From soybeans to tofu: the underlying chemistry

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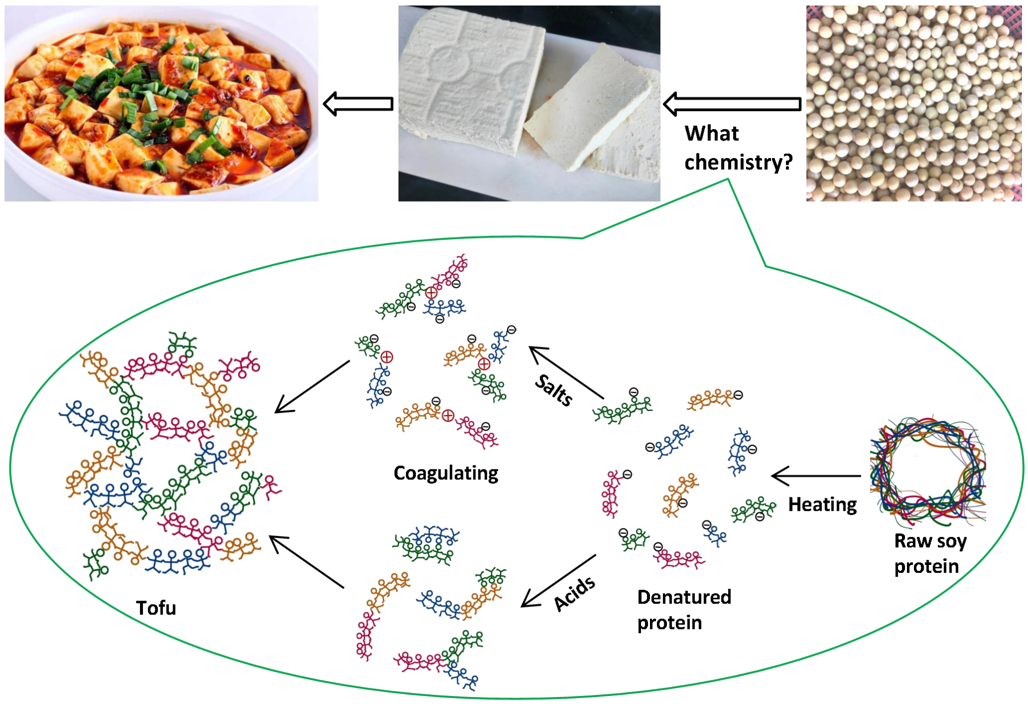
ABSTRACT

Tofu, a traditional Chinese food, is now popular worldwide. However, few people notice the chemistry involved in its production. To shed light on this, we have designed a simple demonstration for lower-level undergraduates in organic chemistry or biochemistry course to understand the chemistry principles underlying the curdling step in tofu processing. Raw soymilk is relatively stable without heating, even with the addition of coagulants. However, heat treatment denatures the soy proteins in the soymilk, making them more amenable to coagulation. Coagulation is promoted with salt coagulants such as calcium gluconate, zinc gluconate, and calcium lactate. Acid coagulants such as white vinegar or grape, orange, and lemon juice can also induce coagulation due to their acidic properties. Based on our results and previous reports, we have illustrated the curdling mechanism. This demonstration can also be used as an at-home experiment during the pandemic and can arouse students' curiosity about the coagulation of other food proteins and making alternative tofu.

GRAPHICAL ABSTRACT



KEYWORDS

Lower-Division Undergraduates, Coagulation of Proteins, Isoelectric Point of Protein, Denaturation, Distance Learning/Self Instruction, Food Science, Soymilk

