

Review

High Pressure, a Unique Tool for Food Texturization

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High pressure is used to texturize food with two different strategies. The first strategy consists of using high pressure of several hundred MPa as a gelling agent *per se* to replace any other gelling or coagulating agents. In the second strategy, a moderate pressure of 100-300 MPa is used as a pretreatment to modify the food components properties in order to improve the efficiency of the traditional texturing treatment such as heat texturization, acid or rennet coagulation of milk, and so on. In both cases, pressure effects depend on many parameters such as the nature and the concentration of the macromolecules, the temperature of pressurization, the holding time and pressure, the pH, and the presence of additives. The behavior of real foods upon pressurization is even more complex compared to that of macromolecular model solutions due to their complex composition and, especially, to the presence of enzymes which are sometimes hardly affected by pressurization. Findings exposed in this article demonstrate that high-pressure technology is a unique tool to texturize food and provide products with unique properties.

Keywords: high pressure, texturization, high-pressure-induced gels, starch gelatinization, food proteins, polysaccharides, food processing

Introduction

The effects of high pressure on the viability of microorganisms (Hite, 1899) and protein coagulation (Bridgman, 1914) have been known for nearly a century. Nevertheless, this technique has been disregarded for a long time in the food industry, probably because of the technical difficulties in building large scale pressure equipment economically.

Since the end of the 80's, renewed interest has appeared. This new impulsion came from Japan, where the potentialities of high-pressure technology in the food industries have been proposed (Hayashi, 1987; 1992). In 1990, the first high-pressure-processed food in the world, a fruit jam, was released by Horie and his group on the Japanese market; since then, about a dozen high-pressure-treated foods have been on sale mainly in Japan (Dumoulin, 1998).

The applications of high pressure are wide: they are not restricted to microbial sanitation (see Cheftel, 1995 for a review) but also cover modification of protein functionalities (see Messens *et al.*, 1997 for a review), selective removal of β -Lg from the WPC (Hayashi *et al.*, 1987; Okamoto & Hayashi, 1990), reduction of the bitterness of grapefruit juice

(Yuge *et al.*, 1993) and so on.

Research on the use of high pressure in food technology was begun only 10 years ago and then does not allow us a full discussion on the principal mechanisms of the observed pressure effects. Therefore, this article mainly focuses on the phenomenological observations of the high-pressure effects on the texturization of biological material including macromolecular model solutions, such as proteins, starches, polysaccharides solutions and their mixture, and also on the texturization of real foods such as milk, fish meat, vegetables and so on.

I. HIGH-PRESSURE EFFECTS ON FOOD MACROMOLECULES

PROTEINS

The effects of high pressure on protein gelation depend on several parameters, such as the type and the concentration of the protein, the pressure level, the pressurization time and temperature, the pH and the presence of some additives in the medium.

Effects of the type and the concentration of protein WPC and HPC could gel upon pressurization (Van Camp & Huyghebaert, 1995a). A minimum operating pressure of 200 MPa for WPC and 300 MPa for HPC combined with a minimum protein concentration of 160 and 120 g/l, respectively, were necessary for network formation to occur. On the other hand, EWP and BPC could not form a gel even by pressurization at 400 MPa with a protein concentration as high as 200 or 170 g/l, respectively.

The visual aspect of high-pressure-induced gels differs from

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Abbreviations: β -Lg, beta-lactoglobulin; BPC, blood plasma concentrate; DSC, differential scanning calorimetry; EWC, egg white concentrate; HM-pectin, high-methoxyl pectin; HPC, haemoglobin protein concentrate; LM-pectin, low-methoxyl pectin; NIL, non-incorporated liquid; SEM, scanning electron microscopy; WPC, milk whey protein concentrate; WPI, milk whey protein isolate.

N.B.: when not specified, pressurization was carried out at room temperature.

that of heat-induced gels. High-pressure-induced gels of WPC were surrounded in the recipient by NIL, whereas all the liquid was incorporated in their heat-induced counterparts (Van Camp & Huyghebaert, 1995a; b). NIL was also observed with high-pressure-induced gels of β -Lg which displayed a sponge-like structure (Zasytkin *et al.*, 1996). In the case of HPC, no color change was noticed before and after pressure treatment, on the contrary, brownish red gels were formed upon heat treatment (Van Camp & Huyghebaert, 1995a).

The minimum protein concentration capable of forming a gel by high-pressure treatment was significantly increased compared to that necessary for heat-induced gelation (Van Camp & Huyghebaert, 1995a; Zasytkin *et al.*, 1996).

The strength and the rigidity of high-pressure-induced gels of WPC and β -Lg increased with a concomitant decrease in the proportion of NIL when the protein concentration increased from 90 to 180 g/l and from 123 to 220 g/l, respectively (Van Camp & Huyghebaert, 1995a; Zasytkin *et al.*, 1996). Increasing the protein concentration increased the pores size of the gel networks (Cheftel & Dumay, 1996). Generally, the probability of intermolecular contacts among polypeptide side chains increases with protein concentration and results in the formation of stronger gels. Increasing the protein concentration markedly increased the formation of both long- and short-term bondings (Van Camp & Huyghebaert, 1995b). However, high-pressure-induced gels of WPC, HPC, β -Lg and soy protein were softer than their heat-induced counterparts at all the protein concentrations tested (Dumay & Cheftel, 1997; Matsumoto & Hayashi, 1990; Okamoto *et al.*, 1990; Van Camp & Huyghebaert, 1995a). The storage modulus (G') and loss modulus (G'') of WPC high-pressure-induced gels were lower than those of their heat-induced counterparts, especially at high protein concentration. This suggests that the number of bondings of heat-induced gels is probably larger than that of high-pressure-induced gels, and it results in the formation of a stronger gel network with higher water-holding capacity. Creep measurements and large deformation experiments showed that high-pressure-induced gels of WPC contained a greater number of weak and short-range interactions among polypeptide side chains than heat-induced gels. Probably, polypeptide strands in high-pressure-induced gels have a higher degree of mobility to rearrange between the crosslinks in comparison to heat-induced gels (Van Camp & Huyghebaert, 1995b).

SEM observations supported the rheological conclusions, indeed, high-pressure-induced gels were characterized by a porous, finely stranded network structure in which the holes around polypeptide chains are presumably filled with NIL, whereas heat-induced gels had a compact structure with more intermolecular bondings among the adjacent polypeptide chains. However, pressurization of a 12% WPC solution at 600 MPa, at about 8°C and pH 7.5 for 20 min, induced a higher degree of aggregation compared with conventional heat-treatment (Walkenström & Hermansson, 1997). The network obtained was more inhomogeneous containing large aggregates and pores resulting in weak gel strength and low elasticity.

Effects of the operating pressure The operating pressure significantly influences the textural properties of high-pressure-induced gels. The strength of WPC gels increased with pressure increase from 200 to 400 MPa, the improvement being more pronounced at high protein concentrations (Fig. 1) (Van Camp & Huyghebaert, 1995a). The hardness of ovalbumin and soy protein gels also increased with the pressure increase (Dumoulin *et al.*, 1998). In the case of HPC, however, pressures higher than the minimum pressure necessary to induce gelation did not form stronger gels (Van Camp & Huyghebaert, 1995a). The porosity of the high-pressure-induced gels of β -Lg increased with pressure from 250 to 450 MPa; in parallel, the protein loss in the gel exudate decreased, indicating more extensive aggregation and gelation under higher pressure levels (Dumay & Cheftel, 1997).

Effects of the pressure holding time The pressure holding time influences the characteristics of the gels obtained. An increase in the duration time from 10 to 60 min for a single compression-decompression cycle (400 MPa, 25°C) significantly improved the strength of WPC and HPC gels (Van Camp & Huyghebaert, 1995a). The hardness and breaking strength of the high-pressure-induced gels of β -Lg (14% w/v, pH 7.20, 800 MPa, 30°C) were not influenced by the pressurization time from 5 to 120 min (Kanno *et al.*, 1997b). The water-holding capacity of high-pressure-induced gels was also not influenced by the pressurization duration, suggesting that the protein-water interaction in gels was not significantly changed. Gel solubility in different dissociating solutions decreased with the increase in pressurization duration, probably by the change in protein-protein interactions (Kanno *et al.*, 1997b). However, it has been described that the pressure holding time did not influence gel porosity but appeared to enhance the matrix density and simultaneously to increase the gel rigidity and reduce exudation (Dumay & Cheftel, 1997). Repeated compression-decompression cycles have been found not to extensively increase the strength of the WPC gels (Van Camp & Huyghebaert, 1995a).

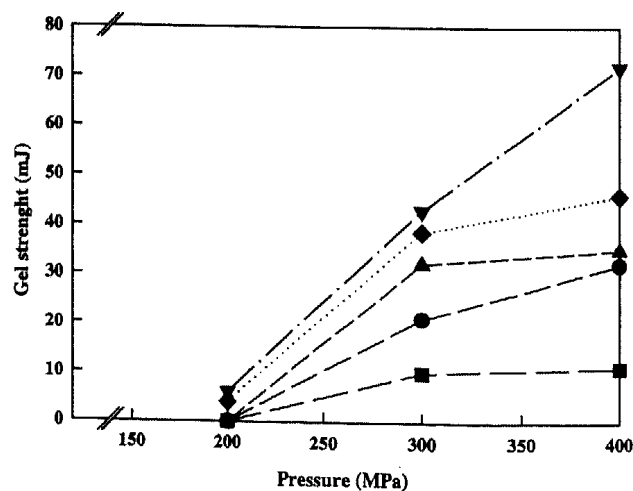


Fig. 1. Effect of pressurization level for 30 min on the high-pressure-induced gel strength obtained at 150 (■), 180 (●), 200 (▲), 220 (◆), and 250 (▼) g/l WPC. The initial pH was set to 7.0 in the presence of 0.05 M phosphate buffer (from Van Camp & Huyghebaert, 1995a).

Effects of the pressurization temperature The combined use of elevated temperatures and pressurization can result in the formation of stronger gels than those obtained upon pressurization at room temperature. This is the case of WPC for which the gel strength increased with an increase in the pressurization temperature (Van Camp *et al.*, 1996). The improvement was more significant with high protein concentrations: indeed stronger gels than heat-induced ones (80°C, 30 min) were obtained by pressurization under 400 MPa at 50°C for 30 min at a protein concentration > 132 g/l. Increase in operating temperature during pressure treatment reduced the minimal protein concentration capable of forming a gel, the NIL and the minimum duration time to form a strong gel; however, it did not reduce the minimal pressure needed to induce the formation of a gel strong enough for texture measurement.

High-pressure effects at elevated temperatures are highly dependent on the kind of protein under study: HPC precipitated when high pressure (300–400 MPa) was applied in combination with high temperature (50°C), and EWC and BPC only formed a weak coagulum under the same conditions (Van Camp *et al.*, 1996).

High-pressure effects at low temperatures is a recent concern of the combined use of high pressure and temperature (Dumoulin *et al.*, 1998). Study of high-pressure-induced gelation under a wide range of pressurization temperatures including sub-zero (from -20 to 50°C) revealed that ovalbumin and soy protein, contrary to egg yolk, could form a gel under the whole range of temperatures (Fig. 2). In the temperature range from -5 to 50°C, the hardness of ovalbumin (15% w/w, pH 4.4) gels obtained showed a minimum when the temperature of pressurization was 25°C. However, operating at low temperatures allowed a reduction in the pressure required to obtain rather hard gels. The temperature dependency of soy protein and egg-yolk gelation showed no minimum: gelation effectiveness progressively and continuously decreased by decreasing the temperature of pressuriza-

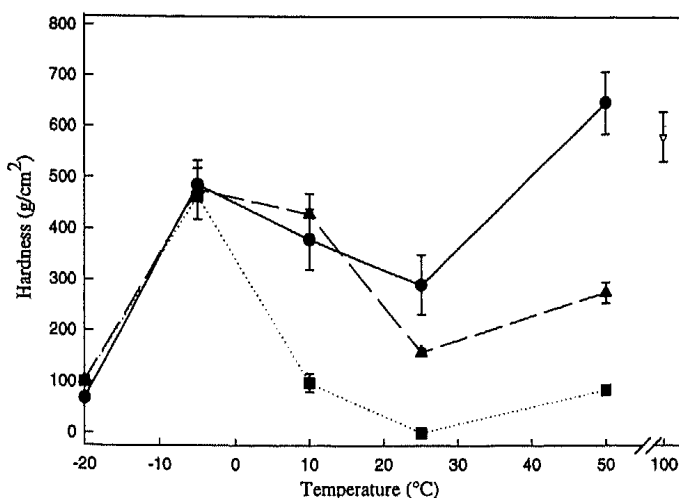


Fig. 2. Effect of pressurization temperature on the hardness of pressure-induced ovalbumin gels obtained at 300 MPa (■), 400 MPa (▲), and 500 MPa (●). Hardness of heat-induced gels obtained at 100°C for 10 min is shown for comparison (▼) (from Dumoulin *et al.*, 1998).

tion. This difference in behavior from that of ovalbumin might be due to the complex composition of these two systems (Dumoulin *et al.*, 1998).

Effects of the pressurization pH The pH of pressurization influences many parameters of the pressure-induced gelation, especially the minimum pressure needed to induce gelation and the optimum pressure needed to induce hard gels (Kanno *et al.*, 1997a). The optimum pH for the formation of high-pressure-induced gels with the highest hardness was 8 and 9 for WPI and WPC, respectively (Kanno *et al.*, 1997a). The hardness of both gels was lower in the acidic pH range than in the neutral and alkaline pH ranges. The same tendency was observed for high-pressure-induced gelation of WPC (Van Camp and Huyghebaert, 1995a; Walkenström and Hermansson, 1997). In the alkaline pH-region, the NIL was significantly reduced with increasing pH values both in the presence or absence of salts (Van Camp & Huyghebaert, 1995a).

Effects of polyols and salts The rheological and morphological properties of high-pressure-induced gels significantly depend on their sugar contents. For example, SEM observations revealed that high-pressure-induced gels of a 20% WPI solution at pH 6.8 under 400–1000 MPa had a microstructure like a honey comb, while those from a 20% WPC solution consisted of granular particles like coral (Kanno *et al.*, 1997a). These differences might be due to the baro-protective effects of lactose, which exists in larger quantity in WPC. Upon storage at 4°C, high-pressure-induced gels of β -Lg (12% protein, 450 MPa, 25°C, 15 min) underwent progressive syneresis and exudation (gel weight was decreased by 30 and 45% after 1 and 24 h of storage, respectively), but this loss of liquid exudate was reduced in the presence of 10% polyol. Moreover, the rigidity and elasticity index of high-pressure-induced gels decreased in the presence of polyol, where glucose exhibited the greatest baro-protective effect followed by sorbitol and sucrose (Cheftel & Dumay, 1996). The presence of sucrose decreased the porosity of β -Lg high-pressure-induced gels in a manner equivalent to the reduction in protein concentration (Dumay & Cheftel, 1997).

The ionic strength and the nature of the salts added before pressurization influence the rheological and morphological properties of high-pressure-induced gels (Kanno *et al.*, 1997a). For example, a 20% WPC solution containing NaCl at an ionic strength of 0.3 formed high-pressure-induced gels with higher hardness and breaking stress than the solution without salts. However, gels formed from a solution at the ionic strength of 0.5 had a lower hardness and breaking force than gels obtained at the ionic strength of 0.3 (values obtained were almost the same as those obtained in the absence of salts). Increasing the ionic strength to 0.5 by adding CaCl₂ increased the breaking stress but not the hardness of high-pressure-induced gels. SEM observations showed that increased NaCl concentration decreased the size of the granular particles accompanied by an increase in their density. However, such a granular structure disappeared with the addition of CaCl₂ and changed to an amorphous and dense structure.

Mechanism of pressure-induced gelation Gel seems to be formed during pressurization and/or during pressure

release, although this has not yet been well documented. A steel ball placed over a freshly prepared high-pressure-induced gel of β -Lg did not fall through the gel upon a second pressurization (Zasytkin *et al.*, 1996). This suggests that the primary gel network is formed under pressure rather than during pressure release. The large-pored structure and sponge-like texture of high-pressure-induced gels may result from volume expansion during pressure release. Pressurization of a 12% β -Lg isolate at 450 MPa and 25°C for 15 min induced the immediate formation of a soft gel, whose proteins were easily solubilized. During subsequent chilled storage (for 20–24 h) the protein solubility decreased due to the accumulation of intermolecular interactions and SS bridges (Cheftel & Dumay, 1996). The importance of SS bonds in the high-pressure-induced gels of β -Lg was also underlined by others (Kanno *et al.*, 1997b).

