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Opportunities and Challenges in High Pressure Processing of Foods

N. K. Rastogi [a]; K. S. M. S. Raghavarao [a]; V. M. Balasubramaniam [b]; K. Niranjan [c]; D. Knorr [d]

[a] Department of Food Engineering, Central Food Technological Research Institute, Mysore, India
[b] Department of Food Science & Technology, The Ohio State University, Columbus, OH, USA
[c] School of Food Biosciences, The University of Reading, Reading, Berkshire, U.K.
[d] Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Berlin, D-14195, Germany

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N. K. RASTOGI, and K. S. M. S. RAGHAVARAO

Department of Food Engineering, Central Food Technological Research Institute, Mysore 570 020, India

V. M. BALASUBRAMANIAM

Department of Food Science & Technology, The Ohio State University, 333 Parker Food Science & Technology Building 2015 Fyffe Road, Columbus, OH 43210, USA

K. NIRANJAN

School of Food Biosciences, The University of Reading, PO Box 226, Whiteknights, Reading RG6 6AP, Berkshire, U.K.

D. KNORR

Department of Food Biotechnology and Food Process Engineering, Berlin University of Technology, Königin-Luise-Strasse 22, D-14195 Berlin, Germany

Consumers increasingly demand convenience foods of the highest quality in terms of natural flavor and taste, and which are free from additives and preservatives. This demand has triggered the need for the development of a number of nonthermal approaches to food processing, of which high-pressure technology has proven to be very valuable. A number of recent publications have demonstrated novel and diverse uses of this technology. Its novel features, which include destruction of microorganisms at room temperature or lower, have made the technology commercially attractive. Enzymes and even spore forming bacteria can be inactivated by the application of pressure-thermal combinations, This review aims to identify the opportunities and challenges associated with this technology. In addition to discussing the effects of high pressure on food components, this review covers the combined effects of high pressure processing with: gamma irradiation, alternating current, ultrasound, and carbon dioxide or anti-microbial treatment. Further, the applications of this technology in various sectors— fruits and vegetables, dairy, and meat processing—have been dealt with extensively. The integration of high-pressure with other matured processing operations such as blanching, dehydration, osmotic dehydration, rehydration, frying, freezing / thawing and solid-liquid extraction has been shown to open up new processing options. The key challenges identified include: heat transfer problems and resulting non-uniformity in processing, obtaining reliable and reproducible data for process validation, lack of detailed knowledge about the interaction between high pressure, and a number of food constituents, packaging and statutory issues.

Keywords high pressure, food processing, non-thermal processing

INTRODUCTION

Consumers demand high quality and convenient products with natural flavor and taste, and greatly appreciate the fresh appearance of minimally processed food. Besides, they look for safe and natural products without additives such as preservatives and humectants. In order to harmonize or blend all these demands without compromising the safety of the products, it is necessary to implement newer preservation technologies in the food industry. Although the fact that "high pressure kills microorganisms and preserves food" was discovered way back in 1899 and has been used with success in chemical, ceramic, carbon allotropy, steel/alloy, composite materials and plastic industries for decades, it was only in late 1980's that its commercial benefits became available to the food processing industries. High pressure processing (HPP) is similar in concept to cold isostatic pressing of metals and ceramics, except that it demands much higher pressures, faster cycling, high capacity,

Address correspondence to N. K. Rastogi, Department of food Engneering, Central Food Technology Research Institute, Mysore 570020, India. E-mail: nkrastogi@cftri.com

and sanitation (Zimmerman and Bergman, 1993; Mertens and Deplace, 1993). Hite (1899) investigated the application of high pressure as a means of preserving milk, and later extended the study to preserve fruits and vegetables (Hite, Giddings, and Weakly, 1914). It then took almost eighty years for Japan to re-discover the application of high-pressure in food processing. The use of this technology has come about so quickly that it took only three years for two Japanese companies to launch products, which were processed using this technology. The ability of high pressure to inactivate microorganisms and spoilage catalyzing enzymes, whilst retaining other quality attributes, has encouraged Japanese and American food companies to introduce high pressure processed foods in the market (Mermelstein, 1997; Hendrickx, Ludikhuyze, Broeck, and Weemaes, 1998). The first high pressure processed foods were introduced to the Japanese market in 1990 by Meidi-ya, who have been marketing a line of jams, jellies, and sauces packaged and processed without application of heat (Thakur and Nelson, 1998). Other products include fruit preparations, fruit juices, rice cakes, and raw squid in Japan; fruit juices, especially apple and orange juice, in France and Portugal; and guacamole and oysters in the USA (Hugas, Garcia, and Monfort, 2002). In addition to food preservation, high-pressure treatment can result in food products acquiring novel structure and texture, and hence can be used to develop new products (Hayashi, 1990) or increase the functionality of certain ingredients. Depending on the operating parameters and the scale of operation, the cost of high-pressure treatment is typically around US$ 0.05–0.5 per liter or kilogram, the lower value being comparable to the cost of thermal processing (Thakur and Nelson, 1998; Balasubramaniam, 2003).

The non-availability of suitable equipment encumbered early applications of high pressure. However, recent progress in equipment design has ensured worldwide recognition of the potential for such a technology in food processing (Gould, 1995; Galazka and Ledward, 1995; Balci and Wilbey, 1999). Today, high-pressure technology is acknowledged to have the promise of producing a very wide range of products, whilst simultaneously showing potential for creating a new generation of value added foods. In general, high-pressure technology can supplement conventional thermal processing for reducing microbial load, or substitute the use of chemical preservatives (Rastogi, Subramanian, and Raghavarao, 1994).

Over the past two decades, this technology has attracted considerable research attention, mainly relating to: i) the extension of keeping quality (Cheftel, 1995; Farkas and Hoover, 2001), ii) changing the physical and functional properties of food systems (Cheftel, 1992), and iii) exploiting the anomalous phase transitions of water under extreme pressures, e.g. lowering of freezing point with increasing pressures (Kalichevsky, Knorr, and Lillford, 1995; Knorr, Schlueter, and Heinz, 1998). The key advantages of this technology can be summarized as follows:

1. it enables food processing at ambient temperature or even lower temperatures;
2. it enables instant transmittance of pressure throughout the system, irrespective of size and geometry, thereby making size reduction optional, which can be a great advantage;
3. it causes microbial death whilst virtually eliminating heat damage and the use of chemical preservatives/additives, thereby leading to improvements in the overall quality of foods; and
4. it can be used to create ingredients with novel functional properties.

The effect of high pressure on microorganisms and proteins/enzymes was observed to be similar to that of high temperature. As mentioned above, high pressure processing enables transmittance of pressure rapidly and uniformly throughout the food. Consequently, the problems of spatial variations in preservation treatments associated with heat, microwave, or radiation penetration are not evident in pressure-processed products. The application of high pressure increases the temperature of the liquid component of the food by approximately 3°C per 100 MPa. If the food contains a significant amount of fat, such as butter or cream, the temperature rise is greater (8–9°C/100 MPa) (Rasanayagam, Balasubramaniam, Ting, Sizer, Bush, and Anderson, 2003). Foods cool down to their original temperature on decompression if no heat is lost to (or gained from) the walls of the pressure vessel during the holding stage. The temperature distribution during the pressure-holding period can change depending on heat transfer across the walls of the pressure vessel, which must be held at the desired temperature for achieving truly isothermal conditions. In the case of some proteins, a gel is formed when the rate of compression is slow, whereas a precipitate is formed when the rate is fast. High pressure can cause structural changes in structurally fragile foods containing entrapped air such as strawberries or lettuce. Cell deformation and cell damage can result in softening and cell serum loss. Compression may also shift the pH depending on the imposed pressure. Heremans (1995) indicated a lowering of pH in apple juice by 0.2 units per 100 MPa increase in pressure. In combined thermal and pressure treatment processes, Meyer (2000) proposed that the heat of compression could be used effectively, since the temperature of the product can be raised from 70–90°C to 105–120°C by a compression to 700 MPa, and brought back to the initial temperature by decompression.

As a thermodynamic parameter, pressure has far-reaching effects on the conformation of macromolecules, the transition temperature of lipids and water, and a number of chemical reactions (Cheftel, 1992; Tauscher, 1995). Phenomena that are accompanied by a decrease in volume are enhanced by pressure, and vice-versa (principle of Le Chatelier). Thus, under pressure, reaction equilibriums are shifted towards the most compact state, and the reaction rate constant is increased or decreased,

depending on whether the "activation volume" of the reaction (i.e. volume of the activation complex less volume of reactants) is negative or positive. It is likely that pressure also inhibits the availability of the activation energy required for some reactions, by affecting some other energy releasing enzymatic reactions (Farr, 1990). The compression energy of 1 litre of water at 400 MPa is 19.2 kJ, as compared to 20.9 kJ for heating 1 litre of water from 20 to 25°C. The low energy levels involved in pressure processing may explain why covalent bonds of food constituents are usually less affected than weak interactions. Pressure can influence most biochemical reactions, since they often involve change in volume. High pressure controls certain enzymatic reactions. The effect of high pressure on protein/enzyme is reversible unlike temperature, in the range 100–400 MPa and is probably due to conformational changes and sub-unit dissociation and association process (Morild, 1981).

For both the pasteurization and sterilization processes, a combined treatment of high pressure and temperature are frequently considered to be most appropriate (Farr, 1990; Patterson, Quinn, Simpson, and Gilmour, 1995). Vegetative cells, including yeast and moulds, are pressure sensitive, i.e. they can be inactivated by pressures of ~300–600 MPa (Knorr, 1995; Patterson, Quinn, Simpson, and Gilmour, 1995). At high pressures, microbial death is considered to be due to permeabilization of cell membrane. For instance, it was observed that in the case of *Saccharomyces cerevasia*, at pressures of about 400 MPa, the structure and cytoplasmic organelles were grossly deformed and large quantities of intracellular material leaked out, while at 500 MPa, the nucleus could no longer be recognized, and a loss of intracellular material was almost complete (Farr, 1990). Changes that are induced in the cell morphology of the microorganisms are reversible at low pressures, but irreversible at higher pressures where microbial death occurs due to permeabilization of the cell membrane. An increase in process temperature above ambient temperature, and to a lesser extent, a decrease below ambient temperature, increases the inactivation rates of microorganisms during high pressure processing. Temperatures in the range 45 to 50°C appear to increase the rate of inactivation of pathogens and spoilage microorganisms. Preservation of acid foods (pH $\leq$ 4.6) is, therefore, the most obvious application of HPP as such. Moreover, pasteurization can be performed even under chilled conditions for heat sensitive products. Low temperature processing can help to retain nutritional quality and functionality of raw materials treated and could allow maintenance of low temperature during post harvest treatment, processing, storage, transportation, and distribution periods of the life cycle of the food system (Knorr, 1995).

Bacterial spores are highly pressure resistant, since pressures exceeding 1200 MPa may be needed for their inactivation (Knorr, 1995). The initiation of germination or inhibition of germinated bacterial spores and inactivation of piezo-resistive microorganisms can be achieved in combination with moderate heating or other pretreatments such as ultrasound. Process temperature in the range 90–121°C in conjunction with pressures of 500-800 MPa have been used to inactivate spores forming bacteria such as *Clostridium botulinum*. Thus, sterilization of low-acid foods (pH > 4.6), will most probably rely on a combination of high pressure and other forms of relatively mild treatments.

High-pressure application leads to the effective reduction of the activity of food quality related enzymes (oxidases), which ensures high quality and shelf stable products. Sometimes, food constituents offer piezo-resistance to enzymes. Further, high pressure affects only non-covalent bonds (hydrogen, ionic, and hydrophobic bonds), causes unfolding of protein chains, and has little effect on chemical constituents associated with desirable food qualities such as flavor, color, or nutritional content. Thus, in contrast to thermal processing, the application of high-pressure causes negligible impairment of nutritional values, taste, color flavor, or vitamin content (Hayashi, 1990). Small molecules such as amino acids, vitamins, and flavor compounds remain unaffected by high pressure, while the structure of the large molecules such as proteins, enzymes, polysaccharides, and nucleic acid may be altered (Balci and Wilbey, 1999).

High pressure reduces the rate of browning reaction (Maillard reaction). It consists of two reactions, condensation reaction of amino compounds with carbonyl compounds, and successive browning reactions including metanoidin formation and polymerization processes. The condensation reaction shows no acceleration by high pressure (5–50 MPa at 50°C), because it suppresses the generation of stable free radicals derived from melanoidin, which are responsible for the browning reaction (Tamaoka, Itoh, and Hayashi, 1991). Gels induced by high pressure are found to be more glossy and transparent because of rearrangement of water molecules surrounding amino acid residues in a denatured state (Okamoto, Kawamura, and Hayashi, 1990).

The capability and limitations of HPP have been extensively reviewed (Thakur and Nelson, 1998; Smelt, 1998; Cheftal, 1995; Knorr, 1995; Farr, 1990; Tiwari, Jayas, and Holley, 1999; Cheftel, Levy, and Dumay, 2000; Messens, Van Camp, and Huyghebaert, 1997; Ontero and Sanz, 2000; Hugas, Garriga, and Monfort, 2002; Lakshmanan, Piggott, and Paterson, 2003; Balasubramaniam, 2003; Matser, Krebbers, Berg, and Bartels, 2004; Hogan, Kelly, and Sun, 2005; Mor-Mur and Yuste, 2005). Many of the early reviews primarily focused on the microbial efficacy of high-pressure processing. This review comprehensively covers the different types of products processed by high-pressure technology alone or in combination with the other processes. It also discusses the effect of high pressure on food constituents such as enzymes and proteins. The applications of this technology in fruits and vegetable, dairy and animal product processing industries are covered. The effects of combining high-pressure treatment with other processing methods such as gamma-irradiation, alternating current, ultrasound, carbon dioxide, and anti microbial peptides have also been described. Special emphasis has been given to opportunities and challenges in high pressure processing of foods, which can potentially be explored and exploited.

EFFECT OF HIGH PRESSURE ON ENZYMES AND PROTEINS

Enzymes

Enzymes are a special class of proteins in which biological activity arises from active sites, brought together by a three-dimensional configuration of molecule. The changes in active site or protein denaturation can lead to loss of activity, or changes the functionality of the enzymes (Tsou, 1986). In addition to conformational changes, enzyme activity can be influenced by pressure-induced decompartmentalization (Butz, Koller, Tauscher, and Wolf, 1994; Gomes and Ledward, 1996). Pressure induced damage of membranes facilitates enzyme-substrate contact. The resulting reaction can either be accelerated or retarded by pressure (Butz, Koller, Tauscher, and Wolf, 1994; Gomes and Ledward, 1996; Morild, 1981). Hendrickx, Ludikhuyze, Broeck, and Weemaes (1998) and Ludikhuyze, Van Loey, and Indrawati et al. (2003) reviewed the combined effect of pressure and temperature on enzymes related to the ity of fruits and vegetables, which comprises of kinetic information as well as process engineering aspects.

Pectin Methylesterase

Pectin methylesterase (PME) is an enzyme, which normally tends to lower the viscosity of fruits products and adversely affect their texture. Hence, its inactivation is a prerequisite for the preservation of such products. Commercially, fruit products containing PME (e.g. orange juice and tomato products) are heat pasteurized to inactivate PME and prolong shelf life. However, heating can deteriorate the sensory and nutritional quality of the products. Basak and Ramaswamy (1996) showed that the inactivation of PME in orange juice was dependent on pressure level, pressure-hold time, pH, and total soluble solids. An instantaneous pressure kill was dependent only on pressure level and a secondary inactivation effect dependent on holding time at each pressure level. Nienaber and Shellhammer (2001) studied the kinetics of PME inactivation in orange juice over a range of pressures (400–600 MPa) and temperatures (25–50°C) for various process holding times. PME inactivation followed a first-order kinetic model, with a residual activity of pressure-resistant enzyme. Calculated D-values ranged from 4.6 to 117.5 min at 600 MPa/50°C and 400 MPa/25°C, respectively. Pressures in excess of 500 MPa resulted in sufficiently faster inactivation rates for economic viability of the process. Binh, Van Loey, Fachin, Verlent, Indrawati, and Hendrickx (2002a, 2002b) studied the kinetics of inactivation of strawberry PME. The combined effect of pressure and temperature on inactivation kinetics followed a fractional-conversion model. Purified strawberry PME was more stable toward high-pressure treatments than PME from oranges and bananas. Ly-Nguyen, Van Loey, Fachin, Verlent, Hendrickx (2002) showed that the inactivation of the banana PME enzyme during heating at temperature between 65 and 72.5°C followed first order kinetics and the effect of pressure treatment

of 600–700 MPa at 10°C could be described using a fractional-conversion model. Stoforos, Crelier, Robert, and Taoukis (2002) demonstrated that under ambient pressure, tomato PME inactivation rates increased with temperature, and the highest rate was obtained at 75°C. The inactivation rates were dramatically reduced as soon as the essing pressure was raised beyond 75°C. High inactivation rates were obtained at a pressure higher than 700 MPa. Riahi and Ramaswamy (2003) studied high-pressure inactivation kinetics of PME isolated from a variety of sources and showed that PME from a microbial source was more resistant to pressure inactivation than from orange peel. Almost a full decimal reduction in activity of commercial PME was achieved at 400 MPa within 20 min.

Verlent, Van Loey, Smout, Duvetter, Nguyen, and Hendrickx (2004) indicated that the optimal temperature for tomato pectinmethylesterase was shifted to higher values at elevated pressure compared to atmospheric pressure, creating the possibilities for rheology improvements by the application of high pressure.

Castro, Van Loey, Saraiva, Smout, and Hendrickx (2006) accurately described the inactivation of the labile fraction under mild-heat and high-pressure conditions by a fractional conversion model, while a biphasic model was used to estimate the inactivation rate constant of both the fractions at more drastic conditions of temperature/pressure (10–64°C, 0.1–800 MPa). At pressures lower than 300 MPa and temperatures higher than 54°C, an antagonistic effect of pressure and temperature was observed.

Balogh, Smout, Binh, Van Loey, and Hendrickx (2004) observed the inactivation kinetics of carrot PME to follow first order kinetics over a range of pressure and temperature (650–800 MPa, 10–40°C). Enzyme stability under heat and pressure was reported to be lower in carrot juice and purified PME preparations than in carrots.

Pectinesterase

The presence of pectinesterase (PE) reduces the quality of citrus juices by destabilization of clouds. Generally, the inactivation of the enzyme is accomplished by heat, resulting in a loss of fresh fruit flavor in the juice. High pressure processing can be used to bypass the use of extreme heat for the processing of fruit juices. Goodner, Braddock, and Parish (1998) showed that the higher pressures (>600 MPa) caused instantaneous inactivation of the heat labile form of the enzyme but did not inactivate the heat stable form of PE in case of orange and grapefruit juices. PE activity was totally lost in orange juice, whereas complete inactivation was not possible in case of grapefruit juices. Orange juice pressurized at 700 MPa for 1 min had no cloud loss for more than 50 days. Broeck, Ludikhuyze, Van Loey, and Hendrickx (2000) studied the combined pressure-temperature inactivation of the labile fraction of orange PE over a range of pressure (0.1 to 900 MPa) and temperature (15 to 65°C). Pressure and temperature dependence of the inactivation rate constants of the labile fraction was quantified using the well-known Eyring and Arrhenius relations. The stable fraction was inactivated at a temperature

higher than 75°C. Acidification (pH 3.7) enhanced the thermal inactivation of the stable fraction, whereas the addition of Ca^{++} ions (1M) suppressed inactivation. At elevated pressure (up to 900 MPa), an antagonistic effect of pressure and temperature on inactivation of the stable fraction was observed. Ly-Nguyen, Van Loey, Smout, Ozcan, Fachin, Verlent, Vu-Truong, Duvetter, and Hendrickx (2003) investigated the combined heat and pressure treatments on the inactivation of purified carrot PE, which followed a fractional-conversion model. The thermally stable fraction of the enzyme could not be inactivated. At a lower pressure (<300 MPa) and higher temperature (>50°C), an antagonistic effect of pressure and heat was observed.

Polygalacturonase

High pressures induced conformational changes in polygalacturonase (PG) causing reduced substrate binding affinity and enzyme inactivation. Eun, Seok, and Wan (1999) studied the effect of high-pressure treatment on PG from Chinese cabbage to prevent the softening and spoilage of plant-based foods such as kimchies without compromising quality. PG was inactivated by the application of pressure higher than 200 MPa for 1 min. Fachin, Van Loey, Indrawati, Ludikhuyze, and Hendrickx (2002) investigated the stability of tomato PG at different temperatures and pressures. The combined pressure temperature inactivation (300–600 MPa/5–50°C) of tomato PG was described by a fractional conversion model, which points to 1st-order inactivation kinetics of a pressure-sensitive enzyme fraction and to the occurrence of a pressure-stable PG fraction. Fachin, Smout, Verlent, Binh, Van Loey, and Hendrickx (2004) indicated that in the combination of pressure-temperature (5–55°C/100–600 MPa), the inactivation of the heat labile portion of purified tomato PG followed first order kinetics. The heat stable fraction of the enzyme showed pressure stability very similar to that of heat labile portion.

Peeters, Fachin, Smout, Van Loey, and Hendrickx (2004) demonstrated that effect of high-pressure was identical on heat stable and heat labile fractions of tomato PG. The isoenzyme of PG was detected in thermally treated (140°C for 5 min) tomato pieces and tomato juice, whereas, no PG was found in pressure treated tomato juice or pieces.

