

# UDC 664.38:635.641.51-026.565.2 THE EFFECT OF HIGH PRESSURE ON SOY PROTEIN FUNCTIONAL FEATURES: A REVIEW

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#### Abstract

The aim of this work is to research the effect of high pressure on soy protein and its main components: 7S and 11S glycinins; changes of soy protein technological and functional features.

Results: soy protein, 7S and 11S glycins functional features processed by high pressure are analyzed, as well as their emulsion features, ability to retain water, gel features. The impact of pressure, time and temperature of the process on soy protein is researched. Structural changes of 7S and 11S glycinins, conformations, technological and functional features are analyzed. Soy protein isolate processing with high pressure improves its rheological, gel features and moisture content. Due to reasonable parameters soy protein and 7S and 11S glycinins processing with a high pressure increased moisture content; improved gel and emulsion features; influenced on non-covalent and covalent bonds and protein conformation; decreased soy protein's allergenicity in food products, including baby formulas. Despite great efforts, the mechanism of soy protein and 7S and 11S glycinins processing is still insufficiently understood, which makes it difficult to get a clear and unambiguous idea of their behavior.

Conclusions. The use of high pressure and soy protein isolate combination can improve functional and consumptional features of soy protein and food safety.

Key words: high pressure; functional properties; soy protein; 7S glycinin; 11S glycinin; allergenicity.

# ВПЛИВ ВИСОКОГО ТИСКУ НА ФУНКЦІОНАЛЬНІ ВЛАСТИВОСТІ СОЄВИХ БІЛКІВ. ОГЛЯД

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#### Анотація

Мета. Дослідити вплив високого тиску на соєвий білок та його головні інгредієнти - гліциніни 7S, 11S та зміни технологічних та функціональних властивості соєвих білків. Результати. Проаналізовано функціональні властивості соєвого білка, 7S та 11S гліцинінів, оброблених високим тиском, їх емульсійні властивості, здатність утримувати воду, властивості гелю. Досліджено вплив значень параметрів тиску, часу та температури процесу на соєвий білок, 7S та 11S гліциніни за змінами їх структури, конформацій, технологічних та функціональних властивостей. Обробка високим тиском ізолята соєвого білка покращує їх реологічні властивості, властивості гелю та вміст зв'язаної вологи. Обробка високим тиском при обгрунтованих параметрах процесу соєвого білка, гліцинінів 7S та 11S збільшила вміст зв'язаної вологи, покращила властивості гелю та емульсії, впливає на нековалентні та ковалентні зв'язки та конформацію білка, а також зменшує алергенність соєвих білків у харчових продуктах, включаючи дитячі суміші. Незважаючи на великі зусилля, механізм обробки високим тиском соєвого білка, 7S та 11S гліцинінів все ще недостатньо зрозумілий, що ускладнює отримання чіткого і однозначного уявлення про їх поведінку. Висновки. Використання технології високого тиску при обробці ізоляту соєвого білка може поліпшити функціональні та споживчі властивості соєвого білка та безпеку харчових продуктів. *Ключові слова:* високий тиск; функціональні властивості; соєвий білок; 7S гліцинін; 11S гліцинін; алергенність

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# ВЛИЯНИЕ ВЫСОКОГО ДАВЛЕНИЯ НА ФУНКЦИОНАЛЬНЫЕ СВОЙСТВА СОЕВЫХ БЕЛКОВ. ОБЗОР

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#### Аннотация

Цель. Исследовать влияние высокого давления на соевый белок и его основные ингредиенты - глицинины 75, 115 и описать технологические и функциональные свойства соевых белков. Результаты. Проанализированы функциональные свойства соевого белка, 75 и 115 глицининов, обработанных высоким давлением, их эмульсионные свойства, влагоудерживающую способность, свойства геля. Исследовано влияние величины давления, времени и температуры процесса на соевый белок, глицинины 75 и 115 по изменению их структуры, конформации, функциональных и технологических свойства Собработка изолята соевого белка высоким давлением улучшает их реологические свойства, свойства геля и содержание связанной влаги. Обработка высоким давлением при рациональных параметрах процесса соевого белка, 75 и 115 глицининов увеличивает количество связанной влаги, улучшае свойства геля и эмульсии, влияет на нековалентные и ковалентные связи и конформацию белка, а также снижает аллергенность соевых белков в пищевых продуктах, включая детское питание. Несмотря на большие усилия, механизм обработки соевого белка, 75 и 115 глицинінов под высоким давлением, все еще недостаточно изучен, что затрудняет получение четкого и однозначного представления об их поведении. Выводы. Использование технологии высоким давлением при обработке изолята соевого белка может улучшить функциональные и потребительские свойства соевого белка и безопасность пищевых продуктов.

