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EFFECT OF pH-SHIFTING TREATMENT ON THE GEL PROPERTIES OF PUMPKIN SEED PROTEIN ISOLATE

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Abstract

pH-shifting treatment is a novel method to modify protein, which can improve the functional properties and application of protein in food products. In the present study, the influence of pH-shifting method on heat induced gel properties of pumpkin seed protein isolate (PSPI) was investigated. PSPI, treated under different pH values (pH 2, pH 4, pH 6, pH 8, pH 10, and pH 12) were labeled as PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12, respectively. The water-absorbing ability, textural analysis (hardness, adhesiveness, and cohesiveness) of the gels formed by different pH-shifting treated PSPI were investigated. Compared with that of control, the water-absorbing ability of PSPI 2, PSPI4, and PSPI 12 showed increased ($p<0.05$) value significantly. The textural analysis of PSPI gels showed that only the hardness of PSPI 6 showed increased ($p<0.05$) value. The cohesiveness of PSPI samples after treating at pH 2, pH 4, pH 6, pH 8, pH 10, and pH 12 showed no significant ($p > 0.05$) difference compared to control. The adhesiveness of PSPI 2, PSPI 4, and PSPI 10 showed significant ($p < 0.05$) decreasement while the adhesiveness of PSPI 6, PSPI 8 and PSPI 12 didn't show any significant ($p > 0.05$) difference compared to control. These results indicated that pH-shifting treatment might be a convenient and economical method to change the gel properties of PSPI, which would provide a fundamental knowledge for further utilization of PSPI in food products.

Keywords: protein isolate; pumpkin seeds; vegetable meal; gel; vegetable proteins; vegetarian food; pH-treatment; modified protein.

ВПЛИВ pH-КОРЕГУЮЧОЇ ОБРОБКИ НА ГЕЛЕУТВОРЮЮЧІ ВЛАСТИВОСТІ ІЗОЛЯТУ ПРОТЕЇНУ НАСІННЯ ГАРБУЗА

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Анотація

Обробка зі зміною pH – це новий метод модифікації білка, який може поліпшити функціональні властивості і застосування білка в харчових продуктах. У цьому дослідженні було вивчено вплив методу зміни pH на властивості гелю, індукованого нагріванням ізолятів білка насіння гарбуза (ІБНГ). Обробка зі зміною pH - це новий метод модифікації білка, який може поліпшити функціональні властивості і застосування білка в харчових продуктах. У цьому дослідженні було вивчено вплив методу зміни pH на властивості гелю, індукованого нагріванням ізолятів білка насіння гарбуза (ІБНГ). ІБНГ, оброблені при різних значеннях pH (pH 2, pH 4, pH 6, pH 8, pH 10 і pH 12), були позначені як ІБНГ 2, ІБНГ 4, ІБНГ 6, ІБНГ 8, ІБНГ 10 і ІБНГ 12 відповідно. Були досліджені водопоглинаюча здатність та текстурний аналіз (твердість, адгезивність і когезивність) гелів, утворених за допомогою ІБНГ, обробленого за допомогою різних змін pH. У порівнянні з контролем, водопоглинаюча здатність ІБНГ 2, ІБНГ 4 і ІБНГ 12 значно збільшилася ($p < 0.05$). Текстурний аналіз гелів ІБНГ показав, що тільки твердість ІБНГ 6 показала підвищений ($p < 0.05$) значення. В'язкість зразків ІБНГ після обробки при pH 2, pH 4, pH 6, pH 8, pH 10 і pH 12 не показала значущої ($p < 0.05$) різниці в порівнянні з контролем. Крім того, адгезія ІБНГ 2, ІБНГ 4 і ІБНГ 10 показала значного ($p < 0.05$) зниження, в той час як адгезійна здатність ІБНГ 6, ІБНГ 8 і ІБНГ 12 не показала якої-небудь значущої ($p < 0.05$) різниці в порівнянні з контролем. Ці результати показали, що обробка зі зсувом pH може бути зручним і економічним методом зміни властивостей гелю PSPI, що дасть фундаментальні знання для подальшого використання PSPI в харчових продуктах.

Ключові слова: ізолят протеїна; насіння гарбуза; рослинний шрот; гель; рослинні протеїни; вегетаріанська їжа; pH-обробка; модифікований білок.