Verlent, Van Loey, Smout, Duvetter, and Hendrickx (2004) investigated the effect of high pressure (0.1 and 500 MPa) and temperature (25–80°C) on purified tomato PG. At atmospheric pressure, the optimum temperature for enzyme was found to be 55–60°C and it decreased with an increase in pressure. The enzyme activity was reported to decrease with an increase in pressure at a constant temperature.

Shook, Shellhammer, and Schwartz (2001) studied the ability of high pressure to inactivate lipoxygenase, PE and PG in diced tomatoes. Processing conditions used were 400, 600, and 800 MPa for 1, 3, and 5 min at 25 and 45°C. The magnitude of the applied pressure had a significant effect in inactivating lipoxygenase and PG, with complete loss of activity occurring at 800 MPa. PE was very resistant to the pressure treatment.

Polyphenoloxidase and Peroxidase

Polyphenoloxidase (PPO) and peroxidase (POD), the enzymes responsible for color and flavor loss, can be selectively inactivated by a combined treatment of pressure and temperature. Gomes and Ledward (1996) studied the effects of pressure treatment (100–800 MPa for 1–20 min) on commercial PPO enzyme available from mushrooms, potatoes, and apples. Castellari, Matricardi, Arfelli, Rovere, and Amati (1997) demonstrated that there was a limited inactivation of grape PPO using pressures between 300 and 600 MPa. At 900 MPa, a low level of PPO activity was apparent. In order to reach complete inactivation, it may be necessary to use high-pressure processing treatments in conjunction with a mild thermal treatment (40–50°C). Weemaes, Ludikhuyze, Broeck, and Hendrickx (1998) studied the pressure stabilities of PPO from apple, avocados, grapes, pears, and plums at pH 6-7. These PPO differed in pressure stability. Inactivation of PPO from apple, grape, avocado, and pear at room temperature (25°C) became noticeable at approximately 600, 700, 800 and 900 MPa, respectively, and followed first-order kinetics. Plum PPO was not inactivated at room temperature by pressures up to 900 MPa. Rastogi, Eshtiaghi, and Knorr (1999) studied the inactivation effects of high hydrostatic pressure treatment (100-600 MPa) combined with heat treatment (0–60°C) on POD and PPO enzyme, in order to develop high pressure-processed red grape juice having stable shelf-life. The studies showed that the lowest POD (55.75%) and PPO (41.86%) activities were found at 60°C, with pressure at 600 and 100 MPa, respectively. MacDonald and Schaschke (2000) showed that for PPO, both temperature and pressure individually appeared to have similar effects, whereas the holding time was not significant. On the other hand, in case of POD, temperature as well as interaction between temperature and holding time had the greatest effect on activity. Namkyu, Seunghwan, and Kyung (2002) showed that mushroom PPO was highly pressure stable. Exposure to 600 MPa for 10 min reduced PPO activity by 7%; further exposure had no denaturing effect. Compression for 10 and 20 min up to 800 MPa, reduced activity by 28 and 43%, respectively.

Rapeanu, Van Loey, Smout, and Hendrickx (2005) indicated that the thermal and/or high-pressure inactivation of grape PPO followed first order kinetics. A third degree polynomial described the temperature/pressure dependence of the inactivation rate constants. Pressure and temperature were reported to act synergistically, except in the high temperature (≥45°C)-low pressure (≥300 MPa) region where an antagonistic effect was observed.

Papain

Gomes, Sumner, and Ledward (1997) showed that the application of increasing pressures led to a gradual reduction in papain enzyme activity. A decrease in activity of 39% was observed when the enzyme solution was initially activated with phosphate buffer (pH 6.8) and subjected to 800 MPa at ambient

temperature for 10 min, while 13% of the original activity remained when the enzyme solution was treated at 800 MPa at 60°C for 10 min. In Tris buffer at pH 6.8 after treatment at 800 MPa and 20°C, papain activity loss was approximately 24%. The inactivation of the enzyme is because of induced change at the active site causing loss of activity without major conformational changes. This loss of activity was due to oxidation of the thiolate ion present at the active site.

Alpha-amylase

Weemaes, Cordt, Goossens, Ludikhuyze, Hendrickx, Heremans, and Tobback (1996) studied the effects of pressure and temperature on activity of 3 different alpha-amylases from *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus licheniformis*. The changes in conformation of *Bacillus licheniformis*, *Bacillus subtilis*, and *Bacillus amyloliquefaciens* amylases occurred at pressures of 110, 75, and 65 MPa, respectively. *Bacillus licheniformis* amylase was more stable than amylases from *Bacillus subtilis* and *Bacillus amyloliquefaciens* to the combined heat/pressure treatment.

Riahi and Ramaswamy (2004) demonstrated that pressure inactivation of amylase in apple juice was significantly (P < 0.01) influenced by pH, pressure, holding time, and temperature. The inactivation was described using a bi-phasic model. The application of high pressure was shown to completely inactivate amylase. The importance of the pressure pulse and pressure hold approach for inactivation of amylase was also demonstrated.

Proteins

High pressure denatures protein depending on the protein type, processing conditions, and the applied pressure. During the process of denaturation, the proteins may dissolve or precipitate on the application of high pressure. These changes are generally reversible in the pressure range 100–300 MPa and irreversible for the pressures higher than 300 MPa. Denaturation may be due to the destruction of hydrophobic and ion pair bonds, and unfolding of molecules. At higher pressure, oligomeric proteins tend to dissociate into subunits becoming vulnerable to proteolysis. Monomeric proteins do not show any changes in proteolysis with increase in pressure (Thakur and Nelson, 1998).

High-pressure effects on proteins are related to the rupture on non-covalent interactions within protein molecules, and to the subsequent reformation of intra and inter molecular bonds within or between the molecules. Different types of interactions contribute to the secondary, tertiary, and quaternary structure of proteins. The quaternary structure is mainly held by hydrophobic interactions that are very sensitive to pressure. Significant changes in the tertiary structure are observed beyond 200 MPa. However, a reversible unfolding of small proteins such as ribonuclease A occurs at higher pressures (400 to 800 MPa), showing that the volume and compressibility changes during denaturation are not completely dominated by the hydrophobic
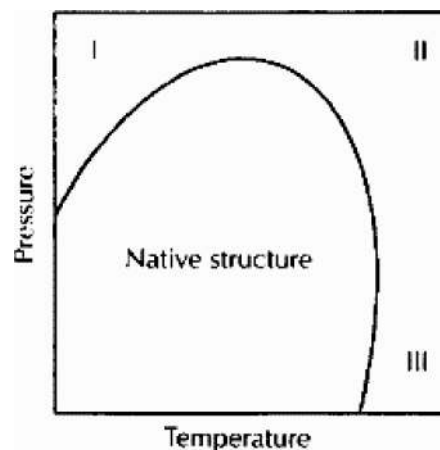

Figure 1 General scheme for pressure-temperature phase diagram of proteins. (from Messens, Van Camp, and Huyghebaert, 1997).

effect. Denaturation is a complex process involving intermediate forms leading to multiple denatured products. Secondary structure changes take place at a very high pressure above 700 MPa, leading to irreversible denaturation (Balny and Masson, 1993).

When the pressure increases to about 100 MPa, the denaturation temperature of the protein increases, whereas at higher pressures, the temperature of denaturation usually decreases. This results in the elliptical phase diagram of native denatured protein shown in Fig. 1. A practical consequence is that under elevated pressures, proteins denature usually at room temperature than at higher temperatures. The phase diagram also specifies the pressure-temperature range in which the protein maintains its native structure. Zone III specifies that at high temperatures, a rise in denaturation temperature is found with increasing pressure. Zone II indicates that below the maximum transition temperature, protein denaturation occurs at the lower temperatures under higher pressures. Zone III shows that below the temperature corresponding to the maximum transition pressure, protein denaturation occurs at lower pressures using lower temperatures (Messens, Van Camp, and Huyghebaert, 1997).

The application of high pressure has been shown to destabilize casein micelles in reconstituted skim milk and the size distribution of spherical casein micelles decrease from 200 to 120 nm; maximum changes have been reported to occur between 150–400 MPa at 20°C. The pressure treatment results in reduced turbidity and increased lightness, which leads to the formation of a virtually transparent skim milk (Shibauchi, Yamamoto, and Sagara, 1992; Derobry, Richard, and Hardy, 1994). The gels produced from high-pressure treated skim milk showed improved rigidity and gel breaking strength (Johnston, Austin, and Murphy, 1992). Garcia, Olano, Ramos, and Lopez (2000) showed that the pressure treatment at 25°C considerably reduced the micelle size, while pressurization at higher temperature progressively increased the micelle dimensions. Anema, Lowe, and Stockmann (2005) indicated that a small decrease in the size of casein micelles was observed at 100 MPa, with slightly greater effects at higher temperatures or longer pressure treatments. At

pressure ≥400 MPa, the casein micelles disintegrated. The effect was more rapid at higher temperatures although the final size was similar in all samples regardless of the pressure or temperature. At 200 MPa and 10°C, the casein micelle size decreased slightly on heating, whereas, at higher temperatures, the size increased as a result of aggregation. Huppertz, Fox, and Kelly (2004a) showed that the size of casein micelles increased by 30% upon high-pressure treatment of milk at 250 MPa and micelle size dropped by 50% at 400 or 600 MPa.

Huppertz, Fox, and Kelly (2004b) demonstrated that the high-pressure treatment of milk at 100–600 MPa resulted in considerable solubilization of alphas1- and beta-casein, which may be due to the solubilization of colloidal calcium phosphate and disruption of hydrophobic interactions. On storage of pressure, treated milk at 5°C dissociation of casein was largely irreversible, but at 20°C, considerable re-association of casein was observed. The hydration of the casein micelles increased on pressure treatment (100–600 MPa) due to induced interactions between caseins and whey proteins. Pressure treatment increased levels of alphas1- and beta-casein in the soluble phase of milk and produced casein micelles with properties different to those in untreated milk. Huppertz, Fox, and Kelly (2004c) demonstrated that the casein micelle size was not influenced by pressures less than 200 MPa, but a pressure of 250 MPa increased the micelle size by 25%, while pressures of 300 MPa or greater, irreversibly reduced the size to 50% of that in untreated milk. Denaturation of alpha-lactalbumin did not occur at pressures less than or equal to 400 MPa, whereas beta-lactoglobulin was denatured at pressures greater than 100 MPa.

Galazka, Ledward, Sumner, and Dickinson (1997) reported loss of surface hydrophobicity due to application of 300 MPa in dilute solution. Pressurizing beta-lactoglobulin at 450 MPa for 15 minutes resulted in reduced solubility in water. High-pressure treatment induced extensive protein unfolding and aggregation when BSA was pressurized at 400 MPa. Beta-lactoglobulin appears to be more sensitive to pressure than alpha-lactalbumin. Olsen, Ipsen, Otte, and Skibsted (1999) monitored the state of aggregation and thermal gelation properties of pressure-treated beta-lactoglobulin immediately after depressurization and after storage for 24 h at 5°C. A pressure of 150 MPa applied for 30 min, or pressures higher than 300 MPa applied for 0 or 30 min, led to formation of soluble aggregates. When continued for 30 min, a pressure of 450 MPa caused gelation of the 5% beta-lactoglobulin solution. Iametti, Tansidico, Bonomi, Vecchio, Pittia, Rovere, and Dall'Aglio (1997) studied irreversible modifications in the tertiary structure, surface hydrophobicity, and association state of beta-lactoglobulin, when solutions of the protein at neutral pH and at different concentrations, were exposed to pressure. Only minor irreversible structural modifications were evident even for treatments as intense as 15 min at 900 MPa. The occurrence of irreversible modifications was time-dependent at 600 MPa but was complete within 2 min at 900 MPa. The irreversibly modified protein was soluble, but some covalent aggregates were formed. Subirade, Loupil, Allain, and Paquin (1998) showed the effect

of dynamic high pressure on the secondary structure of beta-lactoglobulin. Thermal and pH sensitivity of pressure treated beta-lactoglobulin was different, suggesting that the two forms were stabilized by different electrostatic interactions. Walker, Farkas, Anderson, and Goddik (2004) used high-pressure processing (510 MPa for 10 min at 8 or 24°C) to induce unfolding of beta-lactoglobulin and characterized the protein structure and surface-active properties. The secondary structure of the protein processed at 8°C appeared to be unchanged, whereas at 24°C alpha-helix structure was lost. Tertiary structures changed due to processing at either temperature. Model solutions containing the pressure-treated beta-lactoglobulin showed a significant decrease in surface tension. Izquierdo, Alli, Gómez, Ramaswamy, and Yaylayan (2005) demonstrated that under high-pressure treatments (100-300 MPa), the β-lactoglobulin AB was completely hydrolyzed by pronase and α-chymotrypsin. Hinrichs and Rademacher (2005) showed that the denaturation kinetics of beta-lactoglobulin followed second order kinetics while for alpha-lactalbumin it was 2.5. Alpha-lactalbumin was more resistant to denaturation than beta-lactoglobulin. The activation volume for denaturation of beta-lactoglobulin was reported to decrease with increasing temperature, and the activation energy increased with pressure up to 200 MPa, beyond which it decreased. This demonstrated the unfolding of the protein molecules.

Drake, Harison, Apslund, Barbosa-Canovas, and Swanson (1997) demonstrated that the percentage moisture and wet weight yield of cheese from pressure treated milk were higher than pasteurized or raw milk cheese. The microbial quality was comparable and some textural defects were reported due to the excess moisture content. Arias, Lopez, and Olano (2000) showed that high-pressure treatment at 200 MPa significantly reduced rennet coagulation times over control samples. Pressurization at 400 MPa led to coagulation times similar to those of control, except for milk treated at pH 7.0, with or without readjustment of pH to 6.7, which presented significantly longer coagulation times than their non-pressure treated counterparts.

Hinrichs and Rademacher (2004) demonstrated that the isobaric (200-800 MPa) and isothermal (−2 to 70°C) denaturation of beta-lactoglobulin and alpha-lactalbumin of whey protein followed 3rd and 2nd order kinetics, respectively. Isothermal pressure denaturation of both beta-lactoglobulin A and B did not differ significantly and an increase in temperature resulted in an increase in the denaturation rate. At pressures higher than 200 MPa, the denaturation rate was limited by the aggregation rate, while the pressure resulted in the unfolding of molecules. The kinetic parameters of denaturation were estimated using a single step non-linear regression method, which allowed a global fit of the entire data set. Huppertz, Fox, and Kelly (2004d) examined the high-pressure induced denaturation of alpha-lactalbumin and beta-lactoglobulin in dairy systems. The higher level of pressure-induced denaturation of both proteins in milk as compared to whey was due to the absence of casein micelles and colloidal calcium phosphate in the whey.

The conformation of BSA was reported to remain fairly stable at 400 MPa due to a high number of disulfide bonds which are known to stabilize its three dimensional structure (Hayakawa, Kajihara, Morikawa, Oda, and Fujio, 1992). Kieffer and Wieser (2004) indicated that the extension resistance and extensibility of wet gluten were markedly influenced by high pressure (up to 800 MPa), while the temperature and the duration of pressure treatment (30–80°C for 2–20 min) had a relatively lesser effect. The application of high pressure resulted in a marked decrease in protein extractability due to the restructuring of disulfide bonds under high pressure leading to the incorporation of alpha- and gamma-gliadins in the glutenin aggregate. The change in secondary structure following high-pressure treatment was also reported.

The pressure treatment of myosin led to head-to-head interaction to form oligomers (clumps), which became more compact and larger in size during storage at constant pressure. Even after pressure treatment at 210 MPa for 5 minutes, monomieric myosin molecules increased and no gelation was observed for pressure treatment up to 210 MPa for 30 minutes. Pressure treatment did not also affect the original helical structure of the tail in the myosin monomers. Angsupanich, Edde, and Ledward (1999) showed that high pressure-induced denaturation of myosin led to formation of structures that contained hydrogen bonds and were additionally stabilized by disulphide bonds.

Application of 750 MPa for 20 minutes resulted in dimerization of metmyoglobin in the pH range of 6–10, whereas maximum pH was not at isoelectric pH (6.9). Under acidic pH conditions, no dimers were formed (Defaye and Ledward, 1995). Zipp and Kouzmann (1973) showed the formation of precipitate when pressurized (750 MPa for 20 minutes) near the isoelectric point, the precipitate redissolved slowly during storage. Pressure treatment had no effect on lipid oxidation in the case of minced meat packed in air at pressure less than 300 MPa, while the oxidation increased proportionally at higher pressures. However, on exposure to higher pressure, minced meat in contact with air oxidized rapidly. Pressures >300–400 MPa caused marked denaturation of both myofibriller and sarcoplasmic proteins in washed pork muscle and pork mince (Ananth, Murano and Dickson, 1995). Chapleau and Lamballerie (2003) showed that high-pressure treatment induced a threefold increase in the surface hydrophobicity of myofibrillar proteins between 0 and 450 MPa. Chapleau, Mangavel, Compoint, and Lamballerie (2004) reported that high pressure modified the secondary structure of myofibrillar proteins extracted from cattle carcasses. Irreversible changes and aggregation were reported at a pressure higher than 300 MPa, which can potentially affect the functional properties of meat products. Lamballerie, Perron, Jung, and Cheret (2003) indicated that high pressure treatment increases cathepsin D activity, and that pressurized myofibrils are more susceptible to cathepsin D action than non-pressurized myofibrils. The highest cathepsin D activity was observed at 300 MPa. Carlez, Veciana, and Cheftel (1995) demonstrated that L color values increased significantly in meat treated at 200–350 MPa, the meat becoming pink,

and a-value decreased in meat treated at 400–500 MPa to give a grey-brown color. The total extractable myoglobin decreased in meat treated at 200–500 MPa, while the metmyoglobin content of meat increased and the oxymyoglobin decreased at 400–500 MPa. Meat discoloration from pressure processing resulted in a whitening effect at 200–300 MPa due to globin denaturation, and/or haem displacement/release, or oxidation of ferrous myoglobin to ferric myoglobin at pressure higher than 400 MPa.

The conformation of the main protein component of egg white, ovalbumin, remains fairly stable when pressurized at 400 MPa, may be due to the four disulfide bonds and non-covalent interactions stabilizing the three dimensional structure of ovalbumin (Hayakawa, Kajihara, Morikawa, Oda, and Fujio, 1992). Hayashi, Kawamura, Nakasa and Okinada (1989) reported irreversible denaturation of egg albumin at 500–900 MPa with concomitant increase in susceptibility to subtilisin. Zhang, Li, and Tatsumi (2005) demonstrated that the pressure treatment (200–500 MPa) resulted in denaturation of ovalbumin. The surface hydrophobicity of ovalbumin was found to increase with increase in pressure treatment and the presence of polysaccharide protected the protein against denaturation. Iametti, Donnizzelli, Pittia, Rovere, Squarcina, and Bonomi (1999) showed that the addition of NaCl or sucrose to egg albumin prior to high-pressure treatment (up to 10 min at 800 MPa) prevented insolubulization or gel formation after pressure treatment. As a consequence of protein unfolding, the treated albumin had increased viscosity but retained its foaming and heat-gelling properties. Farr (1990) reported the modification of functionality of egg proteins. Egg yolk formed a gel when subjected to a pressure of 400 MPa for 30 minutes at 25°C, kept its original color, and was soft and adhesive. The hardness of the pressure treated gel increased and adhesiveness decreased with an increase in pressure. Plancken, Van Loey, and Hendrickx (2005) showed that the application of high pressure (400–700 MPa) to egg white solution resulted in an increase in turbidity, surface hydrophobicity, exposed sulfhydryl content, and susceptibility to enzymatic hydrolysis, while it resulted in a decrease in protein solubility, total sulfhydryl content, denaturation enthalpy, and trypsin inhibitory activity. The pressure-induced changes in these properties were shown to be dependent on the pressure-temperature and the pH of the solution. Speroni, Puppo, Chapleau, Lamballerie, Castellani, Añón, and Anton (2005) indicated that the application of high pressure (200–600 MPa) at 20°C to low-density lipoproteins did not change the solubility even if the pH is changed, whereas aggregation and protein denaturation were drastically enhanced at pH 8. Further, the application of high-pressure under alkaline pH conditions resulted in decreased droplet flocculation of low-density lipoproteins dispersions.

The minimum pressure required for the inducing gelation of soya proteins was reported to be 300 MPa for 10–30 minutes and the gels formed were softer with lower elastic modulus in comparison with heat-treated gels (Okamoto, Kawamura, and Hayashi, 1990). The treatment of soya milk at 500 MPa for

30 min changed it from a liquid state to a solid state, whereas at lower pressures and at 500 MPa for 10 minutes, the milk remained in a liquid state, but indicated improved emulsifying activity and stability (Kajiyama, Isobe, Uemura, and Noguchi, 1995). The hardness of tofu gels produced by high-pressure treatment at 300 MPa for 10 minutes was comparable to heat induced gels. Puppo, Chapleau, Speroni, Lamballerie, Michel, Anon, and Anton (2004) demonstrated that the application of high pressure (200–600 MPa) on soya protein isolate at pH 8.0 resulted in an increase in a protein hydorphobicity and aggregation, a reduction of free sulfhydryl content and a partial unfolding of the 7S and 11S fractions at pH 8. The change in the secondary structure leading to a more disordered structure was also reported. Whereas at pH 3.0, the protein was partially denatured and insoluble aggregates were formed, the major molecular unfolding resulted in decreased thermal stability, increased protein solubility, and hydorphobicity. Puppo, Speroni, Chapleau, Lamballerie, Añón, and Anton (2005) studied the effect of high-pressure (200, 400, and 600 MPa for 10 min at 10°C) on the emulsifying properties of soybean protein isolates at pH 3 and 8 (e.g. oil droplet size, flocculation, interfacial protein concentration, and composition). The application of pressure higher than 200 MPa at pH 8 resulted in a smaller droplet size and an increase in the levels of depletion flocculation. However, a similar effect was not observed at pH 3. Due to the application of high pressure, bridging flocculation decreased and the percentage of adsorbed proteins increased irrespective of the pH conditions. Moreover, the ability of the protein to be adsorbed at the oil-water interface increased. Zhang, Li, Tatsumi, and Isobe (2005) showed that the application of high pressure treatment resulted in the formation of more hydrophobic regions in soy protein, which dissociated into subunits, which in some cases formed insoluble aggregates. High-pressure denaturation of beta-conglycinin (7S) and glycinin (11S) occurred at 300 and 400 MPa, respectively. The gels formed had the desirable strength and a cross-linked network microstructure.

