African Journal of Biotechnology Vol. 7 (16), pp. 2739-2767, 18 August, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Review

The principles of ultra high pressure technology and its application in food processing/preservation: A review of microbiological and quality aspects

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Accepted 4 July, 2008

Consumers have a growing preference for convenient, fresh-like, healthy, palatable, additive-free, high-quality and microbiologically safe food products. However, as food deterioration is constant threat along the entire food chain, food preservation remains as necessary today as in the past. The food industry has responded by applying a number of new technologies including high hydrostatic pressure for food processing and preservation. In addition, food scientists have demonstrated the feasibility of industrial-scale high pressure processing. High pressure processing is one of the emerging technologies to be studied as an alternative to classical thermal processing of food. This 'clean' technology offers an effective and safe method of modifying protein structure, enzyme inactivation, and formation of chemical compounds. In addition the study of the effects of high pressure on biological materials has received a great deal of attention in recent years. During the last decade, numerous publications that describe the influence of pressure on various constituents and contaminants of foods such as spoilage microorganisms, food pathogens, enzymes and food proteins have appeared in the literature. This paper reviews the literature on high pressure application in food industry most notably it covers various facets of high pressure technology, which is, history, concepts and principles underlying the application of this technology, physicochemical, chemical, microbiological aspects of high pressure in the viewpoint of food technology.

Key words: Ultra-high pressure (UHP), food processing/preservation and new food-processing technologies.

INTRODUCTION

Increasing consumer demand for minimally processed, additive-free, shelf-stable products, prompted food scientists to explore other physical preservation methods as alternative to traditional treatments such as freezing, canning or drying that rely on heating or cooling operations. Although these technologies have helped to ensure a high level of food safety, the heating and cooling of foods may contribute to the degradation of various food quality attributes. The color, flavor and texture of foods processed solely by heating may be irreversibly altered. To ameliorate the undesirable thermal effects on foods, considerable efforts has been made in commercial and academic circles to develop non-thermal technologies other than heating or cooling operation. Investigated technologies are ionization radiation (gamma irradiation), high hydrostatic pressure (HHP), pulsed electrical fields, high pressure homogenization, UV decontamination, pulsed high intensity light, high intensity laser, pulsed white light, manothermosonication (combined ultrasonic, heat and pressure), oscillating magnetic fields, high voltage arc discharge and streamer plasma. Among these emerging technologies, the most promising ones for food application are high-pressure processing.

High-pressure food-preservation and processing technology is being developed to a large extent in reaction to consumers' requirements for foods that are nutritionally healthier, more convenient in use (e.g. easier to store and

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prepare), fresher (e.g. chill-stored), more natural and therefore less heavily processed (e.g. mildly heated), less heavily preserved (e.g. less acid, salt, sugar) and less reliant on additive preservatives (e.g. sulfite, nitrite, benzoate, sorbate) than previously.

Research into the application of HHP (high hydrostatic pressure) processing for food technology began when Hite (1899) demonstrated that the shelf life of milk and other food products could be extended by pressure treatment. This is the technology of applying high hydrostatic pressure to materials by compressing the surrounding water and transmitting pressure throughout the product uniformly and rapidly (Hyashi, 1989). Use of high pressure in food processing is an extension of a technology that is commonly employed in many other industrial processes, notably in the manufacturing of ceramics, diamonds, super-alloys, simulators and sheet metal forming. Similarly, high isostatic pressures are routinely used in the manufacturing of polymeric compounds, such as for the synthesis of low-density polyethylene and in chemical reactors for the manufacturing of quartz crystals. The advances achieved in ceramics and metallurgical industries in the use of HHP techniques during the 1970s and 1980s, has led to the possibility of treating food by this method at industrial level. The first commercial HHP treated products appeared on the market in 1991 in Japan, where HHP processing is now being used for products such as fruit juices, jams, sauces, rice, cakes and desserts. There is increasing worldwide interest in the use of HHP because of the advantages of this technology over other methods of processing and preservation. An important issue in the application of high pressure technology in the food preservation/processing industry is regulatory approval, which focuses upon microbiological and toxicological safety of food products. Generally high pressure preservation processes reduce the microbial load to the same level achieved by traditional technologies, while delivering higher-quality products. Combination of high pressure with other treatments (e.g., mild temperature elevation, refrigerated storage, and acidification) during processing and storage is a likely route the food industry will take, as the inactivation of bacterial spores and some pressure-resistant enzymes at room temperature cannot be achieved by pressure alone.

In high pressure processing of foods, foods are subjected to ultra high hydrostatic pressure (UHHP or UHP), generally in the range of 100-1000 MPa. The processing temperature during pressure treatments can be adjusted from below 0°C to above 100°C with exposure times ranging from a few seconds to over 20 min.

The UHP process is usually carried out with water as a hydraulic fluid to facilitate the operation and compatibility with food materials (Earnshaw, 1996). Pressure primarily reduces the volume of a system. Under equilibrium conditions, according to the Le Chatelier principle, processes associated with volume decrease are encouraged by pressure, whereas processes involving volume increase are inhibited by pressure (Butz and Tauscher, 1998). Due to this fact, at a relatively low temperature (0 - 40°C) covalent bonds are almost unaffected by HP where the tertiary and quaternary structures of molecules which are maintained chiefly by hydrophobic and ionic interactions are altered by high pressure >200 MPa (Hendrick et al., 1998).