INTRODUCTION

Isoelectric point (pI) is a crucial concept for understanding various biochemical problems. However, undergraduates in biology and biochemistry often face difficulties in comprehending this topic. This Journal has previously published several papers related to the pI.[1-4] The pI of a protein is the pH at which the protein does not carry a net charge. At this pH, the proteins are unstable and tend to aggregate or precipitate out of solution. The pI of a protein is determined by its amino acid composition and sequence. By exploring the relationship between protein isoelectric points and food, students can gain a deeper understanding of the chemical properties of proteins and their role in food science. Food has been used as an ideal, practical subject to arouse students' attention to chemistry.[5-8] Thus, utilizing foodstuff to help students understand and apply the concept of the pI is a favorite practice, especially popular food. Tofu, also called "bean curd", is a traditional Chinese food and is well-known worldwide. As a cheese-like product made from soybeans, tofu provides some indispensable nutrients for health owing to its high content of proteins, isoflavones, minerals, lipids, and vitamins.[9-10] Moreover, tofu is believed to have significant potential to reduce the risk of many diseases, such as diabetes, hyperlipidemia, hypertension, and cardiovascular disease.[11-12] Therefore, tofu has become a popular food choice worldwide.

Traditional processing of tofu involves several steps: selecting soybeans, soaking, grinding, filtering of slurry, heating of soymilk, coagulating, pressing, and packing. Of these steps, heating of soymilk and coagulating of the denatured soymilk are the most significant ones.[13-14] Coagulating of soymilk is the most vital step in tofu processing, associated with chemistry. The type of coagulant used in the process has a significant effect on the firmness of the tofu texture, and it can produce different types of tofu,[14-16] including extra firm, firm, soft, and silken tofu. Traditionally, brine (mainly consisting of magnesium chloride) is used as a coagulant, which results in brine tofu. However, the chemistry involved in tofu production is often overlooked. The coagulation process is pH-dependent. The optimal pH for coagulation varies with the coagulant used, but it is usually around the pI of the soy protein, which is approximately pH 4.5-5.5.[17-18] Coagulation is most likely to occur at this pH, resulting in aggregated proteins. If the pH is too high or too low, the coagulation process may produce lower yield and poorer quality tofu.

This paper proposes a simple demonstration for lower-level undergraduates in organic chemistry or biochemistry courses, which explains the chemistry principles underlying the vital steps in tofu production. The demonstration includes a case of tofu processing as an example, which students can follow to make home-made tofu or other kinds of tofu. Most of the materials and instruments used in this demo are common household items or can be found in markets. Furthermore, this demo can also be used as an at-home experiment to address lab closure situations, such as those that arise during a COVID-19 pandemic.[19-20] The hands-on experiments could stimulate and intrigue students' interests in chemistry.[21-22] At the end of this demonstration, students will be able to describe the concept of isoelectric point of proteins, interpret the effect of coagulants and heating on the protein coagulation, and explore the possibility of producing different kinds of tofu or tofu-like products from various foodstuffs based on the chemical principle.

MATERIALS AND EQUIPMENT

Dried soybeans; calcium gluconate tablets (9 % of elemental calcium, Hainan Pharmaceutical Factory Co., Ltd., Hainan province, China); zinc gluconate tablets (14.3 % of elemental zinc, Hainan Pharmaceutical Factory Co., Ltd., Hainan province, China); calcium lactate tablets (12% of elemental calcium, Jilin Aodong Yanbian Pharmaceutical Co., Ltd., Jilin province, China); white vinegar (0.35 g/L, Shanxi Shuita Vinegar Co., Ltd., Shanxi province, China); fresh orange, grape and lemon fruits; grinder (GM-K20, Foshan Shunde Geming Electric Appliance Industrial Co., Ltd., Guangdong province, China); beakers; test tubes; an electric stove; a forced air oven; a pH meter (PHSJ-4A, Shanghai Jingke Industrial Co., Ltd., Shanghai, China); a centrifuge (HDL-4, Jintan Hongke Instrument Factory, Jiangsu province, China).

EXPERIMENTAL PROCEDURE

Preparation of fresh raw soymilk

To avoid the noise caused by blending, it is necessary to prepare the fresh raw soymilk in advance. 60 g of soybeans were first rinsed and soaked overnight in deionized water at ambient temperature before the demonstration day. After this process, the swollen beans were drained and ground for 3 min with 300 mL of deionized water using a grinder. Afterwards, the resulting slurry was filtered through a 200-mesh pure cotton sheet to remove the okara, and finally, the resultant filtrate was designated as fresh raw soymilk.

