OPEN ACCESS Freely available online

Advances in Dairy Research

Review Article

Study on the Effect of Isoelectric Point Shift of Soybean Protein in Acid Precipitation Process Based on Transglutaminase

Yuqiang Shi[1,2*], Rucui Liu[1], Jun Ma[1], Xuejiao Cheng[1], Peng Chen[1], Weigang Yuan[1]

[1]Shandong Yuwang Ecogical Food Industry Co., Ltd., Yucheng 251200, China; [2]Kedong Yuwang Soy Protein Food Co., Ltd., Kedong 164800, China

ABSTRACT

Review Transglutaminase (TG) is mainly used in food modification to adjust food texture and improve protein nutritional value. It works by catalyzing the amide transfer reaction between the γ-formamide group in glutamine and the primary amine group in lysine, thereby crosslinking the two amino acids. Soy protein contains 18 amino acids, and cross-links are formed between soy proteins through TG, thereby forming new protein structures that can cause isoelectric point shifts. In order to investigate the change rule of TG changing the isoelectric point of soybean protein and its influence on the production of soybean protein isolate, the following studies were carried out: The low-temperature skimmed soybean meal was dissolved under alkaline conditions, and soy milk was obtained by centrifugation; TG was added to the soy milk, and then protein isoelectric point adjustment was performed; materials that reach the isoelectric point were centrifuged to obtain soy protein and soy whey; the change trend of soy whey and the shift direction of protein isoelectric points after adding enzyme were analyzed. When different ratios of TG enzyme were added, the shift trend of the isoelectric point of soybean protein was studied. At these isoelectric points, the recovery rate of soybean protein was studied. The results showed that: When the TG was added at a ratio of 1.0% of the dry matter content of the soymilk, the Crude Protein (CP) of the soybean whey was 1.2 percentage points lower than that without TG at the normal protein isoelectric point (pH 4.5). And the pH value of isoelectric point in soymilk with TG was shifted from 4.5 to 3.7, then the CP of the soybean whey was reduced from 23.8% to 20.0% at the new isoelectric point. Under different TG addition, the degree of deviation of the isoelectric point of soybean protein was different, and the pH value of isoelectric point decreased first and then increased with the addition increase. When the addition amount was 1.0%, the pH value of isoelectric point reached the low of 3.7. The recovery of soybean protein increased with the TG addition, and when the addition amount was 1.5%, the recovery was 91.1%. By adding TG to soybean milk, soybean protein could be effectively cross-linked, the pH value of isoelectric point of soybean protein changed, the recovery rate of soybean protein improved, the content of soybean protein in soybean whey reduced, and the utilization of soybean could be improved. It has positive significance for protecting the environment and promoting the development of the soybean protein industry.

Keywords: Low-temperature; Defatted soybean; Transglutaminase; Moisture; Isoelectric point; Recovery rate

INTRODUCTION

Soybean protein isolate has superior food processing properties, including gelling, water-holding, oil absorption, emulsification, film-forming, foaming, etc., and it is widely used in the food industry [1-5]. At the same time, the nutritional and health function of soy protein isolate is also increasingly recognized [6-9]. The process principle of soybean protein isolate production is to dissolve the protein under alkaline condition, add acid to precipitate at the isoelectric point, and then separate to achieve protein purification [10]. The process is after dissolving the low-temperature degreased soybean meal in sodium hydroxide solution, the solid phase and liquid phase are separated to obtain okara and soy milk respectively. Among them, the main components of okara are insoluble dietary fiber and macromolecular protein; the main components of soy milk are soy protein, soy oligosaccharides, soybean dietary fiber and ash. Then, the pH value of soy milk is adjusted to the isoelectric point to realize the aggregation and precipitation of soy protein, and then a centrifuge is used to obtain acidic soy protein. The separation after alkaline solution directly affects the extraction rate of soy protein, while the separation after acid precipitation mainly

Correspondence to: Yuqiang Shi, Shandong Yuwang Ecogical Food Industry Co., Ltd., Yucheng 251200, China, E-mail: slz7142940@hotmail.com

Received: 16-Nov-2023, Manuscript No. ADR-23-28036; **Editor assigned:** 20-Nov-2023, PreQC No. ADR-23-28036 (PQ); **Reviewed:** 04-Dec-2023, QC No. ADR-23-28036; **Revised:** 11-Dec-2023, Manuscript No. ADR-23-28036 (R); **Published:** 18-Dec-2023, DOI: 10.35248/2329-888X.23.11.640

