue. Finally, 40 mL of the distillate from the sample and the blank were titrated with 0.1 N HCl until the solution turned pink. The amount of HCl required for neutralizing the distillate was equivalent to the nitrogen (in the form of NH_4) present in the distillate. The nitrogen content in the protein can then be calculated using the appropriate equation.

$$\%\ \textbf{protein} = \frac{(V_{HCl\ sample} - V_{HCl\ blank})}{\textbf{sample mass (mg)}} \textbf{xN HClx14.008x6.25x100}\% \ldots\ldots\ldots (2).$$

RESULT AND DISCUSSION

Seawater Salinity

Salinity, the total ion concentration in water, was a crucial parameter in our research, indicating salt content from major ions such as sodium, potassium, magnesium, calcium, sulfate, and bicarbonate [3]. We used a hand refractometer to measure the °Brix value, which was then converted to salinity expressed in grams per kilogram or parts per thousand (‰). Our measurements (Table 1) showed that coagulant A (coagulant from pond A) had the lowest salinity due to the fastest evaporation (2 days). Coagulant B showed an increased salinity after 4 days of evaporation, while coagulant C reached the highest salinity of 310 ‰ after 6 days. The evaporation process, driven by solar heat, occurred at the solution's surface, where higher air temperatures provide energy that allowed water molecules to escape as vapor. This increased salinity as water evaporated, concentrating the ions within the solution [3].

Table 1. Salinity of coagulant from different ponds

Sample	Salinity (‰)
Coagulant A	130±0.00
Coagulant B	222±0.00
Coagulant C	310±0.00

Seawater Density

Density reflected the mass of a solute within a given volume and was determined by measuring the mass of the sample for each volume. As the concentration of coagulants increased, the density also rised. This increase in density might be attributed to the higher amount of solute present in the samples of the three coagulants. Variations in the duration of the evaporation process also impact the concentration of the solute; the longer the evaporation, the more water molecules evaporated [18]. The primary solute in the coagulant was salt, which exists in an ionic state while dissolved in water. The salt ions remain in the solution during evaporation because water molecules surround them. As a result, only the water molecules at the solution's surface evaporate, leading to a higher salt concentration in the remaining solution. Coagulant C exhibits the highest density at 1.220 g/mL. Data from Tables 2 indicated a direct relationship between salinity and density: as salinity increased, density also increased. This correlation was due to the higher solute concentration resulting from the evaporation process.

Table 2. Densities of coagulant from different ponds

Sample	Density (g/cm^3)
Coagulant A	1.099±0.059
Coagulant B	1.172±0.104
Coagulant C	1.220±0.073

Tofu Produced using Seawater Coagulant

The process of making tofu demonstrated the principles of salting-out and salting-in, which involve the intriguing precipitation of proteins by adding a salt coagulant (k). In this experiment, the essential coagulant is sourced from three distinct concentration ponds within a salt pond (coagulant A from pond A, coagulant B from pond B, and coagulant C from the depths of pond C). The selection of coagulants from these three sources to determine the most effective coagulant in coagulating soybean extract protein. The use of different volume of coagulant to determine the optimal amount of coagulant that produces the most tofu mass. In tofu-making process was begun by carefully heating soybean extract to a precise temperature range of 70-80°C. This heating step was a crucial as it triggers protein denaturation—a fascinating transformation in which proteins unfold from their compact globular structures [18]. In this solution, proteins were dispersed in water, with hydrophilic side chains interacting harmoniously with surrounding water molecules. Once the soybean extract reached the desired temperature, the coagulant was added and gently stirred while still hot, ensuring a thorough and even mixture. As the coagulant was introduced, it initiated an engaging interaction that attracted water molecules toward the proteins, leading to the aggregation of protein molecules, a phenomenon known as salting-out. The resulting tofu mass, produced from varying volumes of coagulant A, B, and C, was presented in Figure 2.