*Ключевые слова:* высокое давление; функциональные свойства; соевый протеин; 7S глицинин; 11S глицинин; аллергенность.

#### Introduction

Soy has been planted in China for more than 5000 years. Until 1930s, soy cultivation has spread all over the world. Because of it's high yield, high protein content, rich nutrition and good functional characteristics, which has become an important food resource for human beings, and is one of the world's most important economic crops [1; 2].

The rich nutrition of soy protein is mainly reflected in three aspects: (1) The digestibility of soy protein is comparable with that of meat, milk and eggs. The protein digestibility of different soy products is different. The protein digestibility of cooked soy, for example, is only 65 %, that of tofu protein is 93 %, and that of soy protein isolated is 93–97 %. (2) It is well known, soy protein has a low content of sulfur-containing amino acids. The first limiting amino acid is methionine. However, soy protein is rich in lysine, an amino acid that most cereal proteins lack. Soy protein is similar to meat, fish, eggs, milk. It is a full-valent protein. (3) Some studies have shown that the essential amino acid content of soy protein is higher than the recommended intake for all age groups except infants. Soy protein has relatively low levels of sulfur-containing amino acids compared to the recommended intake for infants [3–8].

The physical methods of food processing that are used in modern food technologies lead to changes in the functional properties of products. High pressure processing of raw materials and food products at various stages of their production allows you to effectively manage their functional, technological and consumer properties [9–12].

1. Functional properties of soy protein. The protein content of soy protein isolate is more than 90 %. It is a high-quality plant protein food raw materials. It's functional properties can be divided into three categories: (1) Interface properties, mainly including emulsification and foaming properties. Soy protein is a kind of surfactant, which has both hydrophilic and hydrophobic groups. It can be adsorbed on the oil-water or airwater interface. Once adsorbed at the interface, the protein will form a film, which can prevent small droplets or bubbles from accumulating and help to stabilize the emulsion and bubbles. (2) Hydration properties, including wettability, dispersability, solubility, viscosity and water retention. The hydration of the polar groups on the skeleton of sov protein peptide chain with water molecule is determined by the interaction between protein and water. (3) Properties related to protein-protein interactions, including precipitation, aggregation, and gel properties. Protein molecules are stretched by heat, and the internal hydrophobic groups are exposed. Through hydrophobic interaction, electrostatic interaction, hydrogen bond or disulfide bonds cross-linking, the spatial network structure is formed [13-16]. On the basis, hundreds of countries in the world have developed thousands of food products containing soy protein in recent year. Soy protein and its modified products are widely used in meat products, protein drinks, dairy products, baked products and other foods due to their prominent functional properties. They play an important role in supplementing protein, supplementing the nutrition of multiple types of protein, reducing the intake of animal protein, and giving food health care functions. Therefore, the functional properties of proteins are very important to food manufacturing and processing, they directly affect the quality of products.

The physical methods of food processing that are used in modern food technologies lead to changes in the functional properties of products. High pressure processing of raw materials and food products at various stages of their production allows you to effectively manage their functional, technological and consumer properties [17].

2. Components of soy protein. Soy protein is mainly composed of  $\beta$ -conglycinin (7S globulin) and globulin (11S), accounting for over 70 % of the total protein content [18; 19]. The 7S globulin is a trimer formed by the different combinations of three subunits ( $\alpha'$ ,  $\alpha$  and  $\beta$ ), which are bound by hydrophobic and hydrogen bonds. The molecular weights of  $\alpha'$ ,  $\alpha$  and  $\beta$  are 65 kDa, 62 kDa and 57 kDa, respectively. Each 7S globulin contains a small number of disulfide bonds and is free of sulfhydryl groups [20]. The 11S consists of six subunits. It's weight is 340-375 kDa. Each of which consists of an acidic polypeptide chain (A) and an alkaline polypeptide chain (B) connected by a disulfide bond to form the AB subunit. The 11S molecule contains more disulfide bonds and sulfhydryl groups [21; 22]. The differences in structure between 7S and 11S was affect on the formation of gel. Some studies have reported that 11S has a better gel properties than that of 7S, but the emulsion capacity of 11S is lower [23–25].