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Introduction

Pumpkin seed, which is the matured seed of pumpkin, is abundant in oil, protein, vitamins, and minerals, and other nutrients. Pumpkin seeds have a wide range of resources [1]. It has been recognized as health food and has great development potential due to its high nutritional value. Pumpkin seed is a by-product of pumpkin. It is cheap, and easy to obtain, but mostly abandoned, which caused a waste of resources and environmental pollution. In fact, pumpkin seeds after defatted oil are rich in protein and are a high-quality plant protein resource [2]. Pumpkin seeds have a protein content around 30–40 %, especially for defatted American pumpkin seeds. It can reach up to 66.54 %, which is much higher than the protein content of soybeans (48.32 %). Pumpkin seeds contain a variety of essential amino acids that are well balanced, including eight essential amino acids and histidine for children, and have a proportion of essential amino acids which is like the pattern of amino acid composition required by the human body [3]. The total amino acids of the American pumpkin seeds were 527.1 mg/g, higher than the soybean seeds (456.5 mg/g) and its essential amino acid content is about 180.7 mg/g, which accounted for 34.3 % of the total amino acids. The absorption rate of pumpkin seed protein in the human body can reach 88–97 %, and the physiological valence is 73–86 % [4].

Proteins have many functional properties, such as gel forming ability, emulsifying ability, foaming ability, and so on. Extracting methods have an effect on the functional properties of protein isolate. It has been reported that different preparation methods of cottonseed meals influence the physicochemical and functional properties of the cottonseed protein isolate [5]. High intensity ultrasound (HIUS) has an effect on rheological and structural properties of the sunflower protein isolates [6]. Gel-forming ability is one of the most important properties of proteins. During heating, the internal protein groups might unfold, interact, and then form a network structure of gel. Protein gels can give proteins with high viscosity, elasticity, and plasticity. As a carrier of water and flavor substances, such as sugars and other substances, gels formed by proteins are widely used in food processing. The hardness and water-absorbing ability are two important indicators to describe the gel-forming ability of proteins.

There are some techniques to change the gel properties of proteins, like high pressure method, cold plasma, and pH-shifting treatment. Investigations have been carried out to modify the structure of plant proteins like peanut protein, soybean protein and fish protein to achieve ideal gel properties. For example, high-pressure treatment was conducted to improve the gel property of peanut protein isolate (PPI) [7]. It suggested that the hardness of thermal induced PPI gel was significantly improved [7]. Results indicated that the addition of salt ions can change the crystalline state of gels system [8]. Besides, salt ions, extracting methods, and cellulose nanocrystals were reported to have an influence on the protein gel property. The addition of calcium ion can improve the gel properties and gelation of tilapia (Oreochromis niloticus) protein isolates processed with pH shift method [9]. Heat-induced whey protein isolate gels were improved by cellulose nanocrystals [10]. Furthermore, it has been reported that the water-absorbing ability of the thermal induced PPI gel could be improved by cold plasma treatment [11]. The penetration force of soy protein isolate gel could be increased significantly ($p < 0.05$) by the binding of pH-shifting and heating treatments [12]. Among them, pH-shifting is a convenient way which can make protein in an unfolded state by adjusting pH to an extremely acidic or alkaline environment. As a result, the globular protein will undergo a conformational change, namely “molten globule” structure, which will be refolded to the native state when the pH of the mixture is altered to 7 [13]. As reported, the hardness and water-absorbing ability of peanut protein isolate gel showed significantly improvement after pH-shifting treatment at pH 10 [13]. The gel forming capacity of fish proteins could be also improved by pH-shifting treatment [14]. As it is known to all, soybean protein is widely applied in meat products, such as sausages, meat balls and minced meat. Water-absorbing ability and hardness of thermal induced soybean protein gel have an impact on the texture properties, water, saccharide, and flavor substances holding capacity of meat products [15]. It has been investigated that the gel properties like water-absorbing ability and texture quality of heat-induced proteins have a big influence on the quality of Tofu-type products [16].

In order to apply the pumpkin seed protein into the food products, it is important to analyze and improve the gel-forming ability of the pumpkin seed protein. In this study, the effect of pH-shifting treatment on gel properties of pumpkin seed protein isolate (PSPI) were investigated. PSPI was dealt with a series of pH

values (2, 4, 6, 8, 10, 12) to form a molten globular structure, and then refolded upon adjustment of pH to 7.0. Samples after pH-shifting processing (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12) were determined for water-absorbing ability, and textural analyses in this study, which would provide a fundamental knowledge for further utilization of PSPI in food products.