Citation: Shi Y, Liu R, Ma J, Cheng X, Chen P, Yuan W (2023) Study on the effect of isoelectric point shift of soybean protein in acid precipitation process based on Transglutaminase. J Adv Dairy Res. 11:640.

affects the recovery rate of protein. Soy protein is a complex mixture that mainly contains four types of proteins, namely 2S, 7S, 11S, 15S protein [11]. There are certain differences in the isoelectric points of proteins with different sedimentation coefficients, and according to the optimal recovery rate of soy protein, the conventional soy protein isolate process controls the pH value of isoelectric point at 4.5 [12]. Therefore, there is still a certain proportion of soy protein in the whey, resulting in the waste of soy protein and affecting the full utilization of soy. TG is mainly used in food modification to regulate food texture and enhance protein nutritional value, and its working principle is to catalyze the amide transfer reaction between the γ-formamide group in glutamine and the primary amine group in lysine, thereby crosslinking the two amino acids [13]. Soy protein contains 18 amino acids and contains substrates catalyzed by TG, so cross-linking between soy proteins under the action of TG [14] leads to the formation of new protein structures, resulting in the pH value of the isoelectric point to shift.

There have been no reports of TG treatment of soy protein changing the pH value of isoelectric points. Therefore, the aim of this study was to promote the development of soy protein industry by studying the effect of TG treatment on the isoelectric point of soy protein, improving the recovery rate of precipitated protein, reducing protein loss in soy whey, and improving the utilization rate of soybean.

MATERIALS AND METHODS

Materials

Low-temperature soybean meal was purchased from Kedong Yuwang Soy Protein Food Co., Ltd. (Kedong, Qiqihar, Heilongjiang, China) which has crude protein content of 55 ± 0.5%, NSI of 82 ± 1% and a moisture content of 8.0 ± 0.5%. Concentrated sulfuric acid (analytical reagent) was purchased from Yantai Yuandong Fine Chemicals Co., Ltd (Laiyang, Yantai, Shandong, China). Sodium hydroxide (analytical reagent) was purchased from Beijing Yili Fine Chemicals Co., Ltd. (Beijing, China). Copper sulphate (analytical reagent) was purchased from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). Anhydrous ether (analytical reagent) was purchased from Grice (Tianjin) Pharmaceutical Chemical Technology Co., Ltd (Tianjin, China). 95% ethanol was purchased from Tianjin Jindong Tianzheng Fine Chemical Reagent Factory (Tianjin, China). Acetone (analytical reagent) was purchased from Tianjin Kermel Chemical Reagent Co., Ltd (Tianjin, China). Potassium dichromate (analytical reagent) was purchased from Maoming Xiongda Chemical Co., Ltd (Maoming, Guangdong, China). Tris (hydroxymethyl) aminomethane (analytical reagent) was purchased from Xi'an Tianmao Chemical Co., Ltd (Xi'an, Shaanxi, China). 2-(N-morpholino) ethanesulfonic acid (analytical reagent) was purchased from Yeasen Biotechnology（Shanghai） Co., Ltd (Shanghai, China). TG (enzyme activity 100U/g) was purchased from Jiangsu Yiming Biological Technology Co., Ltd (Taixin, Jiangsun, China).

Apparatus

Electronic balances (AL204-2C) was purchased from Mettler-Toledo Technologies (China) Co., Ltd (Shanghai, China). Constant temperature drying oven (DHG-9051A) was purchased from Shanghai Yiheng Technology Instrument Co., Ltd (Shanghai, China). Thermostatic water bath (HWS-26) was purchased from Shanghai Yiheng Technology Instrument Co., Ltd (Shanghai, China). Vortex oscillator (Vortex 1) was purchased from IKA Works Guangzhou (Guangzhou, Guangdong, China). Homogeneous and emulsification pump (DHX3/185) was purchased from Ningbo Durrex Co., Ltd (Ningbo, Zhejiang, China). Centrifuge (2-16KL) was purchased from Sigma Laborzentrifugen (Osterode am Harz, Germany). High-speed grinder (YF-103B) was purchased from Ruian Yongli PHarmaceutical Machinery Co., Ltd (Ruian, Zhejiang, China). Digital Hand-Held "Pocket" Refractometer (PAL-1) was purchased from ATAGO CO., Ltd. (Tokyo Japan). pH meter (S400-Basic) was purchased from Mettler-Toledo Technologies (China) Co., Ltd (Shanghai, China). Fully automated Kjeldahl analyser (Kjeltec 8200) was purchased from Foss Analytical Co., Ltd (Hilleroed, Denmark).