The function properties of soy protein were affected by the concentration, temperature, pH, and so on [26; 27]. Such as, soy protein isolate concentration is one of the decisive factors in gel formation. The formation of soy protein isolate gel is the result of protein-protein and proteinsolvent interactions, and the balance of attractive and repulsive forces between adjacent peptide chains. When the soy protein isolate concentration is low, protein-solvent interaction dominates, making it difficult for the system to form gel [28]. Therefore, the gel strength is positively correlated with soy protein isolate concentration. However, when the soy protein isolate concentration is lower than 8.0 %, the gel cannot be formed only by heating. However, if the formation concentration of soy protein isolate gel can be changed to a certain extent by adjusting pH value, ion strength or modification, etc [29; 30]. The other, pH and salt addition changed the ionization of functional groups of soy protein isolate and double electric layer thickness, affected the protein-protein interaction [31; 32]. The salt concentration and type have different effects on the gel properties of soy protein isolate. At low ionic strength, salt can reduce the electrostatic repulsion between protein molecules by shielding the charge on the protein, and strengthen the gel strength. The charge on the protein tends to be saturated with the increase of ionic strength, and the properties of water in the solvent change due to the presence of salt, leading to the enhancement of hydrophobic interaction, which becomes the dominant effect, and the gel strength decreases [33-35]. In research [36] studied the changes of gel formation and gel properties of soy protein isolate at different pH and ionic strength, who found that the denaturation of soy protein isolate was occurred under all conditions of pH and ionic strength, such as a low stiffness gel was formed when pH > 6.0, on the contrary, a high stiffness was formed when pH = 5.0. Meanwhile, extensive rearrangements in the network structure took place during prolonged heating when pH = 7.6, whereas at pH3.8 rearrangements did not occur.

Considering the widespread introduction of high-pressure technology in food production of soybeans and soy-containing products, it is of undoubted interest to analyze the dependence of some technological and consumer properties on the parameters of the process of their processing by high pressure.

3. The principle of high pressure processing and high pressure process equipment. High pressure processing (HPP) can be referred to as ultra high pressure technology or hydrostatic technology. The water or other incompressible fluid mediums often act as mediators of pressure. During the high pressure processing, the pressure levels generally not less than 100 MPa, the commonly used range is 100–1000 MPa and can work in the temperature range of -20 °C to 90 °C. After the food is sealed in an elastic container or placed in a pressure system, the non-covalent bonds (hydrogen bonds, ionic bonds and hydrophobic bonds, etc.) are been destroyed or formed at a certain temperature for the appropriate processing time and pressure level, which cited the enzyme in food, protein, starch and other biological high molecular substances

are deactivated, denatured and gelatinized respectively, and kill the microorganism in food biological, so as to achieve the purpose of food sterilization, preservation and processing [37].

*Basic Governing Principles.* As with heat, pressure is a basic thermodynamic variable. Strictly speaking, during HPP the effects of temperature cannot be separated from the effects of pressure. This is because for every temperature there is a corresponding pressure. Thermal effects during pressure treatment can cause volume and energy changes. However, pressure primarily affects the volume of the product being processed. The combined net effect during HPP may be synergistic, antagonistic, or additive [38].

Mathematically, the impact of pressure (p) and temperature (T) can be quantitatively related using Gibbs's definition of free energy G [39]:

$$G \equiv H - TS, \tag{1}$$

where *H* and *S* are the enthalpy and entropy, respectively. Further,

$$H \equiv U + pV, \tag{2}$$

where U = internal energy and V = volume.

It can be deduced from Equations 1 and 2 that

$$d(\Delta G) = \Delta V dp - \Delta S dT.$$
<sup>(3)</sup>

Therefore, reactions such as phase transitions or molecular reorientation depend on both temperature and pressure and cannot be treated separately. The following are some basic governing principles behind HPP.

