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High Pressure Treatments of Soybean and Soybean Products

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1. Introduction

Soybean (*Glycine max*) is an industrial crop extensively cultivated for its oil and protein content. The global demand for soybean has increased dramatically over the last few years. Since the application of high hydrostatic pressure (HHP) to different food systems in the late 1800s (Bridgman, 1914), many researchers today are again applying this promising technology to the processing of foods. There is increasing worldwide interest in the use of HHP because of the advantages of this technology over other methods of processing and preservation. HHP offers homogeneity of treatment at every point in the product due to the fact the applied pressure is instantaneously and uniformly distributed within the HHP chamber. Therefore, processing time is not a function of sample size. Important advantages in using this technology are (1) significant or total inactivation of microorganisms (Knorr, 1993), and (2) better functional and nutritional retention of ingredients in the processed products, with improved food quality parameters (Hayashi, 1989). In addition, there is significant energy economy in comparison to thermal stabilization techniques, because once the desired pressure is reached, it can be maintained without the need for further energy input. Recently, processing of foods with HHP and low to moderate temperatures (less than 70 °C) was introduced as an alternative to thermal preservation. However, it was not until the late 1980s that researchers began investigating ways to commercialize high pressure treatment of foods (Hayashi, 1989).

The main uses of soybeans can be categorized into three groups: industrial, human food and livestock feed. Soybeans for human consumption are processed in many forms. Of major importance in Asian countries are soy foods such as tofu, soy sauce, miso, soy sprouts, and soymilk. These soy foods were recently introduced to the American market as were soy flour and soymilk previously. Soy flour is often mixed with other flours to increase the protein content, and soymilk provides an alternative source of protein for people allergic to the protein in cow's milk (Riaz, 1988). In addition to its versatility, the soybean is a commodity of unique chemical composition. On a mean dry matter basis, soybeans contain about 40% protein and 20% oil. Soybeans contains the highest protein content among food crops, and are second highest with respect to oil content among all the food legumes. Thus, the composition of soy products range in protein content from about 40% for full-fat flours to 95% or more for protein isolates (Wolf & Cowan, 1975).

2. High pressure processing of soybean

Due to the great variety of foods obtained from soybeans, different processing methods are required. Traditional methods include two types of processes: (1) fermentation, which uses fungi to produce fermented products; and (2) soaking and grinding of the soybeans to make bean curd and soymilk. During fermentation, protein is digested into peptides and later into amino acids for increasing digestibility of protein by the human body. Soaking and grinding are usually combined with thermal treatment to inactivate biologically active compounds such as trypsin inhibitors, lipoxygenase, and hemagglutinins, while increasing digestibility of proteins. In addition, thermal treatment (continued steaming) helps to diminish the characteristic beany flavor of raw soy products due to the volatilization of monocarbonyl compounds, which results from oxidation of fatty acids by the enzyme lipoxygenase. However, excessive heating may destroy certain amino acids that are sensitive to heat such as lysine, with losses of possibly more than 50% (Estrada-Giron et al., 2005).

Until recently, little research was being done on the effects of HHP on soybean grains and their sub-products. This is because of the recent interest in the use of HHP as a potential technology to improve the quality of cereals and textured products. These studies include reduction in the microbial population of soymilk curd, commonly known as tofu, to obtain a product with longer shelf life and to avoid secondary contamination. The solubilization of protein from whole soybean grains subjected to different treatments of pressure, time, and temperature was also reported. Additional information is available in a more extensive context about the effects of this technology on the inactivation of pure soybean lipoxygenase and lipoxygenase from some legumes (Estrada-Giron et al., 2005).

2.1 Microbial inactivation

The effects of high hydrostatic pressure on microbial inactivation depend on several factors such as type of microorganism, extent and duration of the high pressure treatment, temperature, and composition of suspension media or food. Therefore, suitable pressure treatment should be applied taking into account these factors to assure microbial inactivation of pathogenic, spoilage, and vegetative cells present in foods. Prestamo et al. (2000) reported that the microbial population of tofu pressurized at 400 MPa and 5 °C for 5, 30, and 45 min decreases from an initial microorganism count of 5.54×10^4 cfu/g to 0.31, 1.56, or 2.38 log units, respectively. Prestamo et al. (2000) also postulated that the effectiveness of HHP treatment to reduce microbial population at 400 MPa largely depends on the exposure time (Fig. 1). In the same study, after HHP treatment of tofu, psychrotrophs were reduced 2 log units from an initial population of 1×10^3 cfu/g. Mesophilic microorganisms were reduced 1 log unit from an initial number of 1.6×10^3 cfu/g, whereas yeast and molds decreased from an initial population of 2.64×10^3 cfu/g to 1×10^2 cfu/g. Other microorganisms such as *Pseudomonadaceae*, *Salmonella*, and Gram-negative bacteria (confirmed before HHP treatment) were not detected after HHP treatment of tofu. *Yersenia enterocolitica* and *Listeria monocytogenes*, which are more resistant to high pressure, were not found before and after HHP treatment. *Hafnia halvei* and *Bacillus cereus* remained active after high pressure treatment of tofu.

In addition to temperature and the extent and duration of high pressure treatment, a factor that significantly influences the effectiveness of HHP treatment on the inactivation and consequently the reduction in microbial population is the medium composition in which microorganisms are dispersed.

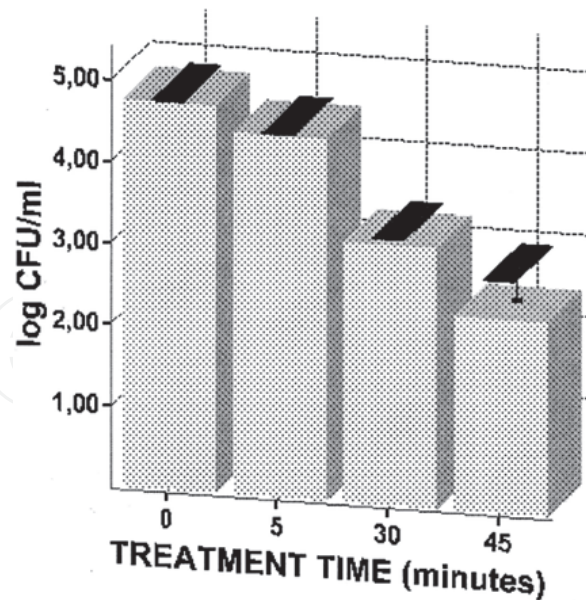


Fig. 1. Viable aerobic mesophilic population in tofu after treatment at 400 MPa and 5 °C for 5, 30, and 45 min (from Prestamo et al., 2000).

Food constituents such as sucrose, fructose, glucose, and salts affect the baro-resistance of microorganisms present in food (Oxen & Knorr, 1993). This effect is often observed since food constituents appear to protect microorganisms from the effects of high pressure. Therefore, a non-nutritive solution can reduce the microorganism's baro-tolerance. The presence of microorganisms such as *Hafnia halvei* and *Bacillus cereus* that remained active after HHP treatment could explain the baro-protective effect that food components exert over the extent on microbial reduction (Prestamo et al., 2000).

2.2 Proteins

Unlike allergenic proteins in cereals such as rice, soybeans contain a large number of proteins with important functional properties (Wolf & Cowan, 1975). Eighty percent of the proteins in soybeans are glycinin and β - and γ -conglycinin, which are globular salt-soluble proteins. On the basis of their sedimentation constants at pH 7.6 and ionic strength buffer of 0.5, the globulins are characterized as 11S or glycinin and 7S or β - and γ -conglycinin (Fukushima, 1991), with other less abundant globulins including 2S or α -conglycinin, 9S globulins, and 15S globulins. Functional properties associated with these kinds of soybean proteins:

1. Hydration properties such as swelling, solubility, and viscosity.
2. Protein-protein interactions resulting in precipitation and gelling.
3. Interfacial properties identified as surface tension and related foam/emulsion stability (Utsumi et al., 1998).

Therefore, the method of processing intact soybeans is important since the retention of proteins in the soybean seed is of special interest because of the high-quality vegetable protein, which also contains most of the essential amino acids (Steinke et al., 1992). When soybeans are immersed in hot water at 50–60 °C for 1 h, a considerable amount of protein solubilized from the soybean seeds is released to the surrounding water (Asano et al., 1989). Later studies identified these solubilized proteins as 7S globulins, which accounted for about 3% of the total protein in mature soybean seeds (Hirano et al., 1992).

In soybean seeds immersed in distilled water and treated at 300 MPa and 20 °C for 0–180 min, the solubilized proteins accounted for 0.5–2.5% of the total seed proteins. No apparent changes in shape, color, and size between treated and untreated soybeans were reported. The solubility of protein in surrounding water increased with increasing pressure, reaching a maximum value at 400 MPa (Fig. 2). Similar to what occurs when heat treatment is applied, SDS-PAGE patterns of high pressure treated seeds exhibited solubilization of 7S globulin, consisting of 27 and 16 KDa bands with staining intensity increasing as pressure increased to 400 MPa. The increase in staining intensity is indicative of the amount of release protein; thus, at higher intensity larger amounts of protein are released. At 700 MPa, 11S glycinin and 2S β -conglycinin also increased their staining intensity (Omi et al., 1996).

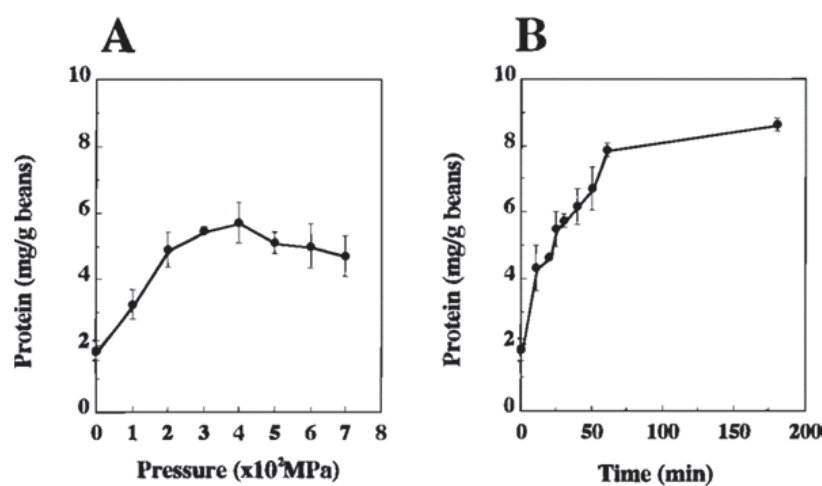


Fig. 2. Effect of high-pressure treatment on the release of proteins from soybean seeds. Water-immersed soybean seeds were pressurized at (A) 0–700 MPa and 20 °C for 25 min and at (B) 300 MPa and 20 °C for 0–180 min (From Omi et al., 1996).

2.3 Enzymes

At present, research regarding the inactivation of enzymes in intact grains and their sub-products is scarce. However, it is well known that high pressure modifies the activities of a whole range of unwanted food enzymes, which can result in a reduction in food quality or cause spoilage during storage. Recent investigations have reported the effect of combined pressure and temperature on soybean lipoxygenase. Lipoxygenase is one of the main anti-nutritional factors in soybean processing, which is also known to occur in other legume seeds, some cereal grains, and oil seeds. At least three types of lipoxygenase are well identified in soybeans as lipoxygenase I, II, and III. These enzymes catalyze the oxidation of unsaturated fatty acids in the presence of molecular oxygen. The presence of lipoxygenase can have detrimental effects on foods, for example:

1. Degradation of the essential fatty acids linoleic, linolenic, and arachidonic acid to yield fatty acid hydroperoxides.
2. Degradation of formed hydroperoxides, resulting in the formation of volatile compounds such as aldehydes, ketones, and alcohols, which cause the development of off-flavors.
3. Production of free radicals that can damage other compounds, including vitamins and proteins (Whitaker, 1972).

In soybean products, off-flavor development is highly dependent on the action of lipoxygenase since subsequent decomposition of the resulting hydroperoxides yields especially rancid flavor and beany aroma. Nevertheless, lipoxygenase is sensitive to heat and is destroyed at 82 °C when processed for 15 min (Baker & Mustakas, 1972). It is well known that thermal processing methods reduce considerably or completely inactivate unwanted enzyme activity, which limits or largely determines the conditions of storage needed to extend shelf life of food products. Although the behavior of enzymes under the influence of heat has been extensively studied, the effects of HHP treatment on enzyme inactivation are not clearly understood.

P/T treatment (MPa/°C)	Total time (min)	Cycling time	Activity retention
350/40	40	1×40	0.709
350/40	40	4×10	0.314
400/35	40	4×10	0.324
400/40	40	4×10	0.300
450/40	40	1×40	0.481
475/10	40	1×40	0.652
475/25	60	4×15	0.160
475/30	60	4×15	0.373
500/15	30	1×30	0.367
500/15	30	3×10	0.018
525/25	20	1×20	0.099
525/25	20	2×10	0.110

Adapted from Ludikhuyze et al. (1998a).

Table 1. Influence of multi-cycling on the inactivation of lipoxygenase in Tris-HCl at pH 9

Thermal inactivation of enzymes at atmospheric pressure occurs in the temperature range 60–70 °C. In contrast, pressure-temperature inactivation occurs in the pressure range 50–650 MPa at temperatures between 10 and 64 °C. Also, depending on the objectives of the research, pressure treatment may be applied in a single cycle or multi-cycles. Multi-cycling is the multiple application of pressure alone or in combination with temperature for the same total treatment time but with various numbers of cycles. Ludikhuyze et al. (1998a) reported the multi-cycling application of pressure to inactivate lipoxygenase. These authors found that in the pressure range 350–525 MPa and thermal treatment at 10–40 °C, the use of multi-cycles exerted an additional inactivation effect on lipoxygenase, compared to single cycle treatments (Table 1). Furthermore, temperature treatments at 10 °C caused an enhanced inactivation of lipoxygenase because the temperature inside the vessel dropped below zero upon depressurization.

In crude green bean extract, irreversible lipoxygenase inactivation was reported in the temperature range 55–70 °C at ambient pressure, whereas at room temperature, pressures around 500 MPa were required to inactivate lipoxygenase. High pressure treatment at 200 MPa and 50 °C resulted in 10% inactivation, while at least 50%, lipoxygenase inactivation occurred at pressures greater than 500 MPa and thermal treatment between 10 and 30 °C (Indrawati et al., 1999). The effect of HHP on enzyme inactivation in food systems is different compared to its effects on pure components dissolved in buffer solutions. As an example, solutions of commercial soybean lipoxygenase type I (100 mg/ml) dissolved in 0.2 M citrate-phosphate (pH range of 4.0–9.0) and 0.2 M Tris buffer (pH range of 6.0–9.0) were

subjected to pressures of 0.1, 200, 400, and 600 MPa for 20 min. Under these conditions, lipoxygenase in citrate-phosphate buffer lost more than 80% of its activity at alkaline pH, whereas it was completely inactivated at acidic conditions and pressure treatment of 400 and 600 MPa (Tangwongchai et al., 2000). In Tris buffer, lipoxygenase activity was significantly inactivated at pH 9.0 and 400 MPa and lost all activity at 600 MPa and all pH values. Similar results were observed by Seyderhelm et al. (1996) who reported that lipoxygenase in Tris buffer pH 7.0 was completely inactivated at 600 MPa and temperatures 45 and 50 °C for 10 min and 5–10 min, respectively.

3. Effect of HHP on technological properties of tofu

Tofu with 0, 2.5 or 5% trehalose was pressurized at 100–686 MPa and approximately -20 °C for 60 min to determine changes in temperature and sensory evaluation of high-pressure-frozen tofu as affected by trehalose.

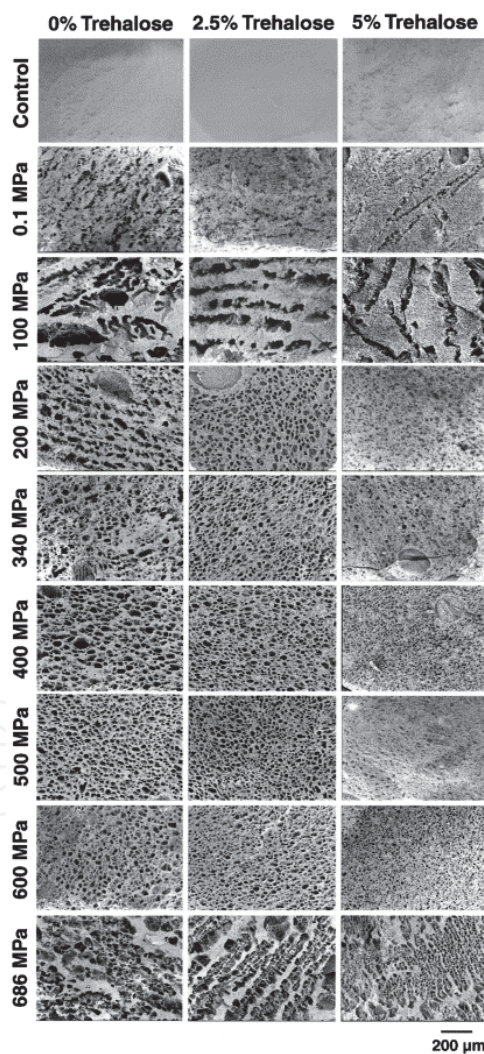


Fig. 3. Cryo-scanning electron micrographs of tofu frozen at high pressure. Tofu was pressurized at 100–686 MPa and approximately -20 °C for 60 min. After decompression, it was left in the pressure medium for 30 min then thawed at 20 °C. Control: unfrozen tofu (From Fuchigami et al., 2002).

Tofu froze during pressurization at 100 or 686 MPa; conversely, tofu did not freeze between 200 and 600 MPa and -20 °C, but it froze rapidly when the pressure was released. It was found that tofu frozen at 0.1, 100 or 686 MPa had larger ice crystals and was firmer (less like unfrozen tofu) than tofu frozen at 200–600 MPa. In the sensory evaluation, results showed that mouth feel (texture of tofu frozen at 400 MPa) was more like the control when 2.5% trehalose was added (Fuchigami et al., 2002).

The micro structure of the tofu gel network high pressure frozen at 686 MPa was compared with untreated tofu (Fig. 3). Tofu (0% trehalose) frozen at 0.1–500 MPa maintained a comparatively coarse network (data not shown), but tofu gel frozen at 686 MPa was compressed. Compression of the protein gel network might have occurred above 600–686 MPa; however, the gel network in tofu frozen at 686 MPa became coarse with the addition of trehalose. This indicates that trehalose with high-pressure-freezing appears to protect against compression (effects of concentration of protein and coagulants on frozen tofu) (Fuchigami et al., 2002).