High pressure can be used in food processing in a similar way as temperature. For instance, hydrostatic pressure can induce gel formation in egg white and yolk, crude carp actomyosin, and a suspension of soy protein by the application of 1000 – 7000 atm pressure at 25°C for 30 min (Farr, 1990). The most unique property of HHP is its ability to be transferred instantly and uniformly throughout food system. Thus, the application of HHP is independent of sample mass and geometry. Other important advantages in using this technology in food industry are:

1. Inactivation of microorganisms and enzymes
2. Modification of biopolymers
3. Quality retention, such as color and flavor
4. Changes in product functionality (Knorr, 1993).

In order to implement this new technology in the food industry, we need to understand the mechanism and kinetics of pressure – induced degradation / denaturation / inactivation of several food compounds (e.g. nutrients, proteins, microorganisms, enzymes) and the way in which the degradation / denaturation / inactivation is induced by other parameters (e.g. temperature, pH).

This review provides a technical description of high hydrostatic pressure technology, along with a discussion of its application in food processing. Also, this review is concerned with the mechanism and kinetics of pressure-induced degradation / denaturation / inactivation of several food compounds and factors influencing the effect of high pressure on this components related to food quality.

HISTORY PERSPECTIVE

The first experiment in food science and technology, the most important work involving microbial inactivation was reported at the end of the 19th century by Hite (1899) and effects of pressure on physical properties of foods were reported soon after, by others such as Bridgman (1914) (on the coagulation of egg albumin); Payens and Hermens (1969) (effects of the pressure on the beta-casein from milk) and Macfarle (1973) (on pressure–tenderization of meat). After a period of lower research in this field, it has received considerable attention since it was rediscovered approximately 20 years ago.

HIGH PRESSURE EQUIPMENT DESIGNS

Although the effects of isostatic pressure on micro-organism inactivation and on protein and polysaccharide

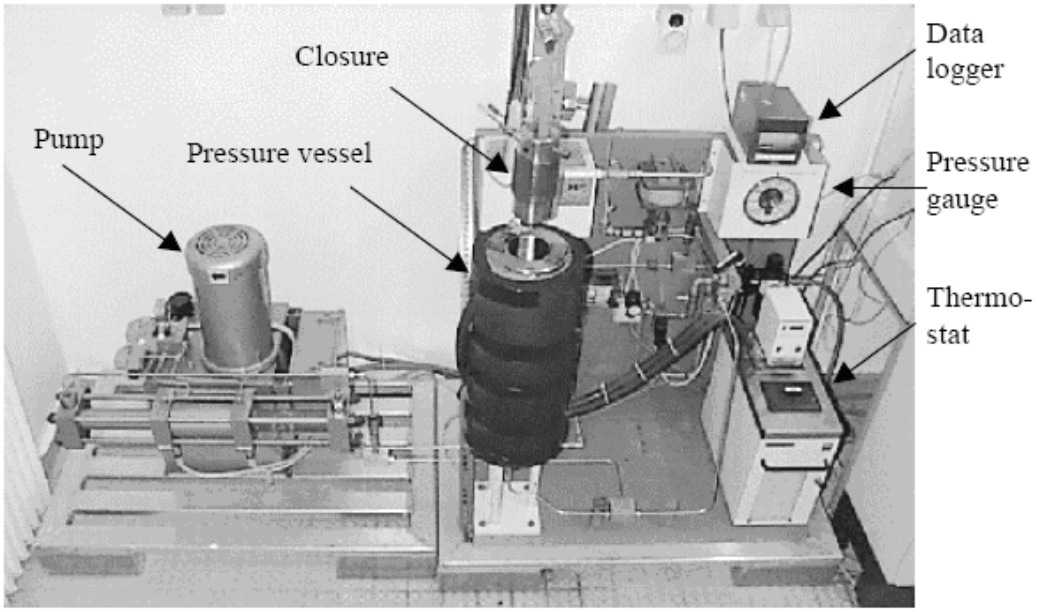

Figure 1.A lab-scale high hydrostatic pressure system. The maximum pressure of the system is 600 MPa with a filling volume of 0.6 L, Temp.: 0℃ to 95℃ (National Forge Europe, Belgium).Redrawn from (Dong-Un Lee ,2002).

denaturation have been known over 100 years ago by Hite, because of technical difficulties and costs associated with high pressure processing units and packaging of materials, the food processing industry did not become interested in applying this technology until recent decade. The interest on the high pressure processing / preservation of foods has been triggered by results of research at universities and institutes and by the efforts of equipment suppliers who recognize a promising new market in high pressure technology. Although high pressure technology is currently more expensive than traditional processing technologies (e.g., high-temperature sterilization), the use of high pressure offers new opportunities for food industry to respond to consumers wishes (Hendrickx et al., 2005a).

GENERATION AND DELIVERY OF HIGH HYDROSTATIC PRESSURE

Pressure is a thermodynamic variable present in the biosphere. In nature, hydrostatic pressure increases by 1 atm pressure for every 10 m depth of water. Thus the pressure at the bottom of Mariana Trench, one of the deepest seas in our planet, reaches up to 116 MPa (Yayanos, 1998). However, HHP in food processing uses the pressure range of 100 - 1000 MPa, which is almost the same level or even 10 times higher than the pressure of the deepest sea. Thus special equipment is needed to generate and endure such high pressures. A typical HHP system consists of a high pressure vessel and its closure, a pressure generation system, a temperature control device (Figure 1). The heart of the HHP system is obviously the pressure vessel, which is in many cases, is a forged monolithic, cylindrical vessel constructed in low alloy steel of high tensile strength. The wall thickness of the monobloc vessel determines the maximum working pressure.