Materials and methods

Materials. Pumpkin seeds were purchased from Alibaba in China. All chemicals used in this experiment were of analytical grade.

Preparation of pumpkin seed protein isolate (PSPI). In the present study, PSPI was obtained from defatted pumpkin seed meal by using the method of alkali solution and acid precipitation. Defatted pumpkin seed meal was suspended in deionized water and the pH of suspension was altered to 10.5. By extracting for 1 h, the pumpkin seed meal suspension was centrifuged (4000 × g) for 15 min. The extracts were acidified to pH 4.5 after filtering. Later, after centrifugation for 20 min at 4000 × g, the protein precipitates were gained and then freeze-dried for further investigation.

pH-shifting treatment of PSPI. The pH of native PSPI suspension was altered to 2, 4, 6, 8, 10, and 12 by 1 mol/L HCl or 1 mol/L NaOH solutions, respectively. All pH-shifting treatments were conducted for 1 h and then adjusted to neutral pH. The mixture was dialyzed and then freeze-dried. In this study, PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12 were applied to indicate the samples, which is processed by pH-shifting treatment for 1 h at pH 2, pH 4, pH 6, pH 8, pH 10 and pH 12, respectively. Except pH- shifting treatment, the control PSPI was achieved using the same steps with PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12. All prepared samples were kept at 4 ℃ for further investigation.

Preparation of PSPI gel. PSPI dispersion (20 %, w/w), prepared with deionized water, was incubated at 75-90°C in a water bath for 1 h. The obtained PSPI gels were kept at 4 ℃ for 24 h. After equilibration at 25 ℃ for 30 min, the PSPI gels were applied to determine the water-absorbing ability (WAA).

Water-absorbing ability (WAA, %). The WAA was analyzed by the reported method [17]. The weighted PSPI gel (W_1) was centrifuged (10,000×g) for 15 min, and subsequently inverted on the filter paper for 20 min. After that, the PSPI gel was weighed (W_2) again. The WAA was calculated as:

$$WAA\ (\%) = W_2/W_1 \times 100\%$$

Textural analysis. The textural analysis was conducted Using TA. XT plus Texture Analyzer with a P 0.5 probe according to a previous method with minor modification. The post-test, test, pre-test speeds, strain, and trigger force were set as 2.0 mm/s, 1.0 mm/s, 1.0 mm/s, 40 %, and 5 N, respectively. By textural analyses, the hardness, cohesiveness, and adhesion were determined. Hardness means the peak force needed to break the gel. All measurements were carried out in sextuplicate.

Statistical analysis. All measurements were performed for three times unless otherwise specified. Results were presented as the mean value ± standard deviation (SD). A one-way analysis of variance (ANOVA) was used to measure the significant difference ($p<0.05$) among independent variables. SPSS software (version 26.0, SPSS Inc., Chicago, IL, USA) was utilized for data processing and analysis.

Results and Discussion

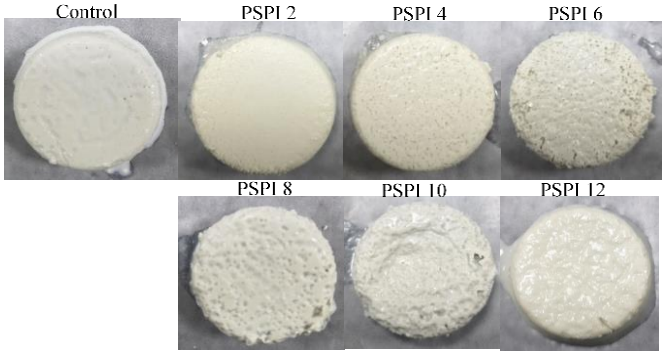

Fig. 1. Pictures of control PSPI gel and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12).

Textural analysis of PSPI gel. Gel is a dispersion system in which molecules are interconnected to form a network structure under specific conditions. Proteins are primarily

responsible for gelation, and therefore play an important role in the formulation of gelled food. Texture is an essential sensory quality attribute when assessing consumer acceptability of food products [18]. Texture profile analysis (TPA), known as "two bite tests", can simulate the mouth's chewing action and can give an insight into the samples' behavior. It is a popular method to determine the textural properties of food products by a double compression test [19]. In this study, parameters including hardness, adhesiveness and cohesiveness were conducted by the texture profile analysis to compare the gels formed by PSPI after pH-shifting treatment [20]. The definition of these three parameters was showed in Table 1. The pictures of control PSPI and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12) were showed in Fig. 1.