*The fundamental principles* of hyperbaric technique are pascaline law and Le Chatelier principle. Pascaline law takes advantage of the compression effect of high pressure on liquids, which means that the pressure applied to the liquid can be transmitted to all parts of the system instantaneously at the same size. Therefore, dry food, powdery food or granular food should not be used high pressure treatment. According to Pascaline law, the effect of high pressure processing is independent of the size, shape and volume of the food. In the process of high pressure processing, the whole food will be treated uniformly, the pressure transfer speed is fast, there is no pressure gradient. Therefore, the high pressure processing of food is simpler, and the energy consumption is also significantly reduced. According to Le Chatelier principle, the external pressure reduces the volume of the pressurized system and vice versa. Therefore, the physical and chemical reactions in food ingredients will be carried out in the direction of the maximum

compression state under the pressure treatment of food. The increase or decrease of the reaction rate constant k depends on whether the "active volume" of the reaction is positive or negative. This means that high pressure processed food will force the reaction system to reduce the volume, affecting not only the reaction balance in the food, but also the reaction rate, including chemical reactions and possible changes in molecular conformation. It is well known that the mechanism of meat proteins unfolded, denaturation and formed gel caused by heat and high pressure is difference. High pressure processing induced meat gels are based on the protein volume decline, while the thermal meat gels is caused by the violent movement of molecules and destruction of non-covalent bonds.

*Principle of microscopic ordering.* At constant temperature, an increase in pressure increases the degree of ordering of molecules of a given substance. Therefore, pressure and temperature exert antagonistic forces on molecular structure and chemical reactions.

*Arrhenius relationship.* As with thermal processing, various reaction rates during HPP are also influenced by thermal effects during pressure treatment. The net pressure-thermal effects can be synergistic, additive, or antagonistic.

High-pressure processing of muscle based products is paying more and more attention in the meat industry, which could prolong the shelf life of meat products, inactivate vegetative microorganisms and enzymes near room temperature, because of the processing allows the decontamination of muscle based products with minimal impact on their nutritional and sensory features. Therefore, The application of high pressure offers some interesting opportunities in the processing of muscle-based food products, such as, the high pressure can affect the texture and gel-forming properties of meat batter and myofibrillar proteins, the tenderize, color and other properties of muscle. The processing effects on muscle based products are highly dependent on the primary effects of pressure, time and temperature on the relevant thermodynamic and transport properties of meat systems. However, the pressure-labile nature of some meat protein systems, such as myosin or myoglobin often limits the range of attractive commercial applications to prefermented and cooked meat products.

*Pressure Generation Means.* Unlike straight processes such as thermal processing, the high-pressure process is independent of the equipment and processed food size and shape. The reason is that the pressure transmission is not mass/time

dependent. Hence, reducing the processing time and scaling up the equipment from the laboratory to commercial size will not touch the efficiency of HPP. In contrast, it helps the HPP applications to develop faster. Two types of the compression processes, direct (piston) or indirect (pump) compression can achieve generation of high pressures in the pressure vessels (Fig. 1).



Fig. 1. Schematics of high-pressure food processing techniques direct (left) and indirect (right) compression 1 - top closure; 2 - pressure medium; 3 - pressure vessel; 4 - product; 5 - piston; 6 - low-pressure pump; 7 tank; 8 - intensifier pump

1) Direct compression This technique uses the vessel ends closure/s to act as a piston to build/release the pressure. This happens by reducing the specific volume inside the vessel until the desired pressure is reached. Although the direct system can achieve a rapid compression, the restrictions of the dynamic seal between the piston and the vessel obstruct the applications of this technique for a small-scale laboratory.

2) Indirect compression. Indirect compression is the method used in the application of much high pressure processing equipment in the food industry. It employs a high-pressure intensifier pump to compress a pressure fluid from its reservoir tank into a pressure vessel, transmitted through high-pressure tubes. This technique is more appropriate for solids and high viscous liquid food.

This method also allows pressure to be released or kept constant at the required level during the treatment time for several minutes.

4. The affects of soy protein by high pressure. The globulin of soy protein has a closely globular structure, molecular weight is small, and active group packages within the molecule, and some methods of modification is difficult to effectively change its structure, improve its functional characteristics. Thus, the function of

11S globulin is worse than the 7S globulin ( $\beta$ conglycinin), it is a key factor that restrict the application of soy protein isolate and modification. How to effectively improve the structure of soy protein isolate becomes the primary factor of soy protein modification [16; 40; 41].

