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Mathematical modeling of optimal coagulant dosage for tofu preparation using $MgCl_2$

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ABSTRACT

To explore the association between the optimal coagulant for tofu and the components of soybeans,30 different kinds of soybeans were selected, and tested for their optimal coagulant $MgCl_2$ content. The optimal amount of coagulant was taken as the dependent variable, and the soybean Composition were taken as independent variables for the correlation analysis. The results showed that there was a positive correlation between the optimal coagulant content and the content of histidine, 7S β-conglycinin, $B_{1a}B_{1b}B_2B_3B_4$ of 11 s glycincin, and α'-subunit of 7S β-conglycinin, negative correlation with lysine. The regression formula is y = -1.186 + 3.457*$B_{1a}B_{1b}B_2B_3B_4$ + 2.304*7S + 0.351*histidine − 0.084*lysine + 4.696*α', and the model is validated to be within 10 % of the error value and has a high degree of confidence. This study provides theoretical support for realizing the green production of traditional soybean products.

Introduction

Tofu is a protein gel with a long history and worldwide popularity, known for its nutritional value(Ali, Tian, & Wang, 2021; Guo et al., 2018; Wang et al., 2020; Yang et al., 2020; Zhu et al., 2019). The formation of tofu using salt coagulants has been attributed to three main theories: ionic bridge theory(Saio et al., 1968), salt precipitation theory (Zhihong & Lite, 2007), and PH reduction theory(J. Y. Lu, Carter, & Chung, 1980), are now generally recognized as ionic bridge theory. Soybeans undergo a series of processes to become tofu, yellow slurry water is a byproduct of the tofu pressing process after the addition of coagulants, currently yellow slurry water tends to be discarded. And the type and amount of coagulant used can significantly impact the nutrient composition and sensory evaluation of tofu(Li et al., 2023; Rui et al., 2016; Chen et al., 2016; Zhao, Chen, Hemar, & Cui, 2020). Additionally, the coagulant used can also affect the content composition of substances in the yellow slurry water.

There are two main stages in the formation of tofu, the first stage is to heat the soybean milk to denature the protein, and the second stage is to add coagulants to make the soybean proteins gather quickly, in the second stage, the excessive coagulants(This article refers to the $MgCl_2$ coagulant) will make the time of protein aggregation shorter, and tofu will be produced faster, but also result in a higher concentration of metal ions in the yellow slurry water. These ions can be released into water bodies through the disposal of yellow slurry water, potentially causing structural changes in the water bodies, pollution containing metal ions entering the land where crops are grown can lead to land salinization. Additionally, yellow slurry water contains nutrients, resulting in waste and pollution(K. Guo, Shang, Gao, Xu, Lu, & Qi, 2018). The wide dispersion and small scale of tofu enterprises, along with the high cost of treatment, such as chitosan flocculation, membrane separation(Chua & Liu, 2019), etc., make it difficult to achieve harmless disposal of yellow slurry water. Given the current recycling technology and the potential environmental impact of yellow slurry water, it is not appropriate to view it as a means of pollution followed by treatment. Instead, a molecular-level understanding of the binding mechanism between coagulants and proteins is needed to determine the relationship between raw material composition and optimal coagulant amount, and preparing low-salt ionized yellow slurry water on this basis is the key to its harmless production. The analysis of the final optimal coagulant content

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and the creation of a mathematical model formula can be a valuable tool for improving the efficiency and effectiveness of the testing process and promoting sustainable practices in the tofu production industry. Firstly, it can reduce the pre-test cycle of tofu industry, which means that the producing process can be completed faster. This can save time and resources for the producing process. Secondly, it can save test costs, which can be a significant expense for the testing process. Thirdly, it can help promote the use of the optimal coagulant for tofu production enterprises, which can reduce pollution at the source. In addition, the production of by-products can be reduced, reducing the cost of treatment.

Yellow slurry water is composed of various functional substances such as soy whey protein(Sorgentini & Wagner, 1999), soy oligosaccharides, vitamins, organic acids, soy isoflavones(H. Wang & Murphy, 1994), and soy saponins, as well as antinutritional factors such as trypsin inhibitors, lectins, and lipoxygenase. Additionally, yellow pulp water is easily utilized by microorganisms, which can be processed into food products such as tofu coagulant, fermented beverages, and condiments. Research finds that as a microbial carbon and nitrogen source for cellulose, astaxanthin, riboflavin, γ-amino butyric acid, and other functional substances prepared is also one of the main uses at this stage. The large amount of metal ions present in yellow slurry water makes it impossible to use it directly as a culture substrate for microorganisms, and at the same time the accumulation of metal ions in the human body without removing them from yellow slurry water can be harmful. After reducing the metal ions in the yellow slurry water, the yellow slurry water containing a large amount of nutrients can be used as a fermentation substrate for microorganisms. It can also be directly converted into foodstuffs such as beverages and condiments.

This study aims to establish a mathematical model to investigate the linear relationship between various factors affecting the quality of tofu, including oil, moisture, protein content, water-soluble protein content, 7S and 11S content, polypeptide chain content, amino acid content, and final coagulant content in soybeans. By analyzing the relationship between the protein structure at all levels and the final coagulant, the study aims to identify the factors related to the optimal coagulant for tofu production. The establishment of this mathematical model provides a valuable guide for the industrial production of traditional soybean products, specifically in the preparation of low-salt ionized yellow slurry water.

Materials and methods

Soybean variety

Thirty soybean varieties were randomly chosen from the seed bank of the Division of Soybean Processing, Soybean Research & Development Center. These varieties were sourced from different provinces in China, including Heilongjiang, Henan, Jilin, Shanxi, and Anhui. These soybeans had no obvious defects in appearance, were all produced within three years of each other, and some soybeans with special traits due to experimentation were removed. Each variety was assigned a number from 1 to 30 for identification purposes.