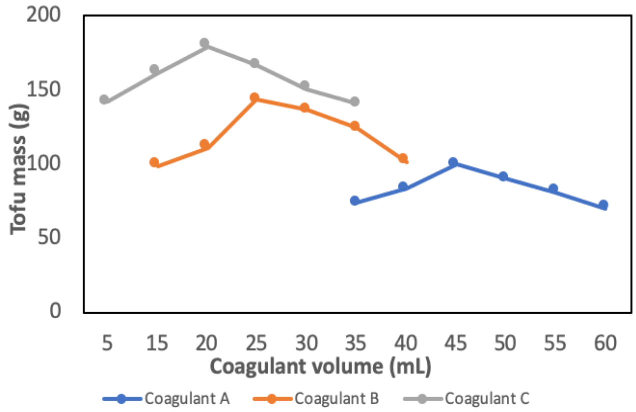

Figure 2 The correlation of coagulant volume and tofu mass produced

The average mass of the tofu, as shown in Figure 2, fluctuated significantly by changing the coagulant volume used in the molds. However, when a minimal amount of coagulant was applied, the tofu fails to form correctly and does not take shape as intended. This issue arises from insufficient coagulant, leaving excess water molecules attached to the proteins. As a result, this excess water prevented the formation of adequate protein aggregates, leading to an inadequate tofu product. As the volume of the coagulant increases, the tofu gradually begins to form, and the mass of the molded tofu rises, due to the more effective application of the salting-out process. However, the crucial moment was the addition of more coagulant, resulting in decreased tofu mass, and prevented it from shaping correctly according to the mold's specifications. This decline indicated the onset of the salting-in process, where the excess coagulant saturated the salt ions, causing water molecules to re-engage with the proteins. The relationship between the mass of tofu and the ideal coagulant volume that enabling proper molding show a consistent pattern across coagulants A, B, and C, as illustrated in Figure 2. Different limit of tofu mass and coagulant volume limits might be attributed to the different salinity levels in coagulants A, B, and C, as detailed in Table 1. Higher salinity indicated the greater ion concentration, significantly improving protein coagulation [19]. Eventually, among the three coagulants, coagulant C is the most effective for optimal coagulation, as proved by the extensive mass of molded tofu produced. Specifically, tofu made with 15 mL of coagulant C weighed an impressive 179.426 grams, achieving this remarkable mass at the lowest volume compared to coagulants A and B. Therefore, coagulant C is the best choice for converting soybean extract protein into high-quality tofu.

Water Content of Tofu with Different Coagulant

Water content refers to the percentage of water present in a sample, significantly impacting the mass of the analyte within that sample. This measurement was conducted on tofu produced based on the specific molds used. The average water content of tofu made with three different coagulants was shown in Figure 3. The water content of tofu using coagulants A, B, and C decreased by the addition of coagulant, and then, at specific points, the addition of coagulant increased by the increasing volume coagulant. The decrease in water content is attributable to an effective salting-out process, where protein aggregates compact under pressure during the tofu printing process, allowing maximum moisture extraction. The observed increase in water content occurs when the salting-in process begins to take effect. Tofu produced with coagulant A exhibited the highest water content, followed by tofu made with coagulant B. In contrast, tofu produced with 15 mL of coagulant C had the lowest water content at 71.152%. This difference is likely due to the higher salinity levels of coagulants A, B, and C, enhancing their ability to coagulate proteins effectively.

Protein Content of Tofu Product

Protein is an essential component of tofu, and understanding the protein content in tofu is vital for assessing quality. The analysis of protein content was performed using the semimicro Kjeldahl method, which consisted of three main stages: destruction, distillation, and titration.

In the destruction phase, the sample was heated with concentrated sulfuric acid, which acts as a strong oxidizer to decompose protein-rich materials. This process required a catalyst, typically a mixture of sodium sulfate (Na_2SO_4) and copper sulfate ($CuSO_4$), to accelerate the decomposition. The destruction stage lasts about 90 minutes, during which the solution turns a clear green color upon completion, indicating that the solid particles have dissolved. This results in the formation of ammonium sulfate $(NH_4)_2SO_4$.