Isoelectric point verification method for soybean protein isolate affected by TG

Low-temperature soybean meal was put into dilute sodium hydroxide (45℃, pH10.5 ± 0.5, dilute sodium hydroxide powder (in mass) =8:1,), then the liquid pH was adjusted to 7.35 ± 0.05 with sodium hydroxide (10% mass fraction). Stirred the liquid (120r/min,30min). Then the liquid was separated into supernatant and okara with centrifugal (4500r/min,5min). The okara was washed with water (45℃, the ratio of water to the original soybean meal was 4:1) under stirring (120r/min,5min). Separation was followed with centrifugal (4500r/min,5min) after the washing, then the two parts of supernatant was mixed. After adding TG to the mixture, stirred for 45min at a speed of 120r/min. Then the soymilk was isoelectric precipitated with hydrochloric acid. The liquid after acid precipitation was separated into whey and protein curd with centrifugal (5000r/min,10min).

Solids (Total) in soybean milk and wheat by direct forced air oven drying

Initial weighing-sample and weigh soybean whey at 38°C ± 1°C. Record all weights to 4 decimal places. Remove metal shelf from oven and place next to balance. Weigh predried weighing dish. Pipet ca 3 g of 38°C ± 1°C pre pared test portion to nearest 0.1 mg directly into preweighed dish. Do not chase drift. Record test portion weight immediately after weight stabilizes and just before start of drift to lower numbers. Remove dish from balance and place on oven shelf for trans port to oven. Check balance zero between weighings. Blanks-Weigh 2 empty, predried dishes. Drying-Pre heat oven to 100°C ± 1°C. Open hot air exhaust vent to let moisture be removed from oven. After test portions are weighed, turn off oven and place shelf plus all dishes (including blanks) into forced air oven. Immediately after loading all test portions in oven, close door and turn oven on again, and dry for 4 h at 100°C ± 1°C. Do not open oven door until 4 h drying is completed. Cooling-Remove dishes from oven and let cool to room temperature in desiccator (≥ 30 min). Weigh dish plus dry soybean whey on same balance that was used for initial weighings.

Calculations: $Total\ solids, \% = \frac{(W_2 - W) - B}{(W_1 - W)} \times 100$

where W=Weight of dish; W_1=Weight of dish+milk test portion; W_2=Weight of dish+dry milk; and B=Mean blank weight.

The whey was as the same

Protein in soybean whey Kjeldahl method

To 25 mL prepared test portion, at 20°C in Kjeldahl flask, add 2-3 mL H_2SO_4 and concentrate to syrupy consistency. Determine N as follows: Place weighed test portion (0.7-2.2 g) in digestion flask. Add

0.7 g HgO,15 g powdered K_2SO_4, and 35 mL H_2SO_4. Place flask in inclined position and heat gently until frothing ceases (add small amount of paraffin to reduce frothing); boil briskly until solution clears and then ≥ 2 h min longer. Cool, add Ca 200 mL H_2O cool, to <25°C, add 25 mL of the thiosulfate solution, and mix to precipitate Hg. Add few Zn granules to prevent bumping, tilt flask, and add layer of NaOH without agitation. (For each 10 mL H_2SO_4 used, or its equivalent in diluted H_2SO_4, add 15 g solid NaOH or enough solution to make contents strongly alkaline.) (Thiosulfate solution may be mixed with the NaOH solution before addition to flask) Immediately connect flask to distilling bulb on condenser, and, with tip of condenser immersed in standard acid and 5-7 drops indicator in receiver, rotate flask to mix contents thoroughly; then heat until all NH3 has distilled (≥ 150 mL distillate). Remove receiver, wash tip of condenser, and titrate excess standard acid in distillate with standard NaOH solution. Correct for blank determination on reagents.