Table 1

The definition of parameters tested by TPA

Parameters	Definitions
Hardness	Mechanical textural attribute correlated with the force needed to compress the sample.
Adhesiveness	Force needed to remove substance adhered to the mouth. Work needed to overcome between the sticky forces of the sample and the probe
Cohesiveness	Mechanical textural attribute correlated with the degree of deformation before food breaks

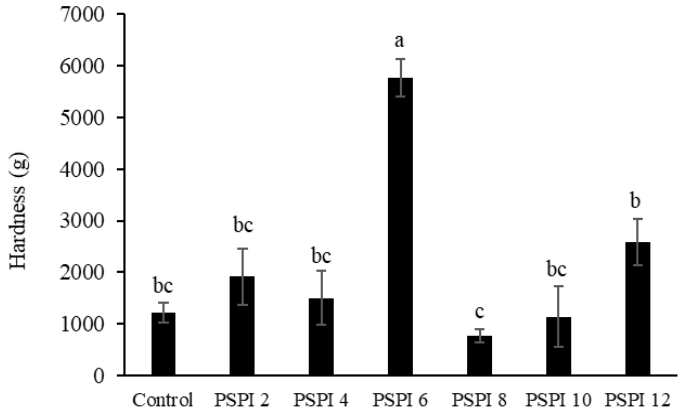

Fig. 2. Hardness of control PSPI gel and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12)

Hardness is described as the force to make a product produce deformation at a given distance. Fig. 2 showed the hardness of the PSPI gels. Meanwhile, no significant ($p > 0.05$) difference was observed at the hardness of PSPI 2, PSPI 4, PSPI 8, PSPI 10, and PSPI 12 compared to that of control, while the hardness of PSPI 6 showed significant ($p < 0.05$) increased value. This may be because that pH-shifting treatment under pH 6 allowed the proteins to partially unfold and exposed more active sites such as free sulfhydryl group, which will promote the protein-protein interaction via hydrophobic associations and disulfide bridging upon heating [21].

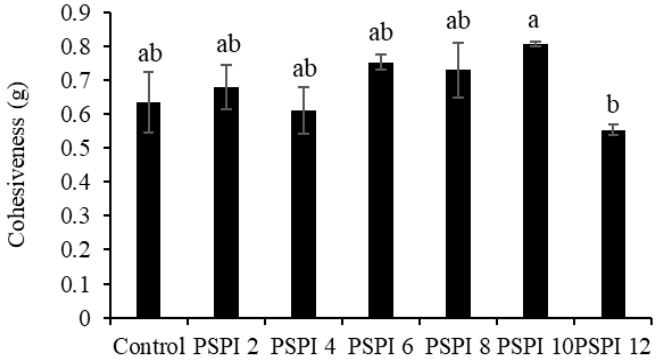

Fig. 3. Cohesiveness of control PSPI gel and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12).

Cohesiveness is supposed to be an indication of the gel's ability to keep an intact network structure. It is a parameter to describe how well the gel endures a second deformation which is bound up with its behavior during the deformation of the first time. As shown in Fig. 3, there was no significant ($p > 0.05$) difference between cohesiveness values of control PSPI and PSPI samples after pH-shifting treatments (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12), which might indicate that pH-shifting treatment can't improve the cohesiveness of gels formed by pumpkin proteins.

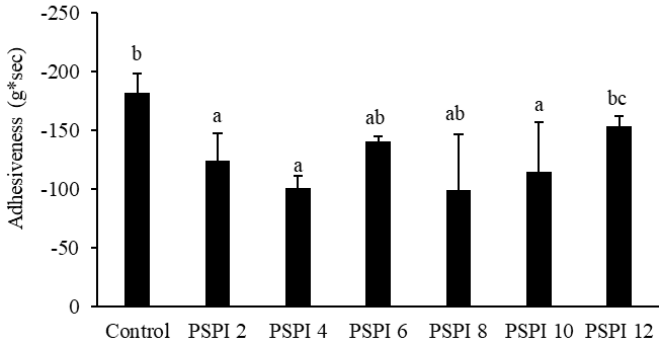

Fig. 4. Adhesiveness of control PSPI gel and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12)

The adhesiveness, which is applied to measure the textural properties of gels, is corresponding to the attractive force to be overcome between the surface of the gel and the surface of the probe. Compared to control, the adhesiveness of PSPI 2, PSPI 4, and PSPI 10 showed significant ($p < 0.05$) decreasement. The adhesiveness of PSPI 6, PSPI 8 and PSPI 12 didn't show any significant ($p > 0.05$) difference, which might indicate that pH-shifting treatment can't improve the adhesiveness of gels formed by pumpkin proteins.