Depending on the internal diameter of the vessel, the use of monobloc vessels is typically limited to maximum working pressure of 400-600 MPa. In case higher pressures are required, pre-stressed vessel designs like multilayer vessels or wire-wound vessels are used (Mertens, 1995). HHP can be generated either by direct compression (Figure 2) and indirect compression (Figure 3). In the case of direct, piston-type compression, the pressure medium in the high pressure vessel is directly pressurized by a piston, driven at its larger diameter end by a low pressure pump. The indirect compression method uses a high pressure intensifier which pumps the pressure medium from the reservoir into the closed and de-aerated high pressure vessel, until the desired pressure is reached. Most of the industrial cold, warm and hot isostatic pressing systems use the indirect pressurization method (Mertens, 1995). The cold isostatic pressing (CIP) process can be either "wet bag (free mould)" in which the mould is filled outside the pressure vessel and then placed into the pressure vessel, and directly immersed in the fluid. These installations are used for pressing larger parts and complex forms. Or "dry bag (fixed mould)" in which case, the mould is fixed inside the pressure vessel and filled *in situ*. It is separated from the pressure fluid by an elastomeric sleeve. These machines are mostly used for smaller parts such

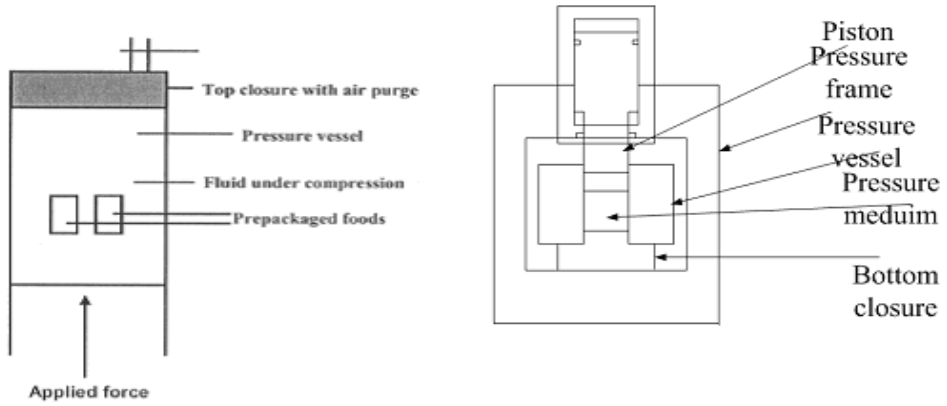

Figure 2. Direct method for generation of high isostatic pressure uses a piston driven by a low-pressure pump.

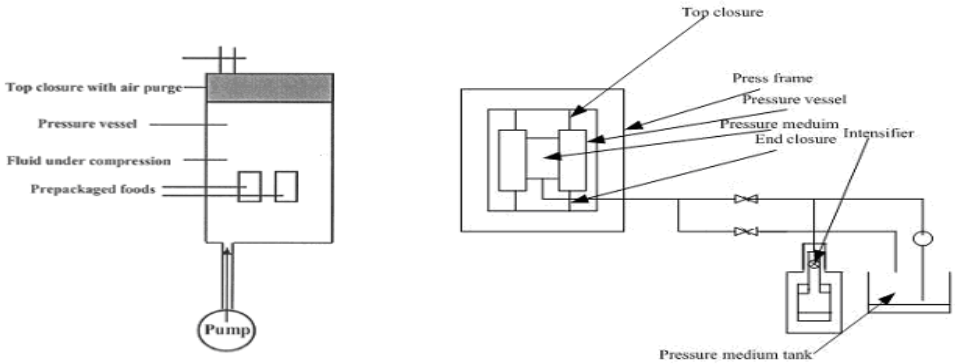

Figure 3. Indirect method for generation of high isostatic pressure uses high-pressure intensifier to pump pressure medium into closed vessel.

as tubes, rods and nozzles. For food processing applications, both dry-bag and wet-bag processes are of interest but wet bag is more suitable for food processing. A variety of flexible packaging may be used to contain samples for high-pressure processing.

Common packaging materials used in food high pressure processing, are ethylene vinyl alcohol copolymer (EVOH) and polyvinyl alcohol (PVOH). Pressure-transmitting fluids are used in the vessel to transmit pressure uniformly and instantaneously to the food sample. Some of the commonly used pressure-transmitting fluids are water, food-grade glycol–water solutions, silicone oil, sodium benzoate solutions, ethanol solutions, inert gases and castor oil. The sealed packages are sufficiently flexible to withstand the compression which occurs during pressurization. The pressure medium and the pack contents are compressed to about 80 – 90% of their original volumes during pressurization in the 400 – 800 MPa pressure range, but of course, return to their original volumes when the pressure is released. Also, because of the low volume contraction of liquids and solids, particulate products such as foods are not mechanically damaged during pressurisation. The elastic capacity of many foods helps them to recover their original structure and shape (Rovere, 2001). There are three major types of high pressure processing of food products: the (conventional) batch systems derived from cold isostatic processing, semicontinuous and continue systems. Batch systems can process both liquid and solid products, but these have to be pre-packed. In-line systems can be applied only to pumpable products (e.g. fruit juice). The product is pumped into the pressure vessel and pressurised using a floating piston, which separates the product from the pressure medium. For batch systems, the overall cycle time is the sum of the number of single steps: filling, closing, pressure built up, pressure holding, pressure releasing, opening, taking out (Figure 4). For liquid products continuous treatment also is possible using tube reactors or special valve systems (Van den Berg et al., 2002). In a packed product immersed in a compressed liquid, the pressure is transferred homogeneously and instantaneously throughout the product. Under these conditions, the product is iso-pressed, since its internal