Demonstration

Calcium gluconate (CG) solutions with different calcium concentrations of 1.88, 3.75 and 7.50 g/L were prepared with deionized water and calcium gluconate tablets. Zinc gluconate (ZG) and calcium lactate (CL) solutions (both with 7.50 g/L concentrations) were also prepared with their respective tablets. Seven clean test tubes named from A to G were set up, with each containing 10 mL of fresh prepared raw soymilk, as shown in Table 1. Tubes A to F were heated in a water bath at 95 °C for 5 min and then removed from the heat source. Next, with stirring, a 3-mL salt coagulant sequentially chosen from one of the above prepared salt solutions was, added to Tubes B to F, respectively. Only Tube G did not undergo heat treatment and was instead treated with 3 mL of the 7.50 g/L CG solution for comparison. All samples were then left at room temperature for 10 min, and the pH values were determined with a pH meter at the end of coagulation. The students should observe and record the effect of the added salts and the heat treatment on the coagulation of the soymilk. Finally, each mixture was separated into a sediment and a supernatant through centrifugation for 10 min at 3500 r/min at room temperature. The amount of the sediment was determined after drying with a forced air oven at 70 ℃ for 15 h.

Table 1. The Labeled Test Tubes with Different Treatments[a]

Label	A	B	C	D	E	F	G
Heating	Yes	Yes	Yes	Yes	Yes	Yes	No
Coagulant[b]	No	CG(1.88)	CG(3.75)	CG(7.50)	ZG(7.50)	CL(7.50)	CG(7.50)

[a]Each test tube contains 10 mL fresh soy milk.
[b]When coagulant is added, the volume of coagulant is 3 mL and the number in parentheses is the concentration in g/L.

White vinegar solutions with different concentrations (0.0175 g/L, 0.0525 g/L, and 0.105 g/L) were prepared by diluting white vinegar with deionized water. Juices were squeezed from grape, orange, and lemon fruits, respectively. Following the same procedure as described above, 2 mL of white vinegar solutions with different concentrations, grape juice, orange juice, and lemon juice, as acid coagulants, were added to each sample with heat treatment, respectively. Furthermore, to compare the effect of heat treatment, 2 mL of 0.105 g/L white vinegar solution was added to an untreated sample.

Benchtop tofu production

Figure 1 illustrates the benchtop tofu production process that students can follow to make their own tofu at home or adapt the coagulants to create other types of tofu. First, 400 g of dried soybeans were rinsed and soaked in tap water at room temperature for about 10 h. The swollen beans were drained and blended with 2000 mL of water for 3 min. Afterward, the resulting mixture was separated into raw soy milk and solid pulp/okara by squeezing through a 200-mesh pure cotton sheet. The raw soy milk was then heated to above 80 ℃ before adding 200 mL of diluted white vinegar solution, with acetic acid at 0.175 g/L, to act as a coagulant. The mixture was allowed to sit for 5 min to coagulate into curd. The curd was later poured into a 14 cm × 11 cm × 9.5 cm (h) cubic mold covered with a layer of 200 mesh pure cotton sheet which wraps the curd. The mold lid was secured, and a weight of about 1 kg was placed on top to help to drain off the carbohydrate-laden whey. After being pressed for 2 h, the tofu was taken out of the mold and could be cut into squares. Video S1 in Supporting Information demonstrates the tofu processing steps.

