



## Advances on physical treatments for soy allergens reduction - A review

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### ABSTRACT

**Background:** Food allergies represent one of the most rapidly increasing and significant human challenges connected with global health. Soybean has high protein and fat quality; however, it is responsible for allergenicity in humans. Despite this, the requirement for protein will increase by 110% in 2050. Traditional methods such as heat treatments (boiling, roasting) are usually exploited to decrease food allergenicity, but they produce modifications in food quality (color, smell, flavor, and texture) due to high temperatures.

**Scope and approach:** Consequently, new potential approaches for reducing soybean allergenicity are described in this review, divided into two categories. The first is related to pressure techniques, such as extrusion, high hydrostatic pressure (HHP), high-pressure homogenization (HPH), and controlled instantaneous pressure drop (DIC). The second is related to waves: gamma irradiation ( $\gamma$ -irradiation), pulsed ultraviolet light (PUV), cold plasma, microwave, and ultrasonication. These techniques have the same common goal: lowering soybean allergenicity of the products with an impact on protein structure.

**Key findings and conclusions:** The review found that physical treatments have better profiles on the food nutritional characteristics and sensory properties, maintaining the color, flavor, and freshness, as compared to the conventional methods (proteolysis, thermal, fermentation). The allergenicity may increase, decrease or persist unchanged depending on treatment intensity and protein structure. However, allergenicity can be minimized if optimal parameters (energy, time, temperature, and frequency) are applied during these processes.

### 1. Introduction

Two hundred and fifty million people are globally affected by food allergies. Infants (5–8%) are more susceptible than adults (1–2%), according to the World Allergy Organization (WAO). Food allergy corresponds to the immune system intermediated by cell or antibody immunoglobulin E (IgE), identifying potential threats from food constituents. The immune response is mainly caused by antigenic proteins, having a molecular weight between 7 and 71 kDa (Wilson et al., 2005). In the first case, with IgE (type 1 or immediate hypersensitivity), reactions quickly develop (in a few minutes up to 2 h). In the second one, the symptoms appear in hours or days (type 4 or delayed hypersensitivity) (De Angelis et al., 2019). In 2014, a scientific opinion reported that around 0.2–0.4% of people were affected by soybean allergenicity (EFSA, 2014).

Soybean (*Glycine max*) is an attractive variety of legumes due to the high quality of protein and fat. Moreover, it is an essential bean in the world for producing different products, being one of the few vegetal

resources that present a better profile in amino acids required for the human body, compared with other plant-based foods. On a dry-weight basis, the chemical composition of soybeans consists of about 40% protein, 35% carbohydrates, 20% fat, and 5% ash (Snyder, 2003).

About 90% of soybean proteins are represented by globulins, the primary storage proteins, which are soluble in salt solutions, with the remaining 10% being water-soluble albumins. By acidification at pH 4.5–4.8, the defatted soybean can be extracted into storage globulin and whey fractions by obtaining the soy protein isolate (SPI), which contains a mixture of proteins, which are 2S, 7S, 11S, and 15S, “S” being the Svedberg sedimentation coefficient, where a higher number suggests a higher molecular weight (Nishinari et al., 2014; Sui et al., 2021).

Soybean proteins are found in the list of essential allergens of the Food and Agriculture Organization of the United Nations (FAO) and the United States Department of Agriculture (USDA) (Thrane et al., 2017). In Europe, this allergen is mentioned with the other common food allergens in Directive 2003/89/EC (EU, 2003). All food packaging has to mention these proteins, and a warning on a separate label must confirm

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the allergens (Thrane et al., 2017).

Additionally, the European Food Safety Authority (EFSA) reported a few data about the amount of protein that can produce severe effects, like anaphylactic incidents. The lowest response registered in people with an allergic reaction to soybean was 0.2 mg (EFSA, 2014).

According to the International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-Committee ([www.allergen.org](http://www.allergen.org)), eight verified allergenic proteins have been registered in soybean seeds: Gly m 1 to Gly m 8 (Verhoeckx et al., 2015).

As an illustration, the two major storage proteins, Gly m 5 and Gly m 6, are correlated with the critical effects in subjects from Europe (Verhoeckx et al., 2015). In Japan, it was found that in children with a reaction to soybean, Gly m 8 (2S albumin) presented a notable value of allergenicity (Besler et al., 2001).

However, literature has shown that many common technologies could be used to reduce allergenicity, such as thermal (dry heat -roasting and moist heat -autoclaving and boiling), enzymatic, and fermentation treatments (Vanga et al., 2017). These treatments require necessary amounts of energy and water, which can lead to high costs, limit the process's sustainability, and decrease food quality (Dong et al., 2020). In this way, the use of physical processes could be a more durable solution in the current context of our society. The present work aimed to show the advances in the potential use of physical treatments to reduce the allergenicity of soybean products.

Recently, Pi, Sun, et al. (2021) published the review article "Effect of processing on soybean allergens and their allergenicity," which contains a general overview of food treatments such as thermal, fermentation, enzyme catalysis, sprouting, glycosylation, irradiation, HHP and discussed more elaborate the molecular viewpoints of soybean (major soybean allergens). However, to our knowledge, detailed descriptions of the effect of physical treatments on soybean allergens are still not available in the literature. The current review intends to discuss the most relevant physical technologies focused on soybean allergenicity individually and in detail, developing mainly technological aspects.

The following sections will also present a description of the treatment history, the principle of the method, its usage, and the effects on soy proteins.

## 2. Allergenicity and food processing

Different studies have been performed on food allergies as a global health problem by studying the connection between immunoreactivity and food processing (Vanga et al., 2017).

This allergenicity can be achieved by changing the protein structure of all the identified allergens. One of the most used methods worldwide for preserving food is thermal treatment (e.g., pasteurization, sterilization) (Dewan, 2020).

However, these traditional thermal treatments have some negative effects on the sensory characteristics of food by losing some nutritional value and degrading the color, smell, flavor, and texture (Hogan et al., 2005).

When people started to cook food from the raw state, they did not think that cooking could impact the allergenicity of the food. However, roasting and boiling are believed to be efficient methods to reduce the allergenicity in some foods (Dong et al., 2020). Cabanillas et al. (2018) described that after 30 min of boiling soybean, the IgE reactivity was not affected by this treatment, but it was partially reduced after 60 min. Also, the authors described that a combination of heat and pressure had the highest impact on soybean allergenicity by a potential fragmentation of the proteins even if some allergens ( $\beta$ -conglycinin) persisted after boiling. Despite these findings, it has been found that under thermal conditions, some allergens found in tree nuts, wheat, celery, and soybean are not altered by heat, being stable at this treatment (Dong et al., 2020).

The mechanism for reducing soybean allergenicity is the denaturation of the peptide bonds, affecting the protein structure. The result is a

change of conformation into a random coil or unfolded proteins. Also, the epitopes can be destroyed, or new ones can appear through thermal treatment. For example, between 55°C and 70 °C the secondary structure of the protein is lost, at 70°C–80 °C the peptide bonds are broken, new interactions are formed at 80°C–90 °C and at 90°C–100 °C, aggregates are created (Wal, 2003).

Compared to thermal treatment, non-thermal procedures presented more advantages, not only in terms of organoleptic properties (by having a better profile on flavor, color, texture, smell) and improving antioxidants and phenolics components but also in decreasing the allergenicity of shrimp, peanuts, and soybean (Dong et al., 2020).

Several types of physical treatments are outlined in this review by describing two groups. One is related to the pressure, such as extrusion, high hydrostatic pressure (HHP), high-pressure homogenization (HPH), and controlled instantaneous pressure drop (DIC). The second group is presented in the form of waves: gamma irradiation ( $\gamma$ -irradiation), pulsed ultraviolet light (PUV), cold plasma, microwave, and ultrasonication. Some of these treatments can be used alone or in combination with thermal treatment. However, all these techniques have the same common goal: lowering soybean allergenicity of the products with an impact on protein structure. Fig. 1 shows an overview of the classification of the treatments, which may influence the markers responsible for the allergic reactions in soybean.

### 2.1. Physical treatments based on pressure for soybean allergy control

#### 2.1.1. Extrusion

Extrusion was used in pasta dough for the first time in 1935, followed by animal feed in 1950. In the '60, more extensive equipment for extrusion treatment was necessary due to the request for pre-cooked cereals and starches. These machines allowed to broaden the spectrum of extruded food: snacks, ready-to-eat cereals, infant formula, and dry food for animals (Gray & Chinnaswamy, 1995).

Under high temperature, pressure, and shear forces, the feed material exposed to extrusion melts by changing phase. The mass passes through a die and, in the end, is bared to the atmospheric conditions. Immediately, the melted feed expands because of the contact with these atmospheric circumstances (Ek & Ganjyal, 2020).

The simultaneous combination of moisture content, external heating, time, pressure, and mechanical shear makes the material porous with an expanded structure. These properties change the color, shape, taste, composition, texture, bulk density of the material (Morantes et al., 2020). Also, a short time and a high temperature induce cooking and a plasticization of the proteins and carbohydrates in the extruder barrel (Moreno et al., 2018; Wilson et al., 2005).

Extrusion impacts soybean allergenicity, particularly in textured soy protein, a vegan meat substitute obtained by extrusion. Parameters such as temperature and pressure can make the ingredients flow by aligning the proteins, followed by expanding and collapsing. In this way, products with a meat-like texture can be obtained (Wilson et al., 2005).

The combination of shear force, temperature, and pressure disintegrate the cell walls, thus affecting the protein structure (Fig. 2). Then, an increase in temperature partially breaks disulfide and hydrogen bonds by spreading the spatial structure of the protein and destroying its molecular structure. An additional increase of extrusion temperature breaks the co-ordinate bonds, carbon-nitrogen, and other chemical bonds, destroying the secondary structure. A higher screw rotation and lower feed speed increase the shear force, which affects the epitopes of antigen protein, impacting the food allergenicity (Arêas, 2009; Moreno et al., 2018; Yin et al., 2019).

The aggregation and denaturation of proteins resulting from the thermomechanical effect lead to a decrease in the allergenicity of soy products (Ek & Ganjyal, 2020).

Starting from 1994, Ohishi et al. used a twin-screw extrusion process with a temperature higher than 66 °C, which reduced the soybean's antigenicity to 0.1% of the initial value. This decrease was due to the

### Classification of the physical treatments

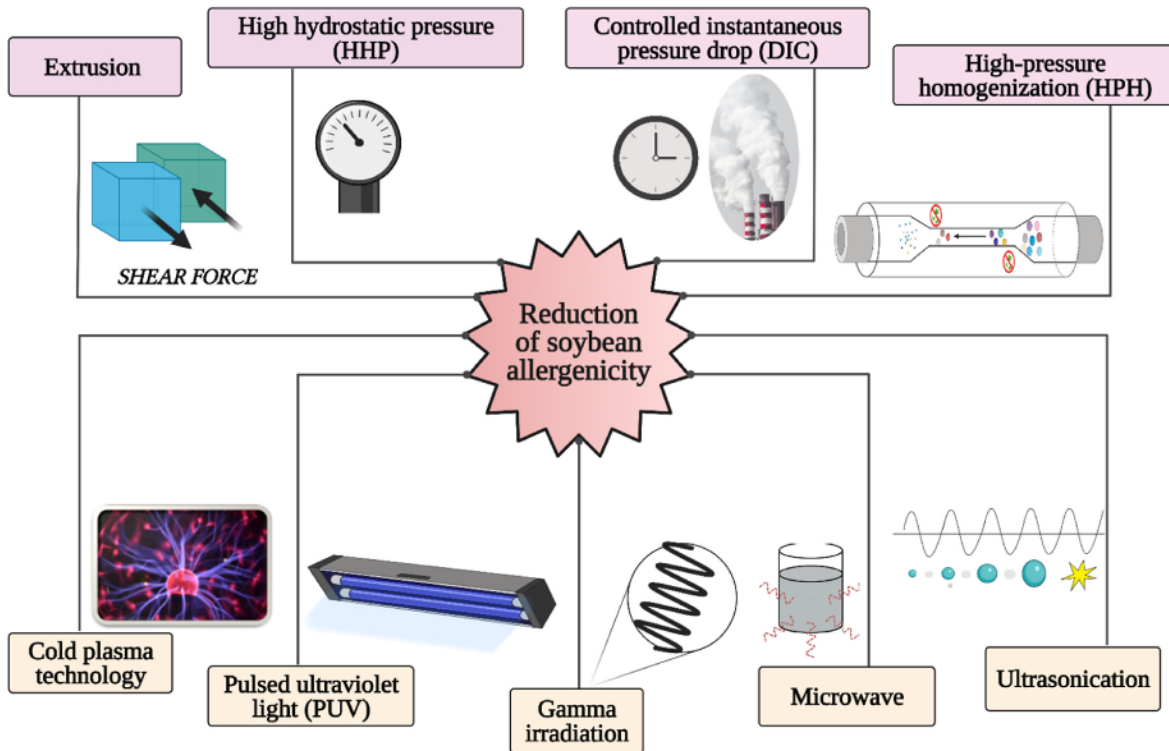


Fig. 1. Reduction of soybean allergenicity using physical processes (created with BioRender.com).