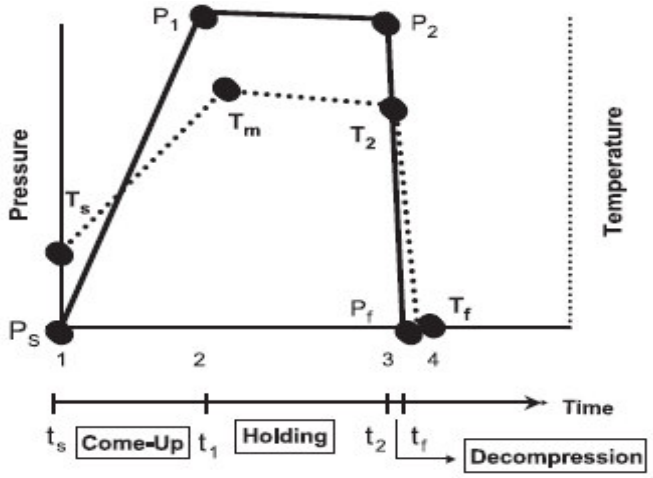

Figure 4. Typical variables (pressure, temperature, and time) to indicate conditions of HPP testing. Ambient pressures Ps and Pf are typically 0.1 MPa. Tm is the maximum temperature at process pressure. The difference between ambient temperatures before and after high-pressure processing. (Ts and Tf) generally indicates the extent of heat loss during testing (with the assumption that the depressurization time is within a few seconds). Redrawn from Balasubramaniam et al 2004

pressure becomes equalised to the external one. In practical terms, the gaseous part of the product nearly disappears completely while the liquids and solids remain according to their compressibility.

MAJOR MANUFACTURERS OF HIGH PRESSURE PROCESSING EQUIPMENT

Worldwide, a number of manufacturers have developed ultra-high pressure equipment, each of them starting from their own area of expertise. Three major players dominate the market:

- Flow International Corporation, (United States and Sweden)
- ACB Pressure System-Alstom, (France)
- Kobe Steel (Japan)

Manufacturers started developing this equipment as early as 40 years ago, mainly focusing on processes such as hot and cold isostatic presses. A decade ago, equipment manufacturers started to develop high pressure equipment dedicated to the treatment of food products. A typical example of the scale of these units is the Avomax high pressure processing facility installed by Flow, which has a capacity of 215 L operating at 600 MPa. Whereas the large equipment manufacturers focus mainly on industrial-scale equipment for the food industry, a large number of smaller companies develop laboratory-scale and pilot units for use at research institutes and universities (Hendrickx et al., 2005a). The French and British governments have recently launched R and D programs on high pressure food processing, while a group of university laboratories and industries headed by Dietrich Knorr of the Technical University of Berlin have received European Community funding for a basic research program on biological effects of high pressure. In addition several European food processors are starting their own R and D program in this area.

ECONOMICS OF HIGH PRESSURE PROCESSING

An important criterion in the evaluation of preservation technologies is the cost per amount of treated product (Ting and Farkas, 1995). Although, high pressure processing is a very promising technique for increasing product shelf life, it is more expensive than conventional methods such as, high temperature sterilization. For this reason, high pressure processing is mainly used for niche products such as fresh fruit juices, sea foods and guacamole. Currently, costs of the high pressure processing range from 10 - 20 cents/L, whereas costs for high temperature treatment may be as low as 2 - 4 cents/L. A commercial scale, high-pressure vessel costs between $500,000 to $2.5M depending upon equipment capacity and extent of automation. Wire winding increases equipment costs leading to the present definition of low cost operations such as oyster shucking requiring 200 – 400 MPa separated by a technology barrier at ~400 MPa from higher cost operations such as guacamole salsa production at ~600 MPa (Hendrickx et al., 2005a).

SAFETY OF HPP EQUIPMENT OPERATION

High pressure equipment design is a mature technology and has its origin in the chemical processing industry. Most high-pressure vessels are manufactured under guidelines established by the American Society of Mechanical Engineers (ASME) boiler and pressure vessel codes. Processors should also ensure that the vessels are manufactured, installed, tested, and operated according to relevant state regulations. With a little training, food plant personnel can learn to safely operate the equipment (Hendrickx et al., 2005).