High pressure processing, can be referred to as ultra high pressure technology or high hydrostatic technology, the water or other incompressible fluid mediums often act as mediators of pressure. Which has the advantages of pressure uniform transmission, instantaneous, efficient, low energy consumption, pollution little dyeing, and no obvious effects of low molecular compounds, such as Vitamins, pigments and flavor substances, etc. During the high pressure processing, the pressure levels generally not less than 100 MPa, the commonly used range is 100-1000 MPa [42; 43]. The ability of high pressure to denature proteins has been known for over one hundred years. In this processing, the non-covalent bonds (ionic bonds, hydrogen bonds, and hydrophobic bonds, etc.) of proteins are been destroyed or formed at a certain temperature for the appropriate processing time and pressure level [44; 45]. Therefore, The high pressure technology can develop the appearance and new types of soy protein with different techno-functional properties will be available in soy protein processing.

The changes of protein spatial structure caused by high pressure processing are the focus of current research. In general, high pressure has not effect on the primary structure of proteins, has some effect on the secondary structure, and has great effect on the tertiary and quaternary structures. The effect of high pressure on protein can be reversible or irreversible. Generally, protein changes are reversible under 100-200 MPa. When the pressure exceeds 300 MPa, protein changes tend to be irreversible, that is, protein permanent denaturation [46-49]. In food applications, the various functional properties of soy protein are mainly realized by the physical and chemical properties of storage proteins, namely, 7S and 11S globulin, which are ultimately determined by the intrinsic physical and chemical properties of proteins based on their molecular structure. In research [50] studied the changes of solubility and rheological properties of soy protein isolate after high pressure treatment (400 MPa, 15min), during the process. The power law modulus was used to measure the apparent viscosity, and the Young's modulus was used to measure the G' and G", who found that the change of molecular structure of soy protein isolate after high pressure treatment was caused the change of its related physical and chemical properties. The solubility of protein in low concentration (4.0 % solution was 4.5 %) soy protein isolate significantly improved after high pressure treatment, leading to the apparent viscosity were increased with the increase of pressures. The values of G' and G" were proportional to the apparent viscosity. In a constant pressure force (400 MPa) or less under the action of high pressure, 11S globular depolymerization of soy protein, protein molecules depolymerized to smaller particles on the unit, and the base unit of a certain degree of stretch further, makes the globular protein within the exposed polar groups and hydrophobic groups, and makes the protein molecules (particles) to strengthen the surface charge of distribution. Then the combined water around the newly exposed polar groups were increased. In research [51] found that high pressure (200–600 MPa) could change the large particles to the smaller. The volume fraction of soy protein isolate occupies in the solution was significantly increased, made the dispersibility of protein sov isolate obviously improved. Meanwhile, the free sulfhydryl content and surface hydrophobicity (H<sub>o</sub>) of soy protein isolate

was significantly increased at pressure treatment of 400–600 MPa for 20 min. The results of SDS-PAGE indicated that the subunits composition of soy protein isolate was greatly changed, which caused the content of 7S and 11S protein was obviously increased.

The pressure level and other factors (time, temperature) were influenced of the properties of soy protein. In research [52] studied the modifications of soy protein in soy milk treated by high pressure. They found that the soy proteins were dissociated into subunits by high pressure. Some of which associated to aggregate and became insoluble. The denaturation of 7S and 11S were occurred at 300 MPa and 400 MPa, respectively, and induced tofu gels was formed with gel strength and a cross-linked network microstructure. In research [53] studied the effect of high pressure processing on textural properties and water holding capacity of 20% protein concentration gels (soy protein isolate, 7S and 11S glycinin). They found that the high pressure induced gels were formed at the range from 300 MPa to 700 MPa; compared to the thermal gels. The adhesiveness and hardness of high pressure induced gels were significant lower. The water holding capacity was improved by high pressure in the gels of 7S glycinin. In research [54; 55] shown that the insoluble aggregate of soy protein isolate was formed at lower pressure level (200 MPa). The insoluble aggregate was transformed into soluble aggregate at higher level (600 MPa), pressure much more homogenous soluble aggregate was generated at 400 MPa or 600 MPa had much less mean molecular weight than that at 200 MPa, and the changes of secondary and tertiary structures were induced by high pressure processing, that is the direct evidence or explanation for high pressure induced modification of soy protein isolate.