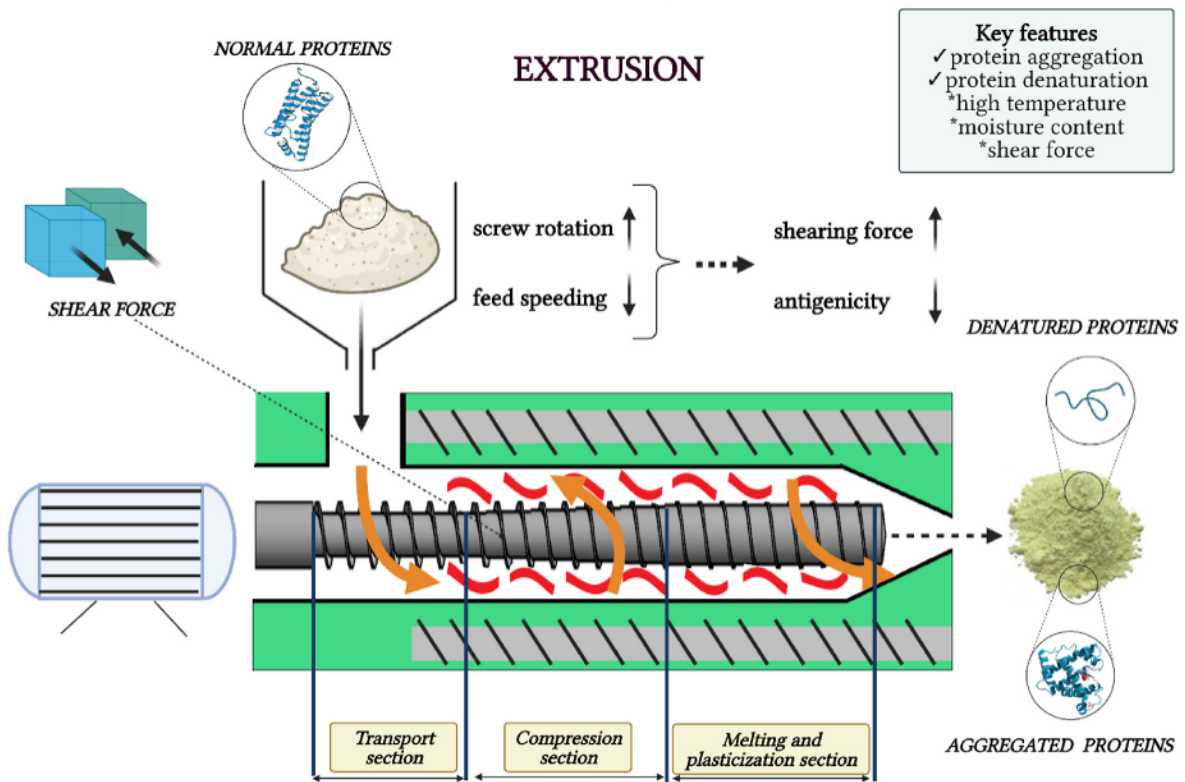


Fig. 2. The inactivation process of soybean allergens treated by extrusion (created with BioRender.com).

degradation of the molecular structure of proteins (especially fractions with high molecular weight) during the extrusion cooking.

A similar study with twin-screw extrusion was done by Saitoh et al., in 2000 on milled soybean hypocotyls (500  $\mu\text{m}$  particle size), where the allergenicity was reduced by 1%. A reduction of urease activity and trypsin inhibitor were also observed. The isoflavones were unchanged.

Franck et al. (2002) compared different soybean products and the allergen P34 (Gly m Bd 30K), which can be eliminated through extrusion. Patients sensitive to soybean responded positively to textured soybean proteins with 38 and 50 kDa molecular weight, which can be related to G1 glycinin acidic and basic chains. Also, in the textured product, the 31–34 kDa band did not bind the IgE, suggesting that Gly m Bd 30K was removed during the extrusion.

The temperature was shown to influence the allergenicity of the  $\beta$ -conglycinin of soybean. The antigenicity was reported to increase at 100 °C. Between 100 °C and 120 °C, the epitopes were relatively blocked or destroyed by the crosslinking and aggregation of  $\beta$ -conglycinin particles. At 140 °C or higher, the antigenicity was lowered by a complete breaking of the  $\beta$ -conglycinin epitopes and molecular structures, without any modifications in the intermolecular connections (Yin et al., 2019).

$\beta$ -conglycinin antigenicity was only 20.06%, under the following optimal extrusion parameters: temperature (130 °C), the feeding speed (35 g/min), and the screw rotation speed (140 rpm). This significant reduction of the allergenicity results from a higher shear force, which can produce depolymerized  $\beta$ -conglycinin polypeptides, breakage of peptide chains, and abolished epitopes (Yin et al., 2019).

The same study presented that an increased water content reduced the antigenicity of soybean (16% water was noted for the lowest antigenicity). The correlation between the last two parameters is related to enhancing viscosity, increasing shearing stress, and reducing antigenicity. Thus, when the temperature increases during extrusion, antigenicity decreases due to protein denaturation (Yin et al., 2019).

In 2020, Zheng et al. reported that the extrusion process decreased allergenic protein activities in two formulas based on corn, one with defatted soybean flour (SF) and the other one with SPI. Enzyme-linked immunosorbent assay (ELISA) technique demonstrated that the extrusion process reduced the immunoreactivities of both SPI-corn (53–68%) and SF-corn products (80%–86%). In addition, extrusion processing decreased the protein solubility of both SPI and SF products by more than 58.7% and 66.3%, respectively, compared to the initial materials. The products obtained with less than 20% moisture content had a slighter decrease in allergenic proteins.

The temperature conditions impact protein solubility in the extrusion process. A higher temperature leads to a decrease in the solubility of protein for both products. It was also shown that after the extrusion,  $\alpha$ -helix decreased, and  $\beta$ -strand structure increased in both formulas. The water hydration capacity of the SPI-corn and SF-corn extrudates was mainly impacted by moisture, which changed from 20% to 40%. As a result, the water hydration capacity was considerably reduced for SPI-corn (from 325.3% to 260.6%) and SF-corn extrudates (from 224.0% to 202.8%). The formula with soy flour and corn was less hydrated than the mixture with corn and soybean isolate (Zheng et al., 2020).

In conclusion, it was shown that the extrusion technique combines a mechanical action (shear force) and a thermal effect that induces aggregation and denaturation of the proteins, which lead to the reduction of allergenicity. Further, the product's viscosity is an essential key because it increases the shearing stress and decreases antigenicity.

### 2.1.2. High hydrostatic pressure (HHP)

In 1899, Hite was the first researcher who used a hydraulic press to inactivate microorganisms and extended the shelf life for at least one day for milk, compared with untreated milk (Hite, 1899). Starting from there, this technology answered questions more about preserving the food by inactivation of bacteria.

Later, the effect of pressure on changing the conformational

structure was evaluated on some different biomolecules, including proteins. More recently, some scientists had the idea to check the impact on food allergenicity, especially on milk allergens, and broaden the food spectrum (Vanga et al., 2017).

*Le Châtelier* and isostatic principles are applied in this treatment. It is a discontinuous process used for liquid and solid packed products, with a pressure dispersed continuously and directly and equally applied in all food directions. Pressurization helps the phenomena (chemical or biochemical reactions, phase transition, and molecular configuration), resulting in reduced volume (Augusto et al., 2018; Muntean et al., 2016).

The pressure applied equally on food products impacts the level of secondary, tertiary, and quaternary protein structure, inducing aggregation (the epitopes can be destroyed or masked) (Jiménez-Saiz et al., 2015; Rahaman et al., 2016).

The primary and secondary bonds suffer an elastic effect (a reversible distortion) under a low pressure. Also, the primary bond suffers a compression of the hydrogen bonds, reducing the internal cavity size of proteins. The tertiary structure and intermolecular interactions are destabilized at around 200 MPa. Proteins can unfold at around 500 MPa (depending on the protein type), and the aspect of the structure can be disordered due to pressure (the pressure stabilizes the membranes and disturbs the nucleic acid) (Somkuti & Smeller, 2013).

On the laboratory scale, the equipment chambers can be filled with 0.02–1.5 L, while at the industrial level, the equipment works with volumes up to 320 L by applying pressure up to 1400 MPa (Augusto et al., 2018). In addition, horizontal and vertical installations are used in industrial food applications (Yamamoto, 2017).

Usually, a pressure between 50 and 1000 MPa is used, with a temperature of between 0 °C and 150 °C, for a few minutes to hours (Augusto et al., 2018).

The National Advisory Committee on Microbiological Criteria for Foods (NACMCF), United States Department of Agriculture (USDA), and U.S. Food and Drug Administration (FDA) approved the HHP as an alternative to the non-thermal pasteurization (Huang et al., 2014).

In other countries besides the European Union (EU), such as the United States of America, there are no specifications applied to high-pressure treatment. The products obtained through this procedure in the EU fall under the legislation for Novel Foods (Regulation (EU) 2015/2283). Also, the pressure vessels have to follow the Pressure Equipment Directive (PED) regulation (2014/68/EU), which indicates the Good Manufacturing Practices (GMP) and security assessment for the maintenance and operation of the vessels (EU, 2014; EU, 2015).

HHP reduces the unwanted modifications on the nutritional, physicochemical, and sensory properties of food (Salazar et al., 2021). Also, sensory characteristics of proteins are modified, and the functional properties of the macromolecules (solubility, gelation, foaming, emulsification, precipitation, hydration, and agglomeration) can vary (Chauvin & Swanson, 2011).

As early as 1996, Omi et al. concluded that soybean seeds immersed in water and pressurized at 300 MPa at 20 °C for 25 min showed decreased basic 7S globulin allergenic protein. Ten years later, research conducted on a by-product resulting from the production of tofu (soybean whey) showed a reduction of the allergenicity of the Gly m 1 by applying a treatment of 100–300 MPa for 15 min (Peñas et al., 2006).

$\alpha$ -helix and  $\beta$ -sheet were damaged with a 10 min treatment at a high pressure of 500 MPa, by changing the conformation into a random coil. Additionally, a complete denaturation of the glycinin was observed when other parameters were applied. In conclusion, it was shown that HHP higher than 300 MPa, caused the denaturation of  $\beta$ -conglycinin, which was fragmented in subunits and other SH groups (Zhang, Li, & Mittal, 2010).

It was observed that the allergenicity of germinated soybean treated with high pressure was lowered by using 300 MPa, for 15 min at 40 °C in a discontinuous machine. The soybean seeds germination took place in darkness, for five days, at 20 °C. After three days, the antigenicity

decreased due to protein degradation, resulting in new peptides (Peñas et al., 2011). Enzymatic hydrolysis occurred during germination, reducing the immunoreactivity because of the increased susceptibility of seed proteins. The obtained nutritional value and the amino acid composition were less affected, the digestibility was higher, and the antigenicity was lower than untreated beans.

However, the HHP treatment did not impact the protein profile or the immunoreactivity of tofu. This clearly shows that this method can lead to variable results depending on the food matrix and its complexity besides the levels of processing parameters. Therefore, further studies should be performed considering different product processing conditions (Peñas et al., 2011).

The addition of NaCl (0.6 mol/L) combined with a high pressure at 600 MPa had an increased impact on the denaturation of the  $\beta$ -conglycinin. At 200 and 400 MPa, the allergen mentioned and glycinin were protected by the different concentrations of NaCl from high-pressure denaturation (Añón et al., 2011).

The allergenicity of a soy protein isolate for infant formula was reduced using HHP, this being a critical application for babies who have allergies to cow milk. The authors noted that SPI treated at 300 MPa for 15 min produced the highest decrease of allergenicity (48.6%) compared to the native SPI (Li et al., 2012).

According to Li, Zhu, et al. (2016), HHP has the highest impact on the soybean allergens compared to high-intensity ultrasound (HIU), microwaving, and high-pressure homogenization (HPH). The allergenicity of soybean protein isolate for infant formula was reduced by 46.6% (HHP treatment) at 350 MPa, for 16 min. The allergenicity of the soybean can not be reduced totally, but it was shown that this technique could inactivate some conformational epitopes.

Soy protein isolate was tested at 200, 300, 400, and 500 MPa, with a temperature of  $20 \pm 2$  °C for 5, 10, 15, and 20 min. It was concluded that pressures ranging between 200 and 400 MPa reduced the antigenicity of  $\beta$ -conglycinin significantly. At 400 MPa for 15 min, the highest decline of antigenicity was 37% (from 92.72% to 55.15%). Additionally,  $\alpha$ -helix1 and  $\beta$ -strand1 were reduced significantly, which caused changes in protein structures with an effect on the allergenicity of soybean (Xi & He, 2017).

A recent study from 2018 described that HHP did not result in any visible allergenicity differences with an increase in pressure (300, 400, and 500 MPa) for the soy protein isolates, but the IgE binding dropped with the increasing of HHP time. The lowest allergenicity was observed at 300 MPa for 15 min, where the allergenicity of the soy protein isolate for infant formula was reduced by 45.5%. The authors reported that western blotting and mass spectrometry, but not the two-dimensional electrophoresis pattern, produced significant changes in 7S (HHP caused the  $\alpha$  and  $\alpha'$  subunits) and 11S globulin (A1 and A1a subunits), hence reducing the allergenicity in infant formula (Li et al., 2018).

Consequently, these treated soy proteins can be utilized as ingredients or sources of peptides with low immunoreactivity in hypoallergenic foods. The decrease or elimination of the protein's antigenicity in soy whey may be due to the combination between the enzymatic hydrolysis and the high-pressure procedure (Lavilla et al., 2020).

In conclusion, besides the ability to decontaminate food and keep the organoleptic properties unmodified, it was proved that high pressure produces conformational modifications in soybean proteins, changing some allergen structures, making them less accessible to antibody receptors. The secondary and tertiary structure of proteins is influenced by the hydrogen, ionic, and hydrophobic bonds affected by the high pressure, decreasing allergenicity and increasing digestibility (Rahaman et al., 2016).

### 2.1.3. High-pressure homogenization (HPH)

The milk homogenization machine was patented in 1899 by Auguste Gaulin, whose discovery involved the action of a pressure pump by streaming the milk through some capillary tubes. Then, in 1909, Manton-Gaulin Manufacturing Company launched the first commercial

homogenizer. At that time, the process was known as a fixation for milk, as a breakdown of the fat globules was observed, and under the microscope, the view was homogenous and continuous. Later this observation brought on a new term, namely homogenization (Osorio-Arias et al., 2021).