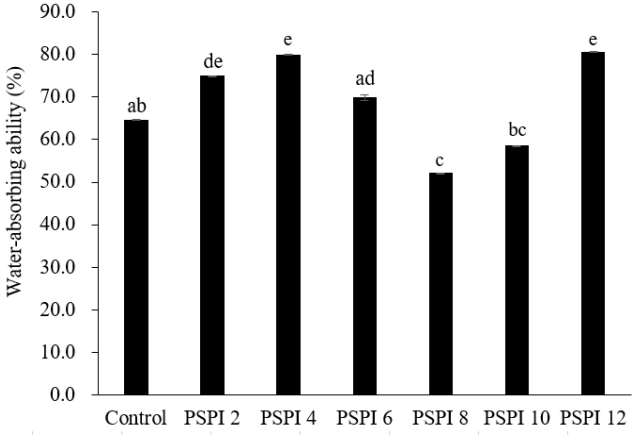

Fig. 5. Water-absorbing ability of control PSPI gel and pH-shifting treated PSPI gels (PSPI 2, PSPI 4, PSPI 6, PSPI 8, PSPI 10, and PSPI 12)

Water-absorbing ability (WAA) of PSPI gel. WAA is a vital indicator to describe the water-absorbing ability of gel. Plant protein with good WAA is more suitable to be added in meat products, such as meat patties, sausages, meatballs and so on. Compared with that of control PSPI, the WAA of PSPI 8 showed significantly decreased ($p<0.05$) value, it might suggest that the gel didn't have a relatively compact structure, which resulted in less trapped water and lowered WAA [13]. Besides, the WAA of PSPI 2, PSPI 4, and PSPI 12 showed significantly increased value, which indicated that the gels of PSPI 2, PSPI 4, and PSPI 12 had a stronger binding ability to water. It has been suggested that appropriate pH-shifting process can expose more active sites and regions of protein, which led to a firmer gel with more compact structure and trapped water [13]. Water-absorbing ability of PSPI varied, depending on the pH-adjustment process applied [22].

Conclusion

Gel-forming ability during heating is one of the most important functional characteristics and has an effect on the quality of gelled food. In the

present study, the heat induced gel properties of PSPI after pH-shifting treatment were investigated by the indicators of water-absorbing ability, hardness, and water state. It showed that the water-absorbing ability of PSPI 2, PSPI 4, and PSPI 12 showed significantly ($p < 0.05$) improved value. The hardness of PSPI 6 also showed significantly ($p < 0.05$) improved value, while the cohesiveness of PSPI samples after treated at pH 2, pH 4, pH 6, pH 8, pH 10, and pH 12 showed no significant ($p > 0.05$) difference compared to control. The adhesiveness of PSPI 2, PSPI 4, and PSPI 10 showed significant ($p < 0.05$) decresement while the adhesiveness of PSPI 6, PSPI 8, and PSPI 12 didn't show any significant ($p > 0.05$) difference compared to control. These results might provide a fundamental knowledge for further utilization of PSPI in food products.

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References

[1] Caili, F., Huan, S., Quanhong, L. (2006). A review on pharmacological activities and utilization technologies of pumpkin. *Plant Foods Hum Nutr*, 61(2), 73–80.

[2] Gao, D. H. A., Duan, Z. (2022). Development of Pumpkin Seed Meal Biscuits. *Eastern-European Journal of Enterprise Technologies*, *2*(11(116)), 36–42.

[3] Vinayashree, S., Vasu, P. (2021). Biochemical, nutritional and functional properties of protein isolate and fractions from pumpkin (Cucurbita moschata var. Kashi Harit) seeds. *Food Chemistry*, 340, 128177.

[4] Longe, O. G., Farinu, G. O., Fetuga, B L. (1983). Nutritional value of the fluted pumpkin (Telfaria occidentalis). *Journal of Agricultural and Food chemistry*, *31*(5), 989–992.

[5] Ma, M., Ren, Y., Xie, W., Zhou, D., Tang, S., Kuang, M., Wang, Y., Du, S. K. (2018). Physicochemical and functional properties of protein isolate obtained from cottonseed meal. *Food Chem*, 240, 856–862.