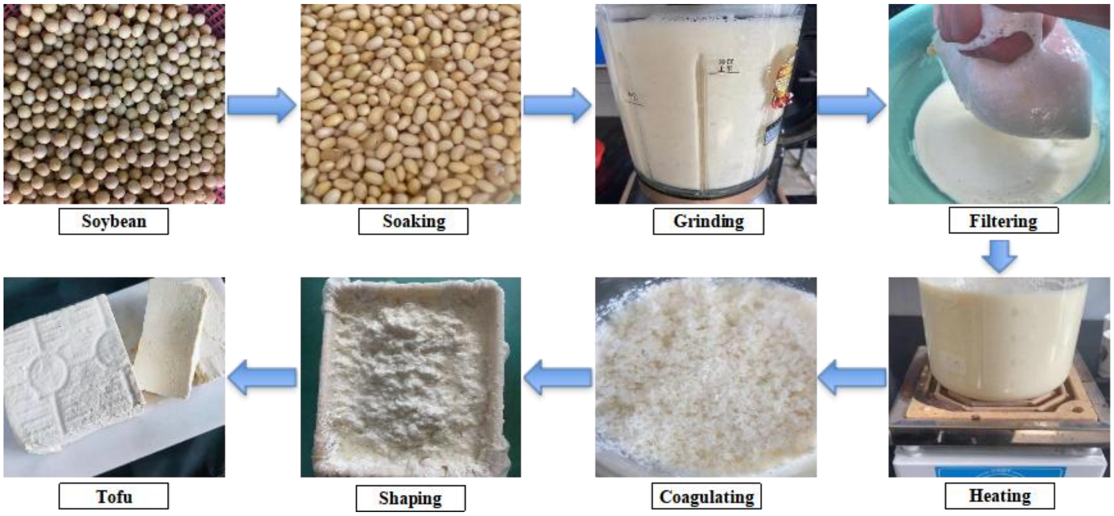


Figure 1. Procedures of tofu processing with white vinegar as coagulant.

HAZARDS AND WASTE DISPOSAL

White vinegar is known to cause irritation and should be handled with care. Operating an oven may cause burns on the operator's skin. Heat-resistant gloves, eye/face protection, and a lab coat should be worn. Chemicals, solution and small aggregates can be disposed of by draining; however, large aggregates should be collected and discarded in appropriate waste containers. It is important to follow these guidelines to ensure the operator's safety and maintain a secure work environment.

RESULTS

Figure 2 displays the effect of various salts on soymilk coagulation. The photo was taken after 10 min the coagulants were added. To provide a comparison, Figure 2A shows fresh raw soymilk, while Figures 2B to 2D demonstrate the consequences of heat treatment with increasing CG concentrations-notably, a rise in the curd amount and supernatant volume. The amount of sediments in samples rises as shown in Figure 3A-D whereas the pH value decreases. Figures 2E and 2F show the coagulation of the soymilk using ZG and CL, respectively. However, without the heat treatment, no curd formation was observed even after the addition of 3 mL CG solution with a calcium cation concentration of 7.50 g/L (Figure 2G). The mixture had a lower pH value and fewer sediment than those with heat treatment, as shown in Figure 3G.

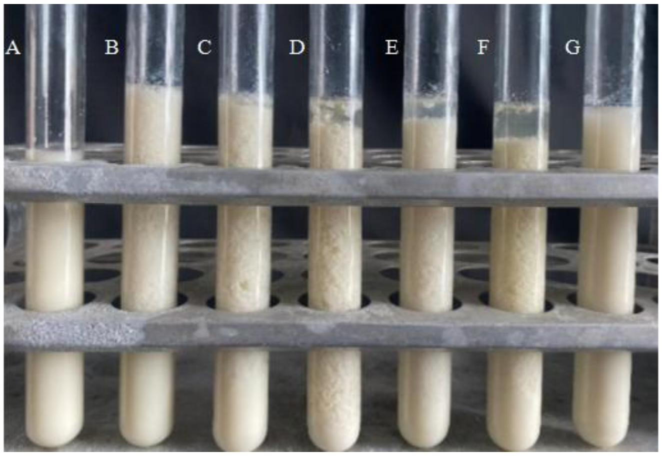


Figure 2. After heat treatment, soymilk without (A) and with addition of 3 mL salt coagulants: CG solutions with different calcium cation concentrations (1.88 g/L (B), 3.76 g/L (C) and 7.50 g/L (D), respectively), ZG solution with zinc cation concentration of 7.50 g/L (E) and CL solution with calcium cation concentration of 7.50 g/L (F). (G) Without heat treatment, soymilk with addition of 3 mL CG solution (calcium cation concentration: 7.50 g/L) for comparison.