Compared with HHP, which is described before, HPH is a continuous treatment that applies a lower pressure, up to 300 MPa, under shear forces and elongation stress, which are defined as a mechanical action. Disaggregation and droplets breaking result from passing the sample through a narrow gap (100  $\mu$ m), with a velocity of 200–300 m/s. The main factors which characterize the process are the shear force, turbulence, and hydrodynamic cavitation (Li et al., 2021; Osorio-Arias et al., 2021). This treatment is applied only to fluids (Augusto et al., 2018).

Nowadays, HPH is used to increase product availability by inactivating the microorganisms and inducing a few modifications to the nutritional value and flavor of the treated product. Additionally, it has applications in protein modification, particle size reduction, cell disruption, emulsification, encapsulation, dispersing, and mixing liquids (Tao et al., 2014).

HPH also seems to impact food allergenicity. Cavitation produces a high mass of energy, resulting in high heating values (at each 10 MPa, the temperature increases by 2–3 °C) and shear stress. This stress dispersed across the product and other components (e.g., proteins) can change the spatial structure by deformation and cleavage of protein. High velocity and shear stress can generate free water radicals, modifying protein structure (Augusto et al., 2018; Li, Zhu, et al., 2016).

One study showed this effect, specifically on soybean. In this respect, more studies are available on HHP than HPH. Thus, Li, Zhu, et al. (2016) observed that HPH reduced soybean allergenicity by 29.8%, using a SPI special for infant formula at two steps at 90 MPa, with a homogenization for 1 min at 10000 rpm.

The mechanical action (shear forces) is responsible for the denaturation or conformation change of the proteins, which reduces the allergenicity of soybeans (Fig. 3). Compared with the HHP, which applies the pressure equally on the product, the HPH uses the shear stress tangentially or parallel to the material (Di Stasio & De Cristofaro, 2010).

### 2.1.4. Controlled instantaneous pressure drop (DIC)

The process of the controlled instantaneous pressure drop (the name in French “Détente Instantané Contrôlée-DIC”) was invented in 1988 by the team of Professor Karim Allaf of the University of Technology of Compiègne, France. Decontamination and drying of the food product were the first procedures, which formed the basis of this treatment. Nowadays, DIC has a large spectrum of applications in the food industry and pharmaceutical area. These include steaming, thermal modifications, extraction of volatile and non-volatile components, decaffeination, swell-drying, decontamination, and de-allergenicity (lupin, soybean, lentils, chickpeas, peanuts) (Hamoud-Agha & Allaf, 2020).

Since 2001, DIC treatment was developed on an industrial and pilot scale by the company ABCAR DIC Process (<https://www.abcar-dic.com>). It is an interesting research topic, the technology is flexible, and powder and solid products are mostly used. At the industrial level, the flow is continuous, and the capacity is 100 kg dry per hour (Abcar DIC Process, 2021).

The product is exposed to steam pressure, up to 0.8 MPa, for a short time of 1–3 min and high temperature, maximum 170 °C. It is mainly applied for biological resources and prevents thermal depreciation, resulting in a quality product (Haddad & Allaf, 2007).

The food exposure to steam, the short time, and the quick pressure drop (about 0.005 MPa) are responsible for the instantaneous evaporation of water and destroying vegetative forms of bacteria. Thus, the treatment has a thermomechanical impact. Additionally, the proteins aggregate, crosslink, and degrade, leading to a decrease in allergenicity (Hamoud-Agha & Allaf, 2020).

Also, protein structure can be altered by heat and pressure, by either losing the conformational epitopes or resulting in hidden epitopes.

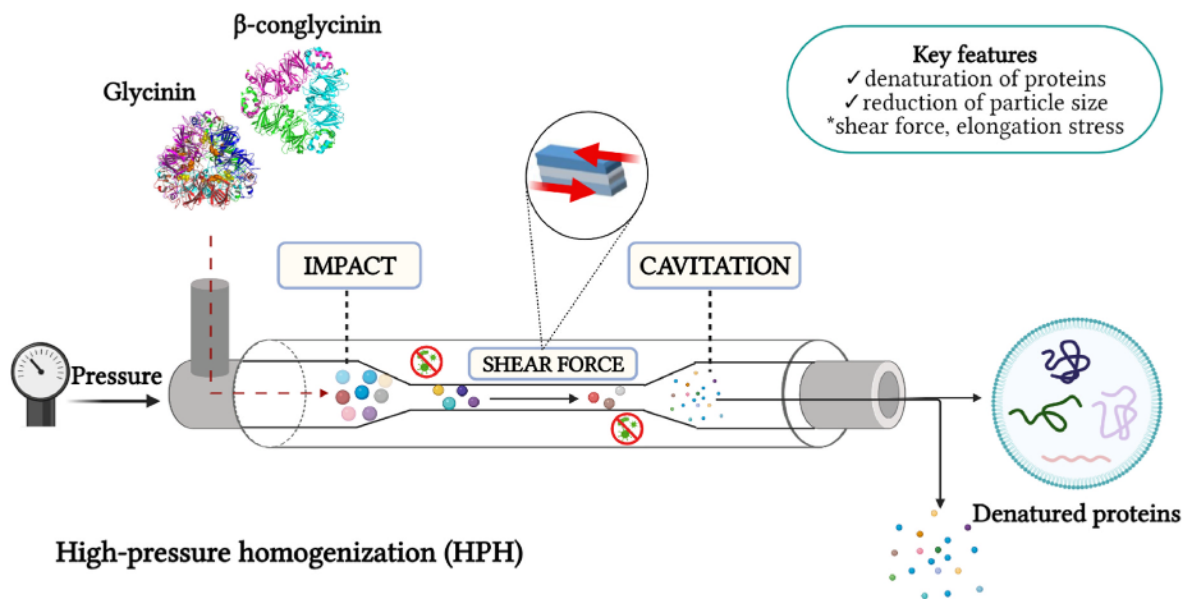


Fig. 3. The effect of high-pressure homogenization treatment on the soybean allergens (created with BioRender.com).

These changes are caused by the damage or reformation of hydrogen bonds and electrostatic and hydrophobic forces. The interactions between disulfide and sulfhydryl bonds are caused by the unfolding of proteins (created under pressure) and produce modifications in their conformation (Boughellout et al., 2013).

Cuadrado et al. (2011) showed a high reduction of the allergenicity of soybean during this treatment. Different pressures and durations were applied (0.3 and 0.6 MPa for 1 and 3 min) for various legumes (peanuts, chickpeas, lentils, and soybeans). A minor decrease of the immunoreactive band was found in the case of soybeans treated for 1 and 3 min at 0.3 MPa. The highest reduction in IgE binding capacity was observed at 0.6 MPa for 3 min. By comparison, the effect created by the DIC treatment is similar to an autoclaving treatment for 30 min at 0.26 MPa.

In a research conducted by Takács et al. (2014), seven spots were found for the control soybean in two-dimensional electrophoresis (2-DE). Two spots were abolished after a 3-min treatment at 0.3 MPa, and the other became less (24% and 65%) or more intense (156–288%) than the untreated control. The treatment was more effective when applied for an extended time (3 min), at a higher pressure (0.6 MPa), because five spots disappeared and two new were present on the gel, but with a lowered intensity (28% and 71%).

In 2007, Haddad & Allaf explained that a DIC treatment of 1 and 6 min reduced trypsin inhibitor activity by 94% and 99%, respectively, being a new advancement for soybean processing. A variety of soybean with an initial trypsin inhibitor content of 41.6 IU/mg was used that was previously soaked in water and mixed in polyethylene bags, at 4 °C for a minimum of 12 h.

Parameters such as steam pressure (up to 0.8 MPa), a short time of 1–3 min, and high temperature, a maximum of 170 °C, can reduce the allergenicity of the soybean. In terms of proteins, these become aggregated, crosslinked, degraded with some modifications in the solubility property.

## 2.2. Physical treatments in the form of waves for soybean allergy control

### 2.2.1. Gamma-irradiation ( $\gamma$ -irradiation)

The radiation process is not a new concept. After the discovery of the X-ray, ionizing radiations were proposed for destroying microorganisms in food. The first commercial irradiation for spices (Germany) and potatoes and grain (Soviet Union) occurred in 1958. After a few years, in 2003, the agencies responsible for food safety (International Atomic

Energy Agency, Food and Agriculture Organization, World Health Organization) announced that ionized food was safe for consumption, without doing any more tests on toxicology. Nowadays, this technique is used for preserving food in more than 60 countries (Bashir et al., 2021).

In the food industry, irradiation serves many purposes. It can be applied in the prevention of foodborne illness, food sterilization, inhibition of sprouting of potatoes or delaying the ripening of fruits, increasing herbs shelf-life, inactivation of bacteria, and controlling insects (Cătunescu et al., 2019; FDA, 2016).

According to Codex Alimentarius, in “General Standard for Irradiated Foods,” the maximum dose permitted for food is 10 kGy, with some exceptions when necessary to use it for a technological reason (Codex Alimentarius, 2003).

Different states from Europe approved an irradiation dose for the treatment. A few examples are: Belgium (5–10 kGy), Norway, Finland, Germany, Croatia, and Estonia are using 10 kGy, Hungary (2–10 kGy), France (1–10 kGy) and Spain, a more precise dose (8.6–9.5 kGy) (Bashir et al., 2021). The maximum dose for soybean protein isolate in China is 8 kGy (Pi, Yang, et al., 2021).

The exposure of food products to electron beams, x-rays produced by an electron accelerator, or  $\gamma$ -rays by using a source of radioisotope (cesium 137 or cobalt 60) is called irradiation. Frequently, doses of 1–10 kGy are used to cold pasteurize fruits and vegetables. Higher doses between 10 and 50 kGy are employed to sterilize products with a low water activity (i.g. spices).  $\gamma$ -rays irradiation is suitable for manufacturing bulky food-stuffs, while electron beams are suitable for treating surfaces (Castagna et al., 2014; Chizoba Ekezie et al., 2018; Sung et al., 2013).

Besides the preservation of food, some researchers tested the effect of  $\gamma$ -irradiation on the allergenicity of food. Thus,  $\gamma$ -rays have shown to impact protein structure through reactions such as fragmentation, unfolding, crosslinking, and development of new reactive units, which may decrease allergens (Castagna et al., 2014; Sung et al., 2013).

Moreover, the reactive oxygen species (ROS) and photons break the covalent bond directly or indirectly. Secondary radicals form when ROS react with molecules, but the main denaturation comes after removing the hydrogen from the amino acid side chain, disulfide bonds, and aromatic compounds. Also, if proteins are irradiated in a solution, the allergenicity is reduced compared with proteins dispersed in a dry formulation due to intermolecular aggregation and crosslinking (Chizoba Ekezie et al., 2018).

Using doses between 2.5 and 30 kGy, the treatment of  $\gamma$ -irradiation on soybeans did not significantly change the intensity of the protein bands of major allergens Gly m 5, Gly m Bd 30 K, Gly m TI, and Gly m 4. Except for a decrease in Gly m TI, irradiation induced no major modifications (Moriyama et al., 2013).

A minor breakdown of Gly m 5 subunits was observed for irradiation doses between 5 and 25 kGy. Gly m 6 was reduced at doses higher than 25 kGy and disappeared altogether at 100 kGy. The peptide bond was cleaved at doses of 50–100 kGy, allowing the detection of aggregates on SDS-PAGE gels (Meinlschmidt et al., 2016).

Thus, an irradiation dose higher than 25 kGy is required to remove allergens effectively. The legislation allows only absorbed doses below 10 kGy for food products, which is insufficient to degrade the soybean proteins.

To conclude, exposure to the  $\gamma$ -rays causes protein structure changes, namely protein aggregation and conformational changes. Additionally, it was observed that irradiation has no significant effect on soybean allergenicity at doses allowed for food processing.

### 2.2.2. Pulsed ultraviolet light (PUV)

The patent for pulsed light used as a sterilization method belongs to Hiramoto (Japan, 1980) with an extension in the United States of America in 1984 (Sterixene, 2018). This discovery is also useful nowadays, being an excellent tool for bacterial inactivation in food manufacturing. Furthermore, the equipment and packaging materials are sterilized with this technique. Starting from 1999, the FDA approved this method for food sterilization (Jan et al., 2017).

Pulsed light (PL) operates with a white light issued as very short and high-power pulses. The light spectrum includes ultraviolet light (54%), infrared light (20%), and visible light (26%) (Shriver and Yang, 2011).

PL has a higher diffusion potential than UV light, and the pulses with increased energy have a better dissipation. A substantial voltage excites xenon (as an inert gas), followed by deexcitation, and then releases photons that are absorbed by the molecules found in the treated foods. In this way, the products are subjected to several thermal, physical, or chemical modifications (Shriver and Yang, 2011). Usually, twenty flashes per second are used for food products (Jan et al., 2017).

Because efficient chromophores sustain photoreactions, protein chromophores absorb the light, submitted at high energy levels. This causes protein modifications to insoluble forms and protein fragmentation through reactions such as oxidation, aggregation, and cross-linking (Chizoba Ekezie et al., 2018).

The heat produced in the product due to the absorption of UV light can contribute to the unfolding of proteins. Also, after the PUV treatment, some residual peptides can re-associate to create protein aggregations. The photothermal, photophysical, and photochemical effects are responsible for the alterations of protein structure, which may reduce allergenicity (Dong et al., 2020; Yang et al., 2010).

In 2010, a study assessed the effect of PUV treatments on soy extracts treated for 2, 4, and 6 min at 13.2 cm from the light source, with energy intensities roughly at 117.6, 235.2, and 352.8 J/cm<sup>2</sup>. The study showed that the treatment reduced the IgE binding by about 20%, 44%, and 50% for the 2, 4, and 6 min-treatment. Treatments of 4 and 6 min had a similar effect on IgE binding. However, 4 min and 235.2 J/cm<sup>2</sup> was considered optimal because it generated fewer proteins and off-flavors. An increase of time led to aggregation, which could be responsible for the allergenicity decrease. Thus, further applications can be developed for soybean products and beverages with fewer allergens using the PUV treatment (Yang et al., 2010).

Pulsed ultraviolet light applied at approximately 8 and 10 cm from the food product reduced the immunoreactivity of soy Gly m 5 and Gly m 6. The SDS-PAGE proved that Gly m 5 and Gly m 6 were no longer visible after 2 and 6 min of treatment, respectively. This phenomenon may be explained by the fact that soy proteins aggregate (by cross-linking) due to exposure to side chains (aliphatic and hydrophobic) (Meinlschmidt et al., 2016).

Furthermore, the same study presented a 91% intense reduction of soy protein immunoreactivity. Modifications of the conformational epitopes were observed for samples treated with PUV for 4 min, at a distance of 8 cm, using the monoclonal antibodies Izimab-Gly m 5-3, Izimab-Gly m 5-4, and Izimab-Gly m 5-5.

On a final note, the PUV treatment causes protein transformation into insoluble forms and protein fragmentation through oxidation, aggregation, and crosslinking, leading to malfunction of the IgE binding. Photophysical, photothermal, and photochemical effects generated by the PUV treatment impact the conformation of soybean allergens.

### 2.2.3. Cold plasma technology

Originally, cold plasma technology was used to improve the properties of polymers (amelioration of adhesion and printing) and in the electronics field. It is a non-thermal treatment and is currently used for bacterial inactivation of food materials, food packaging material, and changing the food properties (water absorption, solubility, thermal characteristics, pasting properties) (Chizoba Ekezie et al., 2017).

When an inert gas is associated with electricity, plasma is produced. The reactive material is charged with photons, different radiations, free radicals, and this formulation represents plasma, the fourth state of matter. The equipment used in this technology contains a power supply (alternating current 60 Hz) and a plasma emitter (a gliding arc system injected with gas) (Fig. 4). The electrodes are attached to the bottom and top of the system, which operates in the open air. Cold plasma is usually used in the food industry, which operates at 30°C–60 °C (Dey et al., 2016).

A study conducted on the toxicity of edible films treated with cold plasma showed that after 14 days, the subjects did not present any critical toxicity reactions. Male and female rats were administered 1000 mg/kg or 5000 mg/kg/day of edible films. After the ingestion, some modifications in blood appeared, but within normal values (Chizoba Ekezie et al., 2017).

Besides the other applications, cold plasma technology may affect the food allergen reactivity by altering conformational and linear epitopes caused by the generation of insoluble protein aggregates. Furthermore, peptide bonds may be split by reactive species, and the formed amino acids can be oxidized, impacting the protein integrity (Chizoba Ekezie et al., 2018).

Moreover, the proteins interact with the reactive species (ions, UV photons, free radicals, excited atoms, electrons) produced by gas ionization and excitation. These species can alter the conformation of proteins through different interactions. The cleavage of disulfide bonds and the attack of reactive oxygen species on amino acids through oxidation may reduce allergenicity (Chizoba Ekezie et al., 2018; Dong et al., 2020). Also, a short plasma treatment causes slight oxidation, resulting in the unfolding of proteins and the breaking of peptide bonds. A long-term plasma treatment produces covalent and noncovalent interactions, causing protein aggregation (Zhang et al., 2021).

Meinlschmidt et al. (2016) proved that cold plasma, applied to soy allergens, reduced the visibility of  $\beta$ -conglycinin and glycinin bands and revealed new proteins with 50 kDa molecular weight. A significant reduction (up to 89–100%) in soy protein immunoreactivity was also identified.

A reduction of immunoreactivity between 91 and 100% was observed when direct cold atmospheric pressure plasma (CAPP) was applied a sinusoidal peak-to-peak voltage at 9 kV<sub>pp</sub>, 10 kV<sub>pp</sub>, and 11 kV<sub>pp</sub> at a frequency of 3000 Hz, for 1–10 min. The operating remote (indirect) CAPP was found to reduce immunoreactivity by 89% after 90 min of treatment, using the monoclonal antibody Izimab-Gly m 5-4. An indirect cold atmospheric pressure plasma is described as a plasma generated by microwave, and direct CAPP uses surface dielectric barrier air discharge (SDBD) (Meinlschmidt et al., 2016).

The level of IgE-binding soybean protein isolate decreased by almost 75%, at 120 Hz for 5 min. At 80 and 120 Hz, the residual allergenicity was reduced slowly with the increasing time of cold atmospheric

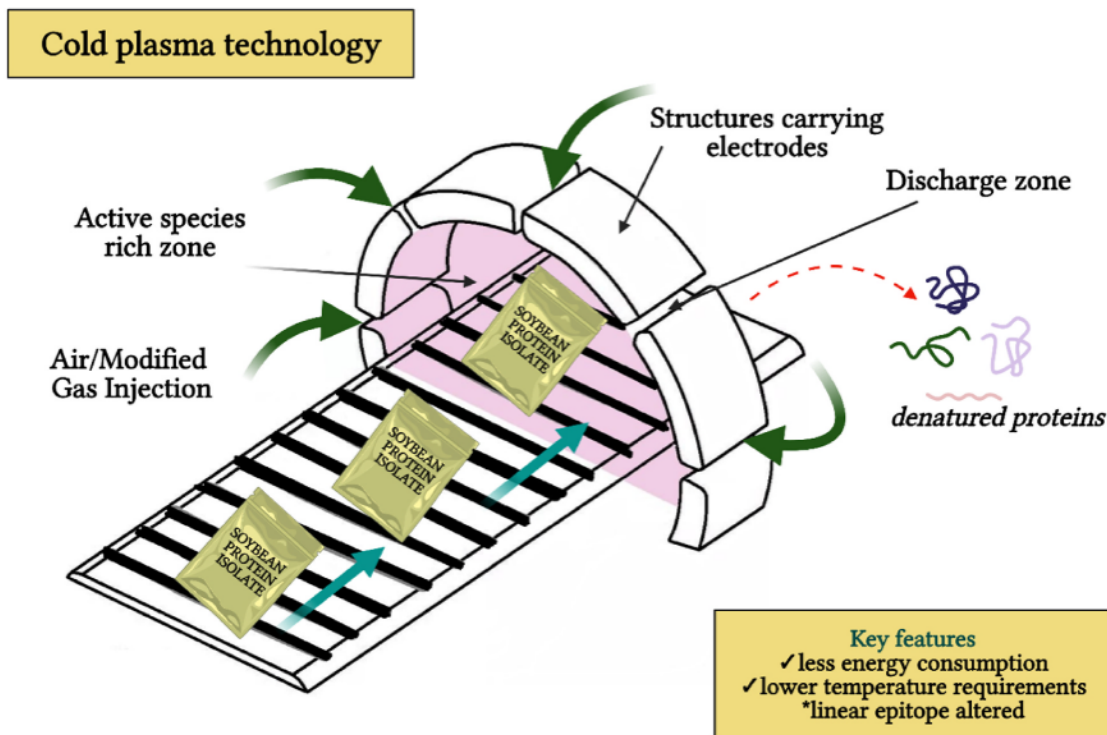


Fig. 4. Conceptual design of cold plasma unit (Misra & Jo, 2017) (created with BioRender.com).

pressure (Zhang et al., 2021).

In conclusion, cold plasma technology may alter the conformational and linear epitopes caused by the generation of insoluble protein aggregates, which reduces soy allergens.

#### 2.2.4. Microwave

Microwave radiation was discovered as a heating method in 1946. Besides the fact that it was used for a long time for heating, the treatment has many applications nowadays (medical, spectroscopy, electronics, food industry, telecommunications, radar, military, and chemistry). In the food area, the microwave is used for pre-cooking, preheating and reheating, drying of potato chips, roasting beans or grains, and so on (Das & Banik, 2021).

The operating principle of the microwave is based on a discontinuous electric field. Due to the very rapid alternation of the electric field (2.45 billion times per second), the polar or charged particles of the material subjected to the microwave will rotate back and forth. Also, the repeated movement of the charged particles with the inevitable friction, as well as their collisions, will cause a quick increase in the temperature of the medium, which leads to the heating of the material (Li et al., 2021). A range of  $3 \times 10^8$  to  $3 \times 10^{11}$  Hz is typical for the frequency applied by electromagnetic waves in the microwave treatment (Ohlsson & Bengtsson, 2001).

The microwave frequency has a particular regulation for preventing interference with other radio waves (Li et al., 2021). Thus, the Industrial and Scientific Medical (ISM) limited the frequency for food products to  $2450 \times 10^6 \pm 50 \times 10^6$  Hz (Ohlsson & Bengtsson, 2001). For the industry, it is usually used at  $915 \times 10^6/2450 \times 10^6$  Hz (Li et al., 2021).

The food particles absorb the energy created by microwaves, which produces heating dependent on the product's geometry and water content. This kinetic energy accelerates food protein denaturation by causing conformational modifications, affecting food allergenicity (Ohlsson & Bengtsson, 2001; Vanga et al., 2017). Additionally, protein digestibility increased after microwave treatment, and the secondary

structure was altered (losses in  $\beta$ -sheet structures and increases in a random coil) (Li et al., 2021). The same researcher mentioned that conduction heating combined with microwave treatment changed the protein profile by altering the hydrogen and ionic bonds.

A microwave treatment was tested on soybean for 25 min, at 700 W. Only 9 allergic people from a panel of 15 presented a response to the soybean allergen, using the enzyme allergosorbent test (EAST) (Besler et al., 2001).

In the study presented by Li, Zhu, et al. (2016), the antigenicity of SPI for babies was reduced by 24.7% when using a microwave oven heated at the power of 600 W for 10 min. No significant modifications of free SH were reported after the microwave treatment. On the other hand, Guan et al. (2011) mentioned that free SH increases or decreases were possible when employing high-power microwaves, resulting in the breaking of protein isolate disulfide bonds.

There are only a few studies on the effect of microwaves on soybean, making it a promising research field. This technique accelerates the denaturation of proteins, with an impact on the secondary structure.

#### 2.2.5. Ultrasonication

Karl Dussik, a neurologist, studied ultrasound for the first time in 1942 to detect brain tumors. In the following years, this technology was used more for medical purposes (Kane et al., 2004). Currently, it is considered an eco-friendly process and has many applications in the food industry. Namely, extraction, drying, emulsification, dehydration of fruits and vegetables, meat tenderization, filtration, inactivation of bacteria, defoaming, and degassing (Rahaman et al., 2016; Zhang et al., 2019). At the industrial level, REUS, a company ([www.etsreus.com](http://www.etsreus.com)) from France, developed equipment up to 1000 L (Chemat et al., 2011).

Waves produced by ultrasonic power form gas bubbles in the media, increasing high pressure and temperature, resulting in bubbles disruption and shear stress forming in the cavitation zone (Fig. 5). The temperature reached can be up to 5500 °C and pressure 50 MPa. The waves generate 20000 Hz or more (Jan et al., 2017; Sango et al., 2014). Also,

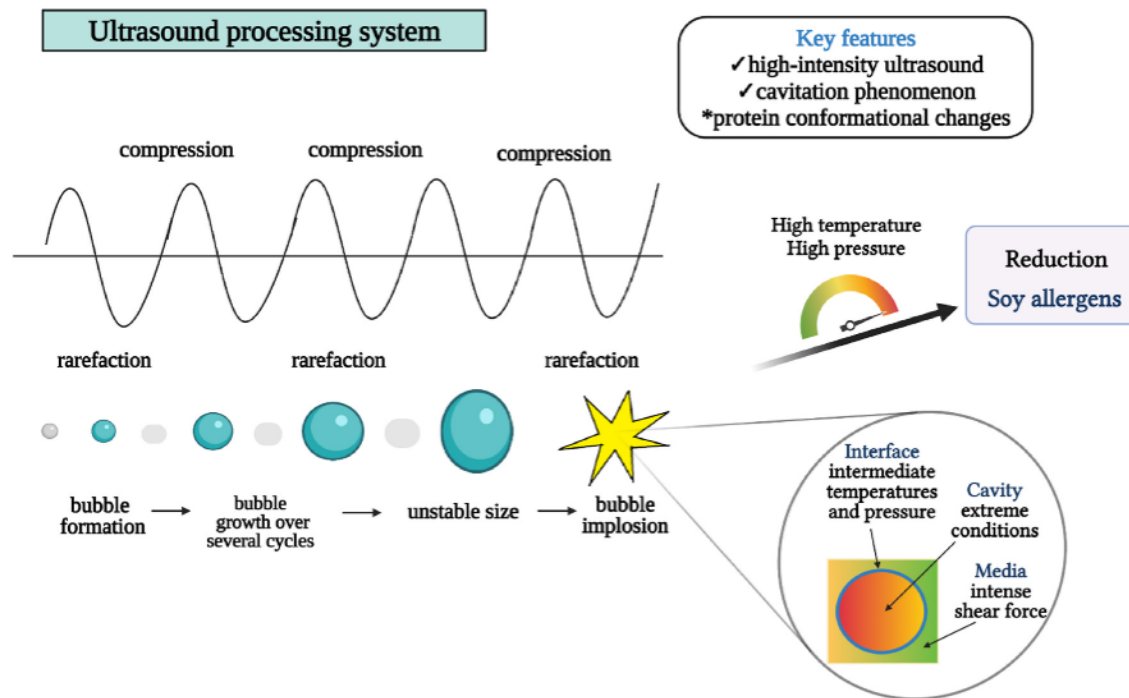


Fig. 5. Mechanism of the ultrasound processing system (Dong et al., 2020) (created with BioRender.com).

waves applied to the treated material may cause an increase in the chemical and structural transformations. These can be influenced by the temperature, pressure gradient, and additional mechanical fragmentation (Chizoba Ekezie et al., 2018).

The spatial structure changes by modifying the main chain of proteins, with a final impact on physicochemical properties. Some authors state that the hydrophobic effect produces the conformation change, while hydrogen bonds are affected by cavitation due to the high impact of shear stress. Also, a high-intensity treatment can induce the refolding of proteins (Lin et al., 2021). Also, the secondary structure can be damaged and the disulfide bond restructured (Chizoba Ekezie et al., 2018).

Yang et al. (2015) applied different ultrasound treatments (0–300 W) to soybean seeds that were later germinated at 30 °C for 5 days in darkness. At 300 W, the IgE-binding capacity was reduced by about 51.39% in the proteins of sprouted soybeans due to protein disruption or epitopes elimination. Additionally, an increase in moisture from 81.42% to 87.26% was observed in treated sprouts. No significant variations were observed between 0 and 200 W. Therefore, secondary and tertiary protein structures are disrupted by sonication at 300 W and local heat treatment.

In 2016, Li et al. observed a reduction of the antigenicity by 18.9% compared to the untreated soybean protein isolate after applying the ultrasonic treatment, at 20000 Hz, 600 W for 15 min.

Furthermore, Zheng et al. (2019) assessed whether ultrasound affected physicochemical characteristics of the soybean protein isolate for 10 or 25 min at 400 W. In a short time, there were no modifications to the secondary structure of the protein. However, the alteration of secondary and tertiary structure (by increasing the  $\alpha$ -helix content and reducing the  $\beta$ -sheet) took place after a treatment of 25 min. Additionally, new aggregates were formed after 10 min. A long time dissociated these new aggregates by reducing the particle size, improving surface hydrophobicity, and increasing solubility. Even if the study was not targeted at allergen, ultrasound impacts the protein structure by altering the secondary and tertiary structure, which may decrease soybean allergenicity.

To conclude, the waves produced by the ultrasonication treatment impact the protein conformation, which is changed by the high intensity and cavitation phenomenon.

In the end, to summarize the review, Table 1 shows a global overview of the physical technologies applied to soy allergens.

### 2.3. Comparison of the advantages and limitations of the physical treatments

After identifying all the physical treatments that may impact soybean allergenicity, all proposed techniques were compared to see which treatment may be the best or the worst for reducing soy allergens. In this way, we summarized the advantages and the limitations of those techniques in Table 2.

Cold plasma,  $\gamma$ -irradiation at high intensities, and PUV seem the most efficient in reducing soy allergenicity. Cold plasma reduces immunoreactivity of Gly m 5 up to nearly 100% by using mouse monoclonal anti-Gly m 5 antibodies (Meinlschmidt et al., 2016). A similar level of reduction is found in  $\gamma$ -irradiation at 100 kGy for the Gly m 6. Unfortunately, the legislation allows only absorbed doses below 10 kGy for food products. At this level, allergens are not removed effectively (Chizoba Ekezie et al., 2018). PUV treatment seems to cause protein crosslinking and aggregation or alter the allergen conformation (for the Gly m 6, a reduction up to 91% of immunoreactivity was observed for samples treated at 8 cm distance for 4 min) (Meinlschmidt et al., 2016). However, extended exposure to PUV can be harmful to industrial workers. Regarding the future perspectives, these technologies are also quite expensive to upscale to an industrial level, with limited productivity.

DIC and extrusion present a good potential to reduce the allergenicity of soybeans (>70%). Nevertheless, at this moment, allergen removal by DIC is at a research level, not an industrial application. Additionally, a decrease of 79–86% of  $\beta$ -conglycinin and glycinin was observed with the extrusion technique for a combination of soybean protein isolate with corn flour. It might be considered a potential treatment in terms of high productivity, low cost of equipment, and

**Table 1**  
Effect of physical treatments on soybean allergens.

Treatment	Food formulation	Allergens	Parameters	Observed effect/Allergenicity	References
Physical treatments based on pressure for soybean allergy control					
Extrusion	Defatted soybean meal	$\beta$ -conglycinin Glycinin	Twin-screw extrusion T = 66 °C-134 °C Screw speed (20 and 40 rpm)	Antigenicity reduced to 0.1% by degradation of protein structures	Ohishi et al. (1994)
	Milled soybean hypocotyls	$\beta$ -conglycinin ( $\alpha$ , $\alpha'$ , $\beta$ subunits) Glycinin (A3-subunit)	Twin-screw extrusion Screw speed = 280 rpm Feed rate = 51.4 kg/h T = 72–143 °C Not mentioned	Antigenicity can be reduced by approximately 1% if soybean hypocotyls are milled about 500 $\mu$ m (degradation of antigen soybean proteins)	Saitoh et al. (2000)
	Texturized soy protein (Soyatop®) Soybean seed	Gly m Bd 30K $\beta$ -conglycinin	T = 130 °C Feeding speed = 35 g/min Screw speed = 140 rpm T = 100 °C and 120 °C	It might be removed during texturization	Franck et al. (2002) Yin et al. (2019)
	Soybean seed	$\beta$ -conglycinin $\beta$ -conglycinin $\beta$ -conglycinin Glycinin	T $\geq$ 140 °C T = 110 °C; 140 °C Screw speed (200; 400 rpm) Moisture (20; 40%) 300 MPa, 25 min	Relatively blocked or destroyed epitopes Lowered antigenicity by a complete breaking of the $\beta$ -conglycinin epitopes Decreased immunoreactivity of SPI-corn and SF-corn (53%–68% and 79%–86%, respectively)	Yin et al. (2019) Yin et al. (2019) Zheng et al. (2020)
	Soybean seeds	Basic 7S globulin	300 MPa, 25 min	A decrease of the allergen	Omi et al. (1996)
	Crude glycinin	Glycinin	400 MPa, 10 min	Completed glycinin denaturation	Zhang et al. (2003) Zhang et al. (2003)
	Soybean whey	Glycinin	500 MPa, 10 min	Destroyed $\alpha$ -helix, $\beta$ -structure and changed into a random coil	Peñas et al. (2006)
	Soybean whey Soybean	Gly m 1 Gly m 1 $\beta$ -conglycinin	100, 200, 300 MPa 100–300 MPa, 15 min $\geq$ 300 MPa	Antigenicity was not detected for the sample under 300 MPa (denaturation and dissociation of some proteins) Reduced immunoreactivity (protein denaturation) $\beta$ -conglycinin might be denatured with fragmentation into subunits	Peñas et al. (2006) Zhang, Li, and Mittal (2010) Añón et al. (2011)
	Soybean protein isolate	$\beta$ -conglycinin Glycinin	200, 400, 600 MPa +0.2–0.6 mol/L NaCl 200 and 400 MPa +0.2–0.6 mol/L NaCl 600 MPa +0.6 mol/L NaCl 300 MPa, 15 min, 40 °C	Glycinin protected by NaCl against denaturation $\beta$ -conglycinin protected by NaCl against denaturation $\beta$ -conglycinin denatured Lower effect of IgE immunoreactivity	Peñas et al. (2006) Zhang, Li, and Mittal (2010) Añón et al. (2011)
	Soybean sprouts Commercial tofu	$\beta$ -conglycinin Glycinin $\beta$ -conglycinin Glycinin	300 MPa, 15 min, 40 °C 300 MPa, 15 min	No impact on the immunoreactivity Decreased allergenicity by 48.6% (changing in allergen structure)	Peñas et al. (2011) Li et al. (2012)
Soybean protein isolate (infant formula)	P34, I.O.X, 130 kDa, 115 kDa, 30 kDa, 28 kDa, $\alpha$ and $\gamma$ subunit of $\beta$ -conglycinin, Chain A and B of glycinin $\beta$ -conglycinin	350 MPa, 16 min	Reduced allergenicity by 46.6% (conformational epitopes inactivated)	Li, Zhu, et al. (2016)	
Soybean protein isolate (infant formula)	Glycinin	400 MPa, 15 min	Decreased antigenicity by 37% ( $\alpha$ -helix and $\beta$ -strand I reduced)	Xi and He (2017)	
Soybean protein isolate	Globulins 7S ( $\alpha$ and $\alpha'$ subunits) 11S (A1 and A1a subunits), P34, I.O.X, 130 kDa, 115 kDa, 30 kDa, 28 kDa, $\beta$ -conglycinin ( $\alpha$ and $\gamma$ subunit), glycinin (chain A and B) Not mentioned	300 MPa, 15 min 90 MPa in two steps 1 and 3 min, 0.3 MPa 3 min, 0.6 MPa	Decreased allergenicity by 45.5% (alteration of subunits protein) Reduced allergenicity by 29.8% (effect on protein conformation) Minor decrease of the immunoreactive band from soybean Higher reduction of immunoreactivity (degradation of proteins)	Li et al. (2018) Li, Zhu, et al. (2016) Cuadrado et al. (2011) Cuadrado et al. (2011)	
High-pressure homogenization (HPH)	Soybean protein isolate (infant formula)				
Controlled instantaneous pressure drop (DIC)	Soybean seeds				

(continued on next page)

Table 1 (continued)

Treatment	Food formulation	Allergens	Parameters	Observed effect/Allergenicity	References
	Soybean seeds	Not mentioned	3 min, 0.3 MPa 3 min, 0.6 MPa	2 IgE spots from 7 eliminated on the immunoblot 5 IgE spots disappeared from immunoblot and 2 small persisted noticeable on the gel	Takács et al. (2014) Takács et al. (2014)
Physical treatments in the form of waves for soybean allergy control					
Gamma- irradiation (γ-irradiation)	Soybean	β-conglycinin Gly m Bd 30K Gly m 4 Gly m 5 Gly m 6	30 kGy 5–25 kGy > 25 kGy 100 kGy	No major modifications Minor breakdown of Gly m 5 subunits Gly m 6 reduced Gly m 6 vanished (cleavage of peptide bond)	Moriyama et al. (2013) Meinlschmidt et al. (2016) Meinlschmidt et al. (2016) Meinlschmidt et al. (2016) Yang et al. (2010)
Pulsed ultraviolet light (PUV)	Soybean extract	β - conglycinin Glycinin	PUV at 2 min, 13.2 cm distance, 117.6 J/cm <sup>2</sup> energy intensity PUV at 4 min, 13.2 cm distance, 235.2 J/cm <sup>2</sup> energy intensity PUV at 6 min, 13.2 cm distance, 352.8 J/cm <sup>2</sup> energy intensity 1–6 min, distance 10 cm	Reduced immunoreactivity by 20% (protein aggregation) Reduced immunoreactivity by 44% (protein aggregation) Reduced immunoreactivity by 50% (protein aggregation) Gly m 5 not detected after 2 min	Yang et al. (2010) Yang et al. (2010) Yang et al. (2010) Meinlschmidt et al. (2016)
	Untoasted soybean/soybean protein isolate	Gly m 5 Gly m 6	1–6 min, distance 10 cm 4 min, distance 8 cm	Gly m 6 vanished after 6 min Reduced immunoreactivity up to 91% (soy proteins aggregation and crosslinking)	Meinlschmidt et al. (2016) Meinlschmidt et al. (2016)
Cold plasma technology	Untoasted soybean/soybean protein isolate	Gly m 5	Direct cold atmospheric pressure plasma (9 kv <sub>pp</sub> –11 kv <sub>pp</sub> ) for 1–10 min, frequency of 3000 Hz Remote cold atmospheric pressure plasma, 90 min, using the monoclonal antibody Izmab-Gly m 5-4 120 Hz, 5 min	Reduced immunoreactivity 91–100% (protein denaturation) Reduced immunoreactivity by 89% (protein denaturation)	Meinlschmidt et al. (2016) Meinlschmidt et al. (2016)
Microwave	Commercial soybean protein isolate Soybean protein isolate (infant formula)	β - conglycinin Glycinin P34, IOX, 130 kDa, 115 kDa, 30 kDa, 28 kDa, (α and γ subunit), glycinin (chain A and B)	600 W, for 10 min microwave oven	Level of IgE-binding SPI diminished by 75% (changing of the conformational structure) Reduced antigenicity by 24.7% (protein denaturation)	Zhang et al. (2021) Li, Zhu, et al. (2016)
Ultrasonication	Soybean sprouts Soybean protein isolate (infant formula) Soybean protein isolate	Main soybean allergens during germination P34, IOX, 130 kDa, 115 kDa, 30 kDa, 28 kDa, β-conglycinin (α and γ subunit), glycinin (chain A and B) It does not target allergens	0–300 W 20000 Hz, 600 W, 15 min 400 W, 10/25 min	IgE -binding reduced by about 51.39% (protein disruption/epitopes elimination) Reduced antigenicity by 18.9% (protein denaturation) 10 min - no modifications on the secondary structure of the protein; aggregates formed; increased particle size 25 min - secondary and tertiary structure altered (increased α-helix content and reduced β-sheet); new aggregates formed before they were dissociated; reduced particle size; the surface hydrophobicity improved; the solubility increased	Yang et al. (2015) Li, Zhu, et al. (2016) Zheng et al. (2019)

**Table 2**  
The advantages and limitations of the physical treatments.

Treatment	Advantages	Limitations	Future perspectives	References
<b>Physical treatments based on pressure for soybean allergy control</b>				
Extrusion	<ul style="list-style-type: none"> <li>- Good reduction of allergenicity (&gt;70%)</li> <li>- Protein digestibility improved (by protein denaturation)</li> <li>- Trypsin inhibitors, hemagglutinins, undesirable enzymes destructed</li> <li>- Natural food colors and flavors maintained</li> <li>- Continuous process and high productivity</li> </ul>	<ul style="list-style-type: none"> <li>- Protein nutritional values can be reduced by the Maillard reaction</li> <li>- The stability of vitamin A and E affected</li> <li>- Several heat-labile vitamins and amino acids are lost</li> <li>- Carotenoids and isoflavones affected</li> <li>- Temperature &gt;200 °C affects the nutritional quality of the product</li> </ul>	<ul style="list-style-type: none"> <li>- The high potential process to reduce the soy allergenicity, but the relationship between nutritional and sensory aspects of the product and the interaction between nutrient retention and extrusion conditions must be considered</li> </ul>	(Arêas, 2009; Moreno et al., 2018; Navale et al., 2016)
High hydrostatic pressure (HHP)	<ul style="list-style-type: none"> <li>- Low cost</li> <li>- Preservation of intrinsic and natural properties of foods</li> <li>- Inactivation of microorganisms</li> <li>- Minimal thermal exposure and reduced processing time</li> <li>- Suitable for liquid and solid packed products</li> <li>- Novel food formulation development</li> <li>- Food safety improvement of SPI in the manufacture of baby food</li> <li>- Positive feedback from the consumer as a physical and environmentally friendly process</li> </ul>	<ul style="list-style-type: none"> <li>- Moderate (about 50%) reduction of allergenicity</li> <li>- Oxidation of vitamins (vitamin C), colorants</li> <li>- Discontinuous process</li> <li>- Limited throughput for fluid products</li> <li>- High cost of pressure vessel (0.5–4 million €)</li> </ul>	<ul style="list-style-type: none"> <li>- Moderate effect on the reduction of food allergenicity</li> <li>- Additional studies are required to identify the impact on food allergens characteristics (functionality, structure, digestibility, etc.) and the actual clinical effects for developing foods that meet a real immunological tolerance and hypersensitivity</li> </ul>	(Augusto et al., 2018; Balasubramaniam et al., 2015; Huang et al., 2014; Lavilla et al., 2020; Muntean et al., 2016)
High-pressure homogenization (HPH)	<ul style="list-style-type: none"> <li>- Low-temperature process</li> <li>- Preservation of the protein nutritional values</li> <li>- Inactivation of microorganisms</li> <li>- Continuous process</li> </ul>	<ul style="list-style-type: none"> <li>- Weak reduction (&lt;30%) of allergenicity</li> <li>- Pressure/shearing affect the preservation of bioactive components and reduces vitamin C at 200 and 300 MPa</li> <li>- Decrease the total tocopherol content (soymilk) 200 and 300 MPa</li> <li>- Cannot be used alone to guarantee the reduction of allergenicity (complementary technology is needed)</li> <li>- High cost of maintenance</li> <li>- Increased lipid oxidation</li> <li>- High cost of the technology</li> <li>- The early state of using at the industrial level</li> </ul>	<ul style="list-style-type: none"> <li>- Limited effect of the reduction of soy allergenicity when the technology is used alone</li> <li>- This technology could have an interest-only if combine with other processes to reduce allergenicity</li> </ul>	(Augusto et al., 2018; Chauhan et al., 2018; Hogan et al., 2005; Osorio-Arias et al., 2021)
Controlled instantaneous pressure drop (DIC)	<ul style="list-style-type: none"> <li>- Good reduction of soy allergenicity (&gt;70%) at a pressure higher than 0.6 MPa</li> <li>- Higher sensory properties (flavor, color, texture)</li> <li>- Inactivation of microorganisms</li> <li>- Meets the highest requirements in terms of food safety</li> <li>- Few thermal degradations</li> <li>- Quick processing time</li> <li>- Environmental preservation</li> </ul>	<ul style="list-style-type: none"> <li>- High cost of the technology</li> <li>- The early state of using at the industrial level</li> </ul>	<ul style="list-style-type: none"> <li>- DIC seems to have a good impact on soy allergenicity</li> <li>- Nevertheless, <i>in vitro</i> tests and <i>in vivo</i> clinical data are needed to check the impact of DIC on the <i>in vivo</i> allergenicity of different legumes (soybean, lupine, chickpea, peanut, lentil)</li> <li>- Few possibilities, at present, to process large volumes of production</li> </ul>	(Burbano & Cuadrado, 2014; Hamoud-Agha & Allaf, 2020)
<b>Physical treatments in the form of waves for soybean allergy control</b>				
Gamma- irradiation ( $\gamma$ -irradiation)	<ul style="list-style-type: none"> <li>- Excellent reduction (&gt;90%) at a higher dose (100 kGy)</li> <li>- Inactivation of microorganism</li> <li>- Successful for different products</li> <li>- Availability of different sources (<math>\gamma</math>-rays, electron beam)</li> </ul>	<ul style="list-style-type: none"> <li>- Weak reduction (&lt;30%) of allergenicity at a low dose (25 kGy)</li> <li>- Long exposure to radiation can be harmful to industry workers</li> <li>- Limitation of legislation – (maximum dose allowed for food is 10 kGy)</li> <li>- Limited consumer acceptability</li> </ul>	<ul style="list-style-type: none"> <li>- <math>\gamma</math> radiations allow reducing allergenicity effectively at high intensities. The level of energy is unfortunately 10 times superior to what is authorized by the legislation</li> <li>- Low intensity treatment reveals a low impact on allergenicity</li> <li>- Further risk assessment studies on irradiated food must also be performed</li> </ul>	(Chauhan et al., 2018; Meinschmidt et al., 2016; Moriyama et al., 2013)
Pulsed ultraviolet light (PUV)	<ul style="list-style-type: none"> <li>- Excellent reduction of allergenicity</li> </ul>	<ul style="list-style-type: none"> <li>- Very high investment cost (300000-800000 €)</li> </ul>		(Chauhan et al., 2018; Meinschmidt et al., 2016)

(continued on next page)

Table 2 (continued)

Treatment	Advantages	Limitations	Future perspectives	References
Cold plasma technology	(>90%) - Continuous treatments - No chemicals are used - Non-heat process - Inactivation of microorganisms - Few impact on flavor, color, aroma - Few impact on the environment - Excellent reduction of allergenicity (>90%)	- Long exposure to the radiation can be harmful to the industry workers  - Slightly color changes induced	- High potential technology to reduce soy allergenicity, but at a very high cost - Consequently, more research is required on the optimization of UV light use  - Very recent technology shows the best reduction in soy allergenicity - This technology has great potential as long as it can evolve towards industrial installations and reduce costs - Further studies are still required to check treatment effects on nutritional and organoleptic profiles	(Chauhan et al., 2018; Chizoba Ekezie et al., 2017; Dey et al., 2016; Niemira, 2012; Zhang et al., 2021)
	- Less impact on sensory properties and nutrients content, as well as the absence of toxic by-products - Increased solubility and water recovery ability in protein isolates  - Residual pesticides in degraded food - Required few amounts of energy and short treatment time - Uniform heating of products	- Very recent technology, which required up-scaling towards industrial scale (low productivity)  - Complexity (vacuum needed) and cost of the equipment  - Commercial scale-expensive due to the use of helium  - Weak reduction (<30%) of allergenicity	- Studies show that microwave has a limited impact on soy allergenicity - The treatment could also affect the product and high productivity is not particularly easy to achieve	(Brewer, 2005; Das & Banik, 2021)
Microwave	- No solvent is needed  - Energy saving - Shorter time of equipment starting and duration of the process - Faster mass and energy transfer	- May affect the appearance, aroma, color, texture, and relative reconstitution capacity (for drying herbs) - Expensive equipment - Unsuitable for upscaling (large scale use)  - Moderate reduction of allergenicity (about 50%)		
Ultrasonication	- Inactivation of microorganisms  - Less environmental impact  - Small batch continuous equipment available	- Off-flavors could appear after treatment  - Impact on food properties at high power intensity (flavor, color, pH, antioxidant capacity, cloudiness) - Scale-up of the technology required (low actual production volume)	- Ultrasonication shows a moderate effect on the reduction of soy allergenicity - Scale-up required research (energy-efficient ultrasound processors) to reach economic feasibility	(Chemat et al., 2011; Li et al., 2021; Ojha et al., 2018)

improved protein digestibility. In terms of bioaccessibility, some components might be affected due to the temperature involved (>200 °C). However, regarding all advantages, extrusion seems to be today the most convenient technology to reduce soy allergenicity.

Further, HHP and ultrasonication express moderate effects (about 50%) in terms of allergenicity reduction. HHP seems to be the most studied treatment. This method better preserves the nutritional and sensory characteristics of the product. It can be used for liquids and solids, but some disadvantages such as discontinuous process and the high cost of the pressure vessel must be considered. Ultrasonication has a similar effect on allergenicity but is still uncertain about reducing the allergenicity to an industrial level, as is the case for other physical treatments.

Finally, HPH and microwave were shown to have less potential (allergenicity reduction <30%). However, these technologies could have an interest if combined with other technologies. In this way, some studies showed that a combination of different techniques (particularly fermentation and enzyme in combination with others) might be optimal to reduce the level of allergenicity because, at this moment, no treatment alone can reduce 100% all the allergens (Chizoba Ekezie et al., 2018; Dong et al., 2020; Pi et al., 2019, 2021; Rahaman et al., 2016).

### 3. Conclusion

All of the treatments reviewed hereby were found to reduce soy allergenicity at different levels. In this way, PUV,  $\gamma$ -irradiation at a high level, cold plasma, extrusion, and DIC have a good potential (reducing allergenicity >70%). The effect of ultrasonication, microwave, HPH, and HHP is much more moderate. Regarding proteins, the most common identified modifications are denaturation, inactivation of conformational epitopes, degradation of structures, alteration of protein subunits, aggregation, and crosslinking. Besides the potential use of these technologies to lower allergenicity, physical treatments better impact the product, nutritional and organoleptic characteristics. These advantages can then increase the shelf-life and added value of the product compared to the conventional methods.

However, some challenges and limitations must be addressed for all technologies before replacing conventional methods entirely. For example, the minimal effect on nutritional and sensory characteristics by HHP, the presence of off-flavors produced after ultrasonication, the negative impact of extrusion on carotenoids and isoflavones, the decrease in the bioaccessibility of different compounds for HPH, the decrease in protein digestibility when employing cold plasma, and the negative effect on product texture produced by microwave processing.

Besides product challenges, implementation, health, and production challenges must also be addressed. Such are the cases of PUV and  $\gamma$ -irradiation where workers and consumer health could be impacted. Therefore, manufacturers and governments need to construct and follow regulations that should aim to reduce their risks while maximizing their advantages (Augusto et al., 2018; Meinschmidt et al., 2016; Moreno et al., 2018; Ojha et al., 2018; Zhang et al., 2021).

To drive forward the aforementioned physical treatments for soybean processing, it is crucial to understand the relationship of allergens with the immune system at a molecular level. This insight will help understand the complexity of soybean allergenicity since it may increase, decrease, or persist unchanged depending on several method parameters such as intensity, time, temperature, pressure, and mechanical force. Therefore scientists and engineers could have more tools for the design and selection of the appropriate process conditions to improve the efficiency of the immunoreactivity reduction in soybean products.

This review hopes to bring more attention to the field by compiling the most recent advances in physical treatments focusing on soybean allergens reduction. Even if thermal processing was the first treatment applied to this intent, promising alternative technologies such as extrusion, high hydrostatic pressure, ultrasonication, controlled instantaneous pressure drop, cold plasma, pulsed ultraviolet light, and gamma-irradiation will have relevant and exciting applications in the food industry in the coming years.