According to the previous studies, 1000 mL water has an adiabatic heating of 19.2 kJ under 400 Mpa. It is the same as the energy of 1000 mL water rises from 20 °C to 25 °C. However, in this study, the denaturation temperature of soy glycine 7S and 11S were about 68 °C and 96 °C, respective. Thus, the changes of soy glycine 7S and 11S were caused by the energy during high pressure processing near 30 °C is titchy compared with that of caused by pressures. In research [56] investigated the effects of heat (20-90 °C) and pressures (0.1-600 MPa) combined on protein denaturation in soy flour mixed with various types of type of aqueous plasticizer (NaCl, sucrose, betaine. and lactobionic acid) using the differential scanning calorimetry, who found that

only a small effect on denaturation of the 7S soy globulin in 50 % (w/w) soy flour-water paste was observed at 200 MPa (20 min, 25 °C). A significant effect on denaturation of both the 7S and 11S soy globulins was showed at 600 MPa. The other, a less-pronounced effect on denaturation of the 11S globulin was observed at 60 °C treated by different pressures, but a similar extent of denaturation of the 11S treated by 600 MPa at 25 °C and 90 °C was observed. The result showed that 7S is sensitive to heat and pressures combined, because it has low denaturation temperature (68 °C); 11S is not sensitive to thermal, and sensitive to pressures, due to it has high denaturation temperature (96 °C). Thus, the application of thermal plus high pressure processing could be used to produce enhanced food quality. In addition, the NaCl, sucrose, betaine, and lactobionic acid had a protective effects on protein denaturation during the high pressure processing treatment at 25 °C.

High pressure and other materials combination was also effected the processing properties of soy protein. In research [57] investigated the effect of high pressure processing (0.1-300 MPa) on soy protein isolate incubated with flaxseed gum at 60 °C for 3 d. The results shown that the solubility of soy protein isolate upon glycation with flaxseed gum was improved. The maximum value reached 86.84 % when treated at pH 8.0 and 200 MPa, accompanied by producing the differences between the secondary structure of the glycated proteins and that of at 200 MPa, such as the  $\alpha$ helix, random coil contents and vibrations of the amide II band. At 100 Mpa the Maillard reactions were significantly promoted, to the contrary, the reactions were significantly suppressed at 200 MPa and over. Overall, proper pressure level can improve the processing properties of soy protein. In research [58] studied the interaction between soybean protein and tea polyphenols under high pressure using circular dichroism, fluorescence spectroscopy and molecular modeling, who found that high pressure processing is a useful tool for improvement the function of tea polyphenols and soybean proteins. The secondary structure of soy proteins was significantly modified at 400 MPa, such as increased the  $\beta$ -sheet content and decreased the  $\alpha$ -helix content, but the  $\alpha$ -helix structure was protected when the 0.1 % (w/v) tea ployphenol was added. The other, the high pressure and tea polyphenols combined could increase the solubility, emulsifying activity and micro-texture, the reason is that the Pi-Pi interaction was formed in the binding of phenolic compounds to 7S or 11S globular protein. In research [59] found that the solubility of ethanol (EtOH)-denatured soy proteins at neutral and alkaline pH as well as low ionic strength was significantly improved treated above 200 MPa. The enthalpy value was increased and the ordered supramolecular structure with stronger intramolecular hydrogen bond was formed.

Meanwhile, the Tyr and Phe residues were exposed, which caused an increase in surface hydrophobicity of 7S glycinin treated by high pressure processing (200-400 MPa), but the surface hydrophobicity was decreased at 500 MPa. In contrast, the progressive unfolding of denatured glycinin was induced with increasing pressure, due to the Tyr and Phe residues were moved to the molecular surface of protein. In research [54] reported that the secondary structure of native soy protein isolate is estimated to be composed of 15 % –16 % alpha-helix, 39 % – 44 % extended strands, 17.5% random coils, and 21 % – 27 % turns. At 200–400 MPa, the intensity and a «red-shift» of these bands were increased. At 600 MPa the band intensity of the amide I' region was further increased, so that, the intensity and absolute area of amide II bands were gradual increases treated by high pressure processing. Meanwhile, the gradual unfolding of secondary and tertiary structure was produced, and the structure of denatured proteins underwent a «rebuilding» process after the release of high pressure.