Soybean whey is a by-product of tofu manufacture. It is a good source of peptides, proteins, oligosaccharides, and isoflavones, and can be used in special foods for the elderly persons, athletes, etc. Prestamo and Penas (2004) studied the antioxidative activity of soybean whey proteins and their pepsin and chymotrypsin hydrolysates. The chymotrypsin hydrolysate showed a higher antioxidative activity than the non-hydrolyzed protein, but the pepsin hydrolysate showed an opposite trend. High pressure processing at 100 MPa increased the antioxidative activity of soy whey protein, but decreased the antioxidative activity of the hydrolysates. High pressure processing increased the pH of the protein hydrolysates. Penas, Prestamo, and Gomez (2004) demonstrated that the application of high pressure (100 and 200 MPa, 15 min, 37°C) facilitated the hydrolysis of soya whey protein by pepsin, trypsin, and chymotrypsin. It was shown that the highest level of hydrolysis occurred at a treatment pressure of 100 MPa. After the hydrolysis, 5 peptides under 14 kDa with trypsin and chymotrypsin, and 11 peptides with pepsin were reported.

COMBINATION OF HIGH PRESSURE TREATMENT WITH OTHER NON-THERMAL PROCESSING METHODS

Many researchers have combined the use of high pressure with other non-thermal operations in order to explore the possibility of synergy between processes. Such attempts are reviewed in this section.

Gamma Irradiation

Crawford, Murano, Olson, and Shenoy (1996) studied the combined effect of high pressure and gamma-irradiation for inactivating *Clostridium sprogenes* spores in chicken breast. Application of high pressure reduced the radiation dose required to produce chicken meat with extended shelf life. The application of high pressure (600 MPa for 20 min at 80°C) reduced the irradiation doses required for one log reduction of *Clostridium sprogenes* from 4.2 kGy to 2.0 kGy. Mainville, Montpetit, Durand, and Farnworth (2001) studied the combined effect of irradiation and high pressure on microflora and microorganisms of kefir. The irradiation treatment of kefir at 5 kGy and high-pressure treatment (400 MPa for 5 or 30 min) deactivated the bacteria and yeast in kefir, while leaving the proteins and lipids unchanged.

Alternating Current

The exposure of microbial cells and spores to an alternating current (50 Hz) resulted in the release of intracellular materials causing loss or denaturation of cellular components responsible for the normal functioning of the cell. The lethal damage to the microorganisms enhanced when the organisms are exposed to an alternating current before and after the pressure treatment. High-pressure treatment at 300 MPa for 10 min for *Escherichia coli* cells and 400 MPa for 30 min for *Bacillus subtalis* spores, after the alternating current treatment, resulted in reduced surviving fractions of both the organisms. The combined effect was also shown to reduce the tolerant level of microorganisms to other challenges (Shimada and Shimahara, 1985, 1987; Shimada, 1992).

Ultrasound

The pretreatment with ultrasonic waves (100 W/cm^2 for 25 min at 25°C) followed by high pressure (400 MPa for 25 min at 15°C) was shown to result in complete inactivation of *Rhodoturola rubra*. Neither ultrasonic nor high-pressure treatment alone was found to be effective (Knorr, 1995).

Carbon Dioxide and Argon

Heinz and Knorr (1995) reported a 3 log reduction of supercritical CO_2 pretreated cultures. The effect of the pretreatment on germination of *Bacillus subtilis* endospores was monitored. The combination of high pressure and mild heat treatment was the most effective in reducing germination (95% reduction), but no spore inactivation was observed.

Park, Lee, and Park (2002) studied the combination of high-pressure carbon dioxide and high pressure as a nonthermal processing technique to enhance the safety and shelf life of carrot juice. The combined treatment of carbon dioxide (4.90 MPa) and high-pressure treatment (300 MPa) resulted in complete destruction of aerobes. The increase in high pressure to 600 MPa in the presence of carbon dioxide resulted in reduced activities of polyphenoloxidase (11.3%), lipoxygenase (8.8%), and pectin methylesterase (35.1%). Corwin and Shellhammer (2002) studied the combined effect of high-pressure treatment and CO_2 on the inactivation of pectinmethylesterase, polyphenoloxidase, *Lactobacillus plantarum*, and *Escherichia coli*. An interaction was found between CO_2 and pressure at 25 and 50°C for pectinmethylesterase and polyphenoloxidase, respectively. The activity of polyphenoloxidase was decreased by CO_2 at all pressure treatments. The interaction between CO_2 and pressure was significant for *Lactobacillus plantarum*, with a significant decrease in survivors due to the addition of CO_2 at all pressures studied. No significant effect on *E. coli* survivors was seen with CO_2 addition. Truong, Boff, Min, and Shellhammer (2002) demonstrated that the addition of CO_2 (0.18 MPa) during high pressure processing (600 MPa, 25°C) of fresh orange juice increases the rate of PME inactivation in Valencia orange juice. The treatment time due to CO_2 for achieving the equivalent reduction in PME activity was from 346 s to 111 s, but the overall degree of PME inactivation remained unaltered.

Fujii, Ohtani, Watanabe, Ohgoshi, Fujii, and Honma (2002) studied the high-pressure inactivation of *Bacillus cereus* spores in water containing argon. At the pressure of 600 MPa, the addition of argon reportedly accelerated the inactivation of spores at 20°C, but had no effect on the inactivation at 40°C.

Microbial Peptides

The complex physicochemical environment of milk exerted a strong protective effect on *Escherichia coli* against high hydrostatic pressure inactivation, reducing inactivation from 7 logs at 400 MPa to only 3 logs at 700 MPa in 15 min at 20°C. A substantial improvement in inactivation efficiency at ambient temperature was achieved by the application of consecutive, short pressure treatments interrupted by brief decompressions. The combined effect of high pressure (500 MPa) and natural antimicrobial peptides (lysozyme, 400 μg/ml and nisin, 400 μg/ml) resulted in increased lethality for *Escherichia coli* in milk (Garcia, Masschalck, and Michiels, 1999).

OPPORTUNITIES FOR HIGH PRESSURE ASSISTED PROCESSING

The inclusion of high-pressure treatment as a processing step within certain manufacturing flow sheets can lead to novel products as well as new process development opportunities. For instance, high pressure can precede a number of process operations such as blanching, dehydration, rehydration, frying, and solid-liquid extraction. Alternatively, processes such as gelation, freezing, and thawing, can be carried out under high pressure. This section reports on the use of high pressures in the context of selected processing operations.

Blanching

Eshtiaghi and Knorr (1993) employed high pressure around ambient temperatures to develop a blanching process similar to hot water or steam blanching, but without thermal degradation; this also minimized problems associated with water disposal. The application of pressure (400 MPa, 15 min, 20°C) to the potato sample not only caused blanching but also resulted in a four-log cycle reduction in microbial count whilst retaining 85% of ascorbic acid. Complete inactivation of polyphenoloxidase was achieved under the above conditions when 0.5% citric acid solution was used as the blanching medium. The addition of 1% $CaCl_2$ solution to the medium also improved the texture and the density. The leaching of potassium from the high-pressure treated sample was comparable with a 3 min hot water blanching treatment (Eshtiaghi and Knorr, 1993). Thus, high-pressures can be used as a non-thermal blanching method.

Dehydration and Osmotic Dehydration

The application of high hydrostatic pressure affects cell wall structure, leaving the cell more permeable, which leads to significant changes in the tissue architecture (Farr, 1990; Dornenburg and Knorr, 1994, Rastogi, Subramanian, and Raghavarao, 1994; Rastogi and Niranjan, 1998; Rastogi, Raghavarao, and Niranjan, 2005). Eshtiaghi, Stute, and Knorr (1994) reported that the application of pressure (600 MPa, 15 min at 70°C) resulted in no significant increase in the drying rate during fluidized bed drying of green beans and carrot. However, the drying rate significantly increased in the case of potato. This may be due to relatively limited permeabilization of carrot and beans cells as compared to potato. The effects of chemical pre-treatment (NaOH and HCl treatment) on the rates of dehydration of paprika were compared with products pre-treated by applying high pressure or high intensity electric field pulses (Fig. 2). High-pressure (400 MPa for 10 min at 25°C) and high intensity electric field pulses (2.4 kV/cm, pulse width 300 μs, 10 pulses, pulse frequency 1 Hz) were found to result in drying rates comparable with chemical pre-treatments. The latter pre-treatments, however, eliminated the use of chemicals (Ade-Omowaye, Rastogi, Angersbach, and Knorr, 2001).
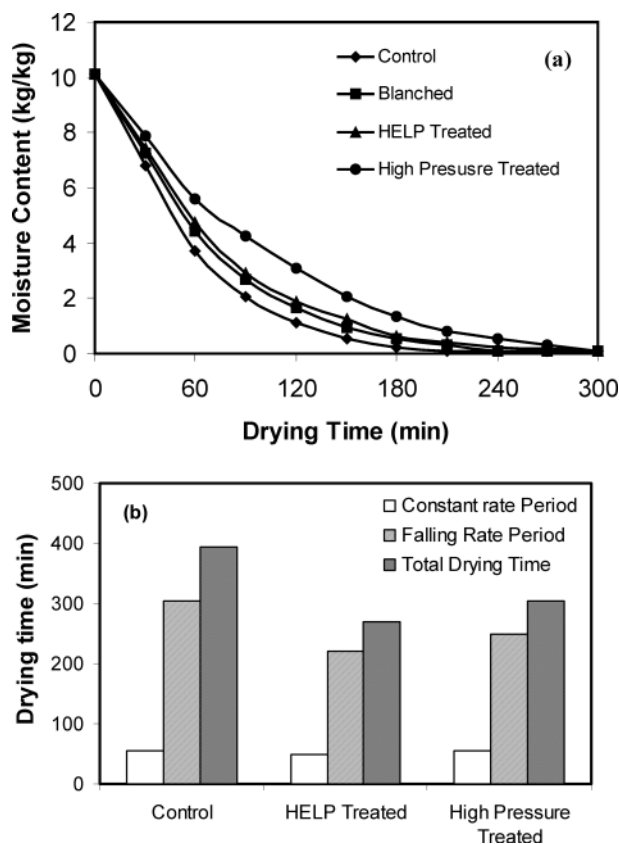
Figure 2 (a) Effects of various pre-treatments such as hot water blanching, high pressure and high intensity electric field pulse treatment on dehydration characteristics of red paprika (b) comparison of drying time (from Ade-Omowaye, Rastogi, Angersbach, and Knorr, 2001).

Figure 3 (a) Variation of moisture and (b) solid content (based on initial dry matter content) with time during osmotic dehydration (from Rastogi and Niranjan, 1998).

Generally, osmotic dehydration is a slow process. Application of high pressures causes permeabilization of the cell structure (Dornenburg and Knorr, 1993; Eshtiaghi, Stute, and Knorr, 1994; Farr, 1990; Rastogi, Subramanian, and Raghavarao, 1994). This phenomenon has been exploited by Rastogi and Niranjan (1998) to enhance mass transfer rates during the osmotic dehydration of pineapple (*Ananas comsus*). High-pressure pre-treatments (100–800 MPa) were found to enhance both water removal as well as solid gain (Fig. 3). Measured diffusivity values for water were found to be four-fold greater, whilst solute (sugar) diffusivity values were found to be two-fold greater. Compression and decompression occurring during high pressure pre-treatment itself caused the removal of a significant amount of water, which was attributed to the cell wall rupture (Rastogi and Niranjan, 1998). Differential interference contrast microscopic examination showed the extent of cell wall break-up with applied pressure (Fig. 4). Sopanangkul, Ledward, and Niranjan (2002) demonstrated that the application of high pressure (100 to 400 MPa) could be used to accelerate mass transfer during ingredient infusion into foods. Application of pressure opened up the tissue structure and facilitated diffusion. However, higher pressures above 400 MPa induced starch gelatinization also and hindered diffusion. The values of the diffusion coefficient were dependent on cell permeabilization and starch gelatinization. The

maximum value of diffusion coefficient observed represented an eight-fold increase over the values at ambient pressure.

The synergistic effect of cell permeabilization due to high pressure and osmotic stress as the dehydration proceeds was demonstrated more clearly in the case of potato (Rastogi, Angersbach, and Knorr, 2000a, 2000b, 2003). The moisture content was reduced and the solid content increased in the case of samples treated at 400 MPa. The distribution of relative moisture (M/M_o) and solid (S/S_o) content as well as the cell permeabilization index (Z_p) (shown in Fig. 5) indicate that the rate of change of moisture and solid content was very high at the interface and decreased towards the center (Rastogi, Angersbach, and Knorr, 2000a, 2000b, 2003).

Rehydration

Most dehydrated foods are rehydrated before consumption. Loss of solids during rehydration is a major problem associated with the use of dehydrated foods. Rastogi, Angersbach, Niranjan, and Knorr (2000c) have studied the transient variation of moisture and solid content during rehydration of dried pineapples, which were subjected to high pressure treatment prior to a two-stage drying process consisting of osmotic dehydration and finish-drying at $25°C$ (Fig. 6). The diffusion coefficients

Figure 4 Microstructures of control and pressure treated pineapple (a) control; (b) 300 MPa; (c) 700 MPa. (1 cm = 41.83 μm) (from Rastogi and Niranjan, 1998).
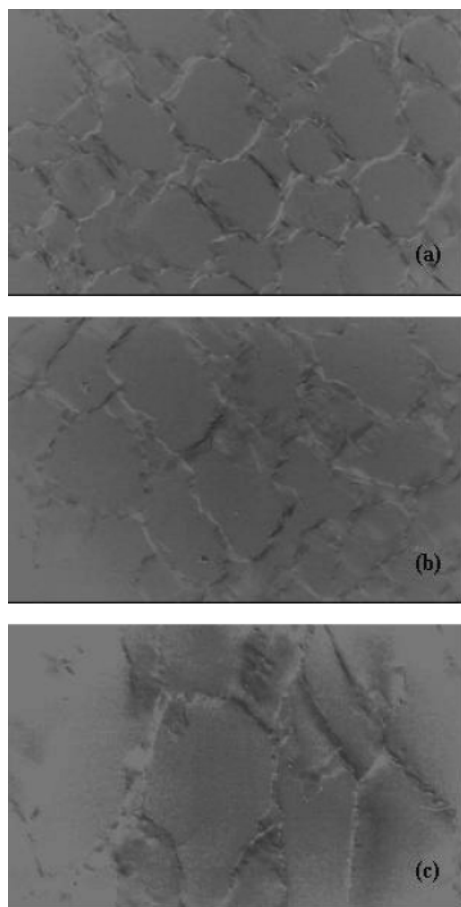
for water infusion as well as for solute diffusion were found to be significantly lower in high-pressure pre-treated samples. The observed decrease in water diffusion coefficient was attributed to the permeabilization of cell membranes, which reduces the rehydration capacity (Rastogi and Niranjan, 1998). The solid infusion coefficient was also lower, and so was the release of the cellular components, which form a gel-network with divalent ions binding to de-esterified pectin (Basak and Ramaswamy, 1998; Eshtiaghi, Stute, and Knorr, 1994; Rastogi Angersbach, Niranjan, and Knorr, 2000c). Eshtiaghi, Stute, and Knorr (1994) reported that high-pressure treatment in conjunction with subsequent freezing could improve mass transfer during rehydration of dried plant products and enhance product quality.

Ahromrit, Ledward, and Niranjan (2006) explored the use of high pressures (up to 600 MPa) to accelerate water uptake kinetics during soaking of glutinous rice. The results showed that the length and the diameter the of the rice were positively correlated with soaking time, pressure and temperature. The water uptake kinetics was shown to follow the well-known Fickian model. The overall rates of water uptake and the equilibrium moisture content were found to increase with pressure and temperature.

Zhang, Ishida, and Isobe (2004) studied the effect of high-pressure treatment (300–500 MPa for 0–380 min at 20°C) on the water uptake of soybeans and resulting changes in their microstructure. The NMR analysis indicated that water mobility in high-pressure soaked soybean was more restricted and its distribution was much more uniform than in controls. The SEM analysis revealed that high pressure changed the microstructures of the seed coat and hilum, which improved water absorption and disrupted the individual spherical protein body structures. Additionally, the DSC and SDS-PAGE analysis revealed that proteins were partially denatured during the high pressure soaking. Ibarz, Gonzalez, Barbosa-Canovas (2004) developed the kinetic models for water absorption and cooking time of chickpeas with and without prior high-pressure treatment (275–690 MPa). Soaking was carried out at 25°C for up to 23 h and cooking was achieved by immersion in boiling water until they became tender. As the soaking time increased, the cooking time decreased. High-pressure treatment for 5 min led to reductions in cooking times equivalent to those achieved by soaking for 60–90 min.

Ramaswamy, Balasubramaniam, and Sastry (2005) studied the effects of high pressure (33, 400 and 700 MPa for 3 min at 24 and 55°C) and irradiation (2 and 5 kGy) pre-treatments on hydration behavior of navy beans by soaking the treated beans in water at 24 and 55°C. Treating beans under moderate pressure (33 MPa) resulted in a high initial moisture uptake (0.59 to 1.02 kg/kg dry mass) and a reduced loss of soluble materials. The final moisture content after three hours of soaking was the highest in irradiated beans (5 kGy) followed by high-pressure treatment (33 MPa, 3 min at 55°C). Within the experimental range of the study, Peleg's model was found to satisfactorily describe the rate of water absorption of navy beans.

Frying

A reduction of 40% in oil uptake during frying was observed, when thermally blanched frozen potatoes were replaced by high pressure blanched frozen potatoes. This may be due to a reduction in moisture content caused by compression and decompression (Rastogi and Niranjan, 1998), as well as the prevalence of different oil mass transfer mechanisms (Knorr, 1999).

Solid Liquid Extraction

The application of high pressure leads to rearrangement in tissue architecture, which results in increased extractability even at ambient temperature. Extraction of caffeine from coffee using water could be increased by the application of high pressure as well as increase in temperature (Knorr, 1999). The effect of high pressure and temperature on caffeine extraction was compared to extraction at 100°C as well as atmospheric pressure (Fig. 7). The caffeine yield was found to increase with temperature at a given pressure. The combination of very high pressures and lower temperatures could become a viable alternative to current industrial practice.
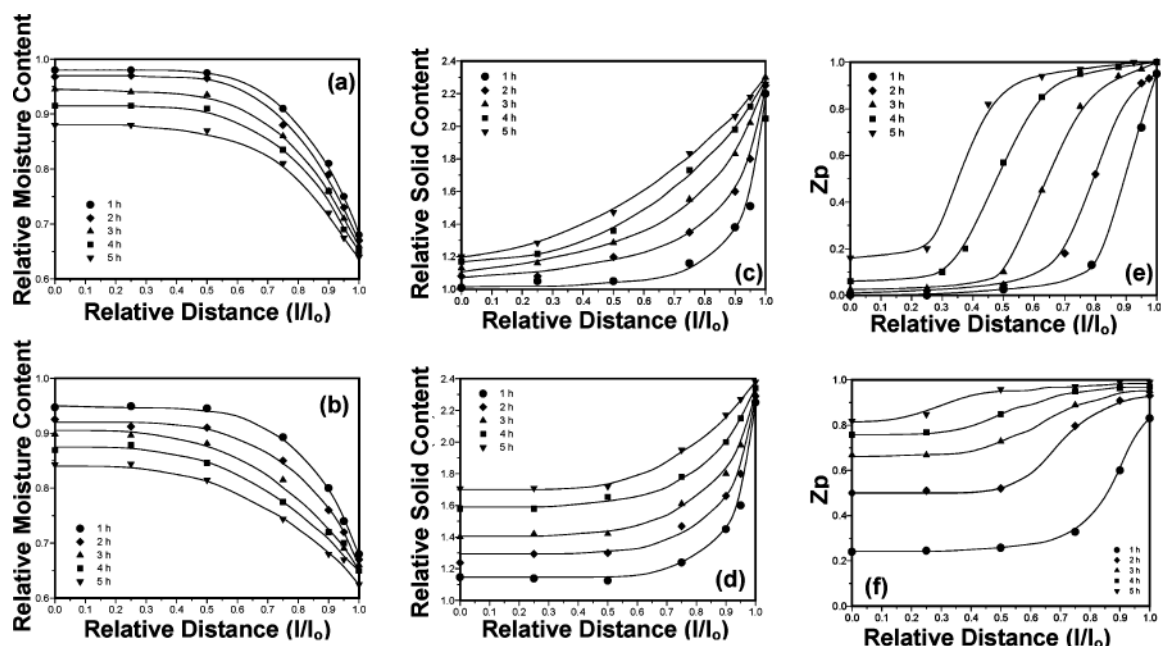
Figure 5 Distribution of (a, b) relative moisture and (c, d) solid content as well as (e, f) cell disintegration index with respect to distance from the center of the potato samples (thickness 10 mm) during osmotic dehydration of control and pressure pretreated at 400 MPa for 10 min. (Osmotic dehydration time ● = 1 h; ♦ = 2 h; ▲ = 3 h; ■ = 4 h; ▼ = 5 h) (from Rastogi, Angersbach, and Knorr, 2000b).
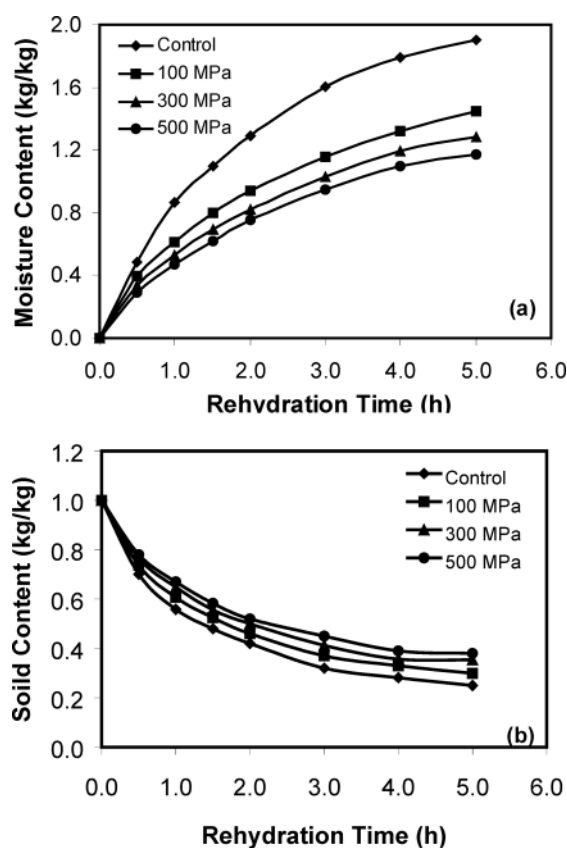
Figure 6 (a) Variation of moisture and (b) solid content with time during rehydration of high pressure treated and osmotically dehydrated sample at 25°C (Rastogi, Angersbach, Niranjan and Knorr, 2000c).

The application of high pressure was explored as a pre-processing step in the extraction and inactivation of trehalose from *Saccharomyces cerevisiae*. The trehalase was inactivated at 700 MPa, whereas the trehalose was resistant to hydrolysis even at pressures of 1500 MPa. A pressure of 700 MPa applied for 10 min at 30°C extracted 12% dry weight of trehalose from *Saccharomyces cerevisiae* (Kinefuchi, Yamazaki, and Yamamoto, 1995)

The effects of high-pressure treatment (200–600 MPa) during the mashing stage in beer production, on sugar extraction were investigated by Perez, Ledward, Reed, and Simal (2002). Pressure application reduced β-glucanase activity in wet-treated malt, but no significant differences were observed in samples subjected to different pressures (200-600 MPa). Disruption of the starch structure began to occur above 400 MPa and became
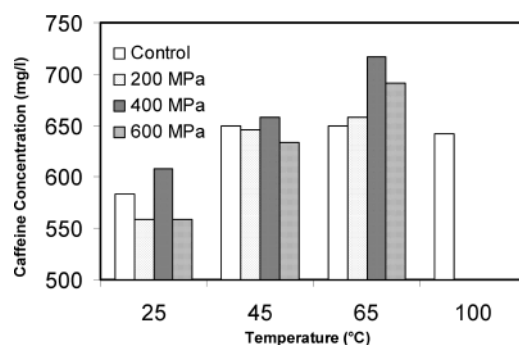
Figure 7 Effect of pressure temperature treatment on caffeine concentration of coffee powder-water mixture (Knorr, 1999).

more significant at 600 MPa. High-pressure treatment of wet-milled malt at 400 or 600 MPa for 20 min resulted in the production of water-soluble sugars, but in quantities lower than those found in the control prepared under standard brewing conditions (65°C, 90 min). High-pressure treatment could, therefore, be a feasible alternative if the stirring of the mash inside the vessel at high pressures is accomplished which may also result in improved sugar extraction and reduced treatment time.

Fernandez, Butz, and Tauscher (2001a) demonstrated that there was no change in the total concentration of beta-carotene due to the application of high pressure, but its lower recovery indicated the changes in the structure of tomato pulp tissue. High-pressure treatment also resulted in an enhanced water binding capacity. The total antioxidant capacity of the water-soluble fraction was unchanged immediately after pressure treatment, but was better compared to the untreated samples when preserved for 21 days at 4°C.

Sanchez, Plaza, Ancos, and Cano (2004) investigated the effects of combining HPP (50–400 MPa) and the use of natural additives (0–0.8% NaCl and 0–2% citric acid) on the lutein, lycopene, lycopene epoxide, gamma- and beta-carotene contents, vitamin A value, and the in-vitro antioxidative activity of tomato puree. The results indicated that moderate pressure treatment (<200 MPa) affected the structure of the cellular tomato matrix such that various carotenes were released differently on the basis of their chemical features and chromoplast location. The total carotenoid content was highest when the pressure increased to 400 MPa without NaCl and citric acid. Guillén, Giménez, and Montero (2005) indicated that the application of high pressure (250 and 400 MPa, for 10 or 20 min) during pre-treatment in acid at 10°C or during extraction in water at 45°C is a useful alternative to the conventional extraction procedure. The resulting gelatins were evaluated in terms of extraction yield, molecular weight distribution by SDS-PAGE, and viscoelastic properties of newly dissolved gelatins after overnight cold maturation. The pressure level and the time of treatment induced noticeable changes in molecular weight distribution and consequently affected viscoelastic properties. Its utility lies basically in that the longest phase of the treatment can be drastically shortened, thus making it possible to produce a gelatin of high gelling quality in only a few minutes.

Pressure Shift Freezing and Pressure Assisted Thawing

Slow freezing may cause extensive structural damage due to the formation of larger ice crystals. It may also result in higher enzyme and microbial activities as well as increased oxidation rates, resulting from increased solute concentration and the insolubility of oxygen in ice. Rapid freezing using cryogens induces cracking because of two effects: the initial decrease of volume due to cooling and the subsequent increase in volume due to freezing (Kalichevsky, Knorr, and Lillford, 1995). The reduction in freezing point under pressure causes super cooling upon pressure release and promotes rapid ice nucleation
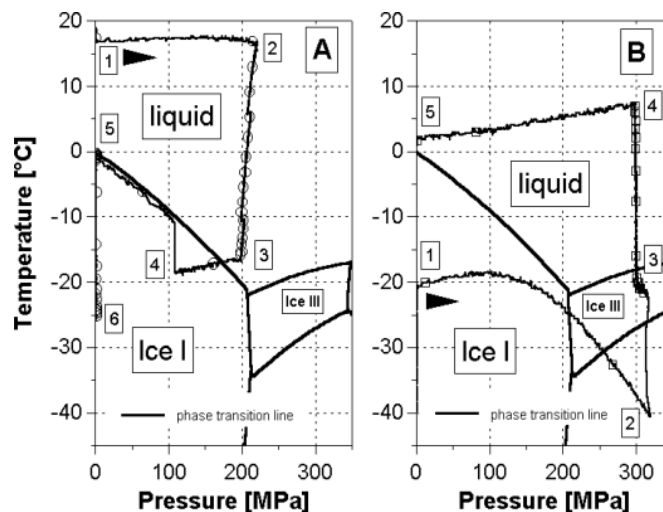
Figure 8 Pressure-shift-freezing (a) and pressure assisted thawing (b) of potatoes at the phase diagram of water (from Kalichevsky, Knorr and Lillford, 1995).

and growth throughout the sample, producing small ice crystals, rather than an ice front moving through the sample. Generally, thawing occurs more slowly than freezing, potentially allowing further damage to the sample. High pressure induced thawing reduces the loss of the water holding capacity and improves color and flavor preservation in fruit. Benet, Schlueter, and Knorr (2004) provided an extensive terminology for freezing and thawing processes including pressure-shift thawing. The phase diagrams for pressure shift freezing and pressure induced thawing are shown in Fig. 8. Table 1 summarizes other key findings in this area.

Gelation and Rheology

High pressure causes gelation of protein as well as polysaccharides. This phenomenon may be used for the modification of functional properties of foods. High pressure induced polysaccharide gels could be created during cold storage of pasteurized kiwi or strawberry puree (Knorr, 1999).

Abbasi and Dickinson (2001) reported pressure-induced gelation of skim milk powder dispersions before and after high-pressure treatment containing 9-15% casein in the presence of various sugars such as sucrose, glucose, and fructose. The gel-like characteristics could be obtained after high-pressure treatment at much lower levels of casein. The gel behavior was independent of the type of sugars, duration, and intensity of pressure and process temperature. Pressure-induced gelation was inhibited at total sugar contents higher than 45–50%. Famelart, Chapron, Piot, Brule, and Durier (1998) showed that no gel formation was observed following high-pressure treatment (200 or 400 MPa for 10 or 30 min) of milk. Ultrafiltered and microfiltered milk concentrate could form gel, but the firmness of gels decreased with an increase in citrate concentration and increased with an increase in protein concentration and the maximum gel firmness was observed at pH 5.9. Whey

Table 1 Key findings in the area of pressure shift freezing and pressure assisted thawing

Product	Conditions	Salient results	References
Gellan gels	200–500 MPa	Better appearance, structure, and texture of gels obtained as a result of high pressure assisted freezing when compared with gels frozen at −20, −30 or −80°C under atmospheric pressure.	Fuchigami and Teramoto (2003a, b)
Agar gels	0.1–686 MPa and −20°C	The texture and structure of the gel produced by pressure-shift-freezing at 200 MPa was better than the gels produced simply by pressure treatment or freezing, say at −20, −30, or −80°C and atmospheric pressure. Further, the addition of sucrose to the gel improved the quality of frozen agar gels.	Fuchigami, Teramoto, and Jibu (2006)
Gelatin gel	0.1, 50, and 100 MPa	High-pressure shift freezing produced better gels due to the homogeneous distribution of small ice crystals.	Fernández, Otero, Guignon, and Sanz (2006)
	100–200 MPa	High-pressure shift freezing process promotes the production of larger number of smaller ice crystals, which help to retain a better texture in the product.	Zhu, Ramaswamy, and Bail (2005a)
Pork and beef muscles	200 MPa	Pressure-induced cold denaturation was complete for actin and very considerable for myosin and other muscle proteins. Connective proteins remained practically unaltered by pressurization and/or freezing. Neither pressurization alone nor pressure-shift freezing was suitable for muscle preservation.	Fernandez, Otero, Solas, and Sanz (2000)
Pork muscle	62–199 MPa, −5 to 20°C	A high-pressure calorimeter was used to evaluate the ratio of ice crystals for pressure-shift freezing of pure water and pork muscle tissue, without knowing the pressure-related properties of the test sample.	Zhu, Ramaswamy, and Bail (2005b)
	100–200 MPa	High-pressure shift freezing resulted in small and regular crystals, which differed along the radial direction. Near the surface, there were many fine and regular intracellular ice crystals with well-preserved muscle tissue. From midway to the center, the ice crystals were larger in size and located extracellularly.	Zhu, Bail, Ramaswamy, and Chapleau (2004)
	100–200 MPa	Application of pressures higher than 150 MPa resulted in color changes in pork muscle during the pressure shift freezing. The pressure shift freezing process reduced thawing drip loss of pork muscle but did not cause obvious changes in total drip loss following thawing and subsequent cooking. Pressures of 150 and 200 MPa resulted in considerable denaturation of myofibrillar proteins and the reduction of muscle toughness.	Zhu, Bail, Chapleau, Ramaswamy, and Lamballerie (2004)
Fish fillets	200 MPa	Organoleptic characteristics of high-pressure thawed fillets were superior to water thawed samples before cooking, due to reduced drip loss, greater protein denaturation than conventional thawing, improved microbial status, and improved textural parameters.	Schubring, Meyer, Schlueter, Boguslawski, and Knorr (2003)
Dogfish and scallops	150 MPa	High pressure thawing of frozen sea foods (dogfish and scallops) had better microbial quality; thawing time and drip volume were also lower than immersion thawed products.	Rouille, Lebail, Ramaswamy, and Leclerc (2002)
Blue whiting	42 or 100 MPa	High pressure thawing was quicker and resulted in lower drip volume	Chevalier, Bail, Chourot, and Chantreau (1999)
Atlantic salmon	100–200 MPa	Treatment at 150 and 200 MPa caused marked color changes in samples, while the texture was markedly modified at 200 MPa. High pressure reduced drip loss in samples subjected to liquid nitrogen freezing.	Zhu, Ramaswamy, and Simpson (2004)
	100–200 MPa, −8.4°C to −20°C	Pressure shift freezing produced a large amount of fine and regular intracellular ice crystals, which were homogeneously distributed. Muscle fibers were better maintained in pressure shift freezing treated salmon tissue in comparison with the frozen muscle structure.	Zhu, Bail, and Ramaswamy (2003)
Salmon mince	207 MPa, for 23 min, up to −25°C	At −21°C, *L. innocua*, *M. luteus,* and *P. fluorescens* were reduced to 1.7, 1.7, and 4.6 log cycle, respectively. Further reduction of temperature up to −25°C under atmospheric condition resulted in further inactivation of the microorganisms.	Picart, Dumay, Guiraud, and Cheftel (2004)
Kinu-tofu (soybean curd)	100–700 MPa, −20°C	The rupture stress and strain of tofu, frozen at atmospheric pressure and 100 MPa increased, but that of tofu frozen at 200 and 340 MPa was similar to untreated tofu. At pressures above 500 MPa, rupture stress increased. The ice crystals in tofu frozen at 200–400 MPa were smaller than in tofu frozen at 100 or 700 MPa, which indicated that high pressure freezing at 200–400 MPa was effective in improving the texture of frozen tofu.	Fuchigami and Teramoto (1997)

Table 1 Key findings in the area of pressure shift freezing and pressure assisted thawing (*Continued*)

Product	Conditions	Salient results	References
Peach and mango	200 MPa, −20°C	The high level of super cooling during high-pressure-shift freezing of peach and mango leads to uniform and rapid ice nucleation throughout the volume of the sample, which largely maintains the original tissue structure. The problems associated with thermal gradients, quality losses due to freeze cracking, or the presence of large ice crystals, were also minimized.	Otero, Martino, Zaritzky, Solas and Sanz (2000)
Eggplants	—	High-pressure-assisted freezing resulted in lower quality damage in comparison with conventional air-freezing techniques.	Otero, Solas, Sanz, Elvira, and Carrasco (1998)
Chinese cabbage (midribs)	100–700 MPa	Texture of samples frozen at 200 MPa, 340 MPa and 400 MPa was comparatively intact in relation to samples frozen at 100 MPa and 700 MPa. Release of pectin and histological damage in midribs frozen at 200 and 340 MPa were less than midribs frozen at 100 and 700 MPa.	Fuchigami, Kato and Teramoto (1998)
Broccoli	180–210 MPa, −16°C to −20°C	The protein content decreased after the high-pressure treatments. Peroxidase and polyphenoloxidase enzymes could not be inactivated. After 30 days of frozen storage at −20°C the flavor of broccoli was not acceptable to consumers, but the texture remained quite firm. The vacuole membrane was destroyed and an internal disorganized cell was observed after pressure shift freezing treatment.	Prestamo, Palomares, and Sanz (2004)
Potato	210–300 MPa	In the pressure range 210–240 MPa, a metastable ice I modification area was observed, as the nucleation of ice (I) crystals in the thermodynamically stable region of ice (III) was reached. A significant degree of supercooling was obtained before freezing the tissue water to ice (III). Phase transition and freezing times for the different freezing paths were compared for the processes such as freezing at atmospheric pressure, pressure-assisted freezing, and pressure-shift freezing.	Schluter, Benet, Heinz, and Knorr (2004)
Carrot	100–700 MPa	Textural properties of carrots pressurized at 200–400 MPa at −20°C were found to be more acceptable, pectin release, and histological damage were also lower in samples frozen at 100 and 700 MPa.	Fuchigami, Miyazaki, Kato, and Teramoto (1997a) Fuchigami, Kato and Teramoto (1997b)
Cheddar cheese	200 MPa, −20°C	Conventional freezing and thawing of Cheddar samples resulted in paste-like body and appearance, whereas pressure-shift freezing and thawing prevented many of these changes. However, the product was different from fresh cheese, it required greater deformation to cause fracture, and had lower hardness, lower springiness and greater cohesiveness.	Johnston (2000)

concentrate formed gels at pH 9.0. The increase in the protein content of whey concentrate had no effect on the firmness of whey concentrate gels whereas increasing the pressure from 200 to 400 MPa resulted in firmer gels. Keim and Hinrichs (2004) indicated that the application of high pressure (600 MPa for 0–30 min at 30°C) formed stable gel of whey protein isolate. These workers also showed that the content of the native whey protein fractions alpha-lactalbumin and beta-lactoglobulin A and B, decreased and the amount of intermolecular disulfide bonds increased with prolonged pressure holding time. The gels became stronger and more elastic with increasing holding time.

Ahmed and Ramaswamy (2003) showed that both pressure and heat-induced glocomarcopeptide samples followed the Herschel-Bulkley model and indicated the presence of yield stress. It exhibited shear-thinning behavior. The consistency co-

efficient and apparent viscosity were reported to increase with pressure up to 300 MPa of and after 300 MPa these values decreased.

HPP of low moisture mozzarella cheese accelerated the increase in the water binding capacity of the casein matrix during ripening and had no significant effects on the extent of casein degradation or on free amino acid generation (O'Reilly, Murphy, Kelly, Guinee, Auty, and Beresford, 2002). Functional properties such as melt time, flowability, and stretchability, were improved by high pressure processing. The application of 225–400 MPa to gouda cheese resulted in less rigid and solid-like cheese, which was more viscoelastic and having less resistance to flow and with no macroscopic breakage. The rheological properties of gouda cheese returned to those of control samples after 42 days of storage of pressurized sample. Such changes are due to the weakening of hydrophobic interactions, which are

restored during ripening (Messens, Walle, Arevalo, Dewettinck, and Huyghebaert, 2000).

An Erl King, Yueh, Chang, and Kwo (1994) indicated that the gel strength of pressure induced gels of soya protein was higher with smoother and finer appearance than that of heat induced gels. Apichartsrangkoon (2003) studied the effect of HPP on the rheological properties of hydrated soy protein concentrates and demonstrated that the shapes of the storage and loss curves appeared to change little with temperature/pressure treatment, however, the overall data indicated that increasing the temperature resulted in more pronounced rheological changes than pressure.

Dong, Heinz, and Knorr (1999) showed that no coagulation of liquid whole egg was observed at pressure 150 MPa, 60 min at 25°C. The increase in pressure higher than 250 MPa and temperature up to 45°C resulted in more or less instantaneous coagulation. Anton, Chapleau, Beaumal, Delepine, and Lamballerie (2001) investigated that the HPP can be used to improve the shelf-life of egg yolk-based emulsions, while decreasing the microbial counts without affecting their physicochemical properties. The pressure treatment had no effect on emulsion characteristics. Ngarize, Adams, and Howell (2005) demonstrated that gels made by heating gave higher gel strength and Young's modulus values, in the case of whey proteins pressure treated at 400–600 MPa for 20 min. In contrast, egg albumen showed no gelation below 500 MPa for 20 min, but there was an increase in both gel strength and Young's modulus at greater pressures, although their values remained lower than those of the heat-induced gels. A mixture of 10:5 whey/egg albumen showed the highest gel strength and Young's modulus for both heated and high pressure treated (400–600 MPa) gels, although, the heated mixture had the highest values.

High-pressure treatment (400–700 MPa, 30 min) of bovine serum albumin before emulsification led to substantial changes in the flocculation and rheological behavior of the emulsion after polysaccharide addition but no discernible change in the emulsion droplet-size distribution prior to addition. Heat treatment at 70–80°C before emulsification led to an increase in the average droplet size and no change in emulsion rheology (following addition of polysaccharide) that is qualitatively different from those found with pressure-treated systems (Dickinson and Pawlowsky, 1996).

Ohshima, Ushio, and Koizumi (1993) presented a review on HPP of fish and fish products along with its benefits as well as shortcomings. The high-pressure treatment of freshwater fish (carp, *Cyprinus carpio*) resulted in the gelling of fish paste, which is useful for product development. Breaking strength of pressure induced carp gels was much lower than that of heat induced carp gels or lizardfish gels. The gel forming ability of myofibrillar proteins was increased by addition of transglutaminase.

An Erl King, Kuo, and Shieh (1997) showed that the gel strength and brightness of the pork paste gel was found to increase with an increase in pressure. The higher the temperature or the sodium chloride concentration used for gelation, the lower is the pressure needed to denature the protein. Chapleau

and Lamballerie (2003) demonstrated that the changes in rheological properties of bovine myofibrillar proteins in solution were influenced by a structural change caused by high-pressure treatment. Further, increase in process pressure and holding time resulted in decrease in viscosity and shift towards the Newtonian flow behavior.