POLYSACCHARIDES

Starch gelatinization

Pressurization at 200–1500 MPa lowered the gelatinization temperature of starch (Muhr & Blanshard, 1982). Thus, by pressure treatment, starch can be gelatinized or changed to amylase-sensitive forms at ambient or moderate temperature (45–50°C) (Douzals *et al.*, 1996a; Ezaki & Hayashi, 1992; Hayashi & Hayashida, 1989; Rubens *et al.*, 1997; Stute *et al.*, 1996).

Effects of pressure, temperature and water content

The pressure range in which the gelatinization occurs depends on the starch source: A- and C-type starches were more sensitive to pressure than B-type starches (Ezaki & Hayashi, 1992; Stute *et al.*, 1996). The proportion of gelatinized starch increased with pressure increase. For example, wheat starch gelatinization occurred between 300 and 500 MPa with 15 and 88% of gelatinized starch, respectively (Douzals *et al.*, 1996a). In comparison, starch gelatinization under atmospheric pressure conditions was completely achieved in a narrow range of temperature at approximately the melting temperature ($\pm 5^\circ\text{C}$) (Muhr & Blanshard, 1982). Therefore, pressure-induced starch gelatinization can be graduated as a function of pressure level, and this behavior could have technological applications (Douzals *et al.*, 1996a).

Increasing the temperature of pressurization allowed a decrease in the pressure needed to induce gelatinization (Hayashi & Hayashida, 1989; Stute *et al.*, 1996).

Pressure-induced gelatinization of starches depends on the water content. A moisture content of 50% was required for wheat starch gelatinization upon pressurization at 600 MPa for 15 min at 20°C. When ethanol or sugar were added, gelatinization could be achieved only by significantly increasing the pressurization temperature even at a high moisture content; therefore, the water has to be free (Stute *et al.*, 1996). The temperature at which high-pressure-induced gelatinization occurs decreased with an increase in the water content (Muhr & Blanshard, 1982).

Effects of starch type on swelling behavior Depending on the swelling behavior of the granules upon high-pressure treatment, starches can be classified in two types: "extensive swelling" starches and "limited swelling" starches (Stute *et al.*, 1996).

The former type of starches, which includes waxy corn starch, was characterized by extensive swelling and almost complete disintegration of the granules (which is usually observed after heat-induced gelatinization) upon pressurization. The properties of the pastes and gels of those starches were very similar to those obtained by heat-induced gelatinization.

The latter "limited swelling" type of starches, which includes most of the starches (*i.e.*, corn, wheat, pea starches), was characterized by a very small swelling upon pressurization (1.5 to about 2 times the original granule diameter) and by the persistence of their granular aspect (Stute *et al.*, 1996). The persistence of the granules has also been reported by others (Douzals *et al.*, 1996a; Ezaki & Hayashi, 1992; Muhr & Blanshard, 1982). The viscosity of a high-pressure-treated 8.6% suspension of "limited swelling" type starches was much lower than that of its heat-treated counterpart and gave upon heating a gel weaker than that obtained with non-pressurized suspensions. Upon pressurization at high concentration (above 25%), these starches can be further classified into those forming pastes with a smooth texture and those forming rigid gels (faba bean, mung bean, lentil) (Stute *et al.*, 1996). The smooth texture of pastes can be easily explained by the granular character of the gelatinized granules, and these pastes are of special interest because they can be used to simulate fat droplet properties (Stute *et al.*, 1996). The formation of rigid gels despite very little swelling and the absence of amylose solubilization has not yet been explained. The gel induced by pressurization of a 30% wheat suspension was softer than its heat-induced counterpart (Douzals *et al.*, 1996b; 1997). It was also more condensed suggesting that the water-starch linkages created under pressure are strong.

Mechanism of high-pressure-induced gelatinization

In situ microscopic study of wheat and potato starch gelatinization upon stepwise pressurization and decompression showed that an increase in the granule size occurred both during pressurization and pressure release (Douzals *et al.*, 1996a). However, a fraction of the granules almost did not swell during the pressure increase and pressure decrease. The volume of granules swelled during a first compression did not change upon a second compression-decompression cycle (a small decrease in volume was observed upon the second compression but, it was recovered upon the pressure release of the second compression to reach the same value as at the end of the first cycle). Also, no further iodine discoloration occurred during the second cycle (Douzals *et al.*, 1996a).

The following mechanism has been proposed for the pressure-induced gelatinization of wheat starch: Above 300 MPa, some granules swell and discolor (Fig. 3), while the compressibility of the suspension became higher than that of pure water at the same pressure. This gelatinization involves a massive water entry into the starch granules and the creation of hydrogen bonds between water and starch enhanced by high pressure. This granule swelling is accompanied by a reduction in the bulk suspension volume, suggesting that water molecules linked with starch occupy a smaller volume than the molecules in pure water. After pressure release, the volume of the suspension remained lower than the initial one (Douzals *et al.*, 1996a).

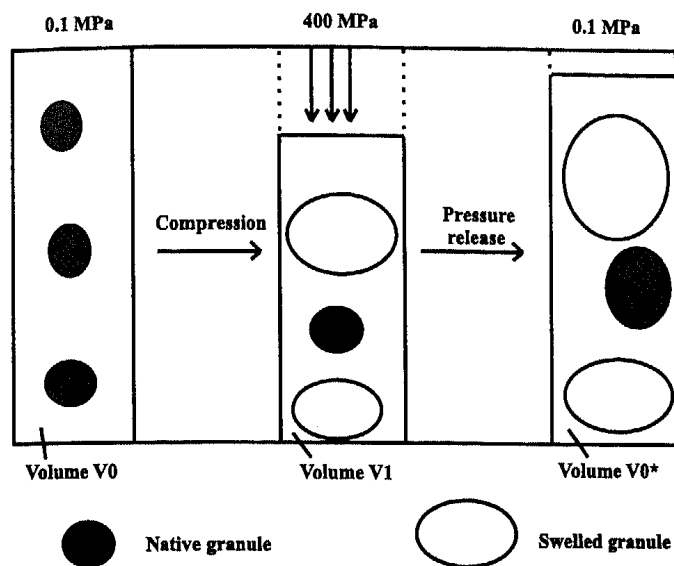


Fig. 3. Behavior of wheat starch granules suspended in water during a 400 MPa treatment. (from Douzals *et al.*, 1996a).

Retrogradation of the high-pressure-induced gelatinized starches The retrogradation speed of pressurized starches seems to be lower than that of heat-gelatinized starches (Douzals *et al.*, 1997; Ezaki & Hayashi, 1992). Retrogradation of pressurized wheat starch (600 MPa, 25°C, 5 min) judged by measuring the elastic modulus of compression and its melting temperature was much lower than that of the heat-induced gelatinized one (85°C, 15 min) upon storage either at 4 or 25°C (Douzals *et al.*, 1997). High-pressure-induced wheat starch gels were dried more slowly than heat-induced ones, confirming their lower susceptibility to modification upon storage (Douzals *et al.*, 1996b). On the contrary, a rapid retrogradation (within or immediately after pressure treatment) was reported for many starches (including wheat, corn and waxy rice starches) treated by 450 MPa or higher, as judged by the apparition of a second peak (at around 50°C) upon DSC analyses (Stute *et al.*, 1996). However, this rapid retrogradation may be different from that observed after heat gelatinization. Indeed, changes reported as retrogradation for pressure-induced gelatinization seemed to occur before the complete gelatinization and within the granule itself because no amylose was leached out. This new structure can be related to that described for corn and wheat starches after long pressurization time, e.g., for 17 h (Hayashi & Hayashida, 1989). Indeed, digestibility of these starches by α -amylase increased up to 2 h at 500 MPa and 50°C and then gradually decreased with longer pressurization times up to 17 h. Formation of a new structure has been postulated to explain this phenomenon, but further experiments under more controllable gelatinization conditions (e.g., *in situ* stirring to make uniform suspension and *in situ* measurement of physical properties during pressurization) have to be undertaken to elucidate the mechanism of this phenomenon.

High-pressure treatment at moderate temperature increased starch digestibility (Hayashi & Hayashida, 1989). This allows interesting applications such as the non-heating process for the fermentation industries, e.g., rice-wine brewing (Miyama *et al.*, 1992).

Pectin gelification

Isolated pectin and pectin compounds in the plant cell wall are affected by high pressure (Gustin *et al.*, 1997; Kuribayashi & Hayashi, 1993). HM-pectin solutions (0.5–1.5% w/w) were gellified by pressurization at 200 and 400 MPa in a narrow sugar concentration (from 55 to 62%). Higher pectin concentration was necessary for the gelification to occur at the extreme sugar concentrations. Pressure up to 400 MPa did not destroy the heat-induced gels of HM-pectin (Gustin *et al.*, 1997). LM-pectin solution (0.5–2% w/w in presence of 0.1% w/w Ca^{2+}) required the same sugar concentration for both heat- and pressure-induced gelations. The high-pressure-induced gel was less sticky than the heat-induced one, and its fracturability, hardness and elasticity were higher. The LM-pectin gel obtained after the pressurization of a heat-induced gel had a new structure; the ball penetration test (see mechanism of pressure-induced gelation p. 101) demonstrated that the structure of heat-induced gel was destroyed and reformed under pressure. The destruction-reformation seemed to be due to a calcium redistribution (Gustin *et al.*, 1997).

A pressure treatment (300–500 MPa, room temperature, 30 min) was effective to extract the pectin from the membrane of mandarin (Kuribayashi & Hayashi, 1991). The pectin thus obtained showed lower viscosity than heat-extracted pectin. The content of methoxyl groups of pressure-extracted pectin was lower than that of heat- or cold-extracted pectin, and its viscosity showed a pH dependence with a maximum at pH 2.7 contrary to that of heat-extracted pectin (Kuribayashi & Hayashi, 1993). Gels prepared according to the boiling acid method from pressure-extracted pectin (1% pectin containing 65% sucrose at pH 2.7) were less hard and adhesive than those prepared from heat-extracted pectin, but they were more easily broken (Kuribayashi, 1992).

β -Glucan

A 5% solution of (1→3)- β -D-glucan formed a gel upon pressurization (1–1000 MPa) at 57.4, 59, 61 or 64.5°C for 10 min (Fuji *et al.*, 1980). All the high-pressure-induced gels exhibited a lower elasticity than heat-induced gels and displayed a more granular microstructure. It has been postulated that these differences might be due to the decrease in the amount of free water under pressure.

Sodium alginate

Sodium alginate formed gels upon pressurization at 900 MPa and room temperature for 10 min in the presence of various calcium salts (Hirano *et al.*, 1993). The texture of the resultant gels varied with the calcium salts and their concentration. Calcium chloride produced an opaque, homogeneous and firm gel, whereas calcium citrate produced a transparent, homogeneous and soft gel. These gels can be utilized for calcium-enriched food gels having a new texture.

MIXED SYSTEMS

Foods are multicomponent systems; therefore, studies of the gelation of mixed systems are of special interest for a better understanding of what happens in real foods. Thus, the effects of high pressure on mixed systems (a mixture of two protein concentrates or of one protein concentrate and one polysac-

charide) has recently become a matter of interest (Fernandes & Raemy, 1996; Tada & Suzuki, 1994; Walkenström & Hermansson, 1997; Zasytkin *et al.*, 1996). The addition of 0.1% κ -carrageenan, xanthan gum or HM-pectin to a 12% whey protein solution (pH 7) induced the formation of gel with higher G' and G'' moduli upon pressurization at 400–800 MPa and 30 or 50°C for 10 min (Fernandes & Raemy, 1996). The increase was much more significant with the addition of κ -carrageenan when the pressurization was performed at 30°C, while the three polysaccharides had almost the same efficiency at 50°C (except at 800 MPa where κ -carrageenan induced a greater improvement). In the case of κ -carrageenan addition, optimal pressure was clearly 600 MPa when pressurization was performed at 30°C, but it was shifted to 800 MPa when the pressurization was carried out at 50°C.

Addition of xanthan (0.9%) was effective in preventing exudation and the formation of a sponge-like texture during pressure-induced gelation of β -Lg (Zasytkin *et al.*, 1996). On the other hand, the presence of xanthan did not lower the minimum concentration of protein capable of forming a self-standing gel upon pressure-induced gelation, although it did in the case of heat-induced gelation. High-pressure- and heat-induced gels of β -Lg are very different (see p. 100); however, in the presence of xanthan, the structure and properties of gels converged whether they were induced by heat or high-pressure treatment.

Microstructural and rheological properties of high-pressure- and heat-induced mixed gels of gelatin and WPC (3% gelatin and 4 or 12% WPC) have been investigated (Walkenström & Hermansson, 1997). In the case of heat treatment, the WPC network was formed first (due to the thermosetting) and then the gelatin network was formed (due to the cold-setting). The use of high pressure allows the inversion of this order: the gelatin network was formed by heating at 40°C and cooling at 4°C prior to the pressure treatment, and the WPC network was then formed by pressure treatment in the presence of the gelatin gel. The rheological properties of high-pressure-induced mixed gels prepared at pH 7.5 indicated a higher degree of gelatin continuity compared with the heat-induced mixed gels, and their microstructural studies showed a dense network in which neither the gelatin nor the WPC network could be identified. However, the high-pressure-induced mixed gels prepared at pH 5.4 formed a phase-separated network, which showed a gelatin continuous phase and a discontinuously distributed WPC phase. The rheological properties of these high-pressure-induced mixed gels were independent of the WPC concentration and were similar to those of pure gelatin.

HIGH-PRESSURE EFFECTS ON HEAT-INDUCED GELS

Finally, high pressure is an effective tool to clarify the mechanism of heat-induced gelation (Gekko, 1992). Experiments are carried out as follows: heat-induced gels of food macromolecules are formed in a test tube containing a steel ball at the bottom, and the test tube is then set bottom up in the light path of a high-pressure vessel which is equipped with a sapphire window. When temperature is increased at a given

pressure, the temperature at which the transmitted light is intercepted by the ball dropping down is taken as the melting temperature. In these experiments, the melting temperature of heat-induced gels of carrageenan, as well as those of ovalbumin and soy protein decreased linearly with increasing pressure. This indicates that these gels were destabilized under pressure. On the contrary, the melting temperature of heat-induced gels of agarose and gelatin increased linearly with increasing pressure, suggesting that these gels were pressure-stabilized. Performing these experiments at different molecular concentrations allows the calculation of the thermodynamic parameters and the elucidation of the gelation mechanism in term of hydration mode and cross-linking junctions.

The microstructure of a 3% gelatin preformed gel (obtained by heating the gelatin suspension at 40°C and further cooling at 4°C) was not affected by pressurization at 600 MPa for 20 min. However, pressurized gels showed a higher shear modulus and a slightly higher melting temperature (Walkenström & Hermansson, 1997).

II. HIGH-PRESSURE EFFECTS ON REAL FOODS TEXTURIZATION

Research and development on the high-pressure effects on real foods including milk, fish meat, animal meat, egg, soy products and vegetables is increasingly reported. In this case, high pressure is often used as a pretreatment to improve or modify the texturing properties of food in addition to the traditional texturing treatment.

HIGH-PRESSURE EFFECTS ON MILK COAGULATION

Use of high pressure as a gelling agent per se Freeze-concentrated raw and market milk containing about 25% solid content formed a gel upon pressurization for 5 min at 5°C and 300 MPa or higher. An increase in pressure induced the formation of stronger and more visco-elastic gels (Kumeno *et al.*, 1993). These gels were characterized by their high whiteness, high brightness and fresh cream-like flavor. Their properties were improved by the addition of sugar, whose optimum concentration was 10%. Freeze-dried and powdered milk, even with a solid content higher than 25%, formed no gels unless sugar was added.

Rennet coagulation under high pressure When pressure was applied just after the addition of rennet to a 20% solution of skim milk powder in 0.02 M CaCl₂, the hydrolysis of casein (the primary phase reaction) was negligibly affected by compression up to 130 MPa (Ohmiya *et al.*, 1987). This result showed both insignificant inactivation of rennet and insignificant change in the digestibility of milk protein by rennet.

Initiation of coagulation (the second phase reaction) seemed to be delayed by pressure increase, although pressure up to 40 MPa may not affect the initiation of milk clotting (Ohmiya *et al.*, 1987). This time has been estimated at 20, 20, 40 and 175 min under pressures of 0.1, 40, 80, and 130 MPa, respectively. For a given pressure holding time, increasing the holding pressure up to 40 MPa resulted in the formation of a more rigid curd. However, when a pressure higher than 60

MPa was applied, the curd rigidity decreased and no coagulation was obtained at a pressure of 100 MPa or higher depending on the holding time.

When high pressure was applied to the sample 15 min after the addition of rennet, the rigidity increased with the increase in both pressure and pressure holding time, suggesting that coagulation (the third phase reaction) was enhanced under high pressure (Ohmiya *et al.*, 1987).

Cheese-making properties of a bovine milk previously pressurized High pressure improves the bovine milk rennet or acid coagulating properties (Desobry-Banon *et al.*, 1994; Johnston *et al.*, 1993; Lopez-Fandiño *et al.*, 1996; Shibauchi *et al.*, 1992). For example, the treatment of milk at 300 MPa for 30 min decreased the coagulation time (r) by 19%, increased the curd-firming rate and the curd firmness by 39 and 58%, respectively, while causing an 80% denaturation of β -Lg and a 13% increase in curd weight. The increase in curd weight was due to a better incorporation of β -Lg into the curd and especially to a greater moisture retention (Lopez-Fandiño *et al.*, 1996). However, these beneficial effects are highly dependent of the pressurization conditions as reported below.

Pressure level and pressure holding time affect differently and in a complex way the various cheese-making properties of milk (Lopez-Fandiño *et al.*, 1996). For milk pressurized at 200 MPa, curd weight and protein loss in the whey did not significantly change by increasing the pressure holding time from 5 to 60 min, although β -Lg denaturation gradually increased up to 42% after 60 min of pressurization. However, for milk pressurized at 400 MPa, both moisture retention and protein recovery in the curd increased as the holding time increased, with greatest changes occurring during the first 20 min of treatment. After the addition of rennet, the coagulating time and the time after which the curd was firm enough for cutting (k_{20}) decreased as the pressure increased up to 200 MPa, but these parameters increased again by pressurization at a pressure higher than 200 MPa to reach values comparable with those of raw milk after pressurization at 400 MPa (Fig. 4A). Curd firmness at cutting (a_{30}) was not significantly changed as the pressure increased, except for milk treated at 300 MPa for 30 min, for which case curd firmness showed an intermediate maximum. Coagulation properties were gradually improved under 200 MPa (decrease in r and k_{20} , increase in a_{30}) with increasing the pressure holding time up to 10 min; no further changes were caused by longer pressurization (Fig. 4B). On the contrary, at 400 MPa, these coagulation properties were also improved during the first 10 min of pressurization, but longer times resulted in an increase in r and k_{20} and decrease of a_{30} .

Somewhat different results have been reported (Desobry-Banon *et al.*, 1994; Shibauchi *et al.*, 1992). Indeed, no change in the clotting time for the rennet coagulation of a reconsti-

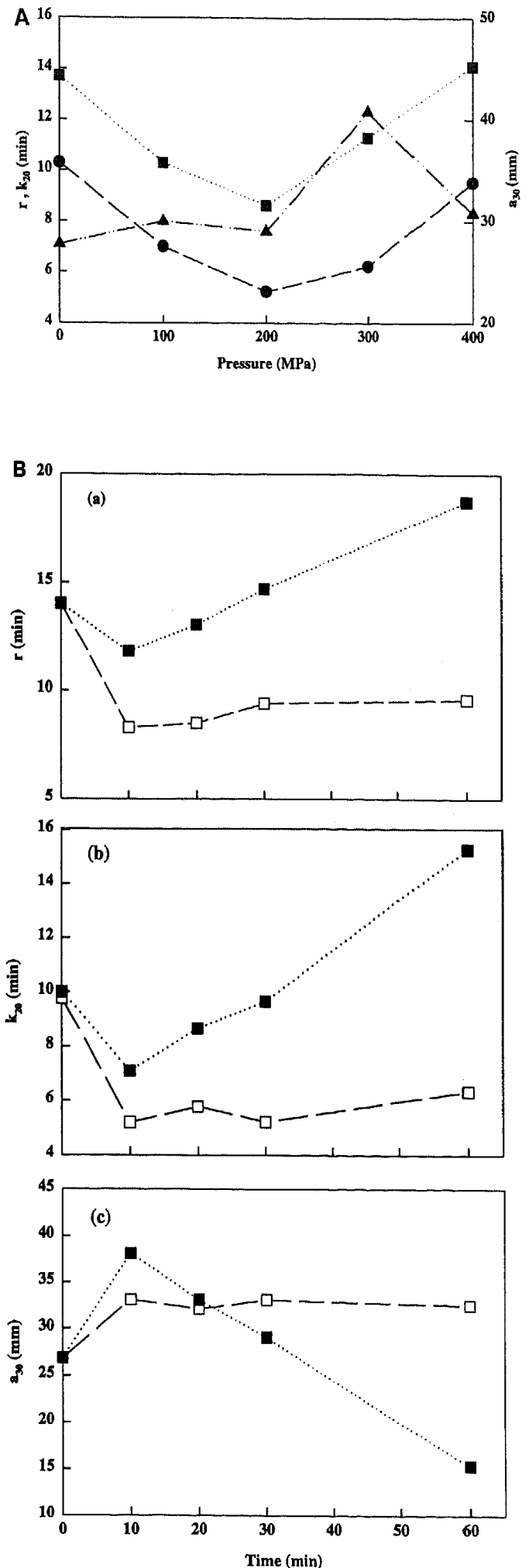


Fig. 4. A) Effect of pressurization for 30 min on the rennet clotting properties of milk: coagulation time, r (■); time when curd is firm enough for cutting, k_{20} (●); and curd firmness, a_{30} (▲). B) Effect of pressurization duration at 200 (□) and 400 MPa (■) on the rennet clotting properties of milk: (a) coagulation time, r ; (b) time when curd is firm enough for cutting, k_{20} ; (c) and curd firmness, a_{30} (from Lopez-Fandiño *et al.*, 1996).

tuted pressurized milk (from a 12% low-heat skim powder) was observed upon pressurization (for 20 min) up to 150 MPa; at higher pressures, this time rapidly decreased to stabilize above 230 MPa (Desobry-Banon *et al.*, 1994). The rennet clotting time of 4 kinds of milk (raw whole milk, raw skim milk, UHT milk and reconstituted skim milk) also decreased upon pressurization (for 5 min at 5°C) between 100 and 400 MPa; pressures higher than 400 MPa did not induce further changes. The decrease was especially drastic for UHT and reconstituted milk (Shibaushi *et al.*, 1992). The time required for rennet coagulation of a cow milk previously treated by a three-1-min cycles at 586 MPa was almost twice shorter than that needed for raw or pasteurized milk (Drake *et al.*, 1997).

The pH at which the acid coagulation starts increased from 5 to 5.3 for milk previously pressurized at 200 or 400 MPa (Desobry-Banon *et al.*, 1994). Acid coagulated gels obtained from pressurized milk were stronger than those formed from non-pressurized milk (Desobry-Banon *et al.*, 1994; Johnston *et al.*, 1993). Gel rigidity and breaking strength increased (up to 8–9-fold and 5-fold, respectively) with increasing pressure level and holding time, but a plateau was reached after 30 min of pressurization at 400 MPa or above (Johnston *et al.*, 1993). The syneresis of acid coagulated gels obtained from pressurized milk was lower than the syneresis of those obtained from non-pressurized milk (Johnston *et al.*, 1993). Gels from high-pressure-treated milk also showed a network structure with a greater number of network strands and smaller pores than gels prepared from unpressurized milk (Johnston *et al.*, 1993). The effects of pressure on gels persisted when the milk was stored up to 8 days at 4°C before gel formation (Johnston *et al.*, 1993).

Generally, it is assumed that pressure higher than about 200 MPa causes a decrease in the casein micelle size (Desobry-Banon *et al.*, 1994). These changes greatly modify the physicochemical and functional properties of milk. They also seem to be further amplified during the acid-induced disintegration and reaggregation process leading to a gel with an improved structure (Johnston *et al.*, 1993). Nevertheless, further studies are needed to fully understand the casein aggregation and curd formation mechanism upon acid and rennet coagulation of pressurized milk.

Properties of mature cheese made from pressurized bovine milk Pressure effects on microbiological and sensorial qualities and yield of mature Cheddar cheese have been investigated (Drake *et al.*, 1997). Cheeses made from pressure-treated milk (three-1-min cycles at 586 MPa) had a higher moisture content. This high moisture content may be due to the reaggregation of casein and milk fat globules following the casein micelle disaggregation induced by pressurization. The protein and fat globules may not reaggregate as closely as they were initially and may allow more moisture to be trapped or held in the cheese. The better whey protein retention in the curd may also contribute to the increase in moisture content. SEM observations of mature Cheddar revealed that the overall microstructure of pressurized-milk cheese was similar to the microstructure of raw-milk cheese. However, upon sensory test, pressurized-milk cheese had a lower texture score than either raw- or

pasteurized-milk cheeses; this texture defect might be attributed to the high moisture content. The flavor of pasteurized- and pressurized-milk cheese was not significantly different and was higher than that of raw-milk cheese; the microbiological quality of these two cheeses was comparable.

HIGH-PRESSURE EFFECTS ON FISH MEAT GELATION

General properties of high-pressure-induced fish meat gels High-pressure-induced gels of fish meat paste are generally formed under pressure at or above 300 MPa depending on the fish species and upon high-pressure processing, the time required for gelation is much lower than that needed for heat-induced gelation (Carlez *et al.*, 1995a; Chung *et al.*, 1994; Gilleland *et al.*, 1997; Ishikawa *et al.*, 1993; Nagashima *et al.*, 1993; Okamoto *et al.*, 1990; Okazaki *et al.*, 1997; Pérez-Mateos *et al.*, 1997; Shoji *et al.*, 1990; Tokutake *et al.*, 1990; Yoshioka *et al.*, 1992).

High-pressure-induced gels of fish meat are generally more deformable and softer than heat-induced ones (Carlez *et al.*, 1995a; Nagashima *et al.*, 1993; Okamoto *et al.*, 1990; Pérez-Mateos *et al.*, 1997). However, higher hardness for high-pressure-induced gels than for heat-induced gels has been reported (Chung *et al.*, 1994; Yoshioka *et al.*, 1992). High-pressure-induced gels are usually more transparent, less white and less yellow than heat-induced gels and contain less air bubble than heat-induced ones (Carlez *et al.*, 1995a; Matahira *et al.*, 1994; Shoji *et al.*, 1990).

As for heat-induced gelation, salts are required to obtain good gels upon pressurization (Carlez *et al.*, 1995a; Ishikawa *et al.*, 1993).

Effect of pressure level, pressure holding time and temperature As judged by the gel strength, the optimum pressure for gelation of Threadfin bream surimi (by pressurization at 5–10°C for 15 min in presence of 1% of NaCl) was 300 MPa; lower or higher pressures gave gels with lower strength (Carlez *et al.*, 1995a). Similar pressure dependence of breaking strength has been observed with Alaska pollack surimi, but with chum salmon meat the breaking strength of high-pressure-induced gels continuously increased with increasing pressure from 0.1 to 500 MPa, although values obtained at 500 MPa were rather low (Okazaki *et al.*, 1997; Shoji *et al.*, 1991). The optimal pressure to obtain the highest work of penetration is a function of the temperature of pressurization (Pérez-Mateos *et al.*, 1997). The optimal pressure at low temperatures was 200–300 MPa (0–15°C), whereas it was around 350–400 MPa at higher temperatures of pressurization (25–30°C). The optimum pressurization time also depends on the temperature of pressurization. The temperature of pressurization differently affects the different textural properties of gels (Fig. 5). In some cases, a correlation between textural properties and pressurization conditions has been found. For example, the cohesiveness of high-pressure-induced gels of Blue whiting muscle proteins was linearly correlated to the pressurization temperature and level; the higher cohesiveness occurred at low temperature and low pressure level (Pérez-Mateos *et al.*, 1997).

Interactions involved in high-pressure-induced gels Studies of the influence of chemical additives have pointed

out a different gelling behavior of heat- and pressure-induced gels; indeed, a given additive often induced different texture modifications depending on the type of gels (Carlez *et al.*, 1995a). For example, in the case of Threafin surimi, a low SDS concentration increased the gel strength and rigidity of high-pressure-induced gels, whereas, it increased the yield deformation and rigidity for heat-induced gels. This strongly suggests that the mechanism of gelation and the proportion of hydrogen bonds, hydrophobic interactions, electrostatic interactions and disulfide bonds are different in the two kinds of gels (Carlez *et al.*, 1995a). A different binding pattern in high pressure- and heat-induced gels was confirmed by studying the solubility of the gel proteins in different dissociating buffers (Pérez-Mateos *et al.*, 1997). More disulfide and other covalent bonds were found in heat-induced (37°C, 30 min plus 90°C, 50 min) than in pressure-induced gels, explaining the higher hardness and lower elasticity of the former ones. Gels formed upon pressurization at low temperature and pressure (200 MPa, 3°C, 10 min) were characterized by a greater presence of ionic and hydrogen bonds, whereas gels obtained under higher pressure and temperature (375 MPa, 38°C, 20 min) were characterized by a higher proportion of hydrophobic interactions (Pérez-Mateos *et al.*, 1997). However, intermolecular disulfide bonding upon pressurization (300 MPa, 10 min, 5°C) has been described to be more important than that which occurs upon heating (90°C, 30 min) (Gilleland *et al.*, 1997).

When Alaska pollack meat was setting at 25°C for 2 h after pressure treatment (300 MPa, 30 min at 5°C), gel with more than 6 times greater gel strength than that of heat-induced gel (90°C, 30 min) was obtained (Gilleland *et al.*, 1997). This improvement has been attributed to the crosslink activity of endogenous transglutaminase, which must have survived the pressure treatment. Pressure-denatured myosin is thought to expose more accessible binding site to the enzyme and then increase the effectiveness of transglutaminase. Therefore, the gelling properties of many types of food proteins might be enhanced by the addition of microbial transglutaminase combined with pressurization (Gilleland *et al.*, 1997). However, the transglutaminase of Whalley pollack was almost completely inactivated by pressurization at 300 MPa or higher at 5°C and 10 min (Shoji *et al.*, 1991). If the setting step (at 37°C for 30 min or at 5°C for 24 h) is done prior to pressurization, it did not modify the texture characteristics of pressure-induced gels, whereas it slightly increased the strength of heat-induced gels (Carlez *et al.*, 1995a).

Effects of different combinations of pressure and heat treatment Gels properties of chum salmon meat were improved by pressurization followed by heat treatment compared to those of high-pressure or heat-induced gels; however, these synergic effects were nonexistent or slight with Alaska pollack (Okazaki *et al.*, 1997; Taguchi, 1990). For both chum salmon and Alaska pollack meat, gels made by pressurization at 60°C exhibited lower breaking strength than that obtained only by heat treatment (Okazaki *et al.*, 1997). Gels of Alaska pollack and Pacific whiting obtained by pressurization at 50°C were also less hard than those obtained by pressurization at a lower temperature (Chung *et al.*, 1994). It was assumed that moderately high pressure (1–2 kbar) may

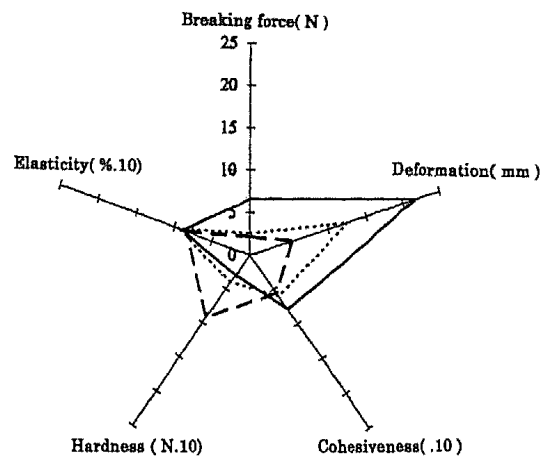


Fig. 5. Rheological properties of Blue whiting muscle protein gels obtained at: 37°C 30 min + 90°C, 50 min (---); 200 MPa, 3°C, 10 min (—); and 375 MPa, 38°C, 20 min (.....) (from Pérez-Mateos *et al.*, 1996).

induce the rupture of the lysosomal membrane and the release of proteases. At 50°C (near the optimal temperature of these fish proteases), the heat-activated proteases which have been released from lysosomes would react with some of the myofibrillar protein and result in weaker gels (Chung *et al.*, 1994).

In the case of sardine surimi, heat treatment (85°C, 20 min) following pressurization (50–400 MPa, 20 min) gave more viscous and elastic gels than heat or pressure treatment alone, pressurization at 100 MPa giving the best results (Ishikawa *et al.*, 1991). Gel elasticity and viscosity increased linearly when the temperature of the heat treatment carried out after pressurization at 100 MPa was increased from 40 to 85°C.

Characteristics of high-pressure-induced gels upon storage Changes in the textural characteristics of high-pressure and heat-induced gels have been followed for 12 days upon storage at 4°C (Carlez *et al.*, 1995a). The yield force and yield deformation of heat-induced gels remained constant throughout the 12 days; on the contrary, these parameters increased in the case of pressure-induced gels (especially, the yield force increased after 8 days). The same tendency has been observed for Alaska pollack, but in the case of chum salmon, the breaking strength only slightly increased upon storage (Okazaki *et al.*, 1997; Shoji *et al.*, 1991). It was assumed that these differences were due to the difference in the chemical bonds that occurred upon pressurization and storage (Okazaki *et al.*, 1997). Formation of harder gels upon storage in the case of Alaska pollack might be due to the polymerization of myosin heavy chains (Shoji *et al.*, 1991). Therefore, pressure-induced gels should be stabilized by heat processing although this may cause a slight decrease in deformation and transparency (Shoji *et al.*, 1991).

HIGH-PRESSURE EFFECTS ON ANIMAL MEAT GELLATION

High-pressure-induced tenderization of meat Pressurization of post-rigor beef skeletal muscle for 5 min at 100 MPa or higher decreased the hardness of the muscle with a

concomitant increase in the myofibril fragmentation. The hardness of pressurized muscle decreased to 60, 20 and 10% of the control at 100, 150 and 300 MPa, respectively, whereas no change in the gel elasticity was observed (Suzuki *et al.*, 1990; Suzuki & Ikeuchi, 1991). The degree of myofibril fragmentation was less than 10% in the untreated meat, whereas it reached 30, 70 and 90% at 100, 150 and 300 MPa, respectively (Suzuki & Ikeuchi, 1991). The degree of fragmentation reached its maximal level after brief exposure (5 min) to the highest pressure (300 MPa). Electron microscopy studies of the pressurized muscle revealed that a marked rupture of the I-band and loss of M-line materials had progressed in the myofibrils with increasing applied pressure. However, degradation of the Z-line in the myofibrils which can be observed naturally in conditioned muscle was not apparent in the pressurized muscle. There was no difference in the electrophoretic pattern of myofibrillar proteins among the control and the pressurized muscle samples in spite of the marked change in the ultrastructure (Suzuki *et al.*, 1990).

High pressure also tenderizes uncooked fermented meat products (Haga & Ueshima, 1997). High-pressure treatment only slightly increased the chicken muscle hardness, whereas high-pressure-treated chicken paste exhibited a significantly lower hardness than its heat-treated counterpart (Yoshioka *et al.*, 1992). The hardness of high-pressure-induced gels of cured or non-cured porcine meat (500 MPa, 10 min, 20°C) was almost three times lower than that of heat-induced gels (75°C, 50 min) (Yoshino & Haga, 1994). When pressurized meat was further heat-cooked, the hardness of cured meat slightly increased, whereas that of non-cured meat markedly increased.

The breaking stress of beef meat increased upon pressurization at or above 250 MPa for 1 h; longer pressurization time (up to 3 h) did not result in higher breaking stress (Nose *et al.*, 1994).

High-pressure treatment (250 MPa, 20°C, 3 h) of pre-roasted beef meat (280°C, 7 min) resulted in a higher tenderness and a lower cooking loss compared to those of non-pressurized roasted beef meat, and the color did not fade; these properties were maintained upon chilled storage (Nose *et al.*, 1994). However, non-cured and cured pork meat were discolored upon pressurization at 300 MPa or higher and 400 MPa or higher, respectively (Suzuki & Tujita, 1990). Beef meat became pink in the 200–400 MPa pressure range, whereas meat turned to grey-brown under 400–500 MPa (Carlez *et al.*, 1995b). Pork slurries pressurized at 200 MPa or higher coagulated and discolored (Ohmori & Shigehisha, 1990).

Upon sensory test, pressurized chicken muscle was judged more juicy, soft and elastic than its heat-induced counterpart (Yoshioka *et al.*, 1992). Pressure treatment of postmortem muscle caused almost the same changes in the components responsible for the flavor of meat as those observed in conditioned meats (Suzuki *et al.*, 1994).

Mechanism of the action of high pressure Investigations with isolated myosin, actomyosin, sarcoplasmic reticulum and intramolecular connective tissue provided some information on the mechanism of high-pressure-induced gelation (Suzuki *et al.*, 1997a; b; Yamamoto *et al.*,

1990; 1993). Myosin solution (10 mg/ml in 0.5 M KCl at pH 6) did not form a gel upon pressurization up to 210 MPa. Its turbidity started to increase under 140 MPa; the increase was due to myosin molecule aggregation. Untreated myosin exists as monomers with two heads per molecule; upon pressurization, the monomers form oligomers via head-to-head interactions as in case of heat treatment. However, the following linkage of aggregates through entangling of tails observed with heat treatment did not occur upon pressure treatment. Because the entangling of the tails is considered to be due to helix-coil transition, the helix structure in the tail is probably retained upon pressurization (Yamamoto *et al.*, 1993). When myosin was pressurized in 0.1 M KCl, 20 mM phosphate at pH 6, it formed a gel under 280 MPa and at a protein concentration above 2 mg/ml. The gel formation is closely related to the structural changes in filaments (disturbance and association) (Yamamoto *et al.*, 1990). The gels strength of pressure-induced gels was almost proportional to the protein concentration, and it was comparable to that of the heat-induced ones. The microstructure of high-pressure-induced gels consisted of a fine network comparable to that of the heat-induced one.

ATPase activities of sarcoplasmic reticulum slightly decreased upon pressurization; simultaneously, the sarcoplasmic reticulum membrane structure was damaged and high-affinity protein released. These two phenomena resulted in a global decrease in the Ca²⁺ uptaking ability, which may be one of the causes of pressure-induced meat tenderization (Suzuki *et al.*, 1997a). SEM observations revealed that changes induced by pressurization in the intramuscular connective tissue were different than those observed with aged muscle (Suzuki *et al.*, 1997b).

HIGH-PRESSURE EFFECTS ON SOY MILK GELATION (tofu)

Soy milk containing 5% protein changed from a liquid to a sol after treatment at 500 MPa at room temperature for 30 min (Kajiyama *et al.*, 1995). After pressurization at higher than 100 MPa, the soy milk could be coagulated by the addition of CaCl₂. The gel formed at a pressure above 300 MPa showed a greater hardness than that of tofu processed traditionally by heat treatment. SS bonds have been found to be unimportant in tofu processed from pressurized soy milk (Kajiyama *et al.*, 1995).

HIGH-PRESSURE EFFECTS ON EGG GELATION

Egg white coagulated at 400–600 MPa, and a gel standing under its own weight was obtained at 600 MPa (Bridgman, 1914; Hayashi *et al.*, 1989). The hardness of egg white gels increased by increasing the pressure, but it was much lower than that of heat-induced gels (Okamoto *et al.*, 1990). Pressurization of old egg white resulted in softer gels (Hayashi *et al.*, 1989).

Egg yolk formed a gel firm enough for texture measurement at 400 MPa and room temperature, and harder gels were obtained by increasing the pressure (Okamoto *et al.*, 1990). The minimum pressure was lowered to 300 MPa, when pressurization was carried out at 50°C, whereas no gel could be formed even under 500 MPa when the temperature of

pressurization was lower than 10°C (Dumoulin *et al.*, 1998). At all temperatures and pressures tested, the high-pressure-induced gels formed were much more softer than heat-induced gels. Moreover, they were more yellow and unbreakable (Okamoto *et al.*, 1990; Dumoulin *et al.*, 1998).

High-pressure treatment increased the protease digestibility, especially that of egg white. Moreover, the taste of pressurized egg white and yolk was that of the raw ones; vitamin and amino acid contents were not modified upon pressurization (Hayashi *et al.*, 1989).

The dynamical properties of egg white during the pressure gelation were investigated (Kanaya *et al.*, 1996). It was found that the rate of protein denaturation was higher than that of protein interaction in the pressure-induced gelation process contrarily to the heat-induced process.

Intermittent treatment (300 MPa, 20°C, 5 times for 2 min) is effective to sanitize the egg white without modifying its technological properties including its gellifying properties (Honma & Haga, 1991).

HIGH-PRESSURE PRETREATMENT EFFECTS ON VEGETABLE TEXTURE

High-pressure treatment modifies the palatability of vegetables. For example, potato and sweet potato became more pliable, the sweetness of sweet potato and fruits increased, and taproot vegetables became more transparent and more pliable upon pressurization (Shimada *et al.*, 1990).

High-pressure treatment prior to cooking is an alternative tool to harden vegetables, fruits and beans to protect their form and texture from overcooking damage (Kasai *et al.*, 1995). The relative hardness (defined by the ratio: hardness of pressure-pretreated sample/hardness of non-pretreated sample) after cooking was increased for pressure-treated Japanese radish as well as that of carrot, burdock and potato; and the increase was greater with increasing applied pressure (Kasai *et al.*, 1995). To be efficient for overcooking protection, the pressure pretreatment should be performed at 400 MPa or higher. For example, the relative hardness of pressure-treated vegetables (400 MPa at room temperature for 120 min) after 30-min cooking was in the range of 2-3. The relative hardness was increased by lengthening the duration of pressure treatment, and it continuously increased as time elapsed up to 24 h even after the release of the pressure. Indeed, the same results were obtained whether the sample was maintained under pressure or stored at atmospheric pressure after a short time of pressurization. High-pressure effects have been explained as follows: First, the cell membrane is collapsed by pressure, then the intracellular water, ions and other cellular components diffuse out. At the same time the degree of pectin esterification decreases, the amount of water soluble and hydrochloric acid soluble pectin decreases, while that of hexametaphosphate soluble pectin increases (Kasai *et al.*, 1995; Yamamoto *et al.*, 1992). Among ions, magnesium ions have a great influence on the hardness of both non-treated and pressure-treated uncooked sample, and calcium ions only affected the hardness of pressure-treated cooked samples. Calcium ions might promote interactions between such components as proteins and hemicellulose (Kasai *et al.*, 1997). The diffusion of the intracellular component and

pectin modification proceeded at almost the same rate under atmospheric (after pressure release) or high pressure (Kasai *et al.*, 1995). An increase in pressurization time expanded the cooking time for optimum hardness compared to the narrow time range of non-treated vegetables, making it easy to avoid overcooking (Fig. 6).

Upon drying, green beans, carrots and potatoes pressurized (600 MPa, 75°C, 15 min) and frozen (-18°C) retained their original color. These products also had good rehydration properties, and once rehydrated, they exhibited a texture very close to that of the raw material, and better than that obtained with blanched products (Esthiaghi *et al.*, 1994).

Pressure-treatment improved the poor cooking properties (high hardness accompanied with a poor stickiness) of aged rice. The best improvement was obtained under 100 MPa, and the improvement might be due to the partial degradation of some biological membranes (Watanabe *et al.*, 1991).

HIGH-PRESSURE FREEZING AND THAWING

The quality of frozen food is partly dependent on the kinetics of freezing and thawing, and because of the unique properties of water, high-pressure technology can improve the qualities of frozen products (Hayashi, 1992). The equilibrium temperature of the solid and liquid of type I ice is lowered as the pressure increases to about 200 MPa (Bridgman, 1912), for example, -4°C under 50 MPa, -8°C under 100 MPa and -20°C under 200 MPa. Derived from this water behavior, three applications have been proposed: non-freezing preservation of food, rapid thawing of frozen food and rapid freezing of food (pressure-shift freezing) (Hayashi, 1992).

Use of moderate high pressure at subzero temperature was effective to store strawberries, tomatoes, raw pork and raw beef without freezing and altering their original flavor and texture and without microbial spoilage (Deuchi & Hayashi, 1990).

Ice prepared at -10, -15, -20 and -30°C completely melted by pressurization at 50 to 200 MPa at 5°C for 30 min,

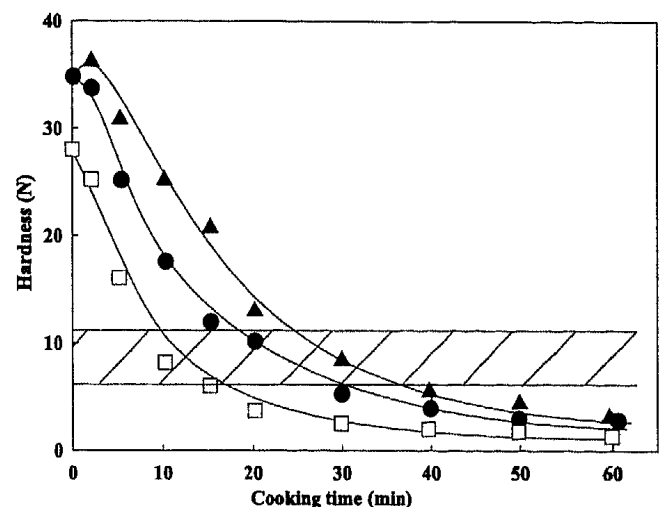


Fig. 6. Softening of Japanese radish during cooking after pressure treatment at 400 MPa. □, non-pressure-treated; ●, pressurized for 10 min; ▲, pressurized for 120 min. Shaded area shows the range of optimum hardness (from Kasai *et al.*, 1995).

whereas only a part of the ice (about 30%) was thawed by standing at atmospheric pressure at 5°C for 30 min (Deuchi & Hayashi, 1991; 1992). Pressure thawing occurred uniformly both outside and inside the ice block. Frozen beef stored at -20°C was pressurized at 50 to 200 MPa and 20°C for 30 min (Deuchi & Hayashi, 1992). As a result, a treatment under 50 MPa thawed it with a low development of drip and without any change in its original color. However, higher pressure gave a whitened meat due to the protein denaturation. A color change and a reduction in the drip upon pressure thawing were also noticed with frozen tuna (Murakami *et al.*, 1992). However, the pressure used for pressure thawing had little sterilizing effect.

Theoretically, the process of pressurization to 200 MPa followed by cooling to -20°C and rapid release of the pressure results in the rapid freezing of food (pressure-shift freezing) (Deuchi & Hayashi, 1992). In tofu frozen by this method, ice crystals were granular in form and had a very small size. When thawed at room temperature, the original shape and homogeneous structure of tofu were restored, and its original taste and texture were regained (Kanda & Aoki, 1993).

The effects of high-pressure freezing on the texture and tissue damages of frozen carrots in relation with ice properties have been investigated. Raw or 3-min-blanching carrots were pressurized at -20°C and 100 MPa (ice I), 200 MPa (liquid), 300 MPa (ice III), 400-600 MPa (ice IV), and 700 MPa (ice VI) for 45 min. When carrots were frozen at 100 MPa, the firmness decreased and the strain increased. The textural values of carrot pressurized at 200, 340 and 400 MPa were acceptable, whereas when pressure higher than 500 MPa was applied, the strain increased (Fuchigami *et al.*, 1997a). When raw carrots were pressurized under the conditions where ice I was formed, their histological structure was extremely damaged due to the volume expansion upon ice formation. Conversely, carrots pressurized at 100 MPa and -10°C (between liquid phase and ice I) or under 200 MPa and -20°C (liquid phase) were not damaged because they were rapidly frozen during the pressure release. No damage was observed under conditions where ice III or V was formed (Fuchigami *et al.*, 1997b). The textural properties and the histological structure of carrots pressurized under 200 or 340 MPa at -20°C were better than those of carrots frozen at -30°C under atmospheric pressure (Fuchigami *et al.*, 1997a). Similarly, it was found that high-pressure freezing at 200-400 MPa was effective to improve the qualities of frozen tofu (Fuchigami & Teramoto, 1997).

Conclusions

Since the pioneering investigations at the end of the 19th century by Hite, and especially within the last decade, much information has been accumulated on the potentialities of high pressure as a tool to modify or improve food texture. Studies on macromolecular model solutions demonstrate the usefulness of high pressure to texturize food macromolecules and to provide pastes or gels with unique properties. The influence of various parameters such as protein concentration, temperature of pressurization, pressurization level and time, pH, effects of salts and sugars on protein gelation has become

clearer. Research on polysaccharides is even more recent and still rare but it underlines the potentialities of high pressure to modify their functional properties. For example, studies on starch suspensions revealed that specific structures, which may develop particular functional properties, could be obtained upon pressurization. Studies on mixed systems are also of special interest since they provide information on what can happen with real foods. Moreover, the use of high pressure with mixed systems allows the reversal of the order of gelation and then gives gels with new structure and properties. However, despite all these new discoveries, additional experimental research is required on food macromolecules systems in the presence of salts, sugars, polysaccharides as well as denaturing reagents for a better understanding and the optimization of the high-pressure effects.

The potentialities of high pressure as a texturing agent *per se* or as a pretreatment method to improve the texturing properties of real foods upon traditional food processing are very wide. Actual knowledge reveals that, as a pretreatment tool, high-pressure technology is effective to improve milk coagulating properties and meat, egg and soy protein gelating properties. It can also constitute a unitary step in food processing to improve the final quality of cooked vegetables after freezing or drying. Nevertheless, the behavior of real foods is complex, especially because of the presence of enzymes, and further studies are needed for a more effective use of high pressure. It is especially important to evaluate the rheological, nutritional and sensorial qualities of pressurized foods in the state they will be eaten (after maturation, cooking and so on).

Organoleptic analysis of a wide range of pressurized products clearly showed that high-pressure-processed food retained the original color and flavor of the raw material (Okamoto *et al.*, 1989). Therefore, high pressure is likely to become a promising technology for the 21st century since consumers increasingly want to eat "natural-like food."

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