5. The affects of soy 7S and 11S by high pressure. 7S and 11S are determined the emulsion capacity, foaming capacity, gel properties of soy protein. Some authors have reported that the changes of soy 7S and 11S were induced by the high pressure processing [60–63]. In research [60] investigated the effect of high pressure processing (200 MPa - 600 MPa) on the emulsion properties on the soy 7S and 11S and soy protein isolate (0.25-0.75 %) at different pHs (7.5 and 6.5). The results showed that the highest emulsifying activity index and surface hydrophobicity of 7S globulin were treated at 400 MPa. The highest emulsifying activity index and surface hydrophobicity of 11S globulin were treated at 200 MPa, implying that the 7S globulin was dissociated into partially or totally denatured monomers at 400 MPa, which enhanced the surface activity; meanwhile, the pressure at 400 MPa induced the unfolding of the polypeptides of the 11S within the hexamer led to aggregation, which lowered the surface hydrophobicity. The glycinin of soy was dissociated into subunits and the conformation of these subunits had been changed after high pressure processing. At 300

MPa and over, the ultraviolet absorbance of hydrophobic regions, sulphydryl groups, and amino acid residues were changed significantly; at 400 MPa for 10 min, the denatured completely of glycinin was observed by DSC analysis; at 500 MPa for 10 min, the  $\alpha$ -helix and  $\beta$ -sheet structures were destroyed and converted to random coil, thus, the pressure level was influence of the conformational of soy glycinin [61]. In research [62] reported that high pressure processing (200, 400 and 600 MPa for 10 min at 10 °C) induced more ability to proteins, and particularly  $\beta$ -7S and A-11S polypeptides, to be adsorbed at the oil-water interface. In research [63] investigated the effect of high pressure treatment (0–600 MPa) on the heat-induced gelation of mixture of actomyosin and soy 11S globulin, found that the firmer mixture gel was formed after high pressure processing, the gel strength and work done values were increased with the increase of pressure levels from 100 MPa to 400 MPa; over 400 MPa, the gel formation dropped dramatically; those indicated that the SH groups play a key factor in the gel of actomyosin and soy 11S under high pressure. 7S and 11S globulin have different emulsion properties treated by high pressure.

In research [56] investigated the effects of heat (20–90 °C) and pressures (0.1–600 MPa) combined on protein denaturation in soy flour mixed with various types of type of aqueous plasticizer (NaCl, sucrose, betaine, and lactobionic acid) using the differential scanning calorimetry, who found that only a small effect on denaturation of the 7S soy globulin in 50 % (w/w) soy flourwater paste was observed at 200 MPa (20 min, 25 °C). A significant effect on denaturation of both the 7S and 11S soy globulins was showed at 600 MPa. The other, a less-pronounced effect on denaturation of the 11S globulin was observed at 60 °C treated by different pressures, but a similar extent of denaturation of the 11S treated by 600 MPa at 25 °C and 90 °C was observed. The result showed that 7S is sensitive to heat and pressures combined, because it has low denaturation temperature (68 °C); 11S is not sensitive to thermal, and sensitive to pressures, due to it has high denaturation temperature (96 °C). Thus, the application of thermal plus high pressure processing could be used to produce enhanced food quality.

In research [64] found that 7S and 11S globulin emulsions (7 %, w/v) behaved differently under

the temperature (20-60 °C) and high pressure (0.1-600 MPa) combined treatments, 7S globulin was responsible for the global properties of sov emulsions, whereas 11S globulin exerted a negligible effect; the 7S emulsions was increased the flocculation and gelation, which caused by aggregation between adsorbed and aqueous 7S proteins. The calcium and high pressure treatment combined was effected the the thermal properties of soy protein isolate, a  $\beta$ -conglycinin-enriched fraction, a glycinin-enriched fraction, and whey protein concentrate. The Td of glycinin was increased for every assayed calcium concentration, high pressure treatment promoted denaturation of  $\beta$ -conglycinin and glycinin, and calcium protected both proteins in β-conglycininenriched fraction and glycinin-enriched fraction at 200 MPa, protected glycinin in soy protein isolate and  $\beta$ -conglycinin-enriched fraction at 400 and 600 MPa [65]. In research [66] reported that the hydrolytic efficiency of Corolase PP was increased and the surface hydrophobicity of the hydrolysates were decreased treated by high pressure processing (80-300 MPa). The higher bioactivities of hydrolysates was observed under 200 MPa for 4 h. The small peptides (< 3 kDa) and the amino acid sequences of these peptides with different inhibitory abilities were increased. Thus, high pressure and Corolase PP combined could be used as a potential technology to produce bioactive peptides from soy protein isolate.