Dickinson and James (1998) studied the effect of high pressure and thermal treatment on flocculation and rheology of model oil-in-water emulsions stabilized by beta-lactoglobulin. HPP induced significant levels of flocculation in the model oil-in-water emulsions and altered the droplet size distribution and rheological behavior. The proportion of unadsorbed protein greatly influenced the extent of flocculation. Elevated pressure treatment (800 MPa for 60 min at ambient temperature) was found to be equivalent to relatively mild thermal treatment (65°C for 5 min). The changes in rheological properties of these systems following high-pressure treatment were attributed to pressure-induced denaturation and gelation of beta-lactoglobulin in the continuous phase of the emulsion (Dickinson and James, 1999). Arora, Chism, and Shellhammer (2003) studied the effect of high pressure treatment on the stability and the rheology of acidified model oil-in-water emulsions containing canola oil, whey protein isolate, polysorbate 60, soy lecithin, and xanthan. Exposure to high pressures up to 800 MPa for 5 min at 30°C did not significantly affect the equivalent surface mean diameter, flow behavior, and viscoelasticity. The pressure treatment had a negligible effect on emulsion stability, whereas the presence of xanthan (0.2% w/w) resulted in improved stability. Soy lecithin-stabilized emulsions resulted in larger mean particles sizes and lower emulsion volume indices than the other emulsions, indicating that the potential instability and application of pressure further destabilized these emulsions. Ahmed and Ramaswamy (2004, 2005) demonstrated that under pressure xanthan gum displayed pseudoplastic behavior with yield stress, and the Herschel-Bulkley model could be used to describe the flow behavior. The application of pressure induced slight structural breakdown in the gum, which exhibited slight thixotrophicity at higher concentration. The consistency coefficient and apparent viscosity were affected by both the applied pressure and the concentration of the gum, whereas the flow behavior index and yield stress were affected by concentration.

Jen and An Erl King (1994) indicated that high-pressure treatment of 1% carrageenan gum at 330 MPa, 45°C resulted in maximum gel strength. Ye, Yang, and Ye (2000) showed that the gelatinization temperature increased with pressures up to 150 MPa, remained stable at 150–250 MPa and then decreased further in the range 250–450 MPa. For pressures greater than 450 MPa, starch was partially gelatinized. Stolt, Stoforos, Taoukis, and Autio (1999) indicated that consistency coefficient increased at a faster rate with increasing pressure up to 550 MPa and weaker gel structure was obtained at high pressure. High pressure can bring out changes in physicochemical properties of foods, which can be appropriately adopted for product development.

Johnston, Rutherford, and Gray (2005) investigated the effects of high pressure (200–600 MPa) on the rheological

properties of gelatinized waxy corn starch-skim milk dispersions. These workers reported that the pre-treatment significantly increased the particle sizes of the milk proteins and also their effective volume fraction in relation to unheated pressurized milk. High-pressure treatment significantly increased the yield stress, the consistency index, and decreased the flow behavior index. Błaszczak, Fornal, Valverde, and Garrido (2005) showed that the application of high pressure (650 MPa, 3–9 min) to waxy corn starch–water suspensions resulted in a significant decrease in the degree of crystallinity with the time of treatment; indeed, a complete loss of crystallinity was reported after 3 min of treatment. The profile of the molecular weight distribution of waxy corn starch pressurized for 6 and 9 min differed significantly from the one obtained for native starch. Błaszczak, Valverde, and Fornal (2005) showed that the application of high pressure (650 MPa, 2–3 min) to potato starch-water suspension resulted in a decrease in gelatinization temperature. The surface of the granule was most resistant to high-pressure treatment, whereas, the inner part was almost completely filled with a gel-like network, with empty spaces growing in diameter towards the center of the granule.

Johnston and Ding (2004) studied the effects of high pressure (200–800 MPa) on the flow characteristics of solutions of guar gum, sodium carboxymethylcellulose (CMC) or xanthan gum (0.5–2.0 g/l) in skim milk, and fitted the data to a power law model. The consistency index of solutions increased with increasing treatment pressure and polysaccharide concentration. The flow behavior index decreased with increasing polysaccharide concentration, indicating increased shear thinning. The protein surface hydrophobicity in the control and pressure-treated milk was shown to decrease by polysaccharide addition, suggesting that hydrophobic bonding contributed to the altered flow characteristics.

Bauer, Hartmann, Sommer, and Knorr (2004) studied in-situ analysis of wheat, tapioca, and potato starch granules using a high pressure cell and an inverse microscope at pressures up to 300 MPa. High-pressure treatment was found to cause a slight discoloration of iodine stained potato starch granules and a complete discoloration of iodine stained tapioca and wheat starch granules. This was attributed to amylose release from the amorphous regions during pressure-induced starch gelatinization. Starch granules from potato and tapioca showed greater resistance to pressure than those from wheat. Bauer and Knorr (2005) studied the applicability of pressure-induced starch gelatinization as a pressure-time-temperature indicator by examining the impact of pressure, temperature, and treatment time on starches of A-type, B-type, and C-type crystallinity. The pressure-induced starch gelatinization was highly sensitive to changes of temperature, pressure, and treatment time. The rate of gelatinization increased with pressure indicating the pressure-induced gelatinization to be a time-dependant process.

Uresti, Velazquez, Vázquez, Ramírez, and Torres (2006) evaluated the effects of i) setting conditions (25°C for 2 h or 40°C for 30 min) and ii) combining microbial transglutaminase with HPP on the mechanical properties of heat induced gels obtained from paste from arrowtooth flounder (*Atheresthes stomias*). They showed that pressurization improved the mechanical properties of gels made from paste treated with transglutaminase and set at 25°C. However, the samples set at 40°C showed poorer mechanical properties, due to the induction of proteolytic degradation in myofibrillar proteins. Montero, Caballero, Mateos, Solas, and Guillén (2005) studied the effect of prior setting at 25°C on the gels produced from horse mackerel (*Trachurus trachurus*) mince induced by high pressure treatment (300 MPa, 25°C, 15 min). The residual transglutaminase activity was significantly reduced after pressurization. Further, the gels were stronger and exhibited a denser and more homogeneous network, when setting preceded high-pressure treatment. Whilst studying the formation of acid milk gels, Anema, Lauber, Lee, Henle, and Klostermeyer (2005) demonstrated that there was an increased cross-linking of the whey proteins, and an increased cross-linking between the whey proteins and caseins, when the transglutaminase treatment was performed under pressure. The introduction of a higher number of intermolecular cross-links resulted in increased acid gel firmness.

HIGH PRESSURE APPLICATION IN SPECIFIC FOOD SECTOR

Fruits and Vegetable Products

High pressure is mainly used in fruits and vegetable processing industries for the inactivation of microorganisms and enzymes, for extending the shelf life, while maintaining better organoleptic, sensory, and nutritional properties. Some of the important finding in this area has been summarized in Table 2.

Dairy Products

The research on application of high pressure on milk was initiated with a view to develop an alternative process for the pasteurization. A number of researchers have studied the pressure inactivation of microorganism (such as *Listeria monocytogenes*, *Staphylococcus aureus* or *Listeria innocua*) either naturally present in milk or introduced in milk (Styles, Hoover, and Farkas, 1991; Erkman and Karatas, 1997; Gervila, Capellas, Ferragur, and Guamis, 1997). High-pressure treatment of milk affects its coagulation process and cheese making properties indirectly through a number of effects on milk proteins, including reduction in the size of casein micelles and denaturation of beta-lactoglobulin, probably followed by interaction with micellar k-casein. Huppertz, Kelly, and Fox (2002) reviewed the effect of high pressure on contents and properties of milk. High pressure induced the disruption of casein micelles and denaturation of whey proteins. High-pressure treatment increased the pH of the milk, reduced the rennet coagulation time of milk, and increased

Table 2 Important findings in the area of fruits and vegetables

Product	Conditions	Salient results	References
Orange Juice			
	350 MPa, 1 min at 30°C	Good quality juice with more than 2 months shelf life under refrigeration condition.	Donsi, Ferrari, and Matteo (1996)
	600 MPa for 1 min, 5°C	Storage up to 20 weeks at 0°C without any change in physicochemical and sensory properties, while minor changes observed after 12 weeks when sample was stored at 10°C.	Takahashi, Pehrsson, Rovere, and Squarcina (1998)
	500 MPa for 1.5 min	Microbial quality of pressure treated juice was similar to thermally pasteurized (below detectable limits). Storage up to 16 weeks under refrigeration with increased flavor retention.	Parish (1998)
	700 MPa for 1 min	Cloud stabilization in freshly squeezed orange juice and shelf life of 90 days under refrigeration conditions.	Goodner, Braddock, Parish, and Sims (1999)
	400 MPa for 10 min	Exhibited acceptable quality during storage for 150 days at room temperature.	Strolham, Valentova, Houska, Novotna, Landfeld, Kyhos, and Gree (2000)
	500 or 800 MPa for 5 min	Storage up to 21 days at 4°C caused no significant difference in antioxidative capacity, vitamin C, sugar and carotene content.	Fernandez, Butz, Bognar, and Tauscher (2001b)
	500 MPa for 5 min, at 35°C	Lower loss of ascorbic acid than in conventionally pasteurized juices at 80°C for 30 s.	Polydera, Stoforos, and Taoukis (2003)
	350–450 MPa, 40–60°C for 1–5 min	Increased extraction of flavanones and retention of potential health-promoting attributes during cold storage.	Sanchez, Plaza, Ancos, and Cano (2004)
	600 MPa for 4 min at 40°C	The rate of degradation of ascorbic acid was lower for orange juice treated with high pressure, which led to a better retention of the antioxidative activity when compared with juice pasteurized in a conventional way using heat.	Polydera, Stoforos and Taoukis (2004; 2005)
	600 MPa for 1 min at 20°C	In case of Navel and Valencia orange juices the population of aerobic bacteria, yeasts, and other fungi was reduced to below detectable levels. Inactivation of Salmonella up to 7-log cycle and marked reduction of PME was also observed. Color, browning index, viscosity, °Brix and titratable acidity, levels of alcohol insoluble acids, ascorbic acid, and beta-carotene were unaffected when stored for 12 weeks at 4 or 10°C.	Bull, Zerdin, Howe, Goicoechea, Paramanandhan, Stockman, Sellahewa, Szabo, Johnson, and Stewart (2004)
	600 MPa for 5 min at 25 or 80°C	The excess ascorbate strongly protected folates against pressure and heat. Freshly squeezed orange juice treated for 5 min at 600 MPa at 25°C showed good retention of folates. Furthermore, treatment at 80°C did not cause large losses in folate, which may be attributed to the presence of intrinsic protective substances in freshly squeezed juice.	Butz, Serfert, Fernandez, Dieterich, Lindauer, Bognar, and Tauscher (2004)
Lemon juice			
	450 MPa, for 2, 5 or 10 min	No fungus growth was detected in the pressure treated sample, whereas the control sample was spoiled by yeast and filamentous fungi after 10 days. Little effects of HPP on the constituents and physicochemical properties.	Donsi, Ferrari, Matteo, and Bruno (1998)
Guava puree			
	600 MPa, 25°C for 15 min	Storage up to 40 days at 4°C without any change in any change in color and pectin cloud and with no loss of ascorbic acid. No change in water soluble, oxalate soluble and alkali soluble pectin with original flavor distribution and viscosity.	Gow and Hsin (1996, 1998, 1999).
Apple juice			
	400 MPa, 10 min	Compared the sensory quality during storage of apple juice subjected to high pressure with that preserved by freezing (−17°C) or pasteurization (80°C, 20 min). The best samples were frozen juice, followed by pressurized, and then by pasteurized, juice with much substantial difference in aroma.	Novotna, Valentova, Stroham, Kyhos, Landfeld, and Houska (1999)
Fresh cut pineapple			
	340 MPa for 15 min	Extended the shelf life, and decimal reductions of surviving bacteria were 3.0, 3.1, and 2.5 at 4°C, 21°C and 38°C, respectively. Pressure treated pineapple pieces had less than 50 cfu/g total plate count as well as yeast and mold counts.	Aleman, Farkas, Torres, Wilhelmsen, and McIntyre (1994)
Strawberry juice			
	200–500 MPa	No major changes in strawberry aroma profiles, whereas a pressure of 800 MPa, induced significant changes in the aroma profile and new compounds were induced.	Lambert, Demazeau, Largeteau, and Bouvier (1999)
	250–400 MPa	Pressurization and depressurization treatments caused a significant loss of strawberry PPO (60%) up to 250 MPa and POD activity (25%) up to 230 MPa, while some activation was observed for treatments carried out in 250–400 MPa range for both the enzymes. Optimal inactivation of POD was using 230 MPa and 43°C in strawberry puree.	Cano, Hernandez, and Ancos (1997)

(Continued on next page)

Table 2 Important findings in the area of fruits and vegetables *(Continued)*

Product	Conditions	Salient results	References
Raspberry puree			
	200–800 MPa for 15 min, 18–22°C	The impact of high pressure on anthocyanin in raspberry puree was evaluated. The highest stability of the anthocyanin was observed when the puree was pressured under 200 and 800 MPa and stored at 4 °C.	Winai, Paul, and Ioannis (2005)
Red and white grape musts			
	300–800 MPa for 1–5 min	Processing at 500 MPa for 3 min sterilized the white grape must, whereas treatment at 800 MPa for 5 min did not fully sterilize red grape must. This is due to higher pressure stability of the natural microflora present in red grape. There were little changes due to high-pressure sterilization on physicochemical properties.	Moio, Masi, Pietra, Cacace, Palmieri, Martino, Carpi, and Dall'Aglio (1994)
White peach			
	400 MPa, 20°C, 10 min	Enzymic formation of benzaldehyde, C6 aldehydes, and alcohols by disruption of fruit tissues was observed in high pressure treated fruit and crushed fruit. The increase in benzaldehyde content in high-pressure treated fruit during storage was caused by residual activity of beta-glucosidase after the treatment.	Sumitani, Suekane, Nakatani, and Tatsuka (1994)
Lychee			
	200–600 MPa for 10 or 20 min at 20–60°C	Pressure treatment caused less loss of visual quality in both fresh and syrup-processed lychee than thermal processing. Pressure treatment at 200 MPa caused a marked increase in POD activity, whereas further increase in pressure did not affect the activity of POD. The combined effect of pressure and temperature on PPO activity was more marked at longer treatment time (20 min) and under more severe treatments. Pressure of 600 MPa at 60°C for 20 min caused extensive inactivation of POD and PPO in fresh lychee, over 50% and 90%, respectively. In case of the sample processed in syrup, these effects were less significant due to baroprotection effect offered by the syrup.	Phunchaisri and Apichartsrangkoon (2005)
Kiwifruit, peaches, pears and melon			
	400 MPa, 5 or 20°C for 30 min	Melon was the most suitable fruit for high pressure processing; peaches and pears underwent browning, which was prevented by addition of ascorbic acid, and kiwifruit became yellow. Texture of all fruits was acceptable after processing. PPO and POD enzymes could not be inhibited by processing, however, activities were higher at 20°C as compared to 5°C.	Prestamo and Arroyo (2000)
Strawberry jam			
	400 MPa at room temperature for 5 min	Explained the production of high-pressure processed jam. The mixture of the powdered sugar, pectin, citric acid and freeze concentrated juice were mixed and then degassed and pressurized. The jam was of bright and red color and retained all the original flavor compounds. The texture of the product was similar to conventionally prepared jam.	Watanabe, Arai, Kumeno, and Honma (1991)
		Pressure treated jam had better quality than heat-treated jam. The pressure treated jam could be stored at refrigeration temperature with minimal loss in sensory and nutritional characteristics up to 3 months.	Kimura, Ida, Yosida, Okhi, Fukumoto, and Sakui (1994)
Lettuce, tomato, asparagus, spinach, cauliflower and onion			
	300–400 MPa	Viable aerobic mesophiles, fungi and yeasts decreased and organoleptic properties were also affected. Loosening and peeling away of the skin of tomato was observed with firm flesh, and no change in color and flavor. Lettuce remained firm but underwent browning and the flavor was unaffected. Slight browning was observed in case of cauliflower. Peroxidase enzyme was displaced to the cell interior, but not inactivated. Low temperature and long treatment time yielded improved inactivation of microorganisms and better sensorial characteristics.	Arroyo, Sanz, and Prestamo (1997, 1999)
White cabbage			
	400 or 500 MPa at 20, 50 or 80°C	High pressure had a marked effect on the distribution of soluble and insoluble fiber in case white cabbage. Pressure application resulted in of reduced fiber solubility up to a temperature of 50°C beyond which the temperature had no effect on both soluble and insoluble fiber contents.	Wennberg and Nyman (2004)
Fruit and vegetable texture			
	100 MPa for 60 min	Dual effect on texture of fruits and vegetables, as characterized by an initial loss in texture, due to the instantaneous pulse action of pressure, followed by a more gradual change as a result of pressure-hold. The extent of the initial loss of texture was more prominent at higher pressures and partial recovery of texture was more prominent at lower pressures. The vegetables treated at were firmer and brighter than the raw product.	Basak and Ramaswamy (1998)

Table 2 Important findings in the area of fruits and vegetables *(Continued)*

Product	Conditions	Salient results	References
	200–500 MPa for 15 min, 20-60°C	High-pressure pretreatment of carrots combined with CaCl₂ infusion improved texture during thermal processing by retarding the rate of thermal softening.	Sila, Smout, Vu, and Hendrickx (2004)
	400 MPa, 60°C for 15 min	High-pressure pretreated carrots resisted the texture loss, when further processed at high temperatures (100-125°C). A significant reduction in the degree of methylation of carrot pectin was observed in high-pressure treated samples, which was correlated with the observed changes in texture. The textural properties were significantly improved when calcium infusion was combined with low-temperature blanching (60°C for 40 min).	Sila, Smout, Vu, Van Loey, and Hendrickx (2005)
Carrot, tomatoes, and broccoli (crushed or liquid extracts)			
	500–800 MPa, 25 or 75°C	Chlorophyll a and b in broccoli, lycopene and beta-carotene in tomatoes and antioxidative activities of water soluble carrot and tomato homogenates were not affected. The water retention and glucose retardation index of tomato pulp was increased and extractability of carotenoids from coarse carrot homogenates was reduced. Structural changes in food matrices were observed but no significant loss of beneficial substances.	Butz, Edenharder, Fernandez, Fister, Merkel, and Tauscher (2002)
Antimutagenic activity of fruit and vegetable juices			
	400–800 MPa, 25–50°C for 10 min	Antimutagenicity of strawberry and grapefruit juices was not affected by the applied heat and pressure treatments. Antimutagenicity of juices of carrot, leek, spinach, kohlrabi, and cauliflower were sensitive to heat treatment and unaffected by pressure. Antimutagenicity of beet and tomato juices was only affected by extreme pressure treatments.	Butz, Edenharder, Fister, and Tauscher (1997)
Tomato puree			
	700 MPa	Natural flora of the tomato puree was reduced below detectable limits. High pressure resulted in higher reduction in spore counts of meatballs inoculated with Bacillus stearothermophilus spores added to the tomato puree, as compared to conventional sterilization, without significant loss of lycopene.	Krebbers, Matser, Hoogerwerf, Moezelaar, Tomassen, and Berg (2003)
	50-400 MPa, 25°C, 15 min.	High pressure was explored as one of the hurdles along with citric acid and NaCl for the manufacture of minimally processed tomato products with optimal sensory and microbiological characteristics. The inactivation of polyphenoloxidase, peroxidase and pectinmethyl esterase increased with combined treatments at high values for pressure and additive concentration.	Plaza, Munoz, Ancos, and Cano (2003)
	100–600 MPa for 12 min at 20°C	High pressure affected the total lycopene content and the percentage of the presumptive 13-cis isomer, both in lycopene solution and tomato puree. At higher storage temperature, the loss of total lycopene and percentage of 13-cis isomer was greater. The highest stability of lycopene was found when tomato puree was pressurized at 500 MPa and stored at 4 ± 1°C.	Qiu, Jiang, Wang, and Gao (2006)

cheese yield, thereby indicating its potential application in the cheese making technology (O'Reilly, Kelly, Murphy, and Beresford, 2001). Table 3 summarizes other key findings in this area of dairy processing.

Animal Products

High pressure induces changes in muscle enzymes, meat proteolysis, and myofibriller proteins, as a consequence of which the structure and texture of meat change. Further, high pressure influences the tenderization and gelation process, color of the product, and the extent of lipid oxidation. Microorganisms in meat can be inactivated by the application of high pressure and the extent of which depends on several parameters such as type of microorganism, pressure level, process temperature and time, and the pH and composition of food or the dispersion medium. In general, gram-negative bacteria are more sensitive

to pressure than gram-positive bacteria, but large differences in pressure resistance are apparent among various strains of the same species. Bacterial spores are highly resistant to pressure (unless pressurization is carried out at temperatures close to 100°C).

Pre-rigor treatment was very effective but its use would require the development of hot boning, a process that is not widely used in industry. Combining pressure with heat does tenderize meat, but the final products have a cooked appearance, and therefore cannot be sold as fresh meat. Such a treatment would also have to compete with other tenderizing processes used for cooked or processed meats. The change in meat color caused by pressure application above 300 MPa, even at low temperature, means products could not be sold as fresh meat. Moreover, in this high-pressure range, the practical benefits for meat texture remain to be demonstrated despite the numerous indirect evidences of tenderization. Table 4 summarizes other key findings in this area of processing of animal products.

Table 3 Important findings in the area of dairy processing

Product	Conditions	Salient results	References
Microorganisms and enzymes in milk			
	~ 680MPa, 10 min, RT	5–6 log cycle reduction in microorganism and combined effect of pressure and temperature (67–71°C) further resulted in increased shelf life.	Hite, Giddings, and Weakly (1914)
	200–250 MPa	The combination of high pressure with bacteriocin such as lacticin resulted in synergistic effect in controlling microbial flora of milk without significantly influencing the cheese making properties.	Morgan, Ross, Beresford, and Hill (2000).
	200–500 MPa, 60 min, 20°C	Periodic oscillation of pressure was very effective for the destruction of pathogen such as Listeria monocytogenes, Escherichia coli and Salmonella enteritidis.	Vachon, Kheadr, Giasson, Paquin, and Fliss (2002)
	250–450 MPa, 0–80 min at 3 or 21°C	Higher pressures, longer holding time, and lower temperature resulted in greater destruction of microorganisms in raw milk. Escherichia coli was more sensitive than indigenous micro flora.	Pandey, Ramaswamy, and Idziak (2003)
	250–500 MPa, 5 min at 20°C.	Combining high pressure and nisin (0, 250 or 500 iu/ml) resulted in a greater inactivation of gram-positive bacteria than when either was applied individually. The gram-negative bacteria were found to be more sensitive to high pressure, either alone or in combination with nisin, than gram-positive bacteria. Such a combination of hurdles may allow lower pressures and shorter treatment times to be used without compromising the product safety.	Black, Kelly, and Fitzgerald (2005)
	200–1000 MPa	High-pressure treatment of pasteurized milk (63°C, 30 min) and pasteurization (63°C, 30 min) of high-pressure treatment resulted in increased reduction in microbial count. Milk enzymes were much less sensitive to pressure. Only alkaline phosphatase and proteinases were completely inactivated at 1000 MPa.	Kolakowski, Reps, and Fettinski (2000)
	300 MPa	The treatment at 300 MPa resulted in 4 log-cycles decrease in microorganisms in milk and subsequently found shelf-life to be 25 days, 18 days and 12 days of milk at 0°C, 5°C, and 10°C, respectively. Further, the kinetics of microorganism destruction, alkaline phosphatase degradation and changes in the color and viscosity was studied in order to establish pasteurization condition for fresh raw milk.	Mussa and Ramaswamy (1997)
	200–600 MPa, 0–120 min	A fuzzy logic model was developed to account for the specific stages of multistep pressure inactivation of L. lactis and to enable prediction of the remaining sublethally damaged cells.	Kilimann, Hartmann, Delgado, Vogel, and Gaenzle (2005)
	400 MPa, 15 min or 500 MPa, 3 min	Treatment of thermally pasteurized milk resulted in increased shelf life by 10 days.	Rademacher and Kessler (1997)
	300–600 MPa	More than 300 MPa had little effect on the beta-lactoglobulin, while levels of beta-lactoglobulin decreased in whey indicating denaturation after 600 MPa.	Pandey and Ramaswamy, 1998; Brooker, Ferragut, Gill, and Needs, 1998.
	400 MPa for 3 min	HPP of milk was shown as a gentle process than conventional procedures for extending the shelf-life of milk, since no significant variation in the content of B_1 and B_6 vitamins was observed	Sierra, Vidal, and Lopez (2000)
	300–500 MPa	Denaturation of beta-lactoglobulin, decrease in plasmin activity, and enhancement of proteolysis were observed possibly due to the increase in the availability of substrate bonds to plasmin which is facilitated by the miceller structure.	Scollard, Beresfold, Needs, Murphy, and Kelly (2000)
	300–400 MPa, 0–180 min	The effect of high-pressure treatment on the activities of lipoprotein lipase and glutamyl transferase of milk was evaluated. Short time pressure exposure resulted in some enhancement in the activity of both the enzymes. In case of lipase, there was no inactivation during the entire pressure hold time (up to 100 min), while glutamyl transferase followed a first order inactivation kinetics.	Pandey and Ramaswamy (2004)
	300–800 MPa, 30 to 65°C	Combined high-pressure thermal inactivation kinetics of plasmin from milk in two model systems was studied. The first system contained both plasmin and plasminogen while in the second system all plasminogen was converted into plasmin using urokinase. For all conditions of pressure and temperature, both the systems showed first order inactivation kinetics. Antagonistic and stabilization effects observed above 600 MPa, which were thought to be related to the disruption of disulfide bonds which stabilized plasmin and plasminogen structure.	Borda, Van Loey, Smout, and Hendrickx (2004)
	300–800 MPa, 25–65°C	Isothermal and high-pressure inactivation of crude plasmin extract prepared from milk at pH 6.7 was described by first order kinetics. The influence of temperature at different pressures on the inactivation rate constant was quantified using the Arrhenius equation. At all temperatures studied, a synergistic effect of temperature and high pressure was observed in the range 300–600 MPa. However, an antagonistic effect of temperature and pressure was observed at pressures greater than 600 MPa.	Borda, Indrawati, Smout, Van Loey, and Hendrickx (2004)

Table 3 Important findings in the area of dairy processing *(Continued)*

Product	Conditions	Salient results	References
Rennet coagulation time			
	200–400 MPa	Decrease in the rennet coagulation time.	Needs, Stenning, Gil, Ferragut, and Rich (2000)
	200 MPa	A lower rennet coagulation time was observed in the case of milk treated at a pressure of 200 MPa.	Kolakowski, Reps, and Fettinski (2000)
	150–670 MPa	Rennet coagulation time remained unchanged for treatments at pressures less than 150 MPa, whereas it decreased at higher pressures.	Derobry, Richard, and Hardy (1994)
	500–600 MPa	Rennet coagulation time of pressure treated milk was higher than pasteurized milk (72°C, 15 s).	Trujillo, Royo, Guamis, and Ferragut (1999a) Needs, Stenning, Gill, Ferragut, and Rich (2000)
	250–600 MPa at 5 or 10°C	Pressure treatment resulted in reduction in rennet coagulation time. in milk without kio_3, the coagulum strength was highest after treatment at 250 or 400 MPa, whereas in milk with kio_3, it was highest after treatment at 400 MPa. This is due to the high-pressure induced association of whey proteins with casein micelles.	Zobrist, Huppertz, Uniacke, Fox, and KELLY (2005)
	100–600 MPa for 0–30 min at at 20°C	Rennet coagulation time of heated milk decreased with increasing pressure and treatment time. The strength of the pressure treated coagulum from heated milk was considerably greater that of unheated unpressurized milk. The yield of cheese curd from high pressure treated heated milk was 15% greater than that from unheated unpressurized milk; the protein content of the whey was 30% lower.	Huppertz, Hinz, Zobrist, Uniacke, Kelly, and Fox (2005)
Curd formation and firming			
	400 MPa	Accelerated rate of curd formation and curd firming of rennet milk.	Ohmiya, Fukami, Shimizu, and Gekko (1987).
	200–400 MPa.	Increase in curd firming rate of milk below 200 MPa and decrease in the pressure range 200–400 MPa. Gel firmness was found to be unaffected below 200 MPa, but increased at 300.	Lopez, Carrascosa, and Olano (1996) and Lopez, Ramos, and Olano (1997)
	400–600 MPa	Rate of curd formation was highest at 200 MPa but slightly decreased on treatment at 400–600 MPa. Higher curd firmness for the milk treated under high pressures.	Needs, Stenning, Gill, Ferragut and Rich (2000) Molina, Alvarez, Ramos, Olano, and Lopez (2000).
	200–500 MPa, 3–21°C, 10–110 min	Decrease in pressure level, temperature and holding time resulted in a decrease in water holding capacity and an increase in gel strength of rennet curd. The processing condition (280 MPa, 9°C, and 40 min) resulted in highest water holding capacity (40%) and high gel strength (0.47 N).	Pandey, Ramaswamy, and St. Gelasis (2000)
	600 MPa for 30 min	High pressure treated LAB-inoculated pasteurized whole milk acidified faster than unpressurized milk due to faster growth of LAB. This was due to increase in the level of non-sedimentable (non-micellar) caseins, in turn enhanced thereby enhancing the supply of accessible nitrogen for bacteria.	Huppertz, Fox, and Kelly (2004e)
	400 MPa, 20 min, 20°C	High pressure treatment of Queso fresco cheese did not impart major textural and compositional changes; however, cheese made from pressure-treated milk contained more moisture and was less firm, less crumbly and more sticky than the control, and decreased in firmness during storage.	Sandra, Stanford, and Goddik (2004)
	345–483 MPa for 3 and 7 min	High-pressure treatment accelerated shredability of Cheddar cheese. Shreds from unripe milled curd Cheddar cheese could be produced with high visual acceptability and improved tactile handling using high pressure.	Serrano, Velazquez, Lopetcharat, Ramirez, and Torres (2005)
Cheese yield			
	300–600 MPa	Increase in cheese yield by high-pressure treatment of cheese milk due to denaturation of whey proteins and increased moisture retention was also found. Higher moisture content of cheese made from high-pressure treated milk due to the fact that casein molecules and fat globules may not aggregate closely and may allow moisture to be trapped or held in cheese.	Drake, Harrison, Asplund, Barbosa, and Swanson, 1997;
	300–600 MPa	Reduced hardness of Cheddar cheese made from high-pressure treated milk may be due to association of whey protein with casein in pressurized milk.	Pandey, and Ramaswamy (1998)
	400 MPa, 15 min, 20°C	Increase in cheese yield (2% on dry basis) and moisture content by adjusting pH (7.0) before pressure treatment.	Arias, Lopez, and Olano (2000)
	200–400 MPa	Pressure treatment of cheese milk increased the yield of low-fat cheese by improving protein and moisture retention. Pressurization of pasteurized milk improved its coagulation properties. Cheese made from pressurized and pasteurized milk showed increased protein and moisture retention as well as improved coagulation properties. The protein degradation and development of texture and flavor was also rapid and the product had lower hardness and cohesiveness and higher sensory scores.	Molina, Alvarez, Ramos, Olano, and Lopez (2000)

(Continued on next page)

Table 3   Important findings in the area of dairy processing *(Continued)*

Product	Conditions	Salient results	References
	400 MPa for 5 min at 21°C	The interaction of high-pressure treatment and storage time on non-expressible serum per gram protein (an index of protein hydration in the cheese) was reported for reduced-fat mozzarella cheese. The interaction levels were higher in high-pressure treated cheese than unpressurized sample. High pressure treatment also decreased the L*, a* and b* -values significantly after storage for 1 day, but no effect was observed after 75 days storage. The composition, pH, proteolysis, rheological properties of the unheated cheese, or flowability and stretchability were not significantly affected.	Sheehan, Huppertz, Hayes, Kelly, Beresford, and Guinee (2004)
	100–800 MPa for 0–60 min	High-pressure treatment up to 250 MPa did not result in any change in curd yield but the moisture content decreased by 5% in comparison with untreated milk. Pressure above 250 MPa increased curd yield and moisture content and decreased protein content of the whey. The curd yield increased by storing milk for 24 h prior to renneting.	Huppertz, Fox, and Kelly (2004f)
Effect on pathogenic and spoilage microorganisms			
	400–500 MPa for 5–15 min at 2, 10 or 25°C	No surviving *Escherichia coli* were detected in the goats' milk cheese sample inoculated with 10^8 cfu/g even after 15, 30, or 60 days of storage.	Capellas, Mor-Mur, Sendra, Pla, and Guamis (1996)
	50–800 MPa, 20 min, 10–30°C	Higher sensitivity of Escherichia coli in cheddar cheese at pressure above 200 MPa was exhibited, possibly due to acid injury during cheese fermentation. In addition to cell death, the recovery of sub-lethally injured pressure treated was demonstrated in high-pressure treated cheese slurries.	O'Reilly, O'Connor, Kelly, Beresford, and Murphy (2000)
	400–700 MPa for 1–15 min	High-pressure treatment was effective in reducing of L. monocytogenes on Gorgonzola cheese rinds without significantly changing its sensory properties.	Carminati, Gatti, Bonvini, Neviani, and Mucchetti (2004)
Ripening of cheese			
	50 MPa, 3 days	Accelerated the ripening of cheddar cheese and increase in proteolysis of milk protein resulted in an increase in free amino acid content. The taste of pressure treated cheese was described as excellent.	Yokohama, Swamura, and Motobyashi (1992)
	50 MPa for 3 days at 25°C	Accelerating the ripening of commercial cheddar cheese by high-pressure treatment due to the degradation of α_{S1}-Casein and accumulation of α_{S1}-1-Casein.	O'Reilly, O'Connor, Murphy, Kelly, and Beresford (2000)
	200 to 800 MPa for 5 min at 25°C	Cheddar cheeses treated at pressures higher than 400 mpa evolved free amino acids at significantly lower rates than the control. No acceleration in free amino acid development was observed at lower pressures. Pressure treatment did not accelerate the rate of texture breakdown. On the contrary, pressure treatment at 800 mpa reduced time-dependent texture changes.	Wick, Nienabert, Anggraeni, Shellhammer, and Courtney (2004)
	500 MPa, 15 min, 20°C	Cheese made from high-pressure treated goat's milk had high pH and salt content, which matured more quickly and developed strong flavors. Small peptides and free amino acids indicated a higher extent of proteolysis in cheese made from high-pressure treated milk.	Trujillo, Royo, Forragut, and Guamis (1999b)
	50 MPa for 3 days or 400 MPa for 5 min	High-pressure treated goats' milk cheese exhibited faster proteolysis and higher pH values than untreated cheese.	Saldo, McSweeney, Sendra, Kelly, and Guamis (2000)
	800 MPa, 15 min, 20°C	Cheese prepared from raw and pressure treated goats' milk was firmer, less fracturable, and less cohesive than pasteurized milk (72°C, 15 s). Pressure treatment resulted in more elastic, regular and compact protein matrix with smaller and uniform fat globules resembling the structure of cheese prepared from raw milk.	Buffa, Trujillo, Pavia, and Guamis (2001)
	500 MPa at 20°C for 15 min	Organic acid levels of cheese made with pressure treated goat's milk rose gradually for 60 days. Organic acids play an integral role in cheese flavor. After the ripening period, there was a lower concentration of organic acids in cheese made from pasteurized milk than in cheeses made from pressure treated or raw milk.	Buffa, Guamis, Saldo, and Trujillo (2004)
	50–500 MPa, 20–200 min or 3 days	Ripening of Gouda cheese was accelerated the by the application of high pressure.	Messens, Dewettinck, and Huyghebaert (1999)
	50 MPa, 72 h	Pressure treatment increased the levels of free amino acid, although the cheese treated at 400 MPa had peptides and casein profiles similar to those of younger non-treated cheese or cheese treated at 50 MPa. Plasmin activity was unaffected by pressure treatments, whereas coagulant activity was decreased by treatment at 400 MPa.	Saldo, McSweeney, Sundra, Kelly, and Gumis (2002)
	400 MPa for 5 min		
	200–500 MPa at 12°C for 10 min	The application of high pressure inactivated undesirable microorganisms and enhanced the proteolysis in ewe's milk cheese at 300 MPa, which was attributed to high-pressure induced cell lysis. Pressure treatment at 400 MPa or greater was proposed as a useful method to slow down or arrest cheese proteolysis.	Ferragut, Guamis, Buffa, and Trujillo (2004)

Table 4 Important findings in the area of processing of animal products

Product	Conditions	Salient results	References
Beef meat (minced)			
	200–400 MPa 20 min at 20°C	Reduction of 5 log cycles or more for *Pseudomonas Jluorescens, Citrobacter freundii, Listeria innocua* was achieved above 200 MPa, 280 MPa and 400 MPa, respectively. Processing at 400 and 450 MPa completely inactivated all the microorganisms such as Pseudomonas, Lactobacillus, Coliforms, except for the total flora, which was reduced by 3 to 5, log cycles. The treatment delayed microbial growth by 2–6 days upon subsequent meat storage at 3°C.	Carlez, Rosec, Richard, and Cheftel (1993; 1994)
Beef meat			
	130–520 MPa, 4.3 min	High pressure for a short time reduced total flora and delayed microbial growth by 1 week, which enabled longer maturation and improved meat tenderness of beef meat. Treatment at 130 MPa improved meat color by increasing redness, which was maintained for the first 3 days of storage at 4°C, without affecting microbiological quality.	Jung, Ghoul, and Lamballerie (2003)
Beef (slices)			
	100 MPa for 10–15 min	Treatment reduced shear strength and the pink color, higher score for pressure treated cooked meat, and lowest exudates.	Jun, Nan, and Che (1999)
Beef (post rigor)			
	520 MPa	Post rigor beef induced significant increases in lysosomal enzyme activities, which were maintained during ageing, but did not improve beef tenderness or reduce the ageing period. Pressurization increased toughness of beef, due to modifications in myofibrillar components as opposed to collagen. Pressurization reduced sarcomere length and increased cooking losses.	Jung, Ghoul, and Lamballerie (2000)
Bovine liver cells			
	100–500 MPa, l0 min, 25°C	Total activities of β-glucuronidase and acid phosphatase of these enzymes in the cytosolic fraction of treated bovine liver cells and of post-rigor treated beef muscles (100–500 MPa, 5 min, 2°C) were found to increase after pressure application. The effect of high pressure on catheptic enzymes, which influence meat tenderization and on acid phosphatase, used as an index of disruption of lysosomal membranes and showed that the pressure-induced increase in the amount of proteinase activity in muscle was due to the release of enzymes from lysosomes.	Ohmori, Shigehisa, Taji, and Hayashi (1992) Homma, Ikeuchi, and Suzuki (1994)
	103 MPa, 30–35°C l-4 min	Pressurization had no significant effects on connective tissue, and pressure-induced tenderization of meat was probably caused by improvement of actomyosin toughness. High-pressure treatment of ovine and bovine muscles resulted in firmness and contraction, however, after cooking, the meat was more tender and with higher moisture content.	Suzuki, Watanabe, Ikeuchi, Saito, and Takahashi (1993); Macfarlane (1973); Kennick, Elgasim, Holmes, and Meyer (1980)
Beef			
	100–300 MPa	High-pressure treatment modulates the proteolytic activities of meat to improve its quality resulting in increased free amino acid content. Tryptic digestibility of the extract of beef was increased at pressure higher than 400 MPa.	Ohmori, Shigehisa, Taji, and Hayashi (1991)
Pre- or post-rigor			
	103.5 MPa, 2 min, 35°C	The muscles undergo an intense contraction, with a length reduction of 35 to 50% and severe disruption in meat structures when pressure is applied in early pre rigor stage. No contraction was induced, but extensive modifications in the sarcomere structure were observed when high pressure was applied in the post-rigor stage.	Macfarlane (1973); Kennick, Elgasim, Holmes, and Meyer (1980); Macfarlane and Morton (1978).
Post-rigor beef muscles			
	150 MPa, 80°C.	Post-rigor beef muscles in a stretched condition resulted in decrease in shear values due to changes in connective tissue.	Beilken, Macfarlane, and Jones (1990)
	Up to 500 MPa	The application of higher pressures may permit meat tenderization without any additional heating. Up to 300 MPa resulted in increased myofibril fragmentation and marked modification in ultrastructure. Further, it led to the conversion of α-connectin to β-connectin the (which was shown to start at 100 MPa and almost complete at 300 MPa), while nebulin decreased more readily and was totally degraded at 200 MPa.	Suzuki, Watanabe, Iwamura, Ikeuchi, and Saito (1990); Suzuki, Kim, Homma, Ikeuchi, and Saito 1992; Kim, Ikeuchi, and Suzuki (1992)
Meat batters with added walnuts			
	400 MPa for 10 min at 10°C	Addition of walnuts and processing by high pressure influenced the physicochemical properties of cooked meat batters. Addition of walnuts resulted in increased fat levels, decreased moisture content of meat batters, and showed good water as well as fat binding properties. The hardness, cohesiveness, springiness, and chewiness of cooked products were also reduced by addition of walnut, but unaffected by application of high pressure.	Ayo, Carballo, Solas, and Colmenero (2005)

Table 4   Important findings in the area of processing of animal products *(Continued)*

Product	Conditions	Salient results	References
Beef and mutton			
	300–700 MPa, for 10–20 min.	Sensory properties (color faded, extension decreased, and flavor emerged) varied considerably after high-pressure treatment. A marked variation could be seen in microscopic structure of myofibrils of cattle and mutton muscle and sarcomere shrinkage occurred with obvious changes. Shear force values of cattle and mutton skeletal muscle were decreased markedly. Thus, with careful choice of pressure, temperature, and processing time, high-pressure treatment can improve tenderness, kill microorganisms, and retain the natural flavor of meat.	Bai, Zhao, Deligersong, and Yang (2004)
Meat products (raw sliced marinated beef loin, sliced dry cured ham, sliced cooked ham)			
	6 min at 600 MPa and 31°C.	Growth of Enterobacteriaceae and yeasts was prevented by high pressure, reducing the potential for off flavor formation in the meat products. The growth of lactic acid bacteria was also delayed by application of the treatment, resulting in slower spoilage. Food safety risks associated with *L. monocytogenes* and *Salmonella* were also reduced in sliced marinated beef loin.	Garriga, Grebol, Aymerich, Monfort, and Hugas (2004)
Beef muscle			
	(200–800 MP at 20–70°C)	The hardness increased with increasing pressure at constant temperature of 20–40°C and increased with increasing temperature at ambient pressure, but decreased significantly with application of 200 MPa pressure at 60 and 70°C. Accelerated proteolysis rather than the structural changes is likely to be the major factor contributing to the loss in hardness of beef.	Han and Ledward (2004)
Ready-to-eat meats (low-fat pastrami, Strassburg beef, export sausage, and Cajun beef)			
	600 MPa for 3 min at 20°C	Counts of aerobic and anaerobic mesophiles, lactic acid bacteria, *Listeria* spp., *Staphylococci, Brochothrix thermosphacta, Coliforms*, and fungi revealed that they were undetectable or low levels of microorganisms when stored at 4°C for 98 days. There was no difference in consumer acceptability and the sensory quality of the product. High pressure can thus extend the refrigerated shelf life of ready-to-eat meats and reduce *L. monocytogenes* by more than 4 log cfu/g in inoculated products.	Hayman, Baxter, O'Riordan, and Stewart (2004)
Fresh raw chicken (minced)			
	400–900 MPa, l0 min, 14–28°C	Treatment of fresh raw chicken mince in sealed polyfilm pouches resulted in extended refrigerated storage life. HPP at 408, 616 and 888 MPa reduced the microbial load by approximately 1.7, 3.4, and 3.7 log cycles, respectively and further storage at 4°C microbial spoilage (10^7 CFU/g) was observed after 27, 70, and > 98 days, respectively.	O'Brien, and Marshall (1996)
Chicken and pork batters			
	200 and 400 MPa, 30 min	Increased water and fat binding properties of chicken and pork batters even at low ionic strength. High pressures resulted in less hard, cohesive, springy, or chewy samples as compared to non-pressurized samples.	Jimenez, Fernandez, Carballo, and Fernandez (1998)
Chicken breast muscle			
	500–800 MPa, 10 min	Pressure treatment of chicken breast muscle at 800 MPa enhanced lipid oxidation to the same extent as heat treatment. Pressure treatment at 600 and 700 MPa resulted in less oxidation. Up to 500 MPa showed no indication of rancidity and was similar to untreated meat during chill storage, suggesting that 500 MPa is a critical pressure for pressure treatment of chicken breast muscle. Increased lipid oxidation was probably related to membrane damage.	Orlien, Hansen, and Skibsted (2000)
	200–600 MPa for 5 min at 10°C	High-pressure treatment at 400–600 MPa led to a substantial increase in secondary lipid oxidation products in the cooked breast chicken when compared to 200 MPa treatment and control. Storage period had considerable influence on the formation of secondary lipid oxidation products, especially in the presence of O_2 in the packs stored for 8 days.	Wiggers, Kroger, and Skibsted (2004)
Foie gras (fatty goose or duck liver)			
	400 MPa, 10 or 30 min, 50°C	Reduced the microbial load while maintaining the unique texture as well as flavor and high product yield. There was no melting or separation of lipids as a result of pressure processing, in contrast to the 15% lipid loss due to thermal pasteurization.	El Moueffak, Cruz, Antoine, Montury, Demazeau, Largeteau, Roy, and Suber (1996)
	350 and 550 MPa, 55 and 65°C, 1–30 min	Aerobic mesophilic flora and heat- and pressure-resistant bacterium *Enterococcus faecalis* on duck foie gras indicated that the combined effect of HPP and temperature could be used to give a product of similar microbiological quality to that obtained by pasteurization.	El Moueffak, Cruz, Antoine, Montury, Demazeau, Largeteau, Roy, and Zuber (2001)

Table 4 Important findings in the area of processing of animal products *(Continued)*

Product	Conditions	Salient results	References
	100–150 MPa, l-5 min, 35°C	Pressurization soon after slaughter of warm pre-rigor meat resulted in a rapid pH decrease and contraction in case of ovine and bovine muscles and white fast-twitch muscle.	Macfarlane, (1973) Horgan (1979; 1981).
Tenderization of meat			
	100–300 MPa, 10 min, RT	Calpain and cathepsins enzymes have their influence on tenderization of meat. The influence of high pressure on the activity of calpains has been mainly studied in the case of pre-rigor treated meat; the level of μ-calpain is markedly reduced during ageing. Total calpain activity was decreased, but acid phosphatase and alkaline phosphatase activities were not significantly different from those of controls.	Qin, Nan, and Che (2001)
	100–200 MPa	Inactivation of calpastatin at 100 MPa was faster than that of calpains. The level of calpain remained in muscle pressure treated up to 200 MPa, and calpastatin levels were decreased by the pressure treatment, whereas calpains resisted pressurization at 200 MPa, but were inactivated at higher than 200 MPa. Thus total activities of calpains in pressurized muscle were increased by pressure treatment, and this resulted in the tenderization of the meat.	Homma, Ikeuchi, and Suzuki (1995)
Rabbit muscles (pre-rigor)			
	200–400 MPa, 5 min	Treatment of an enzymatic extract from pre-rigor rabbit muscles induced a decrease in the activity of calpains. Nevertheless, calpastatin, a specific calpain inhibitor exhibited a greater sensitivity to inactivation by pressure, and this may contribute to the maintenance of a certain level of calpain activity for pressure treatments below 200 MPa.	Deschamps, Cottin, Largeteau, Demazeau, and Ducasting (1992)
Porcine and bovine			
	300–400 MPa	High-pressure treatment of porcine and bovine improved water-holding capacity and reduced thermal drip. Pressurization increased pH values slightly whereas, drip loss was increased.	Kwiatkowska, Jankowska, and Cierach (2002)
Bovine meat			
	450 MPa, 15 min, 10°C	Finely comminuted bovine meats with or without NaCl subjected to high pressure undergo a strong restructuring effect, without any exudation, resulting in gels with smooth cohesive texture and high water retention.	Cheftel, and Culioli (1997); Carballo, Fernandez, and Colmenero (1996)
Salmon spread			
	700 MPa for 3 min	High-pressure treatment of salmon spread extended shelf life from 60 to 180 days at 3 or 8°C without significant chemical, microbiological, or sensory changes. HPP completely inactivated pathogens present in the inoculated sample.	Carpi, Gola, Maggi, Rovere, and Buzzoni (1995)
Blue whiting			
	200–420 MPa, 10–30 min, 0–38°C	High-pressure induced blue whiting gels were having lower adhesiveness, higher water-holding capacity, and less yellowness than heat-induced gels. Combination of pressure and temperature produced more elastic gels, whereas gels made under high pressure at chilling temperature were much harder, more deformable, and more cohesive.	Perez and Montero (2000)
Tilapia fillets			
	50–300 MPa, 12 h.	Tilapia fillets stored at 200 MPa for 12 h having a high freshness index as compared to control. The total plate count of fillets decreased from 4.7 to 2.0 log cfu/g for the fillets stored at 200 MPa.	Wen and Kuo (2001)
	25–150 MPa, 0°C, 5 min	Increased the water-holding capacity of meat homogenates in NaCl solution and increased binding between the meat particles in patties after cooking. The improved binding is due to pressure-induced disaggregation and unfolding of proteins.	Macfarlane McKenzie, Turner, and Jones (1984)
Prawns			
	200–400 MPa	Shelf life of prawns was extended up to 28 and 35 days in samples treated at 200 and 400 MPa, respectively, as compared to 7 days for air-stored samples. The shelf life was extended up to 21 days in vacuum-packaged samples with delayed the onset of blackening, whereas high-pressure treatment aggravated the problem.	Lopez, Perez, Borderias, and Montero (2000)
Cod			
	100–400 MPa	Treatment higher than 400 MPa of cod (Gadus morhua) decreased the oxidative stability of lipids due to the release of metal ions from complexes. Myosin was denatured at 100–200 MPa whereas actin and most sarcoplasmic proteins were denatured at 300 MPa. Several proteinases survived treatment at 800 MPa, although the activity of neutral proteinases decreased at a pressure higher than 200 MPa. The texture of pressure-treated fish differed from that of both raw and cooked fish, being harder, chewier, and gummier than the cooked product.	Angsupanich and Ledward (1998)

Table 4 Important findings in the area of processing of animal products *(Continued)*

Product	Conditions	Salient results	References
Chilled cold- smoked salmon			
	150–250 MPa	Pressure at 250 MPa did not inactivate *L. monocytogenes* but lag phases of 17 and 10 days were observed at 5 and 10°C, respectively. Pressure at 200 MPa had a marked effect on both the color and the texture of chilled cold-smoked salmon.	Lakshmanan and Dalgaard (2004)
	300 MPa for 20 min at 9°C.	The activities of calpains, cathepsin B-like and cathepsin B + L-like enzymes decreased on application of pressure in crude enzyme extracts. At 300 MPa, calpain was almost completely inactivated, but the general proteinase activity was not affected by high pressure. However, higher-pressure levels affected the enzymes in fish flesh, observed for 18 days of storage. After 12 days of refrigerated storage, there was an increase in the activity of cathepsin B + L-like and calpain.	Lakshmanan, Patterson, and Piggott (2005)
Salmon mince			
		Frozen samples at −28°C for 24 h followed by pressurization and fast pressure release resulted in a 2.5 log cycle reduction for *L. innocua*. When the frozen sample was followed by pressure-assisted thawing at 207 MPa at 10°C for 23 min, a reduction of 1.2 log cycle was obtained. None of the combined high pressure-sub-zero temperature treatments was found to induce sub-lethal injury of *L. innocua* dispersed in smoked salmon mince.	Picart, Dumay, Guiraud, and Cheftel (2005)
Cod sausages			
	350 MPa at 7°C for 15 min	The addition of chitosan to cod sausages in dry form led to a noticeable increase in elasticity and yellowness, but higher counts were recorded. When chitosan was added in soluble form, the total volatile basic nitrogen remained stable during 25 days of storage and no appreciable effect of high-pressure was found regarding microbial counts.	Caballero, Guillén, Mateos, and Montero (2005)
Arrowtooth flounder (Atheresthes stomias)			
	600 MPa for 5 min.	The stabilizing effect of sucrose, trehalose, sorbitol, and their mixtures (1:1) on myofibrillar arrowtooth flounder (*Atheresthes stomias*) proteins subjected to pressure treatments was evaluated. Sorbitol at 8 and 12% was found to be the most effective stabilizing agent against high-pressure effects on protein functionality.	Uresti, Velazquez, Vázquez, Ramírez, and Torres (2005)
Squid (Todaropsis eblanae)			
	300 MPa, 7°C, 20 min	Pressure treatment did not modify optimal pH (pH 3) and temperatures but did increase proteolytic activity. The acid cysteine and acid serine proteases were mainly affected by the high-pressure treatment. Myosin was degraded at all the temperatures, but actin was susceptible only to proteolysis in the pressure-treated muscle at 7°C and 40°C.	Hernández, Guillén, Montero, and Mateos (2005).
Sausages			
	500 MPa, 5 or 15 min, 65°C	High pressure processed sausages were less firm, more cohesive, had lower weight loss, and higher preference scores as compared to heat-treated (40 min at 80–85°C) sample, without any effect on the color attributes.	Mor and Yuste (2003)
Turkey thigh muscle			
	Up to 500 MPa, 10°C, 10 and 30 min	The formation of TBA reactive substances in turkey thigh muscle during storage at 5°C was found to depend exponentially on the pressure used for treatment at both 10 and 30 min, and apparent volume of activation are proposed as a parameter for quantification of the effects of pressure on lipid oxidation in meat during subsequent storage.	Dissing, Bruun, and Skibsted (1997)
Raw ham			
	400–600 MPa, 5 min	Vacuum packaged and high-pressure processed samples of raw ham stored processed at 400, 500 and 600 MPa were spoiled by lactic acid bacteria after 40, 60, and 74 days, respectively at 4°C. Shear resistance of control samples were greater than for pressure treated samples with increasing storage time. The sensory shelf life of samples of sliced cooked ham processed at 600 MPa for 5 min was unchanged even after 30 days of storage at 3 or 9°C.	Carpi, Squarcina, Gola, Rovere, Pedrielli, and Bergamaschi (1999)
Pork (homogenates)			
	400 MPa, 10 min, 25°C	At least 6 log cycles reduction in the populations of *Escherichia coli, Campylobacter jejuni, Pseudomonas aeruginosa, Salmonella typhimurium, Yersinia enterocolitica, Saccharomyces cerevisiae*, and *Candida utilis* inoculated at a level of 10^6-10^7 CFU/g.	Shigehisa, Ohmori, Saito, Taji, and Hayashi (1991)
Pork (paste)			
	250 MPa for 20 or 30 min at 20°C	Complete inactivation of Trichinella spiralis. Trichinae were not inactivated by processing at 50 MPa, whereas, the pressure at 100 or 150 MPa achieved complete inactivation at 5°C.	Noeckler, Heinz, Lemkau, and Knorr (2001)

Table 4 Important findings in the area of processing of animal products *(Continued)*

Product	Conditions	Salient results	References
Sliced Parma ham			
	600 MPa for 3, 6 or 9 min	HPP is a useful technique for control of *L. monocytogenes* in sliced Parma ham. The treated sample had less red color and more intense salty taste.	Tanzi, Saccani, Barbuti, Grisenti, Lori, Bolzoni, and Parolari (2004)
Dry-cured Iberian ham and ham slurries			
	200–800 MPa	Application of the low pressure was found to initiate radical formation and promote lipid peroxidation, whereas intermediate to high pressures appeared to promote further reaction and disappearance of free radicals. Hexanal content was found to be significantly affected by pressure treatment in the same way as free radicals. The control samples showed a higher lightness than pressurized samples, and redness decreased with pressure treatment.	Andres, Moller, Adamsen, and Skibsted (2004)
OYSTERS			
	100 and 800 MPa for 10 min at 20°C	HPP killed various pathogens that are commonly found in oysters. Protein and ash contents decreased with increasing treatment pressure, while moisture content increased. Oyster muscles get detached from the shells, resulting in shucking, but the recovered tissue has good shape and is more voluminous and juicy than that of untreated oysters. The pH increased following high-pressure treatment.	Cruz, Smiddy, Hill, Kerry, and Kelly (2004)
	400–700 MPa	The effects of high pressure on bacteria associated with illness in oysters and the influence of a high-salt environment on inactivation were investigated. The higher baroresistance of all bacteria in oysters than in buffer indicated that studies of high pressure induced bacterial inactivation in buffer systems may not predict inactivation in foods.	Smiddy, O'Gorman, Sleator, Kerry, Patterson, Kelly, and Hill (2005)
	350–400 MPa, 8.7–10.3°C for 1 min	Hepatitis A virus can be inactivated by high hydrostatic pressure. Nearly 6 log cycle reduction of the virus was achieved. These results suggested that pressure treatment of raw shellfish is a viable strategy for the reduction of the virus.	Calci, Meade, Tezloff, and Kingsley (2005)

SOME PRACTICAL CHALLENGES

Although HPP offers a number of opportunities, there are several challenges, which have to be addressed before a wider industrial application is considered.

Heat Transfer under High Pressure and Process Inhomogeneities

Most of the high-pressure applications in food are not only pressure dependent but also temperature dependent. In most studies available in literature, the contribution of temperature during the treatment has not been considered. The evolution of temperature is very important on account of its effect on food gelling, protein stability, fat migration, freezing etc. The main difficulty in monitoring or modeling heat transfer in high-pressure processes is the lack of data on thermophysical properties under pressure. Denys, Van Loey, Hendrickx, and Tobback (1997) stated that the temperature history of a product under pressure is essential for the optimization and design of industrial processes.

Thermal Effects during High Pressure Processing

During high pressure processing, the temperature of food material increases as a result of physical compression. The pressure increase during the come-up time from an initial pressure P_s to P_1 increases the temperature (Fig. 9). The magnitude of temperature increase, in part, depends upon the initial temperature, material compressibility and specific heat, and the target pressure. The maximum product temperature at process pressure is independent of the compression rate as the long as heat transfer to the surroundings is negligible. It is further interesting to note that while the rate of temperature increase of the water-like substances is in phase with the change in pressure, fatty substances often exhibit a time delay of 30–60 s before reaching the maximum temperature (T_1). This may be attributed to the difference in their respective molecular structure (Rasanayagam, Balasubramaniam, Ting, Sizer, Bush, and Anderson, 2003). During the pressure holding time (P_1 to P_2), the temperature of the product
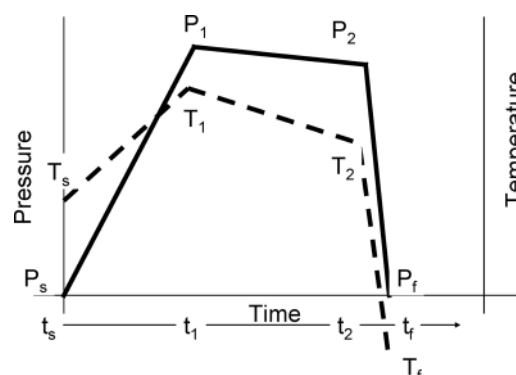

Figure 9 Variation of pressure and temperature in a non-insulated high-pressure vessel.

decreases from T_1 to T_2 due to heat loss through the pressure vessel. Immediately after depressurization, the product temperature returns to a value, slightly lower than the initial temperature. Thus, high pressure offers a unique way to increase the temperature of the product only during the treatment.

Pressure Nonuniformity

Minerich and Labuza (2003) demonstrated that the process of homogeneity in pressure vessels is still an issue that needs attention. Using custom made copper tablets the authors demonstrated that the density of the tablet increased proportionately as the pressure increased between 400 and 600 MPa. The change in density of the tablet placed in the geometric center of a large food product, such as a ham, indicated that the ham received approximately 9 MPa less pressure than the processing system delivered ($P < 0.017$), challenging the assumption that all foods follow the isostatic rule. Authors, finding may have implications when determining the microbial lethality for large food items pasteurized or sterilized using high pressure. More research is needed to evaluate pressure uniformity within a larger pressure volume as well alternative approaches that can verify the above findings.

Compression Heating of Food Materials

All compressible substances change temperature during physical compression and this is an unavoidable thermodynamic effect (Ting, Balasubramaniam, and Raghubeer, 2002). Water has the lowest compression heating values, while fats and oils have the highest. For example, at pressures normally encountered during HPP (400–1000 MPa), under adiabatic conditions near room temperature, water typically changes 3°C for every 100 MPa pressure change. Further, the compression heating value for water increases with temperature. Since water is the main ingredient in most foods, adiabatic temperature changes exhibited by most foods are very similar to that of water, except for oil and alcohol. Fats and oils show the highest compression heating values (6 to 8.7°C per 100 MPa) (Rasanayagam Balasubramaniam, Ting, Sizer, Bush, and Anderson, 2003).

Similarly, the temperature of pressure transmitting fluid will also change after compression depending on its own thermal properties and will influence the temperature of the sample. This process can introduce additional temperature gradients in the product (Denys, Van Loey, and Hendrickx, 2000a). The difference between the temperature of a product (say, meat) and pressuring fluid (water), over a range of pressures under adiabatic compression, is shown in Fig. 10. This difference can also be attributed to the differences in the thermal properties. Balasubramanian and Balasubramaniam (2003) studied the apparent temperature increase of selected pressure-transmitting fluids (food-grade water-based glycol at different concentrations or 2% sodium benzoate solution) during HPP using a pilot scale food processor, together with the effects of these fluids on the inactivation of *Bacillus subtilis*. The highest temperature increase was reported for pressure-transmitting fluid containing 75% glycol,
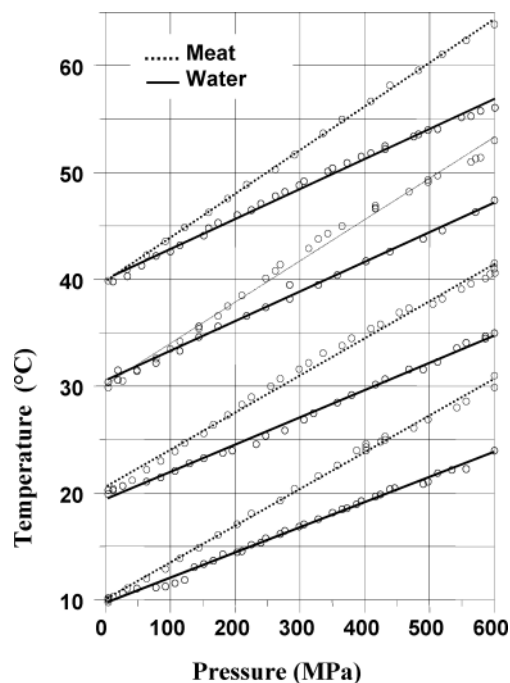

Figure 10 Difference between the temperature of the product (meat) and pressuring fluid (water) over a range of pressures under adiabatic compression (Knorr, 2004).

whereas the fluid containing the highest amount of water (2% sodium benzoate solution) showed the lowest temperature increase, initial temperature, holding time, target pressure, compressibility, and rate of heat loss to the surroundings. Fluid properties such as thermal conductivity, viscosity, and specific heat also affected the temperature change.

Change in pressure transmitting fluid temperature as a result of compression heating and subsequent heat transfer should be considered in HPP microbial inactivation.