[6] Malik, M.A., Saini, C.S. (2018). Rheological and structural properties of protein isolates extracted from dephenolized sunflower meal: Effect of high intensity ultrasound. *Food Hydrocolloids*, 81, 229–241.

[7] He, X. H., Liu, H. Z., Liu, L., Zhao, G. L., Wang, Q., Chen, Q. L. (2014). Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. Food Hydrocolloids, 36, 123-129. (2014). Effects of high pressure on the physicochemical and functional properties of peanut protein isolates. *Food Hydrocolloids*, 2014. 36: p. 123-129.

[8] Wang, W., Shen, M., Liu, S., Jiang, L., Song, Q., Xie, J. (2018). Gel properties and interactions of Mesona blumes polysaccharide-soy protein isolates mixed gel: The effect of salt addition. *Carbohydr Polym*, 192, 193–201.

[9] Zhou, Y., Yang, H. (2019). Effects of calcium ion on gel properties and gelation of tilapia (Oreochromis niloticus) protein isolates processed with pH shift method. *Food Chem*, 277, 327–335.

[10] Xiao, Y., Liu, Y., Wang, Y., Jin, Y., Guo, X., Liu, Y., Xi, X., Lei, H., Xu, H. (2020). Heat-induced whey protein isolate gels improved by cellulose nanocrystals: Gelling properties and microstructure. Carbohydr Polym, 231, 115749.

[11] Ji, H., Dong, S., Han, F., Li, Y., Chen, G., Li, L., Chen, Y. (2017). Effects of Dielectric Barrier Discharge (DBD) Cold Plasma Treatment on Physicochemical and Functional Properties of Peanut Protein. *Food and Bioprocess Technology*, *11*(2), 344–354.

[12] Liu, Q., Geng, R., Zhao, J., Chen, Q., & Kong, B. (2015). Structural and Gel Textural Properties of Soy Protein Isolate When Subjected to Extreme Acid pH-Shifting and Mild Heating Processes. *J Agric Food Chem*, *63*(19), 4853-61.

[13] Li, J., Wu, M., Wang, Y., Li, K., Du, J., Bai, Y. (2020). Effect of pH-shifting treatment on structural and heat induced gel properties of peanut protein isolate. *Food Chemistry*, 325, 126921.

[14] Hultin, H. O., K.S.D. (1999). Process for isolating a protein composition from a muscle source and protein composition: U.S. Patent 6, 005(073), 12–21.

[15] Niu, H., Li, Y., Han, J., Liu, Q., Kong, B. (2017). Gelation and rheological properties of myofibrillar proteins influenced by the addition of soybean protein isolates subjected to an acidic pH treatment combined with a mild heating. *Food Hydrocolloids*, 70, 269–276.

[16] Yasir, S. B. M., Sutton, K. H., Newberry, M. P., Andrews, N. R., & Gerrard, J. A. (2007). The impact of transglutaminase on soy proteins and tofu texture. *Food Chemistry, 104*(4), 1491–1501.

[17] Hu, H., Fan, X., Zhou, Z., Xu, X., Fan, G., Wang, L., Huang, X., Pan, S., Zhu, L. (2013). Acid-induced gelation behavior of soybean protein isolate with high intensity ultrasonic pre-treatments. *Ultrason Sonochem, 20*(1), 187–195.

[18] Yu, B., Ren, F., Zhao, H., Cui, B., Liu, P. (2020). Effects of native starch and modified starches on the textural, rheological and microstructural characteristics of soybean protein gel. *International Journal of Biological Macromolecules*, 142, 237–243.

[19] Lanza, B., Amoruso, F. (2018). Measurement of kinaesthetic properties of in-brine table olives by microstructure of fracture surface, sensory evaluation and texture profile analysis (TPA). *J Sci Food Agric, 98*(11), 4142–4150.

[20] Nishinari, K., Kohyama, K., Kumagai, H., Funami, T., Bourne, M. C. (2013). Parameters of texture profile analysis. *Food Science and Technology Research, 19*(3), 519–521.

[21] Li, Y., Wang, Q., Guo, L., Ho, H., Wang, B., Sun, J., Xu, X., Huang, M. (2019). Effects of ultrafine comminution treatment on gelling properties of myofibrillar proteins from chicken breast. *Food Hydrocolloids*, 97, 105199.

[22] Yongsawatdigul, J., Hemung, B.O. (2010). Structural changes and functional properties of threadfin bream sarcoplasmic proteins subjected to pH-shifting treatments and lyophilization. *J Food Sci, 75*(3), 251-7.