N, %= ((mL standard acid × 2 molarity acid) - (mL standard NaOH × molarity NaOH)) × 1.4007/g sample

N, % × 6.25=% protein

Protein%= ((mL 0.1 M × 2) - (mL 0.1 M base)) × 1.4 × 6.25 × 100)/ (specific gravity × mL sample × 1000)

Detection of isoelectric points of soy protein

The isoelectric point is the pH at which the overall charge of the protein is zero (a neutral charge). At the isoelectric point, proteins do not have the effect of the same charge repelling each other, so they are unstable, the solubility is minimal, and it is easy to quickly combine into larger aggregates by electrostatic attraction. Since soy protein is a complex mixture, in order to achieve maximum protein recovery, in industrial production, isoelectric point generally referred to the pH when the lowest whey protein content was produced after the recovery of precipitated soy protein by centrifugal separation.

Detection of changes in pH value of isoelectric point of soybean milk with different TG addition proportion

According to the method 2.3.1 to extract soy milk and detect the solid content of soy milk. Added TG to the soy milk, the addition proportion was 0.5%~1.5% of solids (total) in soybean milk. Then adjusted pH from 3.5-4.6 to detect the isoelectric points of soy protein. The isoelectric point was defined in this experiment as pH at the lowest solubility of protein under test conditions.

Protein recovery rate calculation in acid precipitation process

$$\text{recovery } \% = \left(1 - \frac{W_1 \times S_1 \times CP_1}{W \times S \times CP}\right) \times 100\%$$

W_1=Weight of soy whey; W=Weight of soy milk; S=Solids(Total) of soy milk; S_1=Solids(Total) of soy whey; CP=Crude protein of soy milk; and CP_1=Crude protein of soy whey.

Data analysis

Number of repetitions of sample preparation were 3 times. Statistical analysis was conducted using SPSS (IBM® SPSS® Statistics version 25). Analysis of variance (ANOVA) and Multivariate Analysis of Variance (MANOVA) followed with Tukey post hoc test were conducted by SPSS. The level of significance was set at $p<0.05$ [15].

RESULTS AND DISCUSSION

Analysis of CP changes in whey from treated soy milk by TG

The conditions were: The isoelectric point of conventional soybean protein isolate production was 4.5, the TG treatment temperature was 45°C, the addition amount was 1.0% of solids (total) in soybean milk, and the mass ratio of soybean meal to water was 1:8 [16]. The changes of CP in soybean whey produced by adding TG process at isoelectric point of conventional process was as Table 1. It was found that the CP of whey from TG-treated soy milk was 1.2 percentage points lower than normal whey. This indicated that TG enzyme-treated soy milk had an improved effect on separation after isoelectric focusing. It was believed that the polymerization of TG prolonged the relative molecular mass of soy protein, improved the overall particle size of aggregates during acid precipitation of soy protein, and was easier to settle and separate in the centrifuge (Table 1).

Table 1: Changes of CP in soybean whey produced by adding TG process at isoelectric point of conventional process.

Process	CP /%(db)
Normal process	23.8±0.19[a]
Process treated by TG	22.6±0.13[b]

The trend of CP in soy whey was shown in Figure 1, when the addition amount was 1.0% of solids (total) in soybean milk. It was shown that the CP in soy whey dropped from 23.8% to 20.00% (the trough), when the pH was adjusted from 3.5 to 3.7. Later, with the increase of pH value, the CP in soy whey slowly increased, and an inflection point appeared at pH 4.5, the CP was 22.2%. When the pH value was 4.6, the amount of residual protein in whey increased rapidly, the CP in soy whey reached to 24.9%. Compared with normal process, the isoelectric point of soy protein with TG was shifted from 4.5 to 3.7, and the CP in soy whey was reduced 3.8 percentage points, it indicated that the TG reacted with soy protein to cause changes in the isoelectric point of soy protein. The analysis concluded that TG cross-linked between soybean protein molecules, and the crosslinking of protein molecules changed the primary structure of the protein, so the isoelectric point shifted; the reason for the change was that when the ε amino group of lysine residues in soybean protein was an amino acceptor, TG catalyzed the formation of γ-(L-glutaminyl)-Lysine isopeptide bonds, resulting in cross-linking between or within protein molecules, when the primary amine was an amino acceptor, TG enzyme catalyzed the acyl transfer reaction between glutamine residues and primary amines [17]. At the macroscopic level, the intermolecular polymerization of soy protein increased the relative molecular mass [18], which increased the volume and density of protein aggregates during acid sedimentation, thereby improving the centrifugal sedimentation effect of soy protein aggregates during centrifugation. The pH shift of the isoelectric point of protein and the reduction of CP in whey provided a theoretical basis for improving the recovery rate of soy protein, reducing the difficulty of whey wastewater treatment, and realizing the full utilization of soybean. On the downside, the isoelectric point shifted to low pH to increase the amount of hydrochloric acid, and the amount of sodium hydroxide needed to be increased when neutralizing, and the ash in soy protein products would increase.

With the TG addition amount was 1.0% of solids (total) in soybean milk without adjusting the isoelectric point (pH=4.5), the CP of soy whey was 22.6%, which decreased to 20.0% after adjusting the isoelectric point (pH=3.7). The change in isoelectric point resulted in an 11.50% decrease in CP of soy whey. At the isoelectric point of the normal process, the CP of soy whey with TG added was reduced by 5.04% compared to the process without TG, protein crosslinking produced by TG was responsible for the change. Therefore, the migration of isoelectric points caused by molecular crosslinking of soybean protein caused by TG has a great influence on protein recovery (Figure 1).

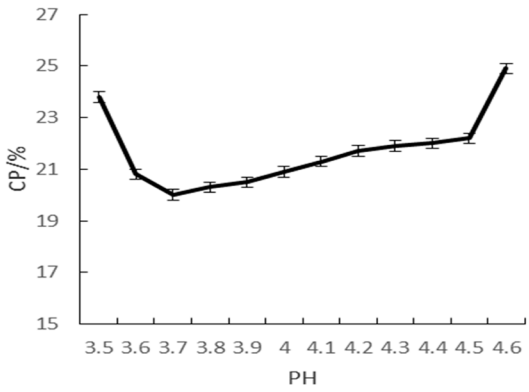

Figure 1: Variation of CP in soybean whey at different pH values.

Changes in pH value of isoelectric point with addition proportion of TG

After the reaction of soymilk with different TG dosages, the pH value changes in isoelectric point of soy protein were shown in Figure 2. With the increase of the proportion of TG addition, the isoelectric point of soy protein first decreased and then increased. The initial stage of decline was slow, and the pH value of the isoelectric point was 4.2 when the addition proportion was 0.5%, and the decrease was 6.67%; when the addition proportion was 1.0%, the pH value of the isoelectric point reached 3.7, and the decrease was 11.90%. As the addition proportion continued to increase, the isoelectric point rose, and when the addition proportion was 1.5%, the pH value of the isoelectric point was 4.0, and the increase amplitude was 8.10%. The analysis concluded that the protein crosslinking produced by TG formed new protein molecular structures, which caused isoelectric point shift, and migrated to low pH when the addition proportion was low, with the increase of the addition proportion, the reaction speed of enzymatic crosslinking increased, the degree of protein crosslinking and the number of crosslinks increased, the degree of protein aggregation increased within the same time, the degree of isoelectric point deviation increased, and the site of TG crosslinking changed dynamically [19]. After exceeding a certain proportion, with the increase of TG, the reaction density of TG with acylaminos of lysine residues increased, resulting in an increase in protein isoelectric point [10]. The increase of isoelectric point pH could reduce the amount of alkali used in neutralization, which was conducive to reducing the amount of inorganic chemical products used in the soybean protein industry and reducing the harm to the environment. Soybean protein isolate production practice needs to balance the cost of TG, alkali amount, product yield, and formulate optimal process conditions.

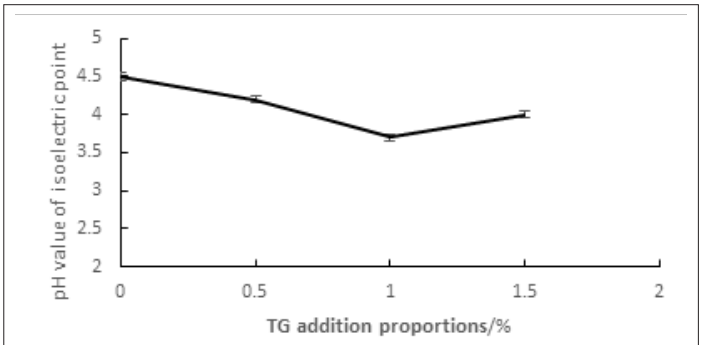

Figure 2: PH Changes of isoelectric point with different TG addition proportions.

Protein recovery at different addition proportion of TG

Milk Soy milk with different concentrations of TG was added, adjusted to the isoelectric point, centrifuged to obtain protein slurry and soy whey, weighed the amount of soy whey, detected the CP in soy whey, and calculated the recovery rate of soy protein in acid precipitation process was shown in Figure 3. With the increase of TG addition proportion, protein recovery rate in acid precipitation process increased: When TG was not added, the recovery rate was 86.1%, When the addition proportion was 0.5%, the recovery rate increased to 89.2%, an increase of 3.1 percentage points, and when the addition proportion was 1.0%, the increase rate slowed down and the recovery rate was 90.8%; when the addition proportion was 1.5%, the recovery rate was 91.1%. Analysis of the reasons for the trend:

- After adding TG, under the premise of adjusting the isoelectric point, the number of protein chain polymerization increased [20].
- Crosslinking reaction occurred between low molecular mass proteins (containing TG reaction sites) that could not be aggregated and precipitated under normal isoelectric points, and the crosslinked proteins formed aggregates that could be precipitated at the isoelectric point and could be separated under corresponding centrifugation conditions during the acid precipitation process.
- Cross-linking between low molecular mass proteins that could not be aggregated and precipitated under normal isoelectric points and soy proteins that could be aggregated and precipitated, and then achieve co-precipitation and be separated and recovered [21].
- At the beginning, with the increase of TG, the reaction density between enzyme and protein increased, the polymerization rate of soy protein increased, and the number of aggregated protein chains increased. After the amount of enzyme added reached a certain proportion, the rate of reaction density increase decreased, indicating that the polymerization site of the enzyme tended to be saturated under the same substrate concentration, and the ability to improve the recovery rate was reduced and the recovery rate stabilized, It was found that the GLN residues acted by TG were located in loosely structured protein molecules [22], and with the increase of TG addition ratio, there was insufficient substrate suitable for enzyme polymerization (Figure 3).

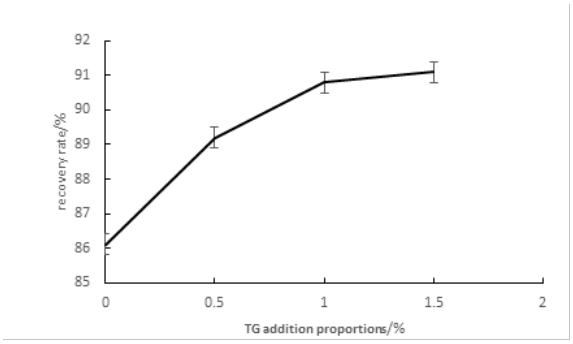

Figure 3: Changes of protein recovery with different TG enzyme addition ratios.

CONCLUSION

The effect of adding different proportions of TG to soy milk in the production of soybean protein isolate on the isoelectric point of acid precipitation was studied. It was found that:

- TG could promote the cross-linking of soy protein and increase the protein sedimentation rate at normal soy protein isoelectric points, which was beneficial to improve the recovery rate of soy protein and reduce the protein loss in soy whey.
- The addition of TG to soy milk could change the pH of the isoelectric point of soy protein, and compared with the isoelectric point pH value of the normal process, the soy protein had lower solubility and less protein loss during acid precipitation.
- Considering the cost and market value of industrial production, it is appropriate to add 1% TG to the production of soy protein isolate, with the development of technology, the cost of TG enzyme will be reduced, and the use of TG enzyme will have room to increase.
- The addition of TG could improve the recovery rate of soy protein, thereby improving the utilization of soybean, reducing the difficulty of whey wastewater treatment, reducing the environmental pollution of the soybean intensive processing industry, and promoting the development of the soybean protein industry. The changes of the primary structure, secondary structure, tertiary structure and quaternary structure of soybean protein formed after TG enzyme treatment, the number of cross-linked protein, and the corresponding functional characteristics of soy protein need to be systematically studied, so as to further optimize the use of TG enzyme in the soy protein industry and promote the sustainable development of the soy protein industry.

DISCLOSURES

Conflict of 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.

Acknowledgements

This work was supported by the Major scientific and technological innovation project in Shandong Province [Grant Number: 2022CXGC010603, 2022], and the Major project of engineering science and technology in Heilongjiang Province [Grant number: SC2021ZX04B0052, 2021]. This project was a collaboration between Shandong Yuwang Ecogical Food Industry Co., Ltd, and the Kedong Yuwang Soy Protein Food Co., Ltd.

REFERENCES

1. Guo Z, Huang Z, Guo Y, Li B, Yu W, Zhou L, et al. Effects of high-pressure homogenization on structural and emulsifying properties of thermally soluble aggregated kidney bean (Phaseolus vulgaris L.) proteins. Food Hydrocoll. 2021; 119(1):106835.
2. Tian R, Feng J, Sui X, Jiang L. Effect of High Intensity Ultrasound on the Conformational and Physicochemical Properties of Soy 7S and 11S Globulin. J Food Sci Technol. 2022; 43(5):87-97.
3. XIE J, Lian JW, Han D, Ren GX, Wang J. Effects of Soy Protein Isolate on Cake Quality and Texture. J Food Sci Technol. 2019; 40(17):19-24.
4. Wang X, Yu M, Wang Z, Luo LS. Effect of Pre-Aggregation with Different Salt Ions on Gel Properties of Soy Protein Isolate Based on Two-Step Gelation Method. J Food Sci Technol. 2022; 40(1):65-75.
5. Luan B, Ma S, Pan W, Zhu ZB. Study on Rheological Properties of Soybean Protein Isolate Based on Kneading and Diffusion Wave Spectroscopy. J Food Sci Technol. 2022; 40(2):45-52.
6. Guo S, Xu J, Liu X, Wu Y. Efficient Utilization and Technological Innovation of Plant-Based Protein Resources in China. J Food Sci Technol. 2019; 37(6):8-15.
7. Li Y, Yan S, Xun J, Xie F, Wu L, Qi B. Effects of Complexation with EGCG on Structural and Functional Properties of Soybean Protein Treated by Ultrasound-assisted Alkali. Trans Chin Soc Agri Mach. 2021; 52(2):364-370.
8. Deng W, Xu J, Guo S, Li Z. Comparative Study on Nutritional Evaluation of Hemp Seed Protein and Soybean Protein. Sci Technol Food Ind. 2021; 42(23):273-279.
9. Shi Y, Liu X, Li S, Ma J, Liu J, Qu L. Flavor Control of Soy Protein Isolate by GC-IMS. Trans Chin Soc Agri Mach. 2021; 52(02):355-363.
10. Wang R, Sun X, Wang Y. Review on the Difference of Structure and Function between Soy Protein and Wheat Protein. Grain Processing. 2016; 41(03):32-36.
11. Chi Y. New technology of soybean protein processing. 2008
12. Li S. Affinity Precipitation Systems with pH/Thermo-Responsive Polymer in Protein Purification and its Molecular Interaction Mechanism Research. East China Univ Sci Technol. 2018.
13. Song P. Study on the Selection Specifity of Transglutaminase on Cross-linking Site. Tianjin Univ Sci Technol. 2020.
14. Sun X, Qiao Y. Extraction and Isoeclectric Point Determination of Protein from Ginkgoes. Food Ind. 2012; 33(9):156-158.
15. Shi Y, He D, Liu J, Lu X, Li C. Effect of glutamine transaminase on production of soybean protein isolate. China Oils Fats. 2018; 43(4):25-27.
16. Li H, Li C, Zhang T, Li h, Yu J. Advance in protein glycosylation catalyzed by transglutaminase. China Oils Fats. 2020; 45(4):18-23.
17. Sun C, Wang Y, Weng L, Liu M, Miao S, Liu G, et al. Effects of transglutaminase on gel properties of buckwheat protein isolate. Sci Technol Food Ind. 2022 43(24):112-122.
18. Gaspar ALC, Favoni SPDG. Action of microbial transglutaminase (MTGase) in the modification of food proteins: A review. Food Chem. 2022; 171(1):315-322.
19. Chen X, Wu W, Zhou J. Protein cross-linking by tyrosinase, laccase and transglutaminase: a comparative study. Chinese J Biopr Eng. 2023; 21(2):176-183.
20. Liu Y. Study on Gelation Mechanism and Gel Properties of Gelatin Cross-linked by Transglutaminase. Tianjin Univ Sci Technol. 2019.

21. Spolaore B, Raboni S, Molina AR, Satwekar A, Damiano N, Fontana A. Local unfolding is required for the site-specific protein modification by transglutaminase. Biochemistry. 2012; 51(43):8679-8689.

22. Song P. Study on the Selection Specifity of Transglutaminase on Cross-linking Site. Tianjin Univ Sci Technol. 2020.