INDUSTRIAL-SCALE HIGH PRESSURE PROCESSING OF FOODS

Although food preservation by pressure was demonstrated by extensions in the shelf-life of milk, fruits and vegetables (Hite et al., 1914), difficulties in applying the technology delayed commercial use for food preservation until the 1980s (Mertens, 1995), when processes were developed for the non-thermal pressure pasteurization of a number of low-pH foods, in which survival of spores was not a problem because they were unable to outgrow under the acid conditions. Currently, food products that

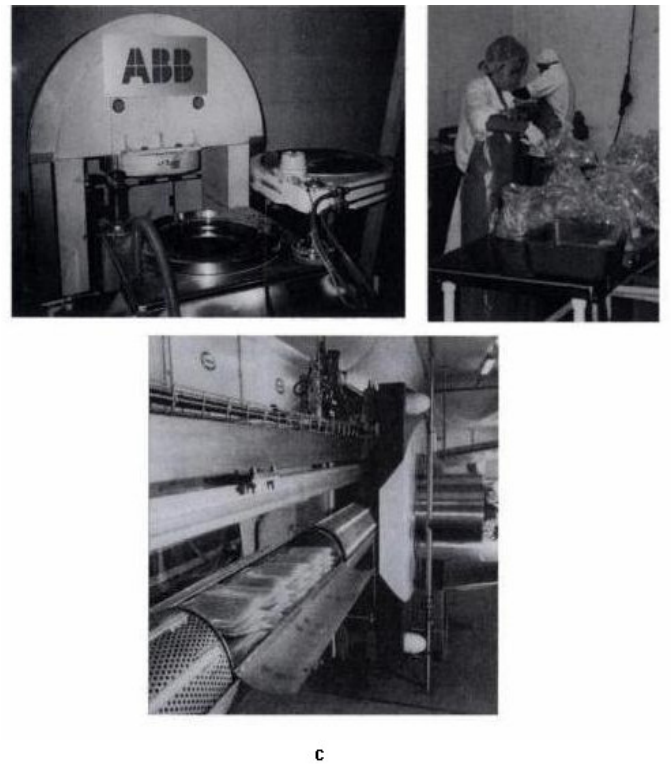

Figure 5. High pressure production of avocado product(A) installed high pressure autoclave at Avomex .Inc.(New Mexico).(B) preparation of avocado paste at Avomex.(C)high pressure processing of cooked ham at Espuna SA,CO, in Olot. Spain. (Redrawn from Hendrickx et al., 2005b.)

have been brought to the market or that employ HHP in their manufacture include fruit jellies and jams, fruit juices, vegetables, milk, yogurt, cheese, fish, pork, beef, pourable salad dressings, raw squid, rice cakes, sliced ham (cured-cooked and/or raw-cooked), cooked ready-to-eat meats and guacamole (avocado puree).

Examples of commercial pressure treated products in Europe and US are: Orange juice by UltiFruit®, Pernod Ricard Company, France; avocado puree (Guacamole) by Avomex Company in US (Texas/Mexico); and sliced ham (cured-cooked and/or raw-cooked) by Espuna Company, Spain (Tewari et al., 1999) (Figure 5).

According to Torres et al. (2005), reports a computer search using Food Science and Technology Abstracts (FSTA) and AGRICOLA revealed ~100 research publications in the 1980's, close to 2000 in the 1990's and >1000 since year 2000. These findings will support existing and lead to new high pressure processing businesses. (Torres et al., 2005).

ADVANTAGES AND LIMITATIONS OF HIGH PRESSURE

Some of advantages and limitations of using hydrostatic pressure method are:

Some advantages of HHP

1. High pressure is not dependent of size and shape of the food.
2. High pressure is independent of time/mass, that is, it acts instantaneously thus reducing the processing time.
3. It does not break covalent bonds; therefore, the development of flavors alien to the products is prevented, maintaining the natural flavor of the products.
4. It can be applied at room temperature thus reducing the amount of thermal energy needed for food products during conventional processing.
5. Since high pressure processing is isostatic (uniform throughout the food); the food is preserved evenly throughout without any particles escaping the treatment.
6. The process is environment friendly since it requires only electric energy and there are no waste products.

Some limitation of HHP

1. Food enzymes and bacterial spores are very resistant to pressure and require very high pressure for their inactivation.
2. The residual enzyme activity and dissolved oxygen results in enzymatic and oxidative degradation of certain food components.
3. Most of the pressure-processed foods need low temperature storage and distribution to retain their sensory and nutritional qualities.

EFFECTS OF HIGH HYDROSTATIC PRESSURE ON MICROORGANISMS

Microbial inactivation is one of the main tasks for the application of high pressure technology. Many reports have demonstrated the inactivation effect of HHP on microorganisms, extending in this way the microbial shelf life and improving the microbial safety of food products. It has been demonstrated that bacterial spores require more extreme conditions of pressure and temperature and longer treatment times to be inactivated (Sale et al., 1970). In general, vegetative cells are inactivated at low pressure levels, around 400 - 600 MPa, while more resistant bacterial spores can survive pressures higher than 1000 MPa (Patterson et al., 1995).

INACTIVATION MECHANISM

High hydrostatic pressure brings about a number of changes in the morphology, cell membrane or biochemical reactions of microorganisms and all these processes are related to the inactivation of microorganisms. For