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## References

- Abcar DIC Process. (2021). *The power of pressure-drop*. <https://www.abcar-dic.com/>. (Accessed 27 June 2021).
- Alimentarius, C. (2003). General standard for irradiated foods. *Codex Standard*, 106–1983. Rev.1-2003.
- Añón, M. C., De Lamballerie, M., & Speroni, F. (2011). Influence of NaCl concentration and high pressure treatment on thermal denaturation of soybean proteins. *Innovative Food Science & Emerging Technologies*, 12(4), 443–450. <https://doi.org/10.1016/j.ifset.2011.06.010>
- Aréas, J. A. G. (2009). Extrusion of food proteins. *Critical Reviews in Food Science and Nutrition*, 32(4), 365–392. <https://doi.org/10.1080/10408399209527604>
- Augusto, P. E. D., Tribst, A. A. L., & Cristianini, M. (2018). Chapter 20 - high hydrostatic pressure and high-pressure homogenization processing of fruit juices. In *Fruit juices: Extraction, composition, quality and analysis* (pp. 393–421). Academic Press. <https://doi.org/10.1016/B978-0-12-802230-6.00020-5>.
- Balasubramaniam, V. M., Martínez-Monteaegudo, S. I., & Gupta, R. (2015). Principles and application of high pressure-based technologies in the food industry. *Annual Review of Food Science and Technology*, 6, 435–462. <https://doi.org/10.1146/ANNUREV-FOOD-022814-015539>
- Bashir, K., Jan, K., Kamble, D. B., Maurya, V. K., Jan, S., & Swer, T. L. (2021). 2.08 - history, status and regulatory aspects of gamma irradiation for food processing. In *Innovative food processing technologies* (pp. 101–107). Elsevier. <https://doi.org/10.1016/B978-0-08-100596-5.23051-5>.
- Besler, M., Steinhart, H., & Paschke, A. (2001). Stability of food allergens and allergenicity of processed foods. *Journal of Chromatography B: Biomedical Sciences and Applications*, 756(1–2), 207–228. [https://doi.org/10.1016/S0378-4347\(01\)00110-4](https://doi.org/10.1016/S0378-4347(01)00110-4)
- Boughellout, H., Choiset, Y., Rabesona, H., Chobert, J. M., Haertle, T., Mounir, S., Allaf, K., & Zidoune, M. N. (2013). Effect of instant controlled pressure drop (DIC) treatment on milk protein's immunoreactivity. *Food and Agricultural Immunology*, 26(1), 71–81. <https://doi.org/10.1080/09540105.2013.864607>
- Brewer, M. (2005). Microwave processing, nutritional and sensory quality. *The Microwave Processing of Foods*, 76–101. <https://doi.org/10.1533/9781845690212.1.76>
- Burbano, C., & Cuadrado, C. (2014). Instant controlled pressure drop (D.I.C.). In T. Allaf, & K. Allaf (Eds.), *Food processing* (pp. 69–82). Springer New York. <https://doi.org/10.1007/978-1-4614-8669-5>.
- Cabanillas, B., Cuadrado, C., Rodriguez, J., Dieguez, M. C., Crespo, J. F., & Novak, N. (2018). Boiling and pressure cooking impact on IgE reactivity of soybean allergens. *International Archives of Allergy and Immunology*, 175(1–2), 36–43. <https://doi.org/10.1159/000485894>
- Cătușescu, G. M., Muntean, M., Marian, O., David, A. P., & Rotar, A. M. (2019). Comparative effect of gamma irradiation, drying and freezing on sensory, and hygienic quality of parsley leaves. *Lebensmittel-Wissenschaft und -Technologie- Food Science and Technology*, 115, Article 108448. <https://doi.org/10.1016/j.lwt.2019.108448>
- Chauhan, N., Singh, J., Chandra, S., Chaudhary, V., & Kumar, V. (2018). Non-thermal techniques: Application in food industries" A review. *Journal of Pharmacognosy and Phytochemistry*, 7(5), 1507–1518.
- Chauvin, M. A., & Swanson, B. G. (2011). Biochemical aspects of high-pressure food processing. *Nonthermal Processing Technologies for Food*, 72–88. <https://doi.org/10.1002/9780470958360.ch6>
- Chemat, F., Zill-E-Huma, & Khan, M. K. (2011). Applications of ultrasound in food technology: Processing, preservation and extraction. *Ultrasonics Sonochemistry*, 18(4), 813–835. <https://doi.org/10.1016/j.ultsonch.2010.11.023>
- Chizoba Ekezie, F. G., Cheng, J. H., & Sun, D. W. (2018). Effects of nonthermal food processing technologies on food allergens: A review of recent research advances. *Trends in Food Science & Technology*, 74, 12–25. <https://doi.org/10.1016/j.tifs.2018.01.007>
- Chizoba Ekezie, F. G., Sun, D. W., & Cheng, J. H. (2017). A review on recent advances in cold plasma technology for the food industry: Current applications and future trends. *Trends in Food Science & Technology*, 69, 46–58. <https://doi.org/10.1016/j.tifs.2017.08.007>
- Cuadrado, C., Cabanillas, B., Pedrosa, M. M., Muzquiz, M., Haddad, J., Allaf, K., Rodriguez, J., Crespo, J. F., & Burbano, C. (2011). Effect of instant controlled pressure drop on IgE antibody reactivity to peanut, lentil, chickpea and soybean proteins. *International Archives of Allergy and Immunology*, 156(4), 397–404. <https://doi.org/10.1159/000324443>
- Das, A., & Banik, B. K. (2021). Foundational principles of microwave chemistry. In *Microwaves in chemistry applications* (pp. 3–26). Elsevier. <https://doi.org/10.1016/B978-0-12-822895-1.00005-9>.
- De Angelis, E., Bavaro, S. L., Pilolli, R., & Monaci, L. (2019). Food and nutritional analysis | allergenic ingredients. In *Encyclopedia of analytical science* (3rd ed., pp. 349–373). Elsevier. <https://doi.org/10.1016/B978-0-12-409547-2.13957-5>.
- Dewan, F. (2020). *Thermal treatment of food preservation*. <https://doi.org/10.13140/RG.2.2.16633.49761/1>
- Dey, A., Rasane, P., Choudhury, A., Singh, J., Maisnam, D., & Rasane, P. (2016). Cold plasma processing: A review. *Journal of Chemical and Pharmaceutical Sciences*, 9(4), 2980–2984.
- Di Stasio, E., & De Cristofaro, R. (2010). The effect of shear stress on protein conformation: Physical forces operating on biochemical systems: The case of von Willebrand factor. *Biophysical Chemistry*, 153(1), 1–8. <https://doi.org/10.1016/j.bpc.2010.07.002>
- Dong, X., Wang, J., & Raghavan, V. (2020). Critical reviews and recent advances of novel non-thermal processing techniques on the modification of food allergens. *Critical Reviews in Food Science and Nutrition*, 61(2), 196–210. <https://doi.org/10.1080/10408398.2020.1722942>
- EFSA NDA Panel (EFSA Panel on Dietetic Products Nutrition and Allergies). (2014). Scientific Opinion on the evaluation of allergenic foods and food ingredients for labelling purposes. *EFSA Journal*, 12(11), 286. <https://doi.org/10.2903/j.efsa.2014.3894>, 2014.
- Ek, P., & Ganjyal, G. M. (2020). Chapter 1 - basics of extrusion processing. In *Extrusion cooking* (2nd ed., pp. 1–28). Elsevier. <https://doi.org/10.1016/B978-0-12-815360-4.00001-8>.
- EU. (2003). *Directive 2003/89/EC of the European Parliament and of the Council of 10 November 2003 amending Directive 2000/13/EC as regards indication of the ingredients present in foodstuffs* (Vol. 308, pp. 15–18). Official Journal of the European Union. <https://eur-lex.europa.eu/legal-content/EN/TXT/HTML/?uri=CELEX:32003L0089&from=EN>.
- EU. (2014). *Directive 2014/68/EU of the European Parliament and of the Council of 15 May 2014 on the harmonisation of the laws of the Member States relating to the making available on the market of pressure equipment* (Vol. 189, pp. 164–259). Official Journal of the European Union. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02014L0068-20140717>.
- EU. (2015). *Regulation (EU) 2015/2283 of the European parliament and of the council of 25 November 2015 on novel foods, amending regulation (EU) No 1169/2011 of the European parliament and of the council and repealing regulation (EC) No 258/97 of the E. Parliament* (Vol. 327, pp. 1–22). Official Journal of the European Union. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:32015R2283>.
- Franck, P., Moneret Vautrin, D. A., Dousset, B., Kanny, G., Nabet, P., Guénard-Bilbaut, L., & Parisot, L. (2002). The allergenicity of soybean-based products is modified by food technologies. *International Archives of Allergy and Immunology*, 128(3), 212–219. <https://doi.org/10.1159/000064254>
- Gray, D. R., & Chinnaswamy, R. (1995). Chapter 13 - Role of extrusion in food processing. In *Food processing* (pp. 241–268). Elsevier. <https://doi.org/10.1016/B978-0-44481500-2/50015-3>.
- Guan, J. J., Zhang, T. B., Hui, M., Yin, H. C., Qiu, A. Y., & Liu, X. Y. (2011). Mechanism of microwave-accelerated soy protein isolate-saccharide graft reactions. *Food Research International*, 44(9), 2647–2654. <https://doi.org/10.1016/j.foodres.2011.05.015>