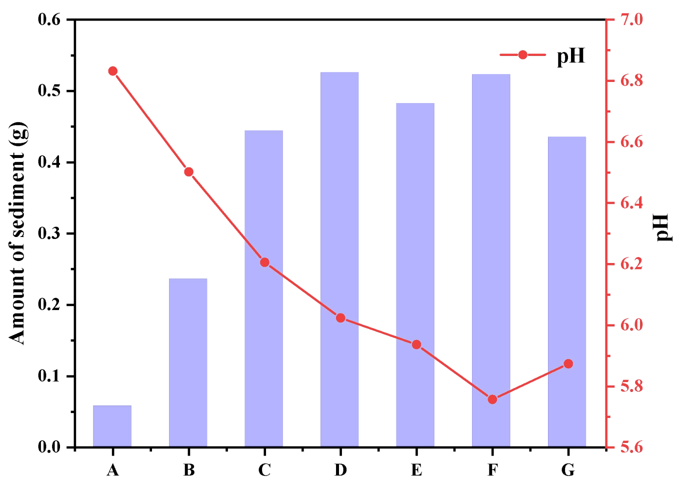


Figure 3. With heat treatment, amount of sediment (light blue histogram) and final pH value (red dot-line plot) of soymilk with addition of CG solutions 0 g/L (A), 1.88 g/L (B), 3.76 g/L (C) and 7.50 g/L (D), ZG solution 7.50 g/L (E) and CL solution 7.50 g/L (F). Without heat treatment, the addition of CG solution 7.50 g/L for comparison (G).

Figure 4 demonstrates that the use of acids as coagulants results in a similar coagulation. The amount of sediment and the final pH values of the mixtures are displayed in Figure 5. When the raw soymilk is subjected to heat treatment and white vinegar is added, curd and supernatant appear, as depicted in Figure 4B-D, compared to fresh raw soymilk displayed in Figure 4A. Furthermore, an increase in the concentration of white vinegar leads to more supernatant and curd appearance. The pH values decrease, and the sediment increases with the concentration of white vinegar, as shown in Figure 5A-D. The curdling of soymilk can also be induced by adding grape, orange, or lemon juice, as illustrated in Figure 4E-G. However, without heat treatment, the addition of white vinegar does not result in obvious curdling of the soymilk, though the presence of supernatant can still be observed (shown in Figure 4H). The sediment is less than that with heat treatment (Figure 5H).

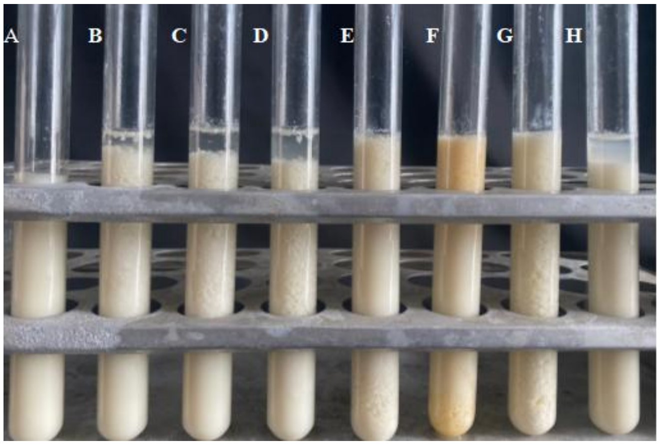


Figure 4. After heat treatment, soymilk without (A) and with addition of 2 mL acid coagulants: white vinegar solutions with different concentration (0.0175 g/L (B), 0.0525 g/L (C) and 0.105 g/L (D), respectively), grape juice (E), orange juice (F) and lemon juice (G). (H) Without heat treatment, soymilk with addition of 2 mL 0.105 g/L white vinegar solution for comparison.

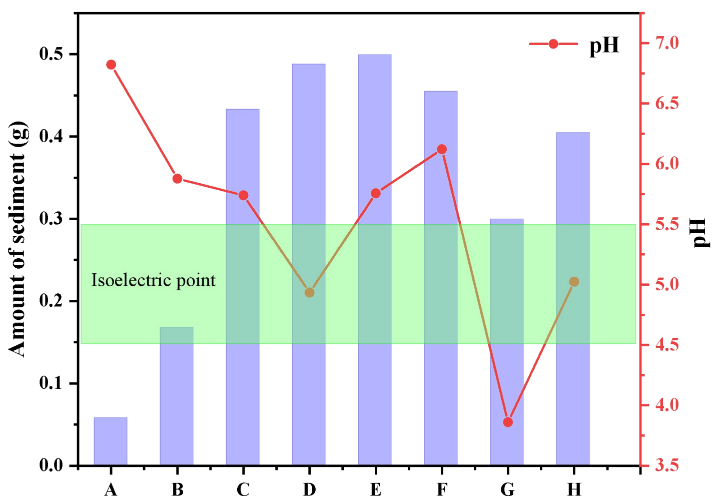


Figure 5. With heat treatment, amount of sediment (light blue histogram) and final pH value (red dot-line plot) of soymilk with addition of white vinegar solutions 0 g/L (A), 0.0175 g/L (B), 0.0525 g/L (C) and 0.105 g/L (D), grape juice (E), orange juice (F) and lemon juice (G). Without heat treatment, the addition of 0.105 g/L white vinegar solution for comparison (H).

DISCUSSIONS

Raw soymilk is a colloidal suspension consisting largely of about 3.6% protein.[23-24] It does not curdle without heat treatment and adding coagulant, as evidenced in Figure 2A and Figure 4A. The concept of the pI of protein can be introduced during the demonstration, presented in the Supporting Information.[2-3] Soy proteins in the colloid are negatively charged[24-25] and most soluble at the neutral pH values that deviate from their pI. According to Coulomb's law, the electrostatic repulsion between negatively charged proteins will decrease protein-protein interactions and their tendency to aggregate and precipitate.

The heat treatment of soymilk is a prerequisite for tofu production. As shown in Figure 2G and Figure 4H, without heat treatment, adding coagulants would not lead to the obvious coagulation of the soymilk. Less sediment was obtained than those with heat treatment, as illustrated in Figure 3G and Figure 5H. The heat treatment results in the rupture of the secondary protein structures and changes the spatial structure. This process exposes hydrophobic groups in denatured soy protein, increasing the hydrophobicity of the protein and making it more prone to aggregation.[26] The addition of coagulants can drastically enhance this aggregation process, leading to the formation of tofu. Additionally, heat treatment helps to destroy microorganisms, inactivates antinutritional factors, and improves flavors in soymilk.[27]

The coagulant addition is regarded as the most important step in tofu processing,[15] as it is responsible for curd formation in soymilk (Figure 2B-F and Figure 4B-G). This process involves the destruction of electrostatic repulsion of negative soy proteins, leading to their coagulation. Once the whey is removed by pressing, the formed gels possess a three-dimensional network structure that is stabilized by different intermolecular interactions. Coagulants are generally classified into three types-salts, acids, and enzymes-based on their coagulation mechanism.[28]

Gypsum (mainly calcium sulfate) and brine (or nigari, mainly magnesium chloride) have traditionally been utilized as salt coagulants in tofu production. Recently, trimagnesium citrate has also been reported as an alternative coagulant.[29] In this demonstration, calcium gluconate, zinc gluconate, and calcium lactate were used as the salt coagulants due to their safety and availability as dietary supplements in pharmacy. Increasing the concentration of CG was observed to increase the formation of floccules in the soymilk (as shown in Figure 2A-D), indicating a direct relationship between the salt concentration and the degree of soymilk coagulation. At the same time, as the concentration of CG increased, the pH value of the mixtures decreased towards the pI of soy protein (around 4.5-5.5), resulting in the increase of sediment (Figure 3A-D). ZG and CL were also observed to curdle the soymilk (Figure 2E-F). Though the pH values dropped further, the continued increase of sediments was not observed (Figure 3E-F), which can be attributed to the effect of both the cations and the anions.[25, 30] While salts have been used as coagulants in traditional tofu processing, their exact mechanism in tofu formation remains inconclusive. Up to now, there exist three major theories: cation bridge theory, salting-out theory, and isoelectric point theory.[26]