Optimum coagulant content

Determination of the optimal amount of coagulant and yellow water preparation

To prepare soybean milk, 100 g of whole soybeans were weighed, cleaned, and soaked for 12 h in water at a ratio of 1:5 and a temperature of 18–21 °C. The soaked beans were then ground in a soybean milk machine at a ratio of 1:7 (dry soybeans to water) to obtain a paste. The paste was sieved through a 120-mesh filter to produce raw soybean milk. The milk was heated in a water bath at 90 °C for 10 min, then boiled on an electric stove for 2 min, and finally cooled to 20 °C for use.

Determination of optimal coagulant concentration -CPCC(critical point of coagulant concentration): 350 mL of cooked soybean milk was weighed and put into a magnetic stirring rotor (8 mmΦ x 50 mm, Φ stands for diameter), and the cooked soybean milk was stirred at a speed of 350 rpm-600 rpm, and 1 moL/L $MgCl_2$ solution was added to the cooked soybean milk by peristaltic pump at a uniform speed, until the vortex disappeared, and the consumption of coagulant was recorded at this time. Keeping the rotor rotating continuously, the vortex reappeared after 1 min, and the optimal amount of coagulant used was calculated by Eq(Y in the formula represents the amount of coagulant added to the soymilk).

$$CPCC = 1000 \times \frac{Y}{350 + Y} \times Molarconcentrationofcoagulant \quad (1)$$

To prepare yellow pulp water, 100 g of whole soybeans were weighed and cleaned. The soybeans were then soaked for 12 h in a 1:5 ratio of soybean water at a temperature of 18 °C~21 °C. After soaking, the soybeans were ground in a soymilk machine at a ratio of 1:9 dry beans to water. To obtain raw soybean milk, the ground soybean milk was filtered through a 120-mesh sieve. The soybean milk was then heated in a water bath at 90 °C for 10 min, and then boiled on an electric stove for 2 min. The cooked soybean milk was stirred at 150 r/min until it reached a temperature of 85 °C. The optimal coagulant dosage and a common coagulant dosage (2.8 g) of magnesium chloride were added, and the mixture was thoroughly mixed. The mixture was then placed in an insulated heat preservation box and held for 12 min. The mixture was then broken down and pressed under 24 lb molds for 15 min, followed by 48 lb molds for another 15 min to collect the yellow pulp water. This process was repeated three times for each soybean variety and coagulant addition.

Soy ingredient composition

Soybean basic indicators

The moisture content, protein content, water-soluble protein content, and oil content of each variety were determined using the soybean tachymeter(CNS-6000E, Changchun Changguangsibo Spectrum Technology Co., Ltd., Changchun, China).

Protein subunit composition

The determination of soybean globulin was conducted according to the laboratory's available test methods. Firstly, the soybean to be tested was ground into powder and passed through a 60-mesh sieve. Acetone was then added to the powder overnight to obtain defatted soybean powder. Next, 0.5 g of defatted soybean powder was taken and mixed with 10 mL of protein extraction solution (pH 8.0, 50 moL/L Tris-HCL with 0.01 moL/L β-mercaptoethanol). The mixture was extracted for 1 h at room temperature and centrifuged at 10,000 r/min for 20 min. The supernatant was collected, adjusted to pH 4.5 with 1 moL/L HCL to precipitate total globulin, and centrifuged again at 5000 r/min for 10 min. The supernatant was discarded, and the precipitate was dried under vacuum and low temperature, which resulted in soy protein. Next, 1.5 mg of precipitate total globulin was dissolved in 500 μL of extraction solution (1 % SDS; 0.01 moL/L β-mercaptoethanol; pH 6.8 0.5 moL/L Tris-HCL; 50 % glycerol; 1 % bromophenol blue), and then heated at 100 °C for 3 min, and then cooled down to room temperature.

Bio-Rad vertical plates were employed for SDS polyacrylamide gel electrophoresis (Mini-PROTEAN Tetra) with a separation gel concentration of 13 % and 10 mA, and a concentrated gel concentration of 5 % and 12 mA. The gel was stained with coomassie brilliant blue R-250 for 40 min, followed by decolorization using a decolorizing solution (water: methanol: acetic acid = 3:1:6) for 40 min, and finally, decolorization was performed with 10 % acetic acid overnight until the bands were visualized. The gel sections were observed using a gel imager (iBright CL1000, Thermo Fisher Scientific, Waltham, Massachusetts, USA), protein content was determined by analyzing the gray scale in the bands by imagej software(National Institutes of Health,USA).

Amino acid composition

The amino acid composition of soybean was determined by the methods of Thiago M.T. do Nascimento(do Nascimento, Mansano, Peres, Rodrigues, Khan, Romaneli, et al., 2020) and Pei-yao Lu(Lu et al., 2020). First, the samples were previously defatted and the appropriate amount was weighed into a 50 mL hydrolysis tube. 20 mL of 1 moL/L HCL was added and the samples were hydrolyzed in an electric blower drying oven at 110 °C for 22 h. After removing the samples and cooling them down, the sample was transferred to a 25 mL colorimetric tube for volume determination. Take 100 μL of the sample into a 15 mL centrifuge tube, and place it in a vacuum drying oven to dry it at 60 °C for 2 h (until all solvent is dried). After drying, the samples were fixed to 0.5 mL with water., mix well, and pass it through a 0.45 μm organic membrane. The amino acid concentration was then determined on a 4.6 mm*100 mm*2.7 μm column at 40 °C, with mobile phase A consisting of 10 mmol/L disodium hydrogen phosphate and 10 mM sodium borate solution, mixed well, and then adjusted to pH 8.2 with hydrochloric acid, the mobile phase B consisted of methanol: acetonitrile: water = 45:45:10, with an injection volume of 37 μL, at wavelengths of 338 nm and 262 nm. The amino acid content (W) was obtained according to the following formula.

$$W = \frac{C - C_0 \times V \times N}{m} \quad (2)$$

In the formula: W-amino acid content in the specimen, unit mg/kg; C-amino acid concentration in the specimen assay solution, unit mg/L; C0-blank control in the target C0 - concentration of the target in the blank control, unit mg/L; V - volume of fixation, unit mL; N - dilution times; m - sampling volume of the specimen, with units of g.

Data statistics and mathematical modeling

The experiments were repeated three times, and statistical calculations (ANOVA) were conducted using the SPSS 22.0 software program (IBM Corporation, Armonk, New York, USA). The significance level was set at $p < 0.05$. The specific results of the three unbiased replicates were presented as the mean ± standard deviation. After obtaining the experimental results, a stepwise regression analysis was performed using the SPSSPRO (Zhongyan Network Technology Co., Ltd, Shanghai, China) software to obtain a mathematical model of multiple factors influencing the optimal coagulant content. After the model was established, it was validated and analyzed.

Results and Discuss

Optimal amount of coagulant for each variety

The optimal coagulant for each variety of soybean was determined by grinding 30 different soybean varieties into soymilk in specific proportions, and the results obtained are shown in Table 1. There was a significant difference in the optimum level of coagulant required for different varieties of soybeans. The protein content, which is closely related to tofu gel formation and directly affects its structure(Cheng, Shimizu, & Kimura, 2005; James & Yang, 2016; Toda et al., 2003), was found to be a possible factor in determining the optimal coagulant content. The 7S β-conglycinin and 11 s glycincin contents, which make up more than 70 % of soy protein, may also be related to the optimal coagulant content. However, further verification is needed to determine whether it is one or both of these factors that are relevant(Taski-Ajdukovic, Djordjevic, Vidic, & Vujakovic, 2010). In addition, the amino acid content is also a non-negligible factor in the formation of tofu gel(Liu et al., 2022), and we need to carefully analyze the amino acids and their effect on tofu gel.

Table 1
Optimal amount of solidifying agent for each variety of soybean.

Species number	Coagulant addition amount (g/L)	Species number	Coagulant addition amount (g/L)
1	1.95 ± 0.08^{klm}	16	2.24 ± 0.01^{defg}
2	2.34 ± 0.04^{bcd}	17	2.48 ± 0.13^{b}
3	2.30 ± 0.03^{cde}	18	2.47 ± 0.05^{b}
4	2.37 ± 0.05^{bcd}	19	2.38 ± 0.11^{bcd}
5	2.45 ± 0.05^{bc}	20	2.77 ± 0.08^{a}
6	2.48 ± 0.10^{b}	21	2.00 ± 0.03^{ijklm}
7	1.99 ± 0.05^{jklm}	22	2.00 ± 0.03^{ijklm}
8	1.94 ± 0.03^{klm}	23	2.13 ± 0.13^{fghij}
9	1.86 ± 0.03^{m}	24	2.12 ± 0.11^{hijk}
10	2.30 ± 0.12^{cde}	25	2.09 ± 0.03^{ghij}
11	2.11 ± 0.09^{ghij}	26	2.15 ± 0.03^{fghi}
12	2.17 ± 0.07^{efgh}	27	1.92 ± 0.03^{lm}
13	2.17 ± 0.08^{efgh}	28	2.28 ± 0.03^{def}
14	2.03 ± 0.09^{hijkl}	29	1.92 ± 0.03^{lm}
15	2.06 ± 0.03^{hijkl}	30	1.62 ± 0.13^{n}

*Different letters in the same column represent significant differences ($p < 0.05$).

Content of main components in soybeans

The results of testing 30 soybeans using the soybean tachymeter are presented in Table 2. The findings revealed that soybean No. 14 had the highest moisture content, soybean No. 10 had the highest protein content, soybean No. 14 had the highest water-soluble protein content, and soybean No. 5 had the highest oil content. Protein content is a crucial factor in tofu formation, but external factors such as temperature changes can affect the basic indicators of soybeans in the tofu production process, potentially impacting the optimal coagulant for tofu gel. Therefore, we need to analyze the data to verify the accuracy of this hypothesis.

Subunit and 7S/11S content

The 30 soybean species were analyzed using SDS-PAGE, and the subunit contents obtained from the analyzed bands are shown in Table 3 along with the 7S/11S contents. From the overall data analysis, it can be seen that the 7S and 11S contents of each type of soybean, as well as the differences in the contents of each subunit, were maintained at a relatively average level. The 7S and 11S globulin subunit composition of soybeans is an important influence on the traits and nutrient composition of tofu(Yu, Woodrow, Shi, & Anderson, 2019; Zheng, Regenstein, Zhou, & Wang, 2022). Furthermore, considering the previously mentioned close relationship between yellow slurry water and tofu, the 7S and 11S globulin subunits may also be important influences on the optimal coagulant. It has been demonstrated that different ratios of 7S globulin and 11S globulin can impact the final quality of the product (Wu, Hua, Chen, Kong, & Zhang, 2017). Furthermore, Yamagishi (YAMAGISHI, TAKAHASHI, KONDO, & YAMAUCHI, 2006) et al. conducted research on the gelation process of soybean 11S globulin and found that the polymerization of its acidic subunit triggers or accelerates the thermal gel formation of soybean globulin. Additionally, Milica (Pavlicevic, Tomic, Djonlagic, Stanojevic, & Vucelic Radovic, 2018) et al. conducted a comprehensive study on the subunit composition of different genotypes of soybean isolate proteins and their gelation properties, which revealed that gels prepared from genotypes with the β-subunit exhibited lower elasticity. A study by Amir(Nik et al., 2011) et al. demonstrated that the type of soybean globulin subunits can influence the aggregation behavior of soybean gels. In summary, subunit composition is undoubtedly a crucial factor in determining the quality of tofu, and it can also be inferred that subunit composition plays a key role in determining the optimal coagulant.

Table 2
Content of main components of various varieties of soybeans.

Number	Moisture (%)	Protein (%)	Water soluble protein (%)	Oil content (%)
1	6.03 ± 0.15^{k}	46.13 ± 0.38^{d}	22.93 ± 0.49^{l}	19.67 ± 0.12^{ab}
2	7.17 ± 0.06^{hi}	42.2 ± 0.26^{ijk}	23.23 ± 0.21^{jkl}	19.17 ± 0.12^{ab}
3	12.67 ± 0.06^{b}	41.63 ± 0.21^{jkl}	32.37 ± 0.06^{c}	18.63 ± 0.23^{ab}
4	6.03 ± 0.12^{k}	43.47 ± 0.40^{g}	20.73 ± 0.15^{n}	18.8 ± 0.30^{ab}
5	7.40 ± 0.20^{h}	39.77 ± 0.21^{n}	20.07 ± 0.45^{h}	22.10 ± 0.00^{a}
6	6.07 ± 0.06^{k}	42.57 ± 0.40^{hi}	21.73 ± 0.23^{m}	18.83 ± 0.15^{ab}
7	7.30 ± 0.10^{hi}	46.37 ± 0.55^{d}	25.83 ± 0.31^{g}	18.43 ± 0.06^{ab}
8	7.67 ± 0.06^{g}	45.70 ± 0.26^{d}	26.30 ± 0.20^{fg}	18.03 ± 0.15^{ab}
9	6.50 ± 0.10^{j}	40.77 ± 0.38^{m}	20.57 ± 0.25^{nh}	19.17 ± 0.15^{ab}
10	6.13 ± 0.12^{k}	50.60 ± 0.26^{a}	26.90 ± 0.20^{e}	17.00 ± 0.26^{abc}
11	7.37 ± 0.12^{hi}	42.27 ± 0.06^{ij}	23.33 ± 0.15^{jkl}	18.83 ± 0.12^{ab}
12	6.03 ± 0.15^{k}	46.03 ± 0.45^{d}	22.90 ± 0.44^{l}	20.23 ± 0.12^{ab}
13	6.13 ± 0.15^{k}	44.23 ± 0.47^{ef}	21.97 ± 0.15^{m}	18.57 ± 0.31^{ab}
14	13.00 ± 0.10^{a}	45.70 ± 0.20^{d}	35.60 ± 0.20^{a}	17.17 ± 0.31^{abc}
15	7.13 ± 0.15^{i}	41.97 ± 0.47^{ijkl}	22.13 ± 0.42^{m}	18.53 ± 0.06^{ab}
16	6.60 ± 0.17^{j}	42.20 ± 0.26^{ijk}	20.93 ± 0.29^{n}	19.77 ± 0.12^{ab}
17	6.20 ± 0.10^{k}	42.13 ± 0.40^{ijk}	21.07 ± 0.15^{n}	19.37 ± 0.06^{ab}
18	6.17 ± 0.15^{k}	46.37 ± 0.80^{d}	23.27 ± 0.38^{jkl}	18.47 ± 0.40^{ab}
19	7.30 ± 0.10^{hi}	43.10 ± 0.44^{gh}	23.13 ± 0.49^{jkl}	20.17 ± 0.15^{ab}
20	5.43 ± 0.06^{l}	49.50 ± 0.53^{b}	23.70 ± 0.36^{j}	18.60 ± 0.20^{ab}
21	7.67 ± 0.12^{g}	41.50 ± 0.36^{klm}	23.53 ± 0.25^{jk}	19.87 ± 0.15^{ab}
22	8.93 ± 0.15^{e}	40.83 ± 0.21^{m}	25.13 ± 0.21^{h}	19.43 ± 0.06^{ab}
23	8.20 ± 0.20^{f}	43.70 ± 0.36^{fg}	24.60 ± 0.26^{hi}	20.63 ± 0.15^{ab}
24	9.30 ± 0.00^{d}	39.93 ± 0.21^{n}	24.40 ± 0.10^{i}	20.17 ± 0.06^{abc}
25	7.37 ± 0.06^{hi}	41.23 ± 0.06^{lm}	22.17 ± 0.15^{m}	20.23 ± 0.06^{ab}
26	9.80 ± 0.00^{c}	48.07 ± 0.15^{c}	33.87 ± 0.15^{b}	16.90 ± 0.10^{ab}
27	9.27 ± 0.12^{d}	44.53 ± 0.32^{e}	29.30 ± 0.35^{d}	12.57 ± 9.41^{c}
28	9.67 ± 0.21^{c}	42.60 ± 0.26^{hi}	26.87 ± 0.12^{ef}	19.73 ± 0.06^{ab}
29	9.60 ± 0.10^{c}	48.67 ± 0.29^{c}	33.33 ± 0.31^{b}	16.30 ± 0.10^{bc}
30	7.70 ± 0.10^{g}	42.40 ± 0.20^{hi}	23.00 ± 0.36^{kl}	20.67 ± 0.06^{ab}

*Different letters in the same column represent significant differences ($p < 0.05$).

Amino acid composition

The results of analyzing the amino acid composition of 30 soybeans are presented in Table 4. From the table, it can be observed that the content of the same amino acid in different soybeans is relatively consistent, with only minor differences, except for methionine and valine. The content of methionine in samples No.28, No.29 and No.30 is significantly higher than that of other soybean varieties, but their content of valine is significantly lower than that of other soybean varieties. Due to this phenomenon, it was hypothesized that methionine and valine were not the influencing factors for the optimal coagulant because their large differences would certainly lead to a large difference in the optimal coagulant. However, in reality, there was no significant difference in the optimal coagulant as expected. Furthermore, it has been demonstrated that alkaline amino acids such as lysine, histidine, and arginine can have an impact on the gel type of protein(Arakawa, Ejima, Tsumoto, Obeyama, Tanaka, Kita, et al., 2007; Chen, Zou, Han, Pan, Xing, Xu, et al., 2016; Gao, Shi, Sun, Li, McClements, & Yuan, 2019; Gao, Wang, Mu, Shi, & Yuan, 2018; X. Y. Guo, Peng, Zhang, Liu, & Cui, 2015; Inoue, Takai, Arakawa, & Shiraki, 2014; S. Li, Li, Zhu, Ning, Cai, & Zhou, 2019; Shukla, Schneider, & Trout, 2011). Therefore, it is possible that the three amino acids used in our experiments also played a role in determining the optimal coagulant for tofu gel.

Modeling

The optimal coagulant content of tofu was used as the dependent variable, aspartic acid, glutamic acid, cysteine, serine, glycine, histidine, arginine, threonine, alanine, proline, tyrosine, valine, methionine, isoleucine, leucine, phenylalanine, lysine, 7S β-conglycinin, 11 s glycincin, 7S β-conglycinin + 11S glycincin, α' of 7S β-conglycinin, α of 7S β-conglycinin, β of 7S β-conglycinin, A3 of 11 s glycincin, $A_{1a}A_{1b}A_2A_4$ of 11 s glycincin, $B_{1a}B_{1b}B_2B_3B_4$ of 11 s glycincin, and the contents of oil, moisture, total protein, and water-soluble protein was used as independent variables in stepwise regression, and the variables retained after stepwise regression were Histidine content, Lysine content, 7S β-conglycinin content, $B_{1a}B_{1b}B_2B_3B_4$ of 11S glycincin content, and α' of 7S β-conglycinin content.

The equation of the model is as follows: $y = -1.186 + 3.457*B_{1a}B_{1b}B_2B_3B_4 + 2.304*7S/Total\ Protein + 0.351*Histidine - 0.084*Lysine + 4.696*\alpha'$ From the analysis of the results of the F-test, it can be obtained that the p-value of significance is 0.001 and the level presents a significance and the original hypothesis that the regression coefficient is 0 is rejected. For the covariate covariance performance, VIF is all less than 10, so the model has no multicollinearity problem and the model is well constructed.

Based on the results of the formulation, the final optimal coagulant was found to be related to one of the factors in the hierarchical structure of the proteins, which was consistent with our pre-experimental predictions. The basic index of soybeans was not found to be a significant factor in determining the optimal coagulant due to the significant changes in the tofu-making process. There may be another reason why the material composition of soybeans changes over time, which can lead to errors in the test. In conclusion, the basic index of soybeans is easily influenced by external factors, and therefore, it does not affect the content of the optimal coagulant. Soymilk production can be classified into raw and cooked methods, with the main difference being the order of heating and filtration. Studies have revealed that there are significant differences between the two methods of tofu production(Huang, He, Zhao, Liu, & Zhou, 2021; Huang, Liu, Zhao, He, Zhou, Chen, et al., 2022; Zhang, Wang, Li, Li, Lin, Chen, et al., 2018), This experiment utilized the raw method, but it is unclear whether the results would be the same if the cooked method was used. Further analysis is required to determine the impact of the cooking method on the results.

It is important to note that the formulas presented in the equation represent only the relationship between the factors under the optimal coagulant, and do not imply that these factors are the only ones involved in the formation of tofu gel. In reality, during the process of tofu gel aggregation, most of the influencing factors mentioned in the article have varying degrees of impact on the formation of tofu gel. As an example, in the soybean soaking and soymilk heating process, the pH and temperature of the water will have an impact on the composition of soybeans, resulting in these ingredients in the optimal coagulant test process did not reflect their role or even the opposite result, but in the existing soybean process, there is no way to solve this problem, perhaps

Table 3
7S/11S content and Subunit content.

Number	7S/total protein (%)	11S/total protein (%)	7S + 11S/total protein(%)	α'(%)	α(%)	β(%)	A3(%)	A1aA1bA2A4 (%)	B1aB1bB2B3B4 (%)
1	23.83 ± 2.91[ab]	34.41 ± 5.09[b]	58.24 ± 3.46[d]	4.96 ± 1.19[ab]	6.53 ± 0.48	12.09 ± 1.32	4.59 ± 0.90	17.13 ± 1.82[hi]	16.19 ± 4.59
2	29.91 ± 5.23[ab]	37.21 ± 4.01[b]	67.11 ± 3.92[abc]	6.69 ± 1.43[ab]	9.16 ± 1.00	13.54 ± 2.92	4.18 ± 1.67	18.18 ± 0.97[hi]	20.51 ± 5.93
3	28.78 ± 6.85[ab]	38.81 ± 5.09[b]	67.59 ± 5.13[abc]	7.09 ± 1.88[ab]	8.23 ± 2.11	13.10 ± 3.14	5.54 ± 1.01	18.97 ± 2.08[ghi]	19.06 ± 4.92
4	29.80 ± 5.71[ab]	37.59 ± 2.91[b]	67.39 ± 3.35[abc]	7.05 ± 2.49[ab]	8.98 ± 0.74	13.18 ± 2.52	5.54 ± 1.82	19.08 ± 1.07[fghi]	16.50 ± 3.87
5	24.86 ± 9.10[ab]	46.21 ± 10.09[ab]	71.07 ± 3.62[abc]	4.12 ± 2.56[ab]	6.83 ± 2.83	13.60 ± 3.74	6.38 ± 2.31	20.37 ± 0.76[efgh]	29.42 ± 11.14
6	27.34 ± 9.29[ab]	40.47 ± 6.91[b]	67.81 ± 5.37[abc]	6.00 ± 2.95[ab]	7.24 ± 2.92	12.82 ± 3.59	5.63 ± 1.52	20.10 ± 2.36[fghi]	20.08 ± 5.93
7	27.56 ± 5.26[ab]	40.51 ± 6.16[b]	68.07 ± 3.23[abc]	7.43 ± 1.34[ab]	8.64 ± 1.66	11.27 ± 2.44	6.15 ± 1.33	21.08 ± 2.77[cdefg]	17.54 ± 5.20
8	26.09 ± 3.49[ab]	36.84 ± 3.01[b]	62.94 ± 0.92 [cd]	6.94 ± 1.26[ab]	6.70 ± 1.67	12.38 ± 1.25	4.44 ± 1.45	20.98 ± 2.29[cdefg]	12.71 ± 1.97
9	25.29 ± 0.37[ab]	38.68 ± 2.19[b]	63.97 ± 1.91[bcd]	6.27 ± 0.79[ab]	7.39 ± 0.32	11.56 ± 0.45	4.25 ± 0.43	17.80 ± 2.48[ghi]	15.24 ± 1.27
10	24.75 ± 0.58[ab]	44.49 ± 2.47[ab]	69.24 ± 2.04[abc]	5.87 ± 0.21[ab]	7.10 ± 0.21	11.84 ± 0.57	5.80 ± 0.63	23.06 ± 1.65[abcde]	15.03 ± 0.27
11	22.57 ± 0.63[ab]	43.46 ± 2.06[ab]	66.03 ± 2.15[abc]	5.77 ± 0.19[ab]	6.70 ± 0.24	10.26 ± 0.62	5.38 ± 0.43	18.84 ± 0.37[ghi]	18.40 ± 1.55
12	20.28 ± 1.30[b]	52.42 ± 2.14[a]	72.70 ± 1.34[a]	3.89 ± 0.35[b]	7.07 ± 0.25	9.58 ± 1.05	6.45 ± 0.29	25.41 ± 1.84[a]	18.69 ± 3.75
13	24.75 ± 0.92[ab]	42.75 ± 2.37[ab]	67.50 ± 2.73[abc]	6.80 ± 0.66[ab]	7.39 ± 0.19	10.66 ± 0.87	4.82 ± 0.69	18.19 ± 2.16[fgh]i	18.06 ± 0.77
14	24.30 ± 2.23[ab]	43.18 ± 0.57[ab]	67.48 ± 2.47[abc]	6.06 ± 0.63[ab]	8.11 ± 0.65	10.22 ± 1.05	5.74 ± 0.38	20.82 ± 0.65[defgh]	16.18 ± 1.35
15	27.78 ± 1.22[ab]	40.51 ± 0.96[b]	68.29 ± 0.81[abc]	7.39 ± 0.24[ab]	8.90 ± 0.61	11.24 ± 0.42	6.80 ± 1.35	16.15 ± 1.64b	16.09 ± 1.17
16	31.17 ± 3.44[ab]	39.28 ± 4.88[b]	70.45 ± 2.19[abc]	5.98 ± 2.77[ab]	9.47 ± 1.00	13.42 ± 0.88	5.51 ± 1.00	22.52 ± 1.46[bcdef]	14.14 ± 3.46
17	28.58 ± 3.03[ab]	37.49 ± 2.70[b]	66.08 ± 2.12[abc]	6.53 ± 1.48[ab]	10.57 ± 3.69	10.48 ± 2.55	5.25 ± 0.72	20.36 ± 1.06[fgh]	14.45 ± 2.96
18	31.97 ± 3.31[ab]	41.64 ± 6.41[ab]	73.61 ± 4.63[a]	9.11 ± 2.43[a]	8.59 ± 0.57	14.20 ± 2.18	6.00 ± 0.65	24.26 ± 3.17[abcd]	14.65 ± 3.79
19	29.18 ± 4.29[ab]	45.22 ± 3.63[ab]	74.40 ± 1.82[a]	4.71 ± 1.64[ab]	9.88 ± 1.06	13.23 ± 1.90	6.15 ± 0.67	26.44 ± 1.05[a]	15.75 ± 3.62
20	29.45 ± 3.90[ab]	42.74 ± 5.76[ab]	72.19 ± 4.43[ab]	3.19 ± 2.38[ab]	8.80 ± 0.98	15.47 ± 1.18	6.06 ± 0.51	24.48 ± 2.47abc	15.48 ± 3.89
21	28.30 ± 3.48[ab]	39.19 ± 3.15[b]	67.49 ± 3.74[abc]	5.71 ± 1.05[ab]	9.29 ± 0.92	12.43 ± 1.75	5.08 ± 1.07	21.86 ± 0.79[cdefg]	14.79 ± 3.26
22	28.91 ± 3.08[ab]	40.03 ± 3.08[b]	68.93 ± 4.25[abc]	6.69 ± 1.36[ab]	10.13 ± 3.29	12.09 ± 1.60	5.30 ± 7.35	21.92 ± 1.54[cdefg]	12.81 ± 0.86
23	29.24 ± 3.13[ab]	40.34 ± 3.13[b]	69.58 ± 4.33[abc]	6.01 ± 1.57[ab]	9.47 ± 0.46	13.76 ± 1.00	4.93 ± 1.77	22.00 ± 0.58[cdefg]	13.41 ± 1.93
24	32.40 ± 3.47[ab]	35.52 ± 3.47[b]	67.92 ± 2.39[abc]	6.73 ± 3.52[ab]	10.01 ± 1.15	15.66 ± 1.23	4.87 ± 1.15	19.73 ± 1.07[fghi]	10.93 ± 0.99
25	28.86 ± 4.22[ab]	41.03 ± 4.22[ab]	69.88 ± 2.58[abc]	5.81 ± 2.53[ab]	8.73 ± 1.62	14.32 ± 1.13	4.21 ± 1.65	22.57 ± 0.66[bcdef]	14.24 ± 1.39
26	25.85 ± 2.01[ab]	45.23 ± 2.01[ab]	71.08 ± 5.97[abc]	3.89 ± 4.14[b]	9.09 ± 0.71	12.87 ± 0.20	5.05 ± 1.13	25.78 ± 0.79[ab]	14.40 ± 3.18
27	27.36 ± 4.13[ab]	40.63 ± 4.13[b]	67.99 ± 5.37[abc]	3.76 ± 1.59[b]	8.34 ± 0.81	15.27 ± 0.90	5.91 ± 2.53	22.50 ± 0.87[bcdef]	12.22 ± 2.19
28	28.38 ± 1.74[ab]	39.10 ± 1.74[b]	67.47 ± 1.05[abc]	5.73 ± 0.73[ab]	8.68 ± 0.54	13.96 ± 0.17	4.62 ± 1.06	21.97 ± 0.92[cdefg]	12.51 ± 0.76
29	26.08 ± 0.48[ab]	45.03 ± 0.48[ab]	71.11 ± 1.93[abc]	4.19 ± 1.85[b]	9.00 ± 0.51	12.89 ± 0.62	5.15 ± 0.58	26.41 ± 0.34[a]	13.48 ± 1.12
30	24.17 ± 0.42[ab]	39.88 ± 0.42[b]	64.05 ± 1.07[bcd]	4.68 ± 1.41[ab]	7.60 ± 1.82	11.89 ± 0.34	4.75 ± 1.40	23.97 ± 1.24[abcd]	11.16 ± 1.25

*Different letters in the same column represent significant differences ($p < 0.05$).

this problem is the next problem to be overcome.

Using the mathematical model of the optimal coagulant for yellow slurry water, the metal ion content of yellow slurry water can be reduced at the source of the tofu industry, and the yellow slurry water can be utilized and transformed while saving a large amount of treatment costs, extending the industrial chain of the tofu industry and enhancing the value chain of the tofu industry.

Validation

After establishing the mathematical model, we validated it by randomly selecting 5 soybean varieties from the laboratory database for basic data analysis. The formula was used to calculate the optimal coagulant dosage for these 5 soybean varieties. The actual determination of the coagulant dosage for these 5 soybeans was completed and compared with the calculated value of the formula Table 5. The validation results showed that the error between the predicted and actual

Table 4
Amino acid composition.

NUMBER	Aspartic acid (mg/g)	Glutamic acid (mg/g)	Cystine (mg/g)	Serine (mg/g)	Glycine (mg/g)	Histidine (mg/g)	Arginine (mg/g)	Threonine (mg/g)	Alanine (mg/g)	Proline (mg/g)	Tyrosine (mg/g)	Valine (mg/g)	Methionine (mg/g)	Isoleucine (mg/g)	Leucine (mg/g)	Phenylalanine (mg/g)	Lysine (mg/g)
1	56.98	89.42	2.60	25.09	20.86	11.64	36.32	18.42	20.14	26.74	16.16	21.95	4.27	22.64	37.93	23.93	27.88
2	54.06	85.36	1.84	22.84	21.05	11.99	36.79	17.87	20.70	19.16	14.79	21.55	3.96	22.65	37.03	24.53	27.07
3	53.78	83.88	2.19	23.88	21.28	11.89	35.16	17.58	19.86	16.95	14.22	21.59	4.32	22.58	35.30	24.69	26.30
4	57.43	90.27	2.29	25.87	21.66	12.88	37.68	19.27	21.54	30.46	15.29	24.66	3.98	24.85	39.39	25.62	29.89
5	54.26	85.77	2.98	23.89	21.07	12.00	37.22	17.92	20.95	25.15	14.39	24.18	3.99	24.10	37.56	25.49	28.85
6	51.53	81.36	2.21	22.73	19.78	11.51	34.23	17.76	18.98	27.99	14.23	22.45	3.85	22.27	34.87	23.20	26.70
7	57.34	92.91	2.42	25.12	21.84	12.10	39.73	18.49	21.14	22.04	15.35	24.40	4.54	25.19	38.32	25.74	29.94
8	52.83	84.07	2.43	22.86	19.72	11.71	37.06	17.19	19.40	36.62	13.81	22.99	3.47	23.07	35.79	24.27	28.92
9	54.09	84.97	2.39	24.03	20.10	11.88	35.72	17.93	19.69	26.64	14.06	21.94	3.83	21.54	36.25	23.61	27.97
10	55.95	91.41	2.79	25.55	21.50	12.26	40.23	18.30	20.82	33.22	15.93	24.25	4.25	23.76	38.43	25.43	28.10
11	54.97	85.90	2.46	24.54	20.87	12.55	39.22	18.37	20.44	27.84	14.56	23.69	3.66	22.98	37.64	24.30	28.31
12	60.30	97.47	3.19	26.60	22.40	13.23	42.75	19.82	21.98	41.24	16.12	25.66	4.23	25.21	41.01	26.80	31.51
13	50.03	79.60	2.14	22.35	20.19	11.54	33.65	17.15	19.22	26.80	14.23	22.08	3.39	22.33	35.23	23.13	26.92
14	57.69	90.50	2.28	24.64	23.12	12.46	42.91	18.86	20.96	29.87	14.85	25.08	5.04	24.86	38.50	26.60	28.28
15	50.24	80.62	1.80	21.95	20.53	11.04	34.03	16.70	18.91	33.39	13.69	22.92	3.83	24.28	34.98	24.51	26.13
16	52.23	85.25	2.41	25.01	20.69	12.01	34.93	18.77	20.09	22.97	14.21	20.92	3.50	19.33	35.89	19.98	28.36
17	52.54	84.06	1.97	24.18	21.29	12.01	36.70	18.58	20.33	18.79	14.56	22.17	3.44	20.34	35.88	20.95	26.20
18	54.39	87.40	2.29	25.07	21.67	11.90	38.40	18.54	20.55	20.87	14.72	22.86	3.78	21.58	36.23	21.71	26.76
19	51.82	84.43	2.43	23.44	20.14	11.33	36.61	17.50	19.43	42.91	13.88	21.63	3.57	20.57	34.25	21.57	26.90
20	58.61	95.08	2.44	26.33	22.91	13.24	39.54	19.06	20.84	37.72	14.63	24.64	3.61	23.58	39.24	25.27	29.73
21	57.48	93.02	2.02	26.39	23.33	12.79	38.99	19.49	21.67	27.95	15.56	24.62	3.81	22.24	38.68	24.29	29.16
22	50.95	83.09	1.97	25.01	20.35	11.20	34.60	18.03	19.80	36.37	14.32	21.07	3.53	18.47	34.05	21.53	27.12
23	55.39	91.54	1.74	26.21	22.21	12.64	38.16	19.76	22.51	41.50	15.54	25.47	3.22	22.79	39.10	24.08	30.81
24	55.39	91.54	1.74	26.21	22.21	12.64	38.16	19.76	22.51	41.50	15.54	25.47	3.22	22.79	39.10	24.08	30.81
25	55.38	90.38	1.59	25.88	23.32	11.86	38.83	19.57	21.98	27.28	16.13	25.30	3.60	21.88	38.36	23.74	28.21
26	63.82	105.75	2.70	29.44	24.17	13.98	50.24	21.13	23.06	43.72	17.83	26.65	5.00	24.46	42.10	27.09	32.96
27	56.64	92.31	1.85	25.87	22.31	12.31	40.65	19.27	21.21	36.89	15.72	23.90	3.33	21.58	38.55	23.93	29.46
28	55.44	87.71	2.04	25.41	22.28	12.28	37.72	19.50	21.86	35.73	15.45	2.83	29.68	21.31	37.98	23.52	27.54
29	57.90	93.61	2.62	26.01	21.44	12.57	44.27	18.78	20.62	28.81	15.44	2.52	29.38	21.32	37.35	22.61	29.19
30	55.04	88.91	2.01	26.28	22.35	11.50	37.12	19.52	21.11	22.43	15.18	2.74	29.67	21.26	37.82	22.12	27.22

Table 5
Five kinds of soybeans were randomly selected for the determination of the best coagulant.

Number	7S content(%)	$B_{1a}B_{1b}B_2B_3B_4$(%)	α'(%)	histidine	lysine	Optimal coagulant prediction	Dosage of coagulant (g/L)	inaccuracy (%)
1	30.31	19.32	7.32	12.37	29.59	2.388	2.21	7.45
2	23.81	27.90	4.81	11.98	29.59	2.383	2.46	2.23
3	25.36	15.48	5.82	11.40	25.41	2.080	1.94	6.73
4	24.75	18.06	6.80	11.54	26.92	2.124	2.17	2.17
5	27.78	16.09	7.39	11.04	26.12	2.044	2.03	0.68

values of the optimal coagulant content of soybeans was less than 10 %, indicating that the model is highly credible. Due to certain objective factors in the coagulant testing process, it is challenging to further reduce the error. Additionally, after solidifying soymilk, there may be a phenomenon of re-spinning, which can also impact the experiment, in this case, the coagulant content tends to be on the large side.

Conclusion

Our study revealed a correlation between the optimal amount of coagulant in tofu and the 7S content, histidine content, lysine content, α' content, and B1aB1bB2B3B4 content of soybeans. The relationship was found to be $y = -1.186 + 3.457 * B_{1a}B_{1b}B_2B_3B_4 + 2.304 * 7S/\text{total protein} + 0.351 * \text{Histidine} - 0.084 * \text{Lysine} + 4.696 * \alpha'$. The experimental data confirmed that there are factors that positively or negatively affect the amount of coagulant used at all levels of soy protein structure. The mathematical modeling effectively eliminated irrelevant factors, resulting in a more precise range of factors affecting the amount of coagulant used. By understanding the relationship between these factors, the tofu industry can be optimized to reduce the pollution problem of metal ions at the source, thereby achieving greening of the tofu industry.

CRediT authorship contribution statement

Jian Chen: Writing – original draft, Software, Methodology, Data curation, Conceptualization. **Lei Cai:** Software, Methodology, Formal analysis, Conceptualization. **Xiaolong Huang:** Software. **Hongling Fu:** Conceptualization. **Ling Sun:** Methodology. **Changwei Yuan:** Software. **Hao Gong:** Software. **Bo Lyu:** Supervision, Software, Funding acquisition, Data curation, Conceptualization. **Zhaohui Wang:** Supervision, Software. **Hansong Yu:** Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2024.101137.

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