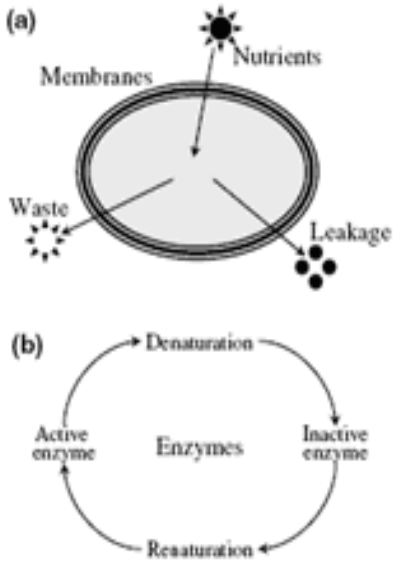

Figure 6. Hydrostatic pressure effects on selected microbial functions: (a) cellular membranes; (b) microbial enzymes. Redrawn from Torres, et al. (2005)

instance, Hamada et al. (1992) noticed changes in colony form after pressure treatment of *Saccharomyces cerevisiae*. Especially, the cell membrane is considered to be the major target for the pressure-induced inactivation of microorganism, and it is generally accepted that the leakage of intracellular constituents through the permeabilized cell membrane is the most direct reason of cell death by high pressure treatment. However, if applied pressure was not severe enough to induce a total permeabilization of cell, the permeabilization took place only in the outer membrane, in the case of gram negative bacteria the permeabilized membrane was rapidly restored after pressure release (Hauben et al., 1996). The fluidity of cell membrane plays an important role in the susceptibility of microorganisms to pressure treatments. Microorganisms with less fluid membranes are more sensitive to HHP (Macdonald, 1992). Conversely, increased membrane fluidity protected against pressure inactivation (Steeg et al., 1999). The denaturation of key enzymes in microorganism by pressure has been regarded by another important reason of cell death. Because intracelluar enzymes seemed not to be a determining factor of pressure resistance (Simpson and Gilmour, 1997), membrane bounded ATPase was considered to such a key enzyme (Wouters et al., 1998). Bacterial spores are known to be pressure resistant, and the inactivation mechanism is different from that of other vegetative microorganisms. It was assumed that pressure caused inactivation of spores by first initiating germination and then inactivating germinated forms (Sale et al., 1970).

Hydrostatic pressures of 100 – 300 MPa can induce spore germination and resultant vegetative cells are more sensitive to environmental conditions. Biochemically, binding of germinant to it's receptor is believed to promote the germination process followed by the efflux of Ca^{2+} and other ions and the influx of water into the spore, which result in the activation of pore specific cortexlytic enzyme (Wuytack et al., 2000). This germination process can be enhanced by pressure treatments (as a general rule (Le Chateler's principle)), because the volume of system decreases during germination as a result of increased solvation of spore's components (Clouston and Wills, 1969; Heinz and Knorr, 1998). It was also suggested that pressure-induced germination involves activation of physiological pathway and is therefore not merely a physicochemical process in which water is forced into the spore protoplast, because inhibitors of nutrient-induced germination also inhibit pressure-induced germination (Wuytack et al., 2000) (Figure 6). However, when high pressure is applied to spores with elevated temperature, the cortexlytic enzymes in spores could be directly inactivated and the inactivation of spores took place without the germination step (Heinz and Knorr, 2001).

The overall pattern of spore inactivation showed a strong pressure–heat synergism. The effect has been confirmed for a wide range of spore types, although the effectiveness of the combination varies greatly in magnitude for different spores (Murrell and Wills, 1977; Kimugasa et al., 1992; Kowalski et al., 1992; Seyerderholm and Knorr, 1992; Hayakawa et al., 1994). The kinetics of pressure inactivation was approximately exponential for spores of *Bacillus pumilus* (Clouston and Wills, 1970), but for spores of *Bacillus coagulans, Bacillus subtilis* and *Clostridium sporogenes* concave-upward curves or long tails were reported (Sale et al., 1970). The fact that, although spores of some species are relatively sensitive to pressure (e.g. *Bacillus cereus*), those of other species, including some of special importance in foods such as *Bacillus stearothermophilus* and *Clostridium botulinum*, are very resistant (Knorr, 1995); has so far prevented the use of pressure to sterilize foods (Hoover, 1993). This situation may change with the development of presses that operate at higher temperature–pressure combinations, or the development of other effective combination techniques. For instance, the presence of bacteriocins such as nisin can amplify the effect of pressure against spores, for example, of *B. coagulans* (Roberts and Hoover, 1996).

INACTIVATION OF MICROORGANISMS IN FOODS

The first report on the HHP processed commercial food product was fruit jams by Horie et al. (1991) from the Meidiya food factory Co. in Japan. Strawberry jams were processed at 294 MPa for 20 min; elimination of yeast was reported as well as bacteria. Nutritionally, the pressure-processed strawberry jam retained 95% of its vitamin C compared to fresh product. The application of

HHP to fruit products has been considered to be the most effective and realistic, because the inherent low pH of fruits can inhibit the growth of most spoilage bacteria. Further, the yeast and mold which survive such low pH range are relative susceptible to HHP (Aleman et al., 1996; Raso et al., 1998; Garcia-Graells et al., 1998; Linton et al., 1999; Zook et al., 1999; Prestamo et al., 1999). In this way, shelf-life extension of fresh-cut pine-apple was achieved by application of 340 MPa/15 min (Alemen et al., 1994). Parish studied HHP to orange juice. For vegetative cells of *S. cerevisiae*, D-values were between 1 – 38 s for treated material at pressures betw-een 500 and 350 MPa. The native flora of the orange juice showed D values ranging from 3 and 74 s (Parish, 1998). HPP application on non-thermal pasteurized rice wine was examined by Hara et al. (1990). No viable lactobacilli and yeasts recovered using a treatment at 294 Mpa/10 min at 25°C.

The spoilage bacteria in vegetables come from soil and the varieties of them are extremely wide. Lettuce and tomatoes were inoculated and pressurized at 20 MPa for 10 min, pressures of 300 and 350 MPa reduced population of gram-negative bacteria, yeasts and molds by at least one cycle but the treatment did cause changes in appearance and structure in tomatoes and lettuce browned (Arroyo et al., 1997). Other examples of HHP applications on vegetable products are the increased shelf life of vegetable juices by HHP (Lee et al., 1996) or fermentation control without the quality loss of fermented vegetable (Sohn and Lee, 1998). However, the limiting parameter of this products processing is often the presence of browning enzymes which need higher pressure levels to inactivate (Eshtiaghi and Knorr, 1993; Seyderhelm et al., 1996).

High pressure treatments of protein rich foods such as egg, meat, or fish are limited because HHP induces protein denaturation. Further, these food materials have fairy high fat content which is known as a common baroprotective component for microbial inactivation (Styles et al., 1991).

Applications of high pressure to liquid egg products are very scarce. *Listeria* and *E. coli* were inoculated in liquid whole egg and pressurized up to 450 MPa (Ponce et al., 1998). Substantial levels of microbial inactivation were accomplished by this pressure treatment. Moreover the kinetic studies on the HHP inactivation of microorganisms of the whole egg were performed at 250 MPa for 886 s or 300 MPa for 200 s at the treatment temperature of 5 and 25°C. These processing conditions ensured the minimum changes in the rheological properties of LWE (Liquid whole egg), and effectively reduced the microbial loads of LWE. The combination processes of HHP with other non-thermal treatments were explored to achieve further microbial inactivation (Dong-Un, 2002).

Fresh minced meat is a highly perishable product, whose shelf life is limited by the growth of different strains of spoilage bacteria contaminated during different steps of processing such as mincing, mixing or packaging (Carlez et al., 1994; O'Brien and Marshall, 1996). Pres-surized minced beef meat and chicken respectively, are found with considerable decreases in mesophile counts and extension of shelf-life. Likewise, high-pressure allowed microbial sanitation of mechanically deboned chicken meat (Rovere et al., 1997).

High pressure treatments inactivated citrobacter, pseudomonas and listeria in minced meats (Carlez et al., 1993). Listeria was the most resistant (D = 330; MPa = 6.5 min) among the three species. Higher (50°C) or lower (4°C) temperature enhanced the effects of pressure treatments. However, partial discoloration of minced beef was observed above 150 MPa. Processed meat product such as spreadable sausage may be more adequate to pressure treatment than fresh meat. Inactivation kinetics of *E. coli* and *Listeria innocua* inoculated in spreadable sausage were investigated and pressure-time contours for 5 log cycle reductions of microorganisms were established (Zenker et al., 2000). Changes in the microbiological quality of vacuum-packaged, minced chicken treated with high hydrostatic pressure at 500 MPa for 15 min at 40 and stored at 3 studied by Linton et al. (2004). Enterobacteriaceae comprised 44% of the microflora in untreated minced chicken after storage for 31 days at 3, but no Enterobacteriaceae were detected at any time in pressure-treated samples. Some authors improved the microbiological quality of nisin added (Yuste et al., 1998; Yuste et al., 2000); combination of nisin and acidification (Yuste et al., 2002) mechanically recovered poultry meat (MRPM) and cooked sausages by means of pressurization. Shigehisa et al. (1991), Patterson et al. (1995) reported the effectiveness of pressure treatment against important food borne pathogens such as *Campylobacter jejuni*, *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus* and *Yersinia enterocolitica*.

The higher content in free amino acids and nitrogenous materials make fresh fish quite susceptible to spoilage microorganisms. A number of studies have demonstrated that HHP can extend the shelf life of fish products such as cod (Ohshima et al., 1993), minced mackerel (Fujii et al., 1994), prawns (Lopez-Caballero et al., 2000), smoked salmon cream (Capri et al., 1995) or surimi (Miyao et al., 1993). It achieves this by controlling or inactivation sea food-related spoilage enzymes, modi-fying texture, and stabilizing colour and lipid oxidation Milk was found to provide the microorganisms protection against HHP. Only 2 log cycles of inactivation at 340 MPa was observed when Listeria was treated in milk whereas almost 7 log cycles of inactivation was observed when the same microorganisms were treated in buffer solution (Styles et al., 1991). The protective effect of milk against HHP was observed again with other species of micro-organisms (Patterson et al., 1998). However, there were no significant difference between skim and whole milk.

HHP of 400 MPa at 7°C reduced aerobic plate counts of whole milk and skim milk equally by 1 log cycle. The pressure treatment did not inactivate the plasmin in milk, since considerable *β* and *α*-casein hydrolysis took place during refrigerated storage after HHP (Garcia-Risco et al., 1998).

All these results indicated that the medium in which the microorganisms are treated is an important determinant factor of the level of inactivation by HHP. Most research to date has concentrated on the application of HHP to inactivate microorganisms in cheese to increase cheese safety and shelf life. Gallot-Lavallee (1998) studied the efficiency of HHP treatment for destruction of *L. monocytogenes* in goat cheese from raw milk finding that 450 MPa for 10 min or 500 MPa for 5 min treatments achieve more than 5.6 log units of reduction of this microorganism without significantly affecting sensory characteristics of cheese. Prestamo et al. (2000) reported that, the microbial population of tofu pressurized at 400 MPa and 5°C for 5, 30, and 45 min decreases from an initial microorganism count of 5.54×10^4 cfu/g to 0.31, 1.56, or 2.38 log units, respectively.

CRITICAL PARAMETERS FOR MICROBIAL INACTIVATION BY HIGH HYDROSTATIC PRESSURE

Primary factors

Besides type of microorganism, composition of suspension media or food, pressure level and treatment time, the critical parameters for high pressure induced microbial inactivation are pH, water activity (a_w) and the treatment temperature. Various combinations of these parameters have been investigated and the general rules are as follows:

1) Microorganisms become more susceptible to pressure at lower pH and recovery of subletally injured cells is reduced. Further, sub lethally injured microorganisms induced by HHP can be reactivated in a nutrition-rich environment, but fail to repair at acidic conditions (Linton, 1999).
2) A reduction of water activity exerts protective effect for microorganisms against pressure treatments (Oxen and Knorr, 1993; Palou et al., 1997).
3) The treatment temperatures above or below room temperature tend to increase the inactivation rate of microorganisms (Knorr and Heinz, 1999).

Secondary factors

Other factors influence the effectiveness of HPP. For example, the redox potential of the pressure menstruum may also play a role in the inactivation for some microorganisms (Hoover, 1993).

In addition to the above, a factor that significantly influences the effectiveness of HHP treatment on the inactivation and consequently the reduction in microbial population is the medium composition in which micro-organisms are dispersed. Food constituents such as sucrose, fructose, glucose and salts affect the baro-resistance of microorganisms present in food (Oxen and Knorr, 1993).

EFFECTS OF HIGH PRESSURE ON CHEMICAL REACTIONS RELATED TO FOOD QUALITY – EFFECTS OF HIGH HYDROSTATIC PRESSURE ON CHEMICAL BONDS

The most basic concept to interpret the effects of pressure on chemical reactions is the Principle of Le Chatelier. Pressure primarily reduces the volume of a system. Under equilibrium conditions, according to the Le Chatelier principle, processes associated with volume decrease are encouraged by pressure, whereas processes involving volume increase are inhibited by pressure (Butz and Tauscher, 1998). Pressure as an important thermodynamic variable can affect a wide range of biological structures and processes. Since the mechanism of UHP is based on decrease in volume, high pressure enhances the rates of chemical and biochemical reactions. Moreover, reactions involving formation of hydrogen bonds are favoured by high pressure because bonding results in a decrease in volume of the molecules (Pothakamury et al., 1995). UHP does not break down the covalent hydrogen, ionic or hydrophobic bonds. Covalent bonds are resistant to pressure, which means that low molecular weight food components responsible for nutritional and sensory characteristics remain intact during pressure treatment, whereas high molecular weight components whose tertiary structure is important for functionality determination are sensitive to pressure (Tewari et al., 1999).

With respect to food systems important characteristics of high-quality foods are texture, flavor, color, and nutritive value. The first three properties are correlated with purchase and consumption quality and determine consumer's acceptance of the product. Nutritive value (i.e. vitamins, minerals, and other nutrients) is a hidden quality. Chemical or biochemical reactions occurring in food products can bring about undesirable changes in or deterioration of these attributes of food quality during preservation / processing treatments and subsequent storage. High pressure allows inactivation of pathogenic / spoilage microorganisms and food-spoiling enzymes while leaving most attributes of food quality intact (Hayashi et al., 1989; Mertens, 1992; Knorr, 1993; Galazka and Ledward, 1995; Thakur and Nelson, 1998). This advantage is attributed to the fact that high pressure keeps covalent bonds in tact and affects only non-covalent bonds (such as hydrogen, ionic and hydrophobic bonds).

THE EFFECT OF HIGH PRESSURE ON TEXTURE, FLAVOR, COLOR AND NUTRITIVE VALUES OF FOOD PRODUCTS

The color retention effects of high pressure for many fruit and vegetable products such as orange juice, fruit jam, tomato juice investigated by some researchers. Chemical and spectrophotometrical analysis showed that high pressure treatment largely preserves fresh color. Also, the effect of high pressure on meat and meat products has been studied by many authors because high pressure processing may offer potential to preserve and restructure meat, provided that, the red color can be maintained. For fruit jams (e.g., strawberry jam) high pressure was found to retain fresh flavor much more than traditional thermal processing (Watanabe et al., 1991; Kimura et al., 1994; Dervisi et al., 2001).

In the experiments carried out by Zabetakis et al. (2000) on the effects of high hydrostatic pressure on strawberry flavour compounds, the highest flavour stability was observed when samples were treated with lower pressures <800 MPa and they were stored at 4 and 30°C. In studies conducted by Rodrigo et al. (2006), no colour degradation of tomato appeared under combined thermal and high pressure treatment (300 – 700 MPa, 60 min, 65 °C). An extensive study on the effect of pressure on the texture of fruits and vegetables has been carried out by Basak and Ramaswamy (1998). They observed that, the change in firmness of treated samples to be dependent on both pressure level and pressurization time.

Suzuki et al. (1994) studied the effect of pressure on water-soluble compounds responsible for taste and meaty flavor. The amount of peptides and amino acids estimated as phenol-reagent-positive materials apparently increase with increasing pressure up to 300 MPa (5 min at 2 °C).

The effect of pressure on the flavor of milk and dairy products has only been studied to a very limited extent. High pressure processes at 400 – 500 MPa for 3 – 15 min followed by refrigeration were found to result in a shelf-life comparable to that of thermal pasteurization; however, this report made no mention of sensory properties of the pressure-processed milk (Rademacher and Kessler, 1996). High pressure treatment only slightly influenced milk viscosity. The building up of milk viscosity can be described by a first-order reaