Soybean Vegetable Protein (Tofu) Preserved with High Pressure

G. Préstamo,*,† M. Lesmes,‡ L. Otero,† and G. Arroyo‡

Instituto del Frío (CSIC), Ciudad Universitaria, 28040 Madrid, Spain, and Departamento de Microbiología III, Facultad de Biología, Universidad Complutense de Madrid, 28040 Madrid, Spain

Tofu is a soybean vegetable protein that Asians have long consumed; its intake is increasing in other countries. Tofu was purchased at a local shop. The tofu samples were already preserved in plastic bags subjected to vacuum and storage (5 °C). Tofu samples were subjected to high pressure (HP) of 400 MPa at 5 °C for 5, 30, and 45 min. Microbial analysis, sensorial evaluation, and structure were determined. HP treatment in tofu reduces the microbial population. Most of the microorganisms found in the initial population belonged to the Enterobacteriaceae family, bacteria Gram-negative (no Enterobacteriaceae), and bacteria Gram-positive. After HP treatment, *Hafnia alvei* and *Bacillus cereus* were found. After HP treatment, tofu is a pasteurized product, which is safer in terms of secondary pathogenic microbial contamination. Sensorial test results revealed that treated tofu was acceptable to consumers. The micrographs on the cryofracture observed with a cryoscanning electron microscope revealed a more compact structure after pressure compared with that of untreated samples, but the aggregates in the treated samples were more disperse.

Keywords: *Tofu; vegetable proteins; high pressure; microorganisms; ultrastructure*

INTRODUCTION

The soybean (*Glycine max*) is an important source of edible vegetable oil (20%) and high-quality vegetable protein (48−50%). The protein from soybean is complete and has all eight essential amino acids (Steinke, 1992). In this respect, soybean is not an ordinary bean. Asians consume soybean in many forms including soy milk, tofu, and fermented products such as miso, soy sauce, and tempeh. Moreover, soy products are being increasingly consumed in other countries because they are a good source of vegetable proteins and low fat content.

Chinese, Japanese, macrobiotic, or vegetarian soybean dishes such as tofu are becoming more popular among people seeking to prevent illness, reduce cholesterol levels, and generally improve their health. The phenolic compound in soybean has this therapeutic effect (Anderson et al., 1999).

Three kinds of functional properties have been reported for soybean proteins: (1) hydration properties (swelling, solubility, and viscosity); (2) protein−protein interaction (precipitation and gelling); and (3) interface properties (surface tension and foam/emulsion capacities) (Utsumi et al., 1998).

The gelation properties of soybean proteins is the reason for their use in imitation cheese (tofu-making) (Garcia et al., 1997).

Tofu preparation follows a procedure similar to that used for cheese. In cheese-making, animal milk is separated into curds and whey. Soy milk is also separated into curds and whey to form tofu. To coagulate the milk, a small amount of salt (Nigari, sea salt with magnesium chloride and calcium salts) has to be added (Shih et al., 1997). The curd consists of aggregated protein particles, and they form a gel capable of retaining water, lipids, sugar, flavors, and other components. Tofu hardness is affected by the ratio of protein to lipid (Taira, 1990).

The major proteins in soybean are globulins (glycinin and β- and γ-conglycinin), which represent 80% of total proteins. On the basis of their sedimentation constants at pH 7.6 and 0.5 ionic strength, they are known as 11S (glycinin) and 7S (β- and γ-conglycinin) globulins (Fukushima, 1991). There are other less abundant ones such as 2S (α-conglycinin), 9S, and 15S globulins. The 7S and 11S globulins interact with each other during heating to form the matrix (Utsumi et al., 1985).

High temperature (75−94 °C) and low pH produce protein denaturation, and this denaturation affects the tertiary and quaternary proteic structure. When denaturation occurs at high protein concentration, the gel appears. The gelation process depends on protein concentration, bonds, pH, and the presence of salts in the medium. This is an irreversible step consisting of a cross-linking through disulfide and hydrogen and hydrophobic interactions among soy proteins (Utsumi and Kinsella, 1985). Textural properties of the gel are related to the networking degree of the matrix.

Tofu is preserved refrigerated under vacuum condition, but without any pasteurization treatment to prevent secondary contamination. We focused the experiment on the microbial, structural, and sensorial aspects before and after high-pressure (HP) treatment. The aim of this work is to study a food treated with HP to produce a safer and pasteurized product with a longer shelf life.

MATERIALS AND METHODS

Plant Material. For the experiment we used tofu purchased from a local vegetarian shop. The tofu was 3 days old (the expiration date was 2 months later). The samples (six packages of 300−350 g) were already preserved in plastic bags subjected to vacuum and storage (refrigerated).

* Author to whom correspondence should be addressed (fax 91 549 36 27; e-mail lupep@if.csic.es).

† Instituto del Frío (CSIC).

‡ Universidad Complutense de Madrid.

10.1021/jf991251y CCC: $19.00
Published on Web 06/13/2000

High-Pressure Treatment. The tofu samples were immersed in a steel container (100 mm diameter, 300 mm height, 2.35 L volume), filled with a low compressibility fluid (water). The water acted as the pressurizing medium, and a thermocouple submerged in the pressure fluid measured the temperature during treatment. The apparatus (ACB GEC Alsthom, Nantes, France) was capable of achieving a maximum pressure of 500 MPa. The temperature was held constant using a water bath. Temperature and pressure were recorded in a Lab Tech notebook program (Laboratory Technologies Corp., Wilmington, MA). The HP conditions used in this work were chosen from previous work (Arroyo et al., 1997, 1999). The samples were subjected to high pressure of 400 MPa at 5 °C for several minutes (5, 30, and 45 min). In each experiment, the indicated pressure was achieved within 2−3 min, held for 5, 30, or 45 min, and then released to atmospheric pressure within 5−8 min. After the pressure was released, the pressurized samples were analyzed.

Microbiological Analysis. An amount of 10 g of each sample was homogenized in a Stomacher (400 Lab Blender) with 90 mL of tryptone soy broth (TSB; Pronadisa, Madrid, Spain). Decimal dilutions were also prepared with TSB, and 0.1 mL of the dilutions was plated on tryptone soy agar (TSA). The following analyses were carried out in triplicate samples: viable aerobic mesophilic bacteria counts, sporulated mesophilic microorganisms counts, psychrotroph counts, mold and yeast counts, and total enterobacteraceae counts; fecal coliform counts and confirmation, fecal enterococci counts and confirmation, sulfite-reducing microorganism spore counts (APHA,-1984); *Staphylococcus aureus* counts and detection (Anderson, 1992); *Pseudomonas aeruginosa* counts and detection (Holt, 1993).

Presence and Isolation of Salmonella shigella. Selective enrichment was carried out in Muller−Kauffman tetrathionate broth and Rappaport−Vassiliadi broth. Selective isolation took place in *S. shigella* Brilliant Green and Hektoen agar.

Identification was achieved using morphological and biochemical tests.

Presence and Isolation of Yersinia enterocolitica. Selective enrichment was performed in Wauters broth and isolation in deoxycholate−citrate agar and Cefsulodin−Irgasam Novobiocin agar.

Presence and Isolation of Listeria monocytogenes. Selective enrichment was carried out in TSB with 12.5 mg/L of acriflavine, 10 mg/L nalidixic acid, and 10 mg/L cicloheximide. Isolation was performed on McBride agar and Palcalm agar.

Analysis of variance together with an F test and Duncan's multiple-range test were used to compare means and to identify significant differences ($P < 0.05$) among treatments. Data analysis was performed using the SPSS 8.0 program (Statistical Program SPSS Inc., Chicago, IL).

Sensorial Evaluation. Changes in taste were evaluated subjectively (Dethmer, 1981). Taste descriptions included "same as untreated samples or different" and as well as good, regular, or bad taste. Evaluations were carried out before and after pressure treatment. The judges liked tofu.

Cryo-SEM. Cryopreservation is an excellent method for determining the real structure of a product. The internal tofu structure can be observed using the Cryo-SEM rapid freezing and fracturing procedure in the optical chamber transmission (OCT) 1500C cryo-trans for scanning electron microscope (Zeiss). The sample processing time was 5 min and the samples can be examined for an extended period in a vacuum without water loss by rapidly cooling and maintaining them below −150 °C. The samples were mounted with OCT compound (Gurr) and mechanically fixed on a sample holder. Rapid freezing of the specimens in the cryostat was achieved by plunging the specimens into nitrogen slush and then transferring them to the preparation unit via an airlock transfer device. The sample was fractured in the preparation unit and transferred directly via a second airlock to the microscope cold stage, where samples were warmed. Greater detail could often be revealed by carefully raising the temperature to a point at which water began to sublime at a controlled rate (−90 to −100 °C for 2 min). This etching process was halted by rapidly lowering the temperature. Finally, it was usually necessary to coat the specimens with a film of conducting metal (gold) at low temperature to prevent charging during examination and photography. After coating, the specimens were transferred again to the SEM cold storage. The specimen was scanned at the cold stage temperature of −135 °C using an accelerating voltage of 15 kv.

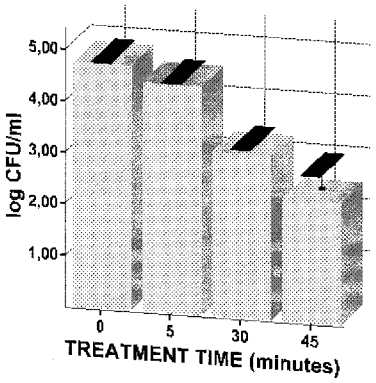

Figure 1. Results of viable aerobic mesophilic population of tofu untreated samples and treated samples at 400 MPa at 5 °C for 5, 30, and 45 min.

Table 1. Microorganisms Isolated on Tofu Control Samples

Enterobacteriacea	*Bacillus* Gram-negative, no
Klebsiella pneumoniae	Enterobacteriacea
Enterobacter intermedius	*Pseudomonas aeruginosa*
Hafnia alvei	*Pseudomonas fluorescens*
Citrobacter freundii	*Chryseomonas luteola*
Cedecea lapagei	*Flavobacterium meningosepticum*
Serratia plymuthica	*Pseudomonas aureofaciens*
Escherichia coli	*Pseudomonas putida*
Salmonella arizonae	*Bacillus* Gram-positive
Coccus	*Bacillus cereus*
Staphylococcus aureus	*Bacillus subtilis*
Enterococcus faecalis	

RESULTS AND DISCUSSION

Effect of Pressure on Tofu. The microbial analysis showed that the initial viable aerobic mesophilic population of untreated samples was 5.54×10^4 colony-forming units (cfu)/g. The microorganisms identified in the samples before pressurization are shown in Table 1. The results showed that the population decreased after HP treatment (400 MPa, at 5 °C for 5, 30, and 45 min). This decrease is in accordance with the treatment time. At 5 min the population decreased 0.31 log unit and at 30 min, 1.56 log units. Under the same conditions but with increased time (45 min) the population decreased 2.38 log units (Figure 1).

The statistical results indicate that treatment efficiency in reducing the microbial population at 400 MPa pressure was largely dependent on its duration. Significant differences were found among all treatments (Duncan's multiple-range test, $P < 0.05$). Thus, the microbial population decreased after all treatments proportionally to their duration.

The initial sporulated mesophilic microorganisms number was 1.6×10^3 cfu/g; after HP treatment, the population decreased 1 log unit. The initial psycrotroph microorganisms was 10^3 cfu/g; after HP treatment, the values were 2 log units less. In yeast and molds the initial population was 2.64×10^3 cfu/g, and after HP treatment, the final value was 10^2 cfu/g.