6. The affects of soy protein in meat products by high pressure. Soy protein isolate, as a replacer of lean meat, is a commonly useful in the meat industry. It has a good water holding capacity, structuring behaviour and excellent gelling. The effect of soy protein isolate on the holding capacity, techno-functional water properties and protein conformation of lowsodium (1 % salt) pork meat batters treated by high pressure was studied. The pork meat batters with 0%, 2%, 4% soy protein isolate were prepared under 200 MPa for 10 min. The result showed that the cooking yield was significantly increased when added the soy protein isolate, but they were not significantly differences when added 2 % and 4 %. Meanwhile, the cooked pork batters with soy protein isolate could reduce the water mobility, and increase the water holding capacity and bounded water content. These indicated that added 2 % soy protein isolate and high pressure combinations enabled to improve the water holding capacity [67]. Soy protein isolate and high pressure combinations also affected the color, rheological property and protein secondary structure of pork meat batters. The pH, emulsion stability,  $L^*$  and  $b^*$  values of pork meat batters were significantly improved when added soy protein isolate under 200 MPa for 10 min. Added soy protein isolate and high pressure processing combined could delay the thermal denaturation of meat protein and declined the pre-gel effects generated, as well as induced the  $\alpha$ helix structure changed into  $\beta$ -sheet,  $\beta$ -turn and random coil structures [68]. Hence, soy protein isolate and high pressure processing combined can improve the techno-functional and texture properties of low-salt meat batters.

7. The affects of allergenicity from soy protein by high pressure. High pressure treatment could reduce allergenicity of soy protein isolate for infant formula. Recently, soybased infant formula, as a replace of milk for the lactose intolerant and cows' milk allergic infants, is being consumed more commonly, accounting for increased uptake all over the world [69-71]. However, soy protein isolate contents some antigenic component, such as glycinin,  $\alpha$ conglycinin,  $\beta$ -conglycinin and  $\gamma$ -conglycinin. Some studies have reported the use of high pressure processing to reduce allergenicity of soy seeds, whey protein, condensed soy glycinin and soy protein isolate [72-76]. The soy whey protein, as a by-product from the manufacture of tofu, has the antibodies against Gly m 1, which is an important allergen of soybean that causes allergy by inhalation. The immunoreactivity was decreased under 100-300 MPa for 15 min [72]. The article [74] showed that the soy glycinin with twelve disulphide linkages displays extensive unfolding at low to intermediate solid levels (30-60 %, w/w), but it largely maintains native conformation at 70 % and 80 % (w/w) solids showing about 20% denaturation under 600 MPa for 15 min at ambient temperature, as compared to the thermal transition of native counterparts. The article [77] studied the effects of high pressure processing treatment (200-700 MPa) on antinutritional factors phytate and trypsin inhibitor content in 5 % soy protein isolate (SPI) solution, who found that the phytate was efficient to eliminate treated by high pressure processing, but the trypsin inhibitor content was not changed. In research [75] found that the processing pressure and duration time could significantly influence the allergenicity reducing efficiency. Such as, the allergenicity of soy protein isolate decreased 48.6 % compared to the native under 300 MPa and 15 min. The reason is that the free SH content and hydrophobicity of soy protein isolate

were significantly increased under 200-300 MPa for 5-15 min. The two interactions were progressively decreased treat by the levels above 300 MPa for 15 min. The secondary structure of soy allergens were interfered and the allergenicity by modifying conformation of allergenic epitopes were decreased after high pressure treatment. In research [76; 77] utilized the method of proteomics to confirm allergen subunit differences of soy protein isolate between control and high pressure, found that the allergenicity was decreased by 45.5 % at 300 MPa for 15 min, and altered the allergenicity of  $\alpha$  and  $\alpha'$  subunits of 7S globulin and A1 and A1a subunits of 11S globulin, so that, the use of high pressure processing could improve the safe of soy protein in infant formula.

## Conclusion

It is well established that high pressure processing was improved the properties of soy protein, 7S and 11S glycinins. The proper pressure treatment of soy protein, 7S and 11S glycinins was increased the water holding capacity, gel and emulsion properties through affected the noncovalent bond, covalent bond and protein conformation, and also reduced the allergenicity of soy proteins in infant formula. In spite of great efforts, the mechanism of high pressure processing on soy protein, 7S and 11S glycinins has not yet been obtained, which leads to get a clear understanding of their behaviour is difficult. Even so, the use of high pressure processing and soy protein isolate combined could improve the soy protein techno-functional properties and security in foods.

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