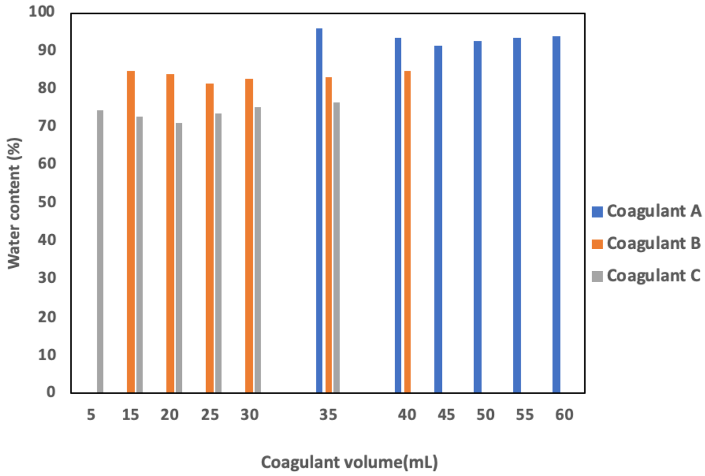

Figure 3. Water content of tofu product from different coagulant

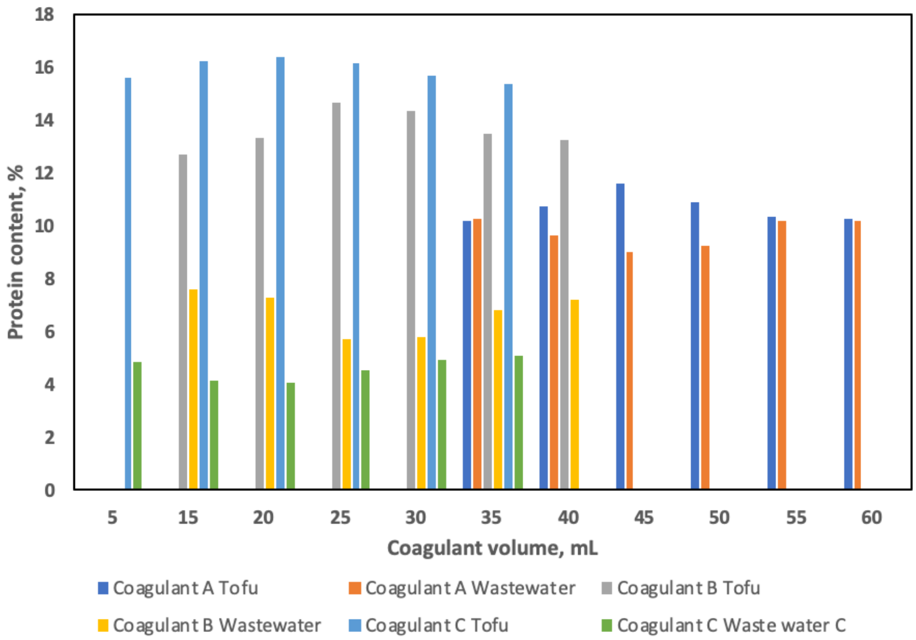

Figure 4. The protein content of tofu and tofu wastewater from different coagulant.

Following destruction, the sample proceeds to the distillation stage, where it is combined with distilled water and a 40% sodium hydroxide (NaOH) solution to create a basic environment. In this stage, NaOH decomposes ammonium sulfate into ammonia (NH_3). The ammonia gas is then collected in an Erlenmeyer flask containing boric acid, which captures the ammonia, and bromocresol green-methyl red (BCG-MR) indicator, which changes color from pink to blue in basic conditions. The distillation stops when the distillate turns blue, signaling the presence of ammonia.

The final stage, titration, involved titrating the distilled solution with hydrochloric acid to determine the nitrogen content in the sample. The amount of HCl used for neutralization correlated with the nitrogen content, and the equivalence point was marked by a color change from blue to pink as excess HCl created an acidic environment.

Using the total nitrogen measured, the protein content of the sample can be calculated by multiplying by a conversion factor of 6.25 for soy protein. The analyzed samples, which included soybean extract, tofu, and tofu wastewater, revealed a consistent pattern in protein content influenced by the different coagulants used in the tofu-making process (Figure 4). Notably, there was an inverse relationship between protein content in tofu and tofu wastewater: as the protein content in tofu increased, that in the wastewater decreased, signifying effective coagulation processes. Conversely, low protein content in tofu can indicate inefficiency in coagulation, possibly due to excessive coagulant addition that leads to an imbalance of salt ions and water molecules interacting adversely with the proteins, resulting in reduced protein content.

The most effective coagulant for protein coagulation is coagulant C, as demonstrated by the protein content in the resulting tofu. Coagulant C produces tofu with the highest protein content, while the tofu water contains the lowest protein content. This can be attributed to coagulant C having the highest salinity compared to coagulants A and B. The increased salinity leads to a greater total ion concentration, which enhances the coagulation process [20]. Tofu made with 15 mL of coagulant C resulting a protein content of 16.409%, met the SNI 01-3142-1998 quality standard. The relationship between ion mass and protein mass is illustrated in Figure 5.

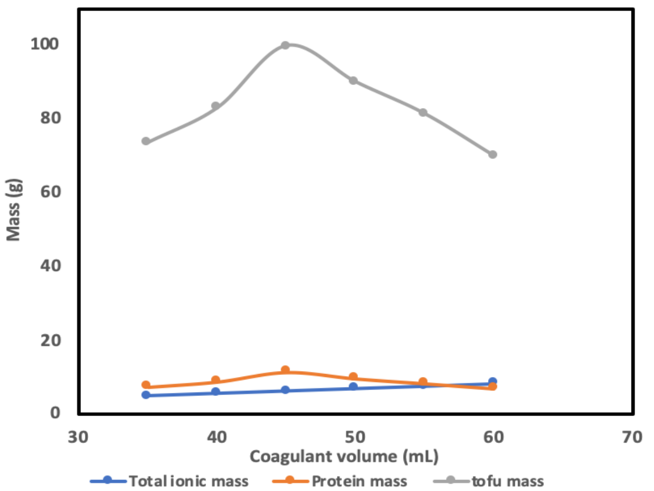

Figure 5. Correlation of volume coagulant with protein mass, tofu mass, and total ionic mass

These figures indicated that the protein mass initially increases and decreases, suggesting both salting-out and salting-in processes occur. Significantly, a high total ion mass does not always correlate with a high mass of coagulated protein. The concentration of the coagulant significantly affects the coagulation process by influencing the ability of ions to attract water molecules that surround the protein molecules.

CONCLUSION

According to the research findings, the highest levels of salinity and density were recorded in pool C, with measurements of 310 ‰ and 1.220 g/cm³, respectively. The salinity in each pool used for the coagulant in tofu production increased, resulting in a higher mass of protein that could be coagulated. Additionally, the volume of coagulant required to form tofu decreased progressively from pools A to C. The highest protein content in the tofu was achieved with the addition of coagulant C, which had a salinity of 310 ‰ and a volume of 15 mL, resulting in a protein content of 16.409%.

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