The total Enterobacteraceae was 1.4×10^3cfu/g; after HP treatment, the total amount was 1.1×10^2 cfu/g and

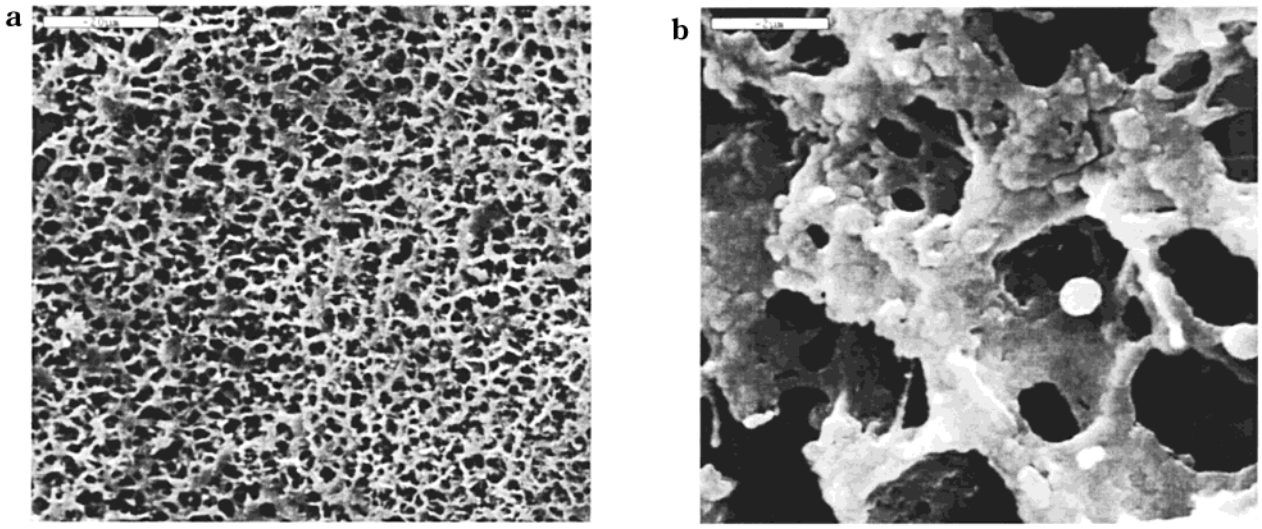

Figure 2. Tofu freeze fracture SEM: (a) untreated tofu ultrastructure, bar = 20 μm; (b) higher magnification, bar = 2 μm.

with biochemical assays (Holt, 1993), and some of them were identified (Table 1).

The fecal coliforms amount was >2500 microorganisms/g, as was the number of fecal enterococci. After HP treatment, the microorganisms amount was 250 microorganisms/g. No sulfite reductor spores were found.

The presence of *Escherichia coli* and *Enterococcus faecalis* was confirmed (APHA, 1984) before HP treatment (Table 1). The initial staphylococci amount was 10^3 cfu/g. The biochemical assays (Anderson, 1992) confirmed the presence of *Staphylococcus aureus* before HP treatment (Table 1).

The initial number of Pseudomonadaceae was 1.7×10^3 cfu/g, and with biochemical assays some of them were confirmed before the HP treatment but not after HP treatment (Table 1).

The *Salmonella* assay was positive, identifying *Salmonella arizonae*. However, after HP treatment this species was not detected.

No *Yersinia enterocolitica* and *Listeria monocytogenes* were found before or after HP treatment.

At 45 min, *Hafnia alvei* and *Bacillus cereus* were the only remaining active microorganisms found; the others were inactivated.

One of the advantages of using HP treatment in food preservation is that it pasteurizes the product; the microbial population decreases, and as a result of this their shelf life increases (Knorr, 1993).

The degree of microorganism inactivation depends on different factors: microorganism type, pressure−time−temperature applied, and composition of the dispersion medium. When Gram-negative bacteria, molds, and yeast pure cultures were subjected to pressures of 350 or 400 MPa for 30 min at 5 °C, the microbial population decreased and no viable cells were found on plate agar (TSA). Gram-positive bacteria were more resistant to the high pressure, showing a viable cell number closer to 10^2 cfu/mL (Arroyo et al., 1999). However, the same experiment was carried out on vegetables (lettuce, tomato, asparagus, spinach, cauliflower, and onion) also by Arroyo et al. (1999), and the viable aerobic mesophilic bacteria, molds, and yeasts still presented viable cells after treatment at 400 MPa for 30 min at 5 °C. The presence in food of endogenous microorganisms in comparison to pure culture could be explained due to the protective effect of some food components. The medium composition where microorganisms are dispersed at the moment of pressurization also influences significantly the efficiency of inactivation (Patterson et al., 1995). For this reason, several microorganisms present in food show a baroresistant effect to HP treatment. The presence of sugars (sucrose, glucose, and fructose) and salts also has a baroresistant effect to microorganisms that are polluting food (Horie et al., 1991; Tokahashi et al., 1993; Oxen and Knorr, 1993).

Styles et al. (1991) also reported that milk subjected to HP treatment has a baroprotective effect to microorganisms, and pressure of 340 MPa at 23 °C for 80 min was needed to inactivate *Listeria monocytogenes* in milk. The same baroprotective effect was shown in fat. In meat products the microorganisms are more resistant to pressure than in pure culture (Carlez et al., 1994). This HP tolerance of some food components could explain the presence of some active microorganisms, such as viable mesophilic, enterobacteriaceae, pseudomonadaceae, or staphylococci.

The Gram-positive bacteria spores are very resistant to HP, pressures up to 1000 MPa being necessary to inactivate them (Cheftel, 1995). All of this could explain tha viable mesophilic microorganisms sporiforming, as well as *B. cereus* detection after HP treatment. The baroprotective effect depends not only on the level of pressure applied and treatment time but also on interactions with other intrinsic and extrinsic variables that condition responses by microorganisms (Knorr, 1993, 1995; Rovere and Maggi, 1995; Palou et al., 1997). The psychrotrophic strains may have adaptations in the cell membrane and fluidity levels that may increase their tolerance to pressure (Lanciotti et al., 1996).

Tofu as a processed food can be contaminated by secondary contamination during packaging. However, the use of HP treatment prevents the product from secondary pathogenic contamination (Préstamo et al., 1999).

To study the network of the tofu matrix and its behavior after HP treatment, we analyzed the tofu structure. The cryofractures of the untreated and pressurized tofu samples in the cryo-SEM micrographs were compared. The results of the untreated tofu samples revealed a fine network structure as depicted in Figure 2a. At higher magnification of the SEM graphs large compact aggregate formation with cavities was observed

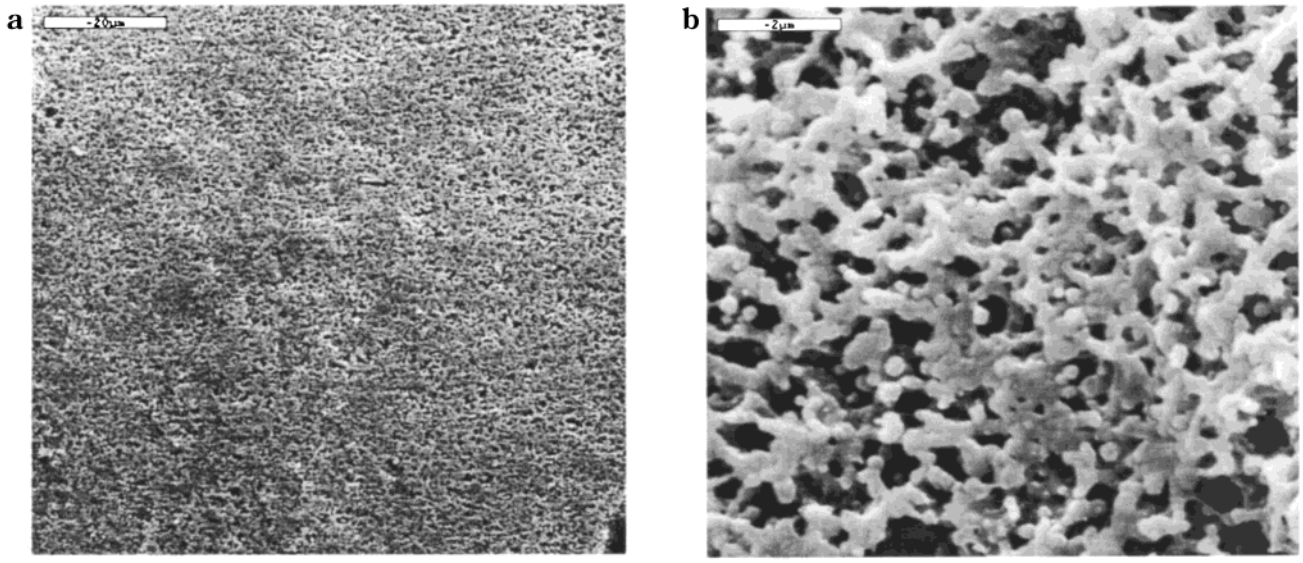

Figure 3. Tofu freeze fracture SEM: (a) treated tofu ultrastructure with HP, bar = 20 μm; (b) higher magnification, bar = 2 μm.

(Figure 2b). When tofu was treated at 400 MPa for 30 min at 5 °C, the internal structure changed, making it more compact (Figure 3a). At higher magnification we observed that these aggregate particles were desegregated, more disperse, and more accessible (Figure 3b). The cavities observed among the aggregate particles were smaller than in the untreated samples.

The sensorial test results revealed that the product after HP treatment in all samples was acceptable to consumers and the taste of the pressurized samples was in the range of good as the control samples. The samples treated with HP were slightly less beany (astringent) than the control ones.

After HP treatment, changes in the structure occurred as Préstamo and Arroyo reported (1998) with tofu; we also observed changes after HP treatment. In tofu the globulins 11S (glycinin) and 7S (β- and γ-conglycinin) are the major soybean proteins, and they are affected by the thermal treatment applied (Utsumi et al., 1985). A preliminary assay made in our laboratory on tofu proteins by differential scanning calorimetry (DSC; data not shown) showed that the proteins were denatured, and this is in accordance with study by Sorgentini et al. (1995), who reported that at 100 °C both 7S and 11S proteins were totally denatured.

The network of the formed matrix in tofu protein aggregation is strong as a result of hydrophobic interactions, high temperature, and the high degree of denaturation reached. As a result of this, the water imbibing capacity (WIC) of the insoluble fraction increased with the degree of protein denaturation (Yao et al., 1988).

In the process of making tofu the soluble fractions were also modified in their Mg^{2+} content, and there was induced aggregation capacity as a consequence of thermal denaturation. This aggregation is related to the accessibility of polar groups of 11S proteins, which are able to form saline bridges with the Mg^{2+} and induce aggregation.

After the pressure treatment, tofu presented an internal structure different from that in the untreated tofu. The aggregate was more compact as we observed in Figure 3a, but at the same time the unions among the particles were more desegregated and disperse, as we can observe at higher magnification in Figure 3b. These aggregates could be subunits of 11S and 7S that disperse after pressurization. This effect is important from the point of view of digestibility because this dispersion could increase tofu digestibility, but this has to be confirmed.

It is well-known that dry beans are a little indigestible. Soybeans are also heavy legumes for digestion, and they have to be cooked well to prevent indigestion. As a result of the HP treatment we observed changes in the tofu aggregate on the micrographs: the network matrix particles were more disperse. This means that they are more accessible, although in the stomach the aggregate particles will also be more accessible to the acid and enzyme digestion action. This is in accordance with the study of Schöberl et al. (1998), who reported that soybean treated with pressure had better digestibility than the untreated samples. We could postulate that for several products, such as tofu, HP treatment could make the product more digestible.

In cheese it has been reported by Casal and Gomez (1999) that a significant hydrolysis of the bitter fragment of β-casein (C-peptide) was found in pressure-incubated curds. This could explain the decrease in the slightly bitter flavor in tofu pressurized samples due to the hydrolytic action of HP treatment, in comparison with untreated tofu. This high peptidolytic activity observed in pressurized cells might be due to increased cell lysis or enhancement of membrane permeability (Casal and Gomez, 1999).

Carotenoids with antioxidant capacity have been related to the reduction of degenerative human disease. The carotenoid content was unchanged after HP treatment (De Ancos and Cano, 1999). This means that HP treatment maintains the phenolic content after pressurization. Although the healthy attributes of tofu are due to the phenolic compounds, it seems that they remain in the product after HP treatment. More work has to be done to be completely sure about it.

CONCLUSIONS

HP treatment in tofu reduces the microbial population. After HP treatment, tofu is safer in terms of secondary pathogenic microbial contamination. After HP treatment, only *H. alvei* and *B. cereus* were found. Sensorial test results revealed that treated tofu was

acceptable to consumers. The micrograph in the cryofracture observed with cryo-SEM revealed more compact structure after HP treatment, but the aggregate particles were more disperse.

ACKNOWLEDGMENT

We thank to Fernando Pinto for all of his help with the SEM.

LITERATURE CITED

Anderson, P. R. *Microbiologia Alimentaria: Metodología Análitica para Alimentos y Bebidas*; Diaz de Santos: Madrid, Spain, 1992.

Anderson, J. J. B.; Anthony, M.; Messina, M.; Garner, S. C. Effects of phyto-oestrogens on tissues. *Nutr. Res. Rev.* **1999**, *12*, 75−116.

APHA. *Compendium of Methods for the Microbiological Examination of Foods*, 2nd ed.; Speck, M. L., Ed.; APHA: Washington, DC, 1984.

Arroyo, G.; Sanz, P. D.; Préstamo, G. Effect of high pressure on the reduction of microbial population in vegetables. *J. Appl. Microbiol.* **1997**, *82*, 735−742.

Arroyo, G.; Sanz, P. D.; Préstamo, G. Response to high-pressure, low-temperature treatment in vegetables: determination of survival rates of microbial population using flow cytometry and detection of peroxidase activity using confocal microscopy. *J. Appl. Microbiol.* **1999**, *86*, 544−556

Carlez, A. Traitements par hautes pressions d'aliments d'origine musculaire: destruction microbienne, modifications de couleur, gelification protique. Ph.D. Thesis, Université Montpelier II, 1994.

Casal, V.; Gomez, R. Effect of high pressure on the viability and enzymatic activity of mesophilic lactic acid bacteria isolated from caprine cheese. *J. Dairy Sci.* **1999**, *82*, 1092−1096.

Cheftel, J. C. Effects of high hydrostatic pressure on food constituents: an overview. In *High Pressure and Biotechnology*; Balny, C., Hayashi, R., Heremans, K., Msson, P., Eds.; INSERM and John Libbey: Paris, France, 1992; Vol. 24, pp 195−209.

Cheftel, J. C. Review: high pressure, microbial inactivation and food preservation. *Food Sci. Technol. Int.* **1995**, *1*, 75−90.

De Ancos, B.; Cano, M. P. Effects of high-pressure treatment on pigment composition of persimmon products with antioxidant capacity. In *XXXVII EHPRG Meeting of the European High-Pressure Research Group*, Sept 9−11; Université Montepellier II: Montpellier, France, 1999.

Dethmer, A. S. Sensory evaluation guide for testing food and beverage products. *Food Technol.* **1981**, *53*, 50−59.

Fukushima, D. Recent progress of soybean protein foods: chemistry, technology, and nutrition. *Food Rev. Int.* **1991**, *7*, 323−351.

Garcia, M.-C.; Torre, M.; Marina, M. L.; Laborda, F. Composition and chraracterization of soybean and related products. *Crit. Rev. Food Sci. Nutr.* **1997**, *37* (4), 361−391.

Holt, J. G. *Bergey's Manual of Determinative Bacteriology*, 9th ed.; Williams and Wilkins: Baltimore, MD, 1993.

Horie, Y.; Kimura, K.; Ida, M.; Yosida, Y.; Ohki, K. Jam preparation by pressurization. *Nippon N'geikagaku Kaishi* **1991**, *65*, 975−980

Knorr, D. Effects of high hydrostatic pressure processes on food safety and quality. *Food Technol.* **1993**, *47*, 156−161.

Knorr, D. Hydrostatic pressure treatment of food microbiology. In *New Methods of Food Preservation*; Gould, G. W., Ed.; Blackie: London, U.K., 1994; pp 159−175.

Lanciotti, F.; Gardini, M.; Sinigaglia, M.; Guerzoni, M. E. Effects of growth conditions on the resistance of some pathogenic ands spoilage species to high-pressure homogenization. *Lett. Appl. Microbiol.* **1996**, *22*, 165−168.

Oxen, P.; Knorr, D. Baroprotective effects of high solute concentration against inactivation of *Rhodotorula rubra*. *Lebensm. Wiss. -Technol.* **1993**, *26*, 220−223.

Palou, A. L.; Lopez-Malo, G. V.; Barbosa-Canovas, J.; Welti-Chanes, J.; Swanson, B. G. Effect of water activity on high hydrostatic pressure inhibition of *Zigosaccharomyces bailii*. *Lett. Appl. Microbiol.* **1997**, *24*, 417−420.

Patterson, M. F.; Quinn, M.; Simpson, R.; Gilmour, A. The sensitivity of vegetative pathogens to high hydrostatic pressure treatment in phosphate buffered saline and foods. *J. Food Prot.* **1995**, *58*, 529−542.

Préstamo, G.; Arroyo, G. High hydrostatic pressure effects on vegetable structure. *J. Food Sci.* **1998**, *63* (5), 878−881.

Préstamo, G.; Sanz, P. D.; Fonberg-Broczek, M.; Arroyo, G. High-pressure response of fruit jams contaminated with *Listeria monocytogenes*. *Lett. Appl. Microbiol.* **1999**, *28*, 313−316.

Rovere, P.; Maggi, A. Approcio alle alte presioni: una nuova tecnologia s disposizione dell'industria alimentare. *Ind. Conserve.* **1995**, *70*, 45−54.

Schöberl, H.; Rub, W.; Meyer-Pittroff, R.; Roth, F. X.; Kirchgessner, M. Comparative studies concerning to digestibility of raw, heated and high-pressure treated foods in young pigs and in vitro. In *Advances in High-Pressure Bioscience and Biotechnology*; Ludwing: Heidelberg, Germany, 1998; pp 385−388.

Shih, M. C.; Hou, H. J.; Chang, K. C. Study on the process optimization of soft tofu. *J. Food Sci.* **1997**, *62*, 833−837

Sorgentini, D. A.; Wagner, J. R.; Añon, M. C. Effects of thermal treatment of soy protein isolated on the characteristics and structure function relationship of soluble and insoluble fractions. *J. Agric. Food Chem.* **1995**. *43*, 2471−2479.

Steinke, F. H. Nutritional value of soybean protein foods. In *New Protein Foods in Human Health: Nutrition, Prevention, and Therapy*; Waggle, D. H., Steinke, F. H., Volgarev, M. N., Eds.; CRC Press: Boca Raton, FL, 1992; pp 59−66.

Styles, M. F.; Hoover, D. G.; Farkas, D. F. Response *Listeria monocytogenes* and *Vibrio parahamolyticus* to high hydrostatic pressure. *J. Food Sci.* **1991**, *56*, 1404−1407.

Taira, H. Quality of soybeans for processed foods in Japan. *J. Agric. Res. Q.* **1990**, *24*, 224−230.

Takahashi, K.; Ishii, K.; Ishikawa, H. Sterilization of bacteria and yeasts by hydrostatic pressure at low temperature: effect of temperature, pH and the concentration of proteins, carbohydrates and lipids. In *High-Pressure Bioscience and Food Science*; Hayashi, R., Ed.; San Ei Publising: Kyoto, Japan, 1993; pp 244−249.

Utsumi, S.; Kinsella, J. E. Forces involved in soy protein gelation: effects of various reagents on the formation, hardness and solubility of heat-induced gels made from 7S, 11S and soy isolate. *J. Food Sci.* **1985**, *50*, 1278−1282.

Utsumi, S.; Matsumura, Y.; Mori, T. Structure−functions relationships of soy proteins. In *Functional Food*; Mazza, G., Ed.; Technomic Publishing: Lancaster, PA, 1998.

Yao, J. J.; Wei, L. S.; Steinberg, M. P. Water-imbibing capacity and rheological properties of isolated soy proteins. *J. Food Sci.* **1988**, *53*, 464−467.

Received for review November 19, 1999. Revised manuscript received March 22, 2000. Accepted March 22, 2000. This work was funded by the CSIC, ALI 97-0759 Project.

JF991251Y