Properties of Food Materials under Pressure

The determination of properties of foods under high pressure is a complex task and practically no data exists in this regard. Otero, Molina, and Sanz (2002a) compiled thermophysical properties of liquid water and ice over a range pressure and temperature. Denys, Van Loey, and Hendrickx (2000b) estimated the density of apple sauce and tomato paste under pressure by using the displacement method. Denys, Van Loey, and Hendrickx (2000a) and Denys, Ludikhuyze, Van Loey, and Hendrickx (2000b) studied thermal expansion coefficient of various products such as apple sauce, tomato paste, and agar gel. This was measured by noting the increase in temperature following a known pressure increase. Denys and Hendrickx (1999) used the line heat source probe for determining the thermal conductivity of food up to 400 MPa.

During a pressure assisted thawing process the phase transition occurs under constant pressure by increasing the temperature and in the pressure induced thawing process the phase

transition is initiated by a pressure change and continued at constant temperature. During pressure build up, the sample temperature increases due to the work of compression. Thawing occurs under pressure in a manner similar to that at atmospheric pressure, ruled by thermal gradients between the sample and the pressurizing fluid. However, phase transition does not occur under constant pressure. A pressure decrease is observed due to the volume decrease associated with liquid transition to ice I (Denys, Van Loey, and Hendrickx, 2000c). The advantages of using pressure are two-fold: (a) as a consequence of the depression in the melting point of water under pressure, the heat flux during thawing is enhanced due to the greater temperature difference between the pressurizing fluid and the phase-change front (Chourot, Cornier, Legrand, and Le Bail, 1996); and (b) the latent heat values are lower with increasing pressure up to 210 MPa (Bridgman, 1912; Hobbs, 1974). Both these effects accelerate thawing in comparison to atmospheric pressure thawing processes occurring at identical temperatures (Takai, Kozhima, and Suzuki, 1991; Chevalier, Bail, Chourot, and Chantreau, 1999). After thawing under pressure, the temperature of the sample must be increased in order to prevent ice crystallization during the expansion. Further research is needed to characterize the combined effects of pressure and temperature on thermal and physical properties of food materials.

Kowalczyk, Hartmann, Luscher, Pohl, Delgado, and Knorr (2005) studied the high-pressure phase change of food by means of numerical simulation and experimental techniques. The strong influence of high pressure on the kinetics of phase transition was reported as well as the thermophysical properties of food at high pressure were also determined. Kubásek, Houška, Landfeld, Strohalm, Kamarád, and Žitný (2006) estimated the thermal diffusivity of olive oil using numerical analysis of the experimental data of the temperature drop under pressure. It was shown that temperature dependence of the apparent thermal diffusivity is a function of the starting temperature and therefore influenced by convection in the oil.

Mathematical Modeling of Process Uniformity

While the temperature of a homogeneous food will increase uniformly due to compression, a temperature distribution can develop during the holding period due to heat transfer between the food and the pressurizing fluid as well as heat transfer across the walls of the pressure vessel (Farkas and Hoover, 2001). After pressurization, the temperature of the product will normally be greater than the temperature of the pressure-vessel. As a result, heat will be lost to the vessel wall and the regions of the product close to the vessel wall may not achieve the final temperature reached at the center of the vessel (De Heij, Van Schepdael, and Van der Berg, 2001; Ting, Balasubramaniam, and Raghubeer, 2002).

The distribution of pressure–temperature–time profiles within a product can result in differential enzyme/microbial inactivation levels, as well as nutritional/sensorial quality degradation (Denys, Van Loey, and Hendrickx, 2000b). It is important to note the thermal related process nonuniformity is primarily having a major impact on pressure sterilization where a combination of elevated pressures and modest temperatures are used for the sterilization of low-acid foods. Denys, Van Loey, and Hendrickx (2000a,b) adopted a numerical approach for modeling conductive heat transfer during batch processing under high pressure, and demonstrated experimentally the non-uniform temperature distribution and resulting non-uniform enzymatic inactivation. The model includes the estimation of the temperature rise/fall during the period of the pressure buildup/release.

Hartmann (2002) and Hartmann, and Delgado (2002) analyzed the thermodynamic and fluid-dynamic effects of high-pressure treatment of fluid foods. The authors studied the spatial and temporal evolution of temperature and fluid velocity fields and its importance on high-pressure induced conversions (inactivation of *Bacillus subtilis* alpha-amylase). They showed that the uniformity of the high-pressure effect could be disturbed by convective and conductive heat and mass transfer which are influenced by parameters such as the compression rate, the size of the pressure chamber, or the solvent viscosity. Hartmann and Delgado (2003) modeled the heat transfer effects during HPP of packaged foods. The changes in thickness and properties of the packaging material were considered by modifying the heat transfer coefficient. Makita (1992) emphasized that the formation of temperature gradients in a product were inevitable, even if the pressure vessel was fitted with a thermostatic device. The only way to avoid these gradients was by stirring the pressure medium, which would be technically difficult. Denys, Van Loey, and Hendrickx (2000b) proposed applying a heat source at the boundary of the product to prevent the temperature gradients (which functioned by anticipating the temperature increase of the compressed product). In this context, their heat transfer model can be used to calculate the temperature rise of the product upon compression.

Otero, Molina, Ramos, and Sanz (2002b) developed a model taking into account all the thermal exchanges in occurring the system, which included the sample, the pressurizing fluid, the pressure vessel, and the thermo-regulating system. This enabled an assessment of the relative importance of various heat transfer processes. In addition, their calculation includes the temperature variation during compression and expansion.

Modeling phase change, even at atmospheric pressure, is a difficult task because of the heterogeneous nature of food where water is not totally available for freezing. When phase change occurs under pressure or by a fast release of pressure, the modeling process can be considerably complicated. A number of analytical models (Plank, 1941; De Michelis and Calvelo, 1983; Chung and Merritt, 1991) or numerical models (Cleland, Cleland, Earle, and Byrne, 1987) have been developed to predict freezing and thawing times at atmospheric pressure, but there are very few papers dealing with high pressure processes. Schluter, Heinz, and Knorr (1998) modeled thermal exchanges in potato cylinders subjected to high-pressure assisted freezing. The radially symmetrical one-dimensional heat conduction problem was solved using a finite difference method. The moving freezing

front was modeled by using a finite difference scheme. A temperature dependent specific heat and thermal conductivity in the vicinity of the freezing front was introduced into the heat balances equations. Due to the unavailability of thermal property values under pressure, the authors regressively estimated them by fitting experimental freezing curves to those deduced by the model. Burke, George, and Bryant (1975) found that the ice nucleation rate increased approximately 10-fold for each 1°K super cooling. High pressure shift freezing is particularly interesting because super cooling due to pressure release results in nucleation throughout the product and not only on the surface, which led to the formation of homogeneously distributed small and granular shaped ice crystals. Otero, Sanz, de Elvira, and Carrasco (1997) found that 36% of water converted to ice instantaneously after an expansion from 210 MPa to atmospheric pressure. Recently, Chevalier, Le Bail, and Ghoul (2001) following along the same lines, calculated the ice fraction formed when pure water subjected to different initial conditions of pressure was depressurized.

Denys, Van Loey, Hendrickx, and Tobback (1997) employed a numerical solution for a two-dimensional heat transfer to predict heat conduction during freezing and thawing of a tylose cylinder. The method is based on an energy balance that assumes that the sensible heat required for the temperature increase in the expansion is subtracted from the total enthalpy of the sample. This model also took into account the temperature decrease of the high-pressure medium during adiabatic expansion. To deal with the problem of phase change, these authors considered temperature-dependent apparent volumetric specific heat and thermal conductivity. Sanz and Otero (2000) presented a comprehensive account of the heat transfer process involved in high-pressure shift freezing, taking into account the metastable conditions reached at atmospheric pressure after rapid expansion. They also produced a mathematical model to predict the freezing times for a model cylindrical food (agar gel), which allowed comparing high-pressure shift freezing and atmospheric pressure freezing processes. Pre-cooling and tempering stages were solved using the transient heat transfer equations for finite cylinders presented by Chung and Merritt (1991) and the thermophysical properties under pressure for the pre-cooling stage (Otero, Molina, and Sanz, 2002a). Le Bail, Chourot, Barillot, and Lebas (1997) did not take into account the sensible heat of instantaneously frozen water, the temperature dependency of heat capacities at atmospheric pressure or the decrease in sample temperature after the expansion. Later, Barry, Dumay, and Cheftel (1998) presented a modified balance considering the sensible heat absorbed by ice crystals just formed, but they did not take into account the decrease in sample temperature after the expansion. Denys, Van Loey, and Hendrickx (2000c) proposed a numerical heat transfer model for predicting product temperature profile during high pressure thawing. The inclusion of pressure dependence of latent heat resulted in improvement in predictive capacity of the model. Carroll, Chen, and Fletcher (2003) developed a general analytical solution for temperature profiling within a pressure vessel during HPP that could be used

to extract thermal expansivity and diffusivity data for any pressurized fluid, and the heat transfer coefficient for any vessel, from an experimentally determined cooling curve obtained during a pressure hold.

Chourot, Cornier, Legrand, and Le Bail (1996) and Chourot, Boillereaux, Havet, and Le Bail (1997) modelled the heat transfer during high-pressure assisted thawing by using a Crank–Nicolson finite difference method (apparent specific heat formulation) to solve the problem and appropriate thermophysical properties of water under pressure including phase change temperature and latent heat (Bridgman, 1912). Schluter, George, Heinz, and Knorr (1999) modeled pressure assisted thawing of potato cylinders in a similar way as they did for high-pressure assisted freezing. They used a finite difference method and implemented a heat balance at each volume element to adjust the time-dependent temperature field. Due to lack of thermophysical property data for potatoes under pressure (apparent specific heat and thermal conductivity), the authors employed modified values of these properties at atmospheric pressure to fit correctly the experimental thawing process. Denys, Van Loey, and Hendrickx (1997) presented a numerical solution for a two-dimensional heat transfer to model heat conduction during high-pressure freezing and thawing of a tylose cylinder. Later, Denys, Van Loey, and Hendrickx (2000c) presented an improved model taking into account the pressure dependence of the latent heat of the product obtained. Finally, to avoid the effect of convection currents, a sample holder with the maximum radial dimension allowed by the internal volume of the high-pressure vessel, was employed. A finite "overall" surface heat transfer coefficient was introduced in the model accounting for heat transfer through the wall of the sample holder and through the thin layer of high-pressure medium situated between the sample holder and the inner wall of the high-pressure vessel. Thermal gradients, established after compression, caused inhomogeneities in the pressure pursued effect (inactivation of microorganisms, enzymes).

Modeling of heat transfer under the above conditions can be a useful tool for optimizing the operating conditions that would minimize non-uniform effects on the product.

Packaging Requirements of High Pressure Processed Foods

High-pressure technology involves different packaging considerations, based on whether a product is processed in-container or packaged after processing. Continuous or semi-continuous systems are used in the case of pumpable products, which are aseptically packaged after pressure treatment. On the other hand, flexible or partially rigid packaging is best suited for batch in-container processing. The effectiveness of HPP is greatly influenced by the physical and mechanical properties of the packaging material. The packaging material must be able to withstand the operating pressures, have good sealing properties, and the ability to prevent quality deterioration during the application of pressure. At least one interface of the package should be flexible enough to transmit the pressure. Thus, rigid metal, glass,

or plastic containers cannot be used. The headspace must be also be minimized while sealing the package, in order to ensure efficient utilization of the package as well as space within the pressure vessel. This also minimizes the time taken to reach the target pressure.

Nachamansion (1995) showed that film barrier properties and structural characteristics of polymer based packaging material were unaffected when at pressures of 400 MPa, when exposed for 30 min at 25°C. Masuda, Saito, Iwanami, and Hirai (1992) examined the effect of high pressure on water vapor and oxygen permeability, tensile strength, and heat seal performance of gas barrier composite films.

Dobias, Voldrich, Marek, and Chudackova (2004) examined the suitability of several homogeneous and multi-layered packaging for: changes in mechanical properties (tensile and seal strengths), transparency, water vapor permeability, migration characteristics into fatty food simulants, and transfer of water and olive oil into the materials; a pressure of 600 MPa was applied for 60 min. HPP was particularly found to affect the sealability of single layered films and the overall migration.

Schauwecker, Balasubramaniam, Sadler, Pascall, and Adhikari (2002) investigated the migration of 1,2-propanediol (PG) through selected food packaging films exposed to HPP. No detectable PG migration into the Polyester/Nylon/Al/PP meal-ready-to eat (MRE) type pouches was observed. PG migration into the Nylon/EVOH/PE (EVOH) pouches was similar at 30, 50, and 75°C after ten minutes under atmospheric pressure. However, the PG migration into the EVOH pouches significantly decreased when treated with high pressure at 30, 50, and 75°C. At 75, and 50°C, the PG migration was significantly higher than the amounts detected at 30°C. Visible signs of delamination between the polypropylene (PP) and aluminum (Al) layers were observed in the MRE pouches processed at ≥200 MPa and 90°C for ten minutes. This delamination appeared to occur between the PP and Al layers. The Differential scanning calorimetric analyses and Fourier Transform Infrared (FTIR) spectra were similar for the high-pressure treated pouches when compared to their respective controls. This indicated that there was no HPP induced molecular changes to the treated pouches.

Caner, Hernandez, Pascall, and Riemer (2003) used C mode scanning acoustic microscopy (C SAM) and scanning electron microscopy (SEM) to examine structural damage to films and found no marked changes. However, structural damage to the metallized PET was identified. Goetz and Weisser (2002) studied the permeation and migration of volatile compounds through plastics used in food packaging. The extent of permeation and migration was found to depend on pressure and time; some reversible structural changes were also detected.

Caner, Hernandez, and Pascall (2000) studied the permeance and transmission rates of water vapor, CO_2, and O_2 of various laminated flexible films exposed to high pressure processing. Prolonged exposure had a greater effect on the permeance of inorganic layers in some films, than lower pressure/time combinations. Metallized PET was most adversely affected by high pressure, the water vapor transmission being more severely af-

fected than gas transmission. The increase in permeance of all most films was less than 11%, making them suitable for use in high pressure processing. The permeance of metallized PET, on the other hand, increased by 150% hence it was deemed to be unsuitable. Caner, Hernandez, Pascall, Balasubramaniam, and Harte (2004) studied the effect of high-pressure processing on the sorption behavior of D-limonene in selected packaging materials such as monolayer polypropylene, multilayer polyethylene/nylon/ethylene vinyl alcohol/ polyethylene, and metallized PET/ethylenevinyl acetate/linear LDPE films. It was shown that with the exception of the metallized PET/ethylenevinyl acetate/linear LDPE films, high-pressure processing did not markedly affect D-limonene in the liquid simulated foods or the packaging films. Caner, Hernandez, and Harte (2004) reviewed the effects of high-pressure processing on packaging materials, the commercial applications of HPP in food processing, packaging materials suitable for high pressure processing, effects of HPP on barrier properties of packaging films, and mechanical properties of flexible packaging films after high pressure processing.

Lambert, Demazeau, Largeteau, Bouvier, Laborde, and Cabannes (2000) studied the effect of high pressure on tensile strength, heat seal strength, delamination, film structure, oxygen barrier permeability, water vapor barrier permeability, and migration characteristics. They observed that the package prepared by cast coextrusion was susceptible to de-lamination, whereas the packages prepared by tubular extrusion were more robust in terms of barrier properties, migration, and overall integrity.

Kuebel, Ludwig, Marx, and Tauscher (1996) showed that systems containing the aroma compounds p-cymene and acetophenone were quickly absorbed by packaging films, leading to a rapid reduction in aroma concentration. It was observed that the distribution of aroma compounds was a function of polarity. Under elevated pressures, the concentration of aroma compounds decreased less than under atmospheric pressure. High pressure raised the diffusion barrier of polymers, probably due to the transition of the material into a glassy state.

Rubio, Lagarón, Muñoz, Almenar, Catalá, Gavara, and Pascall (2005) studied the effect of high-pressure treatments (400 and 800 MPa, 5 and 10 min, 40, and 75°C) on EVOH-based packaging materials and they were compared with the morphological effects produced by sterilization (120°C, 20 min). The oxygen barrier and morphological properties of the treated packaging structures were analyzed and compared with those of the untreated samples. The results indicated that high-pressure treatment scarcely affects packaging materials, especially when compared to the detrimental consequences of retorting.

Reproducibility of Results

There is often difficulty in comparing results of experiments produced in different laboratories. Several factors play a part in this, including the lack of detailed description of the methodologies used as well as variations in procedures used to prepare

the samples, e.g. the inocula in enumerating microorganisms. Further, the equipment used in various laboratories has different design features as well as configurations. This is clearly illustrated in the study by Hugas, Garriga, and Monfort (2002).

It is necessary to provide an adequate description of the HPP equipment such as vessel size, chamber dimensions, material of construction, wall thickness, pressure-transmitting fluid, heating and cooling system, power specification, data acquisition system and any other pertinent information necessary to reproduce the results should also be provided. It is important to document thermal conditions and temperature distribution within the processed volume (Balasubramaniam, Ting, Stewart, and Robbins, 2004). Kinetic parameters and models can be used to compare the impact of different process technologies on reduction in microbial populations. The variation in parameters such as come-up time, processing time, and decompression time led to different results. A slow come up rate might induce a stress response and hence make the process less effective. A fast depressurization rate might contribute to a fast inactivation rate. In an unheated/non-insulated pressure vessel, it is recognized that the coldest region is located near the wall or near vessel closures. However, temperature sensors are often located in the axial center of the pressure vessel. This may not be the coldest region of the chamber. It is essential to specify the location of the thermocouple sensor in relation to sample tested (Balasubramanian and Balasubramaniam, 2003). The temperature of the pressure vessel and pressure-transmitting fluid temperature, and the estimated sample temperature at the start of pressurization may also be reported. Adding thermal insulation to the inside of the pressure vessel can reduce the rate of heat loss. However, in reality, a true isothermal test is difficult to achieve.

Effect of High Pressure on Toxins, Allergens, and Nutrients

Information relating to the effects of high pressure on toxins, allergens, and nutrients are rare. There are no published reports available on the toxicity of high-pressure processed foods. It is well known that high pressure processed food can modify the activity of some enzymes and the structure of some proteins. Although covalent bonds are not affected, hydrogen bonds as well as hydrophobic and intermolecular interactions may be modified or destroyed. Allergenicity is a key concern in the safety assessment of novel foods. The incidence of food allergies is rapidly increasing, as is their severity and the number of foods involved. In heat-treated products, protein denaturation reduces the allergenicity of many foods, but heat-denatured proteins can also present new antigenic sites. New studies on the putative allergenicity of high-pressure processed foods may be needed.

Regulatory Aspects

Developing methods and techniques for validating any process can be challenging. For example, the U.S. Food and Drug regulations for pasteurization (21 CFR 131.3 and 21 CFR 1240.61) and sterilization (21 CFR 108, 113 and 114) primarily stipulate minimum temperature and time requirements for processing foods. Such information does not exist for high-pressure processed products and it is important to establish microbiological criteria for safe production of foods by HPP (Sizer, Balasubramaniam, and Ting, 2002). In the United States, the Food, Drug, and Cosmetic Act (FD&C Act), which requires all foods to be processed, packaged, and held under sanitary conditions, is the basis by which FDA promulgates specific regulations. Currently, high pressure pasteurized products (such guacamole and oysters etc.), distributed under refrigerated conditions. Similar to thermally pasteurized products, high pressure pasteurized products are required to be processed under GMP conditions and relevant commodity specific regulations (e.g., juice HACCP, Pasteurized Milk Ordinance (PMO), Sea Food HACCP etc). The potential for temperature abuse during refrigerated storage and distribution has to be carefully evaluated and minimized. Processors must also work with equipment vendors to ensure that any part of the pressure vessel, which may have incidental contact with the food, is only made from approved materials. Currently, high pressure sterilized low-acid shelf stable products are not commercially sold in the United States. However, various laboratories world wide are conducting research which can aid in establishing criteria for the production of safe high pressure processed low-acid foods.

In EU countries, the national regulations relating to the application of the precautionary principle for new products have been replaced by a EU regulation for novel foods and ingredients (CE 258/97), which came into force in 1997. This legislation for "novel foods" establishes an evaluation and a license system, compulsory for all new foods and processes. High pressure processed foods are deemed to be "new" as well as "novel." In order to simplify the regulation, it was recently admitted that any new product could be treated at a national regulation level, if it is possible to show that the product is substantially equivalent to a product already on the market.

CONCLUDING REMARKS

The range of high-pressure processed products available in international markets (fruits juices, rice cakes, raw squids in Japan; orange and apple juice in France and Portugal; guacamole and oysters in the United States, indicates future potential of the technology. In the coming years, HPP is likely to be used commercially before the underlying science and its full potential are comprehensively understood. Destruction of microorganisms and inactivation of enzymes at low or moderate temperatures without changing organoleptic and nutritional properties shows that high-pressure technology has the potential to be used in the development of a new generation of value added foods. HPP is not likely to replace traditional processing methods. It may complement such methods or find niche applications. Nevertheless, their novel physico-chemical and sensory properties offer

exciting opportunities for industry. The combination of HPP with other processing options such as heat, gamma-irradiation, ultrasound, carbon dioxide, and anti-microbial peptides, can lower the pressures required. The process can be integrated to other processes such as blanching, dehydration, osmotic dehydration, rehydration, frying, extraction, gelation, freezing, and thawing. High capital expenditure may limit its application initially but this will be offset by lower operating costs since the energy used to pressurize is less than the energies used in thermal processing and other benefits with respect to product originality. With further progress of technology and its commercialization, it is expected that the cost of the equipment will come down in the near future and the high-pressure processed safe and nutritious products will be available to all consumers at an affordable cost.

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