- Haddad, J., & Allaf, K. (2007). A study of the impact of instantaneous controlled pressure drop on the trypsin inhibitors of soybean. *Journal of Food Engineering*, 79(1), 353–357. <https://doi.org/10.1016/j.jfoodeng.2006.01.066>
- Hamoud-Agha, M. M., & Allaf, K. (2020). Instant controlled pressure drop (DIC) technology in food preservation: Fundamental and industrial applications. In *Food preservation and waste exploitation* (pp. 1–17). IntechOpen. <https://doi.org/10.5772/intechopen.83439>
- Hite, B. H. (1899). The effect of pressure in the preservation of milk: A preliminary report. In *West Virginia agricultural and forestry experiment station bulletins*. <https://doi.org/10.33915/agnic.58>
- Hogan, E., Kelly, A. L., & Sun, D. W. (2005). 1 - high pressure processing of foods: An overview. In *Emerging technologies for food processing* (pp. 3–32). Elsevier. <https://doi.org/10.1016/B978-0-12676757-5/50003-7>
- Huang, H. W., Hsu, C. P., Yang, B. B., & Wang, C. Y. (2014). Potential utility of high-pressure processing to address the risk of food allergen concerns. *Comprehensive Reviews in Food Science and Food Safety*, 13(1), 78–90. <https://doi.org/10.1111/1541-4337.12045>
- Jan, A., Sood, M., Sofi, S., & Norzom, T. (2017). Non-thermal processing in food applications: A review. *International Journal of Food Sciences & Nutrition*, 2(6), 171–180.
- Jiménez-Saiz, R., Benedé, S., Molina, E., & López-Expósito, I. (2015). Effect of processing technologies on the allergenicity of food products. *Critical Reviews in Food Science and Nutrition*, 55(13), 1902–1917. <https://doi.org/10.1080/10408398.2012.736435>
- Kane, D., Grassi, W., Sturrock, R., & Balint, P. V. (2004). A brief history of musculoskeletal ultrasound: "From bats and ships to babies and hips. *Rheumatology*, 43(7), 931–933. <https://doi.org/10.1093/rheumatology/keh004>
- Lavilla, M., Puértolas, E., & Orcajo, J. (2020). Chapter 5 - HPP impact to reduce allergenicity of foods. In *Present and future of high pressure processing* (pp. 113–138). Elsevier. <https://doi.org/10.1016/b978-0-12-816405-1.00005-4>
- Li, H., Jia, Y., Peng, W., Zhu, K., Zhou, H., & Guo, X. (2018). High hydrostatic pressure reducing allergenicity of soy protein isolate for infant formula evaluated by ELISA and proteomics via Chinese soy-allergic children's sera. *Food Chemistry*, 269, 311–317. <https://doi.org/10.1016/j.foodchem.2018.07.001>
- Lin, D., Zhang, Q., Xiao, L., Huang, Y., Yang, Z., Wu, Z., Tu, Z., Qin, W., Chen, H., Wu, D., Zhang, Q., & Li, S. (2021). Effects of ultrasound on functional properties, structure and glycation properties of proteins: A review. *Critical Reviews in Food Science and Nutrition*, 61(15), 2471–2481. <https://doi.org/10.1080/10408398.2020.1778632>
- Li, W., Wang, Y., Zhao, H., He, Z., Zeng, M., Qin, F., & Chen, J. (2016). Improvement of emulsifying properties of soy protein through selective hydrolysis: Interfacial shear rheology of adsorption layer. *Food Hydrocolloids*, 60, 453–460. <https://doi.org/10.1016/j.foodhyd.2016.04.019>
- Li, S., Zhang, R., Lei, D., Huang, Y., Cheng, S., Zhu, Z., Wu, Z., & Cravotto, G. (2021). Impact of ultrasound, microwaves and high-pressure processing on food components and their interactions. *Trends in Food Science & Technology*, 109, 1–15. <https://doi.org/10.1016/j.tifs.2021.01.017>
- Li, H., Zhu, K., Zhou, H., & Peng, W. (2012). Effects of high hydrostatic pressure treatment on allergenicity and structural properties of soybean protein isolate for infant formula. *Food Chemistry*, 132(2), 808–814. <https://doi.org/10.1016/j.foodchem.2011.11.040>
- Li, H., Zhu, K., Zhou, H., Peng, W., & Guo, X. (2016). Comparative study of four physical approaches about allergenicity of soybean protein isolate for infant formula. *Food and Agricultural Immunology*, 27(5), 604–623. <https://doi.org/10.1080/09540105.2015.1129602>
- Meinlschmidt, P., Ueberham, E., Lehmann, J., Reineke, K., Schlüter, O., Schweiggert-Weisz, U., & Eisner, P. (2016). The effects of pulsed ultraviolet light, cold atmospheric pressure plasma, and gamma-irradiation on the immunoreactivity of soy protein isolate. *Innovative Food Science & Emerging Technologies*, 38, 374–383. <https://doi.org/10.1016/j.ifset.2016.06.007>
- Misra, N. N., & Jo, C. (2017). Applications of cold plasma technology for microbiological safety in meat industry. *Trends in Food Science & Technology*, 64, 74–86. <https://doi.org/10.1016/j.tifs.2017.04.005>
- Morantes, G., Ek, P., & Ganjyal, G. M. (2020). Chapter 16 - food safety in extrusion processing. In *Extrusion cooking* (2nd ed., pp. 507–521). Elsevier. <https://doi.org/10.1016/b978-0-12-815360-4.00016-x>
- Moreno, C. R., Fernández, P. C. R., Rodríguez, E. O. C., Carrillo, J. M., & Rochín, S. M. (2018). Changes in nutritional properties and bioactive compounds in cereals during extrusion cooking. In *Extrusion of metals, polymers and food products* (pp. 103–124). IntechOpen. <https://doi.org/10.5772/intechopen.68753>
- Moriyama, T., Yano, E., Kitta, K., Kawamoto, S. I., Kawamura, Y., & Todoriki, S. (2013). Effect of gamma irradiation on soybean allergen levels. *Bioscience Biotechnology & Biochemistry*, 77(12), 2371–2377. <https://doi.org/10.1271/bbb.130487>
- Muntean, M.-V., Marian, O., Barbieru, V., Cătușescu, G. M., Ranta, O., Drocas, I., & Terhes, S. (2016). High pressure processing in food industry – characteristics and applications. *Agriculture and Agricultural Science Procedia*, 10, 377–383. <https://doi.org/10.1016/j.aaspro.2016.09.077>
- Navale, S. A., Swami, S., & Thakor, N. J. (2016). *Extrusion Cooking Technology for Foods: A Review*, 2(3), 66–80.
- Niemira, B. A. (2012). Cold plasma decontamination of foods. *Annual Review of Food Science and Technology*, 3(1), 125–142. <https://doi.org/10.1146/ANNUREV-FOOD-022811-101132>
- Nishinari, K., Fang, Y., Guo, S., & Phillips, G. O. (2014). Soy proteins: A review on composition, aggregation and emulsification. *Food Hydrocolloids*, 39, 301–318. <https://doi.org/10.1016/j.foodhyd.2014.01.013>
- Ohishi, A., Watanabe, K., Urushibata, M., Utsuno, K., Ikuta, K., Sugimoto, K., & Harada, H. (1994). Detection of soybean antigenicity and reduction by twin-screw extrusion. *Journal of the American Oil Chemists' Society*, 71(12), 1391–1396. <https://doi.org/10.1007/BF02541361>
- Ohlsson, T., & Bengtsson, N. (2001). Microwave technology and foods. *Advances in Food & Nutrition Research*, 43, 65–140. [https://doi.org/10.1016/S1043-4526\(01\)43003-8](https://doi.org/10.1016/S1043-4526(01)43003-8)
- Ojha, K. S., Tiwari, B. K., & O'Donnell, C. P. (2018). Effect of ultrasound technology on food and nutritional quality. *Advances in Food & Nutrition Research*, 84, 207–240. <https://doi.org/10.1016/BS.AFNR.2018.01.001>
- Omi, Y., Kato, T., Ishida, K. I., Kato, H., & Matsuda, T. (1996). Pressure-induced release of basic 7S globulin from cotyledon dermal tissue of soybean seeds. *Journal of Agricultural and Food Chemistry*, 44(12), 3763–3767. <https://doi.org/10.1021/jf960231i>
- Osorio-Arias, J. C., Vega-Castro, O., & Martínez-Monteagudo, S. I. (2021). Fundamentals of high-pressure homogenization of foods. *Innovative Food Processing Technologies*, 118, 244–273. <https://doi.org/10.1016/b978-0-08-100596-5.23021-7>
- Peñas, E., Gomez, R., Frias, J., Baeza, M. L., & Vidal-Valverde, C. (2011). High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. *Food Chemistry*, 125(2), 423–429. <https://doi.org/10.1016/j.foodchem.2010.09.023>
- Peñas, E., Préstamo, G., Polo, F., & Gomez, R. (2006). Enzymatic proteolysis, under high pressure of soybean whey: Analysis of peptides and the allergen Gly m 1 in the hydrolysates. *Food Chemistry*, 99(3), 569–573. <https://doi.org/10.1016/j.foodchem.2005.08.028>
- Pi, X., Sun, Y., Fu, G., Wu, Z., & Cheng, J. (2021). Effect of processing on soybean allergens and their allergenicity. *Trends in Food Science & Technology*, 118, 316–327. <https://doi.org/10.1016/j.tifs.2021.10.006>
- Pi, X., Wan, Y., Yang, Y., Li, R., Wu, X., Xie, M., Li, X., & Fu, G. (2019). Research progress in peanut allergens and their allergenicity reduction. *Trends in Food Science & Technology*, 93, 212–220. <https://doi.org/10.1016/j.tifs.2019.09.014>
- Pi, X., Yang, Y., Sun, Y., Wang, X., Wan, Y., Fu, G., Li, X., & Cheng, J. (2021). Food irradiation: A promising technology to produce hypoallergenic food with high quality. *Critical Reviews in Food Science and Nutrition*, 1–16. <https://doi.org/10.1080/10408398.2021.1904822>
- Rahaman, T., Vasiljevic, T., & Ramchandran, L. (2016). Effect of processing on conformational changes of food proteins related to allergenicity. *Trends in Food Science & Technology*, 49, 24–34. <https://doi.org/10.1016/j.tifs.2016.01.001>
- Saitoh, S., Urushibata, M., Ikuta, K., Fujimaki, A., & Harada, H. (2000). Antigenicity in soybean hypocotyls and its reduction by twin-screw extrusion. *Journal of the American Oil Chemists' Society*, 77(4), 419–424. <https://doi.org/10.1007/s11746-000-0067-8>
- Salazar, F. A., Yildiz, S., Leyva, D., Soto-Caballero, M., Welti-Chanes, J., Anubhav, P. S., Lavilla, M., & Escobedo-Avellaneda, Z. (2021). HPP influence on food quality and bioactive compounds: A review of the last decade. In *Innovative food processing technologies* (pp. 87–111). Elsevier. <https://doi.org/10.1016/b978-0-08-100596-5.22984-3>
- Sango, D. M., Abela, D., Mcelhatton, A., & Valdramidis, V. P. (2014). Assisted ultrasound applications for the production of safe foods. *Journal of Applied Microbiology*, 116(5), 1067–1083. <https://doi.org/10.1111/jam.12468>
- Shriver, S. K., & Yang, W. W. (2011). Thermal and nonthermal methods for food allergen control. *Food Engineering Reviews*, 3(1), 26–43. <https://doi.org/10.1007/s12393-011-9033-9>
- Snyder, H. E. (2003). Soy (Soya) beans. The crop. In *Encyclopedia of food sciences and nutrition* (2nd ed., pp. 5379–5383). Elsevier. <https://doi.org/10.1016/b0-12-227055-x/01109-3>
- Somkuti, J., & Smeller, L. (2013). High pressure effects on allergen food proteins. *Biophysical Chemistry*, 183, 19–29. <https://doi.org/10.1016/j.bpc.2013.06.009>
- Sterixene. (2018). *History of pulsed light for sterilization*. <https://en.sterixene.com/news/history-of-pulsed-light-for-sterilization/>. (Accessed 25 June 2021).
- Sui, X., Zhang, T., & Jiang, L. (2021). Soy protein: Molecular structure revisited and recent advances in processing technologies. *Annual Review of Food Science and Technology*, 12(1), 3–4. <https://doi.org/10.1146/annurev-food-062220-104405>
- Sung, N. Y., Byun, E. B., Song, D. S., Jin, Y. B., Kim, J. K., Park, J. H., Song, B. S., Jung, P. M., Byun, M. W., Lee, J. W., Park, S. H., & Kim, J. H. (2013). Effect of gamma irradiation on mistletoe (Viscum album) lectin-mediated toxicity and immunomodulatory activity. *Federation of European Biochemical Societies Open Bio*, 3, 106–111. <https://doi.org/10.1016/j.fob.2013.01.003>
- Takács, G., Guillamon, E., Pedrosa, M. M., Cuadrado, C., Burbano, C., Muzquiz, M., Haddad, J., Allaf, K., Maczók, A., Polgár, M., & Gelencsér, É. (2014). Study of the effect of instant controlled pressure drop (DIC) treatment on IgE-reactive legume-protein patterns by electrophoresis and immunoblot. *Food and Agricultural Immunology*, 25(2), 173–185. <https://doi.org/10.1080/09540105.2012.759539>
- Tao, Y., Sun, D.-W., Hogan, E., & Kelly, A. L. (2014). High-pressure processing of foods. In *Emerging technologies for food processing* (2nd ed., pp. 3–24). Elsevier. <https://doi.org/10.1016/B978-0-12-411479-1.00001-2>
- Thrane, M., Paulsen, P. V., Orcutt, M. W., & Krieger, T. M. (2017). Chapter 2 - soy protein: Impacts, production, and applications. In *Sustainable protein sources* (pp. 23–45). Elsevier. <https://doi.org/10.1016/B978-0-12-802778-3.00002-0>
- U.S. Food and Drug Administration. (2016). *Food irradiation*. <https://www.fda.gov/food/buy-store-serve-safe-food/food-irradiation-what-you-need-know/>. (Accessed 27 June 2021).
- Vanga, S. K., Singh, A., & Raghavan, V. (2017). Review of conventional and novel food processing methods on food allergens. *Critical Reviews in Food Science and Nutrition*, 57(10), 2077–2094. <https://doi.org/10.1080/10408398.2015.1045965>
- Verhoecx, K. C. M., Vissers, Y. M., Baumert, J. L., Faludi, R., Feys, M., Flanagan, S., Herouet-Guichenny, C., Holzhauser, T., Shimjo, R., van der Bolt, N., Wichers, H., & Kimber, I. (2015). Food processing and allergenicity. *Food and Chemical Toxicology*, 80, 223–240. <https://doi.org/10.1016/j.fct.2015.03.005>

- Wal, J.-M. (2003). Thermal processing and allergenicity of foods. *Allergy*, 58(8), 727–729. <https://doi.org/10.1034/j.1398-9995.2003.00225.x>
- Wilson, S., Blaschek, K., & Mejia, E. G. (2005). Allergenic proteins in soybean: Processing and reduction of P34 allergenicity. *Nutrition Reviews*, 63(2), 47–58. <https://doi.org/10.1111/j.1753-4887.2005.tb00121.x>
- Xi, J., & He, M. (2017). High hydrostatic pressure (HHP) effects on antigenicity and structural properties of soybean  $\beta$ -conglycinin. *Journal of Food Science & Technology*, 55(2), 630–637. <https://doi.org/10.1007/s13197-017-2972-2>
- Yamamoto, K. (2017). Food processing by high hydrostatic pressure. *Bioscience Biotechnology & Biochemistry*, 81(4), 672–679. <https://doi.org/10.1080/09168451.2017.1281723>
- Yang, W. W., Chung, S. Y., Ajayi, O., Krishnamurthy, K., Konan, K., & Goodrich-Schneider, R. (2010). Use of pulsed ultraviolet light to reduce the allergenic potency of soybean extracts. *International Journal of Food Engineering*, 6(3). <https://doi.org/10.2202/1556-3758.1876>
- Yang, H., Gao, J., Yang, A., & Chen, H. (2015). The ultrasound-treated soybean seeds improve edibility and nutritional quality of soybean sprouts. *Food Research International*, 77, 704–710. <https://doi.org/10.1016/j.foodres.2015.01.011>
- Yin, H., Jia, F., Huang, J., Zhang, Y., Zheng, X., & Zhang, X. (2019). Effect of extrusion on the structure and antigenicity of soybean  $\beta$ -conglycinin. *Grain & Oil Science and Technology*, 2(3), 67–72. <https://doi.org/10.1016/j.gaost.2019.09.003>
- Zhang, Q., Cheng, Z., Zhang, J., Nasiru, M. M., Wang, Y., & Fu, L. (2021). Atmospheric cold plasma treatment of soybean protein isolate: Insights into the structural, physicochemical, and allergenic characteristics. *Journal of Food Science*, 86(1), 68–77. <https://doi.org/10.1111/1750-3841.15556>
- Zhang, H., Li, L., & Mittal, G. S. (2010). Effects of high pressure processing on soybean beta-conglycinin. *Journal of Food Process Engineering*, 33(3), 568–583. <https://doi.org/10.1111/J.1745-4530.2010.00607.X>
- Zhang, H., Li, L., Tatsumi, E., & Kotwal, S. (2003). Influence of high pressure on conformational changes of soybean glycinin. *Innovative Food Science & Emerging Technologies*, 4(3), 269–275. [https://doi.org/10.1016/S1466-8564\(03\)00043-2](https://doi.org/10.1016/S1466-8564(03)00043-2)
- Zhang, Z.-H., Wang, L.-H., Zeng, X.-A., Han, Z., & Brennan, C. S. (2019). Non-thermal technologies and its current and future application in the food industry: A review. *International Journal of Food Science and Technology*, 54(1), 1–13. <https://doi.org/10.1111/ijfs.13903>
- Zheng, T., Li, X., Taha, A., Wei, Y., Hu, T., Fatamorgana, P. B., Zhang, Z., Liu, F., Xu, X., Pan, S., & Hu, H. (2019). Effect of high intensity ultrasound on the structure and physicochemical properties of soy protein isolates produced by different denaturation methods. *Food Hydrocolloids*, 97, Article 105216. <https://doi.org/10.1016/j.foodhyd.2019.105216>
- Zheng, H., Yan, G. S., Lee, Y., Alcaraz, C., Marquez, S., & de Mejia, E. G. (2020). Effect of the extrusion process on allergen reduction and the texture change of soybean protein isolate-corn and soybean flour-corn mixtures. *Innovative Food Science & Emerging Technologies*, 64, Article 102421. <https://doi.org/10.1016/j.ifset.2020.102421>

## Glossary

- 2-DE: Two-dimensional electrophoresis  
 CAPP: Cold atmospheric pressure plasma  
 DIC: Controlled instantaneous pressure drop  
 EAST: Enzyme allergosorbent test  
 EFSA: European Food Safety Authority  
 ELISA: Enzyme-linked immunosorbent assay  
 EU: European Union  
 FAO: Food and Agriculture Organization of the United Nations  
 FDA: U.S. Food and Drug Administration  
 GMP: Good manufacturing practices  
 HHP: High hydrostatic pressure  
 HIU: High-intensity ultrasound  
 HPH: High-pressure homogenization  
 IgE: Immunoglobulin E  
 ISM: Industrial and Scientific Medical  
 IU: International unit  
 IUIS: International Union of Immunological Societies  
 Izimab: Generated mouse monoclonal antibodies  
 kDa: Kilodalton  
 kGy: Kilogray  
 kv<sub>pp</sub>: Practical peak kilovoltage  
 NACMCF: National Advisory Committee on Microbiological Criteria for Foods  
 P34: Gly m Bd 30K  
 PED: Pressure Equipment Directive  
 PL: Pulsed light  
 PUV: Pulsed ultraviolet light  
 ROS: Reactive oxygen species  
 Rpm: Rotation per minute  
 S: Svedberg sedimentation coefficient  
 SDBD: Surface dielectric barrier air discharge  
 SDS-PAGE: Sodium dodecyl sulphate–polyacrylamide gel electrophoresis  
 SF: Defatted soybean flour  
 SPI: Soy protein isolate  
 TI: Trypsin inhibitor  
 USDA: United States Department of Agriculture  
 UV: Ultraviolet (light)  
 WAO: World Allergy Organization  
 WHO: World Health Organization  
 $\gamma$ -irradiation: Gamma-irradiation  
 $\gamma$ -rays: Gamma-rays