4. High pressure inactivation of soybean lipoxygenase

The high pressure inactivation of lipoxygenase in soy milk and crude soybean extract was studied in the pressure range 0.1–650 MPa with temperature varying from 5 to 60 °C.

T (°C)	Soy milk	Crude soybean extract
63	0.55 ± 0.02 ^a r ² = 0.993	0.68 ± 0.03 r ² = 0.995
65	1.35 ± 0.05 r ² = 0.995	1.54 ± 0.05 r ² = 0.996
67	3.57 ± 0.09 r ² = 0.998	4.31 ± 0.12 r ² = 0.997
69	13.25 ± 0.50 r ² = 0.994	14.43 ± 0.42 r ² = 0.997
71	47.72 ± 4.64 r ² = 0.972	53.40 ± 2.74 r ² = 0.987
E _a (kJ/mol)	538.78 ± 29.04 r ² = 0.991	526.94 ± 29.54 r ² = 0.991

^a Standard error. (From Wang et al., 2008).

Table 2. Estimated inactivation rate constants ($\times 10^{-2} \text{ min}^{-1}$) for the isothermal inactivation of lipoxygenase in soy milk and in crude soybean extract

For both systems, the isobaric–isothermal inactivation of lipoxygenase was irreversible and followed a first-order reaction at all pressure–temperature combinations tested. In the entire pressure–temperature area studied, the lipoxygenase inactivation rate constants increased with increasing pressure at constant temperature for both systems; the rate constants were somewhat smaller in soy milk system than in crude soybean extract. At constant elevated pressure, lipoxygenase exhibited the greatest stability around 20 °C in both systems, indicating that the Arrhenius equation was not valid over the entire temperature range. For both systems, the temperature dependence of the lipoxygenase inactivation rate constants at high temperature decreased with increasing pressure, while the highest sensitivity of the lipoxygenase inactivation rate constants to pressure was observed at about 30 °C. The

pressure-temperature dependence of the lipoxygenase inactivation rate constants was successfully described either using an empirical mathematical model or using a thermodynamic kinetic model for both systems. On a kinetic basis, neither the reaction order of inactivation nor the pressure and temperature sensitivities of the inactivation rate constants were influenced by the different levels of food complexity between the two systems (Wang et al., 2008).

In 63 to 71 °C temperature range, isothermal inactivation of soybean lipoxygenase followed first-order kinetics, allowing inactivation rate constants (k) to be determined from plots of the natural logarithm of relative residual activity, as a function of inactivation time. The estimated k values, together with standard errors and regression coefficients, are summarized in Table 2. Over the entire temperature domain studied, lipoxygenase was less thermostable in crude soybean extract than in soy milk and the temperature sensitivity of the rate constants for lipoxygenase inactivation in both systems could be estimated using the Arrhenius relation (Wang et al., 2008). First-order kinetics for thermal inactivation of soybean lipoxygenase has been frequently reported in the literature (Indrawati et al., 1999; Ludikhuyze et al., 1998b). Ludikhuyze et al. (1998a, 1998c) investigated the thermal inactivation kinetics of commercial soybean lipoxygenase in Tris-HCl buffer (0.01 M, pH 9) at two different concentrations (0.4 and 5 mg/ml) over the temperature range 60–70 °C (Table 3).

T (°C)	Lipoxygenase concentration	
	0.4 mg/ml ^b	5 mg/ml ^c
60		2.09 ± 0.13 $r^2 = 0.978$
62	2.02 ± 0.09 ^a $r^2 = 0.987$	4.86 ± 0.21 $r^2 = 0.991$
64	4.94 ± 0.16 $r^2 = 0.993$	10.8 ± 0.61 $r^2 = 0.984$
66	9.18 ± 0.32 $r^2 = 0.992$	29.1 ± 3.37 $r^2 = 0.949$
68	15.5 ± 0.52 $r^2 = 0.992$	
E_a (kJ/mol)	319.8 ± 27.3 $r^2 = 0.986$	408.2 ± 14.7 $r^2 = 0.997$

^a Standard error. ^b Ludikhuyze et al. (1998c). ^c Ludikhuyze et al. (1998a). (From Wang et al., 2008).

Table 3. Inactivation rate constants ($\times 10^{-2} \text{ min}^{-1}$) for the isothermal inactivation of commercial soybean lipoxygenase in 0.01 M, pH 9 Tris-HCl buffer

Lipoxygenase in soy milk or in crude soybean extract exhibited a higher thermal stability with the corresponding smaller inactivation rate constants. The two activation energy values derived from the plots of the natural logarithm of inactivation rate constants, as a function of the reciprocal of the absolute temperature were larger, pointing to higher temperature sensitivity of the k values. Likewise, kinetic inactivation of lipoxygenase from many different sources, such as green peas, green beans, potatoes, asparagus, wheat germ, and germinated barley, have also been studied (Bhirud & Sosulski, 1993; Ganthavorn et al., 1991; Guenes & Bayindirli, 1993; Hugues et al., 1994; Indrawati et al., 1999; Park et al., 1988; Svensson & Eriksson, 1974). Indrawati et al. (1999) reported that thermal inactivation of

lipoxygenase in green bean juice could be described by a two-fraction first-order inactivation model, referring to the existence of two fractions (isozymes) with different thermal stability. However, in their study, this phenomenon was not observed.

Van Loey et al. (1999) studies soybean lipoxygenase inactivation [0.4 mg/mL in Tris-HCl buffer (0.01 M, pH 9)] quantitatively under constant pressure (up to 650 MPa) and temperature (-15 to 68 °C) conditions and kinetically characterized by rate constants, activation energies, and activation volumes. The irreversible lipoxygenase inactivation followed a first-order reaction at all pressure-temperature combinations tested. In the entire pressure-temperature area studied, LOX inactivation rate constants increased with increasing pressure at constant temperature. On the contrary, at constant pressure, the inactivation rate constants showed a minimum around 30 °C and could be increased by either a temperature increase or decrease. On the basis of the calculated rate constants at 102 pressure temperature combinations, an iso-rate contour diagram was constructed as a function of pressure and temperature. The pressure-temperature dependence of the LOX inactivation rate constants was described successfully using a modified kinetic model (Van Loey et al., 1999)

5. Immunoreactivity and nutritional quality of soybean products

Penas et al. (2011) reported that sprouts obtained from HHP-treated soybean seeds demonstrated an important reduction in immune-reactivity. Furthermore, they were a good source of proteins and essential amino acids, with Met and Cys corresponding to the limiting amino acids, as indicated by the chemical score (CS), and a high essential amino acid index (EAAI) (Table 4). These results suggested that HHP could constitute an important technological approach for the industrial production of hypoallergenic and nutritive soybean sprouts.

The HHP treatment of raw seeds (PRS) produced lower Gly and Cys levels than raw seeds (RS), while no significant differences ($P \leq 0.05$) were observed for total EAA (Table 4). The germination process resulted in a significant decrease in Glu, Trp and Cys, while no changes were observed in the other amino acids, compared to RS. The total EAA content showed a 4% reduction compared to RS. Pomeranz et al. (1977) found only minor differences in the amino acid composition of germinated and ungerminated soybean, while Mostafa et al. (1987) observed a marked increase in the relative contents of both EAA and NEAA after germination. The levels of sulphur amino acids in germinated soybean seeds remained almost constant, whereas Asp increased compared to raw seeds. Discrepancies between the data reported by other authors and those reported in the present work could be attributed to differences in the germination conditions and seed varieties.

The application of HHP treatment to seeds prior to germination (GPS) led to a reduction in Glu and Ala as NEAA and Trp, Met and Cys in EAA in comparison with GS, and also Pro, and Ile compared to PRS. GPS showed similar statistical ($P \leq 0.05$) values of total EAA content to GS (32 and 34 g/100 g protein, respectively), whilst significant ($P \leq 0.05$) differences were found compared to RS and PRS (34 g/100 g protein) (Table 4).

In another study, the effects of HPP on soybean cotyledon as a cellular biological material were investigated from the viewpoints of the cell structure and enzyme reaction system (Ueno et al., 2010). Damage to cell structure was evaluated by measuring dielectric properties using the Cole–Cole arc, the radius of which decreased as pressure level increased. Results suggested that cell structure was damaged by HPP. The distribution of

free amino acids was measured after HPP (200 MPa) of soybean soaked in water or sodium glutamate (Glu) solution. HPP resulted in high accumulation of free amino acids in water-soaked soybean, due to proteolysis. HPP of soybean in Glu solution caused higher accumulation of γ -aminobutyric acid, suggesting that both proteolysis and specific Glu metabolism were accelerated by HPP. They concluded that HPP partially degraded cell structure and accelerated biochemical reactions by allowing enzyme activities to remain. These events were described as “high-pressure induced transformation” of soybean.

	RS	PRS	GS	GPS	Whole egg protein ^B
Protein (% d.w.)	45.0 ^a	44.3 ^a	47.3 ^b	48.6 ^c	
<i>Non-essential amino acids</i>					
Glu	15.9 ^c	15.7 ^c	14.8 ^b	14.0 ^a	
Asp	7.25 ^a	7.26 ^a	7.09 ^a	6.80 ^a	
Arg	5.44 ^{ab}	5.60 ^b	5.05 ^a	5.27 ^{ab}	
Pro	4.26 ^b	4.25 ^b	3.79 ^{ab}	3.71 ^{ab}	
Ala	4.05 ^b	3.97 ^{ab}	4.04 ^b	3.71 ^a	
Ser	3.87 ^a	3.94 ^a	3.92 ^a	3.68 ^a	
Gly	3.50 ^b	3.07 ^a	3.15 ^{ab}	3.11 ^a	
<i>Essential amino acids</i>					
Leu	6.10 ^a	6.18 ^a	6.52 ^a	5.68 ^a	8.6
Lys	4.32 ^a	4.40 ^a	4.18 ^a	4.13 ^a	7.0
Val	4.19 ^a	4.26 ^a	4.26 ^a	4.11 ^a	6.6
Phe	4.13 ^{bc}	4.17 ^c	3.70 ^{ab}	3.90 ^{abc}	9.3 (Phe+Tyr)
Ile	4.12 ^b	4.22 ^b	3.78 ^{ab}	3.75 ^{ab}	5.4
Tyr	2.91 ^c	2.67 ^{abc}	2.81 ^{bc}	2.57 ^{ab}	
Trp	2.26 ^c	2.29 ^c	1.99 ^b	1.71 ^a	1.7
Thr	2.89 ^b	2.52 ^{ab}	2.50 ^{ab}	2.32 ^a	4.7
His	2.03 ^a	1.93 ^a	1.96 ^a	1.85 ^a	2.2
Met	1.32 ^b	1.36 ^b	1.32 ^b	1.16 ^a	5.7 (Met+Cys)
Cys	1.24 ^c	1.08 ^b	1.06 ^b	0.94 ^a	
Total EAA	35.5 ^b	35.1 ^b	34.1 ^{ab}	32.1 ^a	
CS	45.0	42.8	41.8	37.0	
EAAI	72.4	70.9	68.5	65.6	

^A Data are the mean of three independent results. Different superscripts in the same row mean statistically significant differences ($P \leq 0.05$). PRS: HHP-treated soybean seeds; RS: raw soybean seeds; GPS: germinated PRS, HHP-treated soybean seeds; GS: germinated soybean seed. EAA: essential amino acid; CS: chemical score; EAAI: essential amino acid index. ^B (FAO/WHO/UNU, 1985). (From Penas et al., 2011).

Table 4. Effect of HHP and/or germination on the total protein and amino acids (g/100 g protein) content of soybean seeds and sprouts during germination.^A

6. Inactivation of soymilk trypsin inhibitors

Protease inhibitors (PIs) are generally considered the main anti-nutritional factors in soybeans. Soybean PIs belong to a broad class of proteins that inhibit proteolytic enzymes,

such as trypsin and chymotrypsin. Both compounds are important animal digestive enzymes for splitting proteins to render dipeptides and tripeptides (Scheider, 1983). However, the specificity of these inhibitors is not necessarily restricted to trypsin and chymotrypsin but also to elastase and serine proteases for which serine constitutes the active site. Nevertheless, the literature reports two main types of soybean PIs, specifically called trypsin inhibitors (TIs). The Kunitz soybean inhibitor, with a molecular weight of 20,000 and two disulfide bridges, exhibits specificity to inhibit trypsin. The Bowman-Birk inhibitor, on the other hand, with a molecular weight ranging from 6000 to 10,000 and seven disulfide bonds, exhibits specificity to inhibit chymotrypsin (Liener, 1994).

Residual trypsin was measured in soymilk subjected to selected pressures, temperatures and holding times. Treatment combination at higher pressures and temperatures, for selected holding times resulted in an increased inhibition rate of trypsin inhibitors in soymilk. It was not possible to obtain inactivation rate parameters for treatments at 550 MPa and 80 °C because the data did not fit a first order kinetics model. However, a clear increase of residual trypsin was observed as treatment times increased. Soaking of soybeans in sodium bicarbonate solution, prior to preparation of soymilk, resulted in smaller inhibition rates of trypsin at the working selected pressures, combined with thermal treatment and holding times, than in soybeans soaked in distilled water. The use of sodium bicarbonate, as soaking medium of soybeans, did not result in a significant increase in the percentage of residual trypsin in soymilk treated at 550 MPa and 80 °C for the selected holding times (Fig. 4).

Ven et al. (2005) also evaluated HPP as an alternative for the inactivation of TIs in soy milk and also studied the effect of HPP on in whole soybeans and soy milk. For complete lipoxygenase inactivation either very high pressures (800 MPa) or a combined temperature/pressure treatment (60 °C/600 MPa) was needed. Pressure inactivation of TIs was possible only in combination with elevated temperatures. For TIs inactivation, three process parameters, temperature, time, and pressure, were optimized using experimental design and response surface methodology. A 90% TIs inactivation with treatment times of <2 min can be reached at temperatures between 77 and 90 °C and pressures between 750 and 525 MPa.

7. Conclusions

High hydrostatic pressure (HHP) processing is an innovative technology for processing of soybean which is an important food from nutritional point of view. HHP enables the inactivation of pathogenic bacteria at ambient temperatures. It also showed an increase in protein solubility and staining intensity due to the release of more protein after application of HHP. It also favored the inactivation of quality deteriorating enzymes such as lipoxygenase at room temperatures. Treatment of soybean with HHP improved the bio-availability of nutrients such as amino acids and the reduction of immune-reactivity. HHP also favored the activity of proteases, probably by reducing the activity of their inhibitors. It can be inferred that soybean and its products which are valuable food commodities can be effectively processed using this innovative processing technology, however more research needs to be done on HHP optimization and its effects on various physicochemical properties of soybean and different soy-foods.

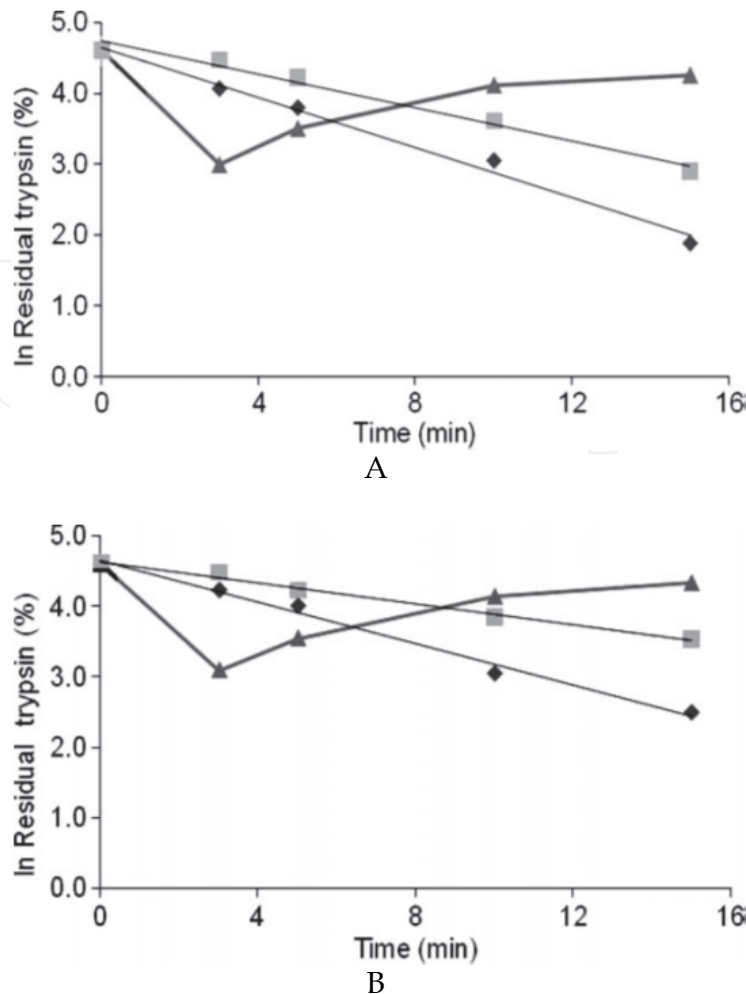


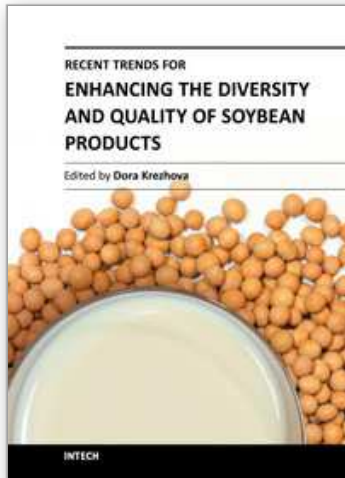
Fig. 4. Residual trypsin in HHP-processed (550 MPa) soymilk from soybeans previously soaked in water (A) and sodium bicarbonate solution (0.5%) (B). Temperatures: 19 (◆), 65 (■), and 80 °C (▲) (From Guerrero-Beltrán et al., 2009).

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This book presents new aspects and technologies for the applicability of soybean and soybean products in industry (human food, livestock feed, oil and biodiesel production, textile, medicine) as well as for future uses of some soybean sub-products. The contributions are organized in two sections considering soybean in aspects of food, nutrition and health and modern processing technologies. Each of the sections covers a wide range of topics. The authors are from many countries all over the world and this clearly shows that the soybean research and applications are of global significance.

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