Although various acids have been investigated on their coagulating effects in tofu production, glucono-δ-lactone (GDL) has recently become a popular option due to its ability to spontaneously hydrolyze in water to generate gluconic acid.[27, 31] In this demonstration, white vinegar was utilized as an acid coagulant, as shown in Figure 1 in the example of tofu production and Figure 4A-D. The charge of proteins is related to the pH value.[1] With the increasing concentration of white vinegar, the pH value of the soymilk falls within the isoelectric point range of 4.5-5.5, forming more curdling and sediment (Figure 4A-D and Figure 5A-D). The addition of an acidic coagulant to the soymilk provides protons that alter the net charge on the soy protein molecules. This causes the pH of soymilk to drop to the nominal pI of soy protein, which results in neutralizing the negative charges on the soy protein molecules.[32] This neutralization declines the electrostatic repulsion between protein molecules, resulting in intermolecular aggregation through either hydrophobic interactions or short-range interactions. Organic acids such as tartaric acid and malic acid, present in grape (the most abundant constituents in grape),[33-34] citric acid, and malic acid found in orange and lemon,[35] respectively, have also been used as acid coagulants. The addition of grape, orange, and lemon juice in the soymilk coagulates the protein, as shown in Figure 4E-G. Here, the pH value close to the pI of soy protein may not necessarily induce more coagulation or sediment (Figure 5E-G). The presence of anions or other components in the juices may also affect the coagulation of soymilk.[25, 30] In the previous paper, bilimbi and lime juices have also been used as acid coagulants in tofu production.[36]

In general, the coagulation of tofu is believed to comprise mainly two processes: the heat-induced denaturation of soy protein, followed by the coagulation promoted by coagulants.[16] Raw soymilk is comparatively stable due to electrostatic repulsion of negatively charged proteins, which hinders protein-protein interactions. Additionally, the soybean protein molecule offers a hydrophobic group inside and a hydrophilic group on the surface, which declines its aggregation tendency. Upon heat treatment, the secondary structures break down in raw soy protein, resulting in unfolded and denatured soy protein. The addition of coagulants results in the neutralization of negative charges on denatured protein, either via cation bridge formation or through pH reduction to the pI of soy protein. Consequently, the neutralized protein molecules gather due to the reduced electrostatic repulsion. The further aggregation of soy proteins intermolecularly takes place through hydrophobic interactions[31] or short-range interactions[23] to form three-dimensional network structures, which give rise to tofu gel. Figure 6 illustrates the curdling mechanism of soymilk, where the cation bridge theory is used for salt coagulants. This theory sates that cations connect two or three denatured soybean proteins like a bridge, accelerating the protein gelation.[28]

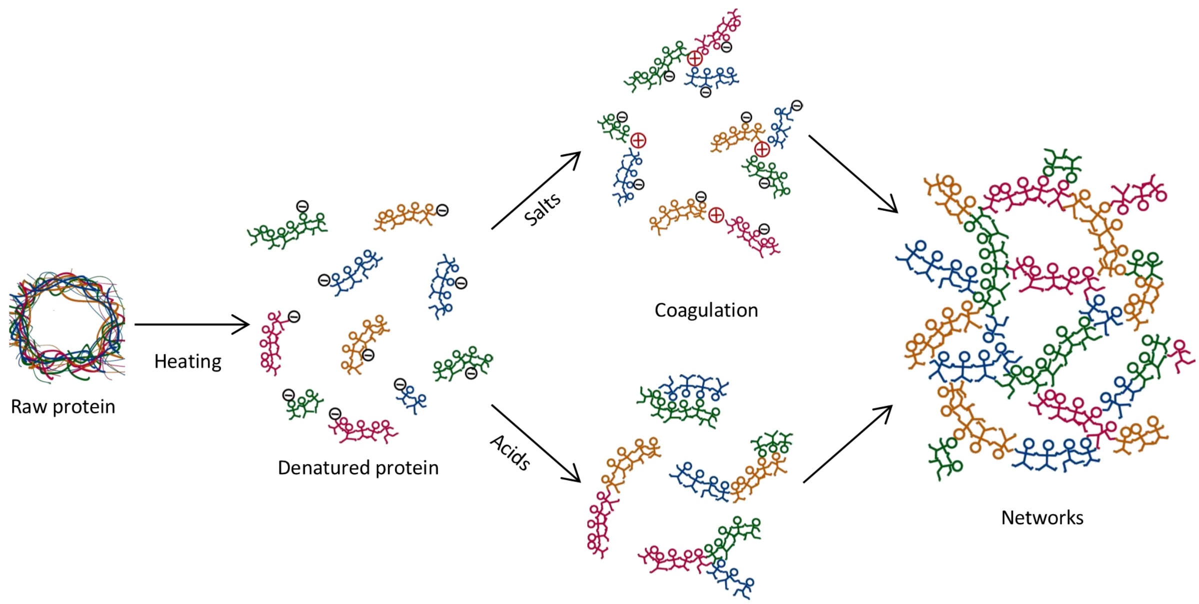


Figure 6. Schematic of the curdling mechanism of soymilk.

CONCLUSION

To understand the chemistry principle behind the curdling step in tofu processing, a simple demonstration was designed. The results showed that thermal treatment is essential for the coagulation of soymilk. Addition of coagulants such as salts or acids further promotes the coagulation of soy proteins. Soy proteins exhibit amphoterism, which means they can become negatively or positively charged in a neutral solution. The electrostatic repulsion of these charged proteins contributes to the stability of the soymilk. The coagulation of proteins occurs when the electrostatic repulsion decreases due to the formation of cationic bridges or a decrease in pH value to the pI. Based on previous finding reported in the literatures, this paper presents the curdling mechanism. This mechanism can be used as a basis for students to further explore the coagulation of other proteins and produce alternative tofu. Furthermore, the majority of the materials and equipment necessary for this experiment can be found at home or most common stores. Therefore, it is a good option as an at-home experiment in case of lab closure due to unforeseen situations such as COVID-19 pandemic. The hands-on experiments can stimulate and intrigue students' interests in the chemistry of tofu production.

The students, who were sophomores in food or agricultural science from an ordinary college in China and were enrolled in organic chemistry course, were evaluated by means of a questionnaire before and after the demonstration, which is presented in the Supporting Information. Results from the questionnaire indicated that before the demonstration, only 26.2% of students had a strong interest in the chemistry involved in tofu processing, whereas even fewer students (19.7%) understood the reason for the stability of soymilk. However, after the demo, more than 50% of students showed improved understanding in these areas. Furthermore, around 17% more students were able to identify what the salt coagulants react with, and the role of acid coagulants in tofu processing. Additionally, there was an increase in the percentage of students grasping the concept of isoelectric point of protein, from 25.7% before the demo to 43.4% after the demo. However, it is important to note that the ratio of the students who fully understood this concept remained low due to their weak educational background. The non-chemistry majors from an ordinary college in China are not expected to have a strong biochemistry/chemistry background. To help instructors more effectively explain the concept of isoelectric point of proteins to students during the demonstration, the concept is provided in the Supporting Information. Therefore, this demonstration can potentially help undergraduates (potentially nonmajors) learn chemistry. The questionnaire responses indicated that 88.2% of students found the demonstration to be either very helpful or helpful in understanding chemistry concepts involved in tofu processing.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.XXXXXXX. [ACS will fill this in.] Example brief descriptions with file formats indicated are shown below; customize for your material.

Instructor notes, students handout, and outcomes of questionnaire (PDF, DOCX)

Video S1 presenting the tofu processing with white vinegar as coagulant (MP4)

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Note

The authors declare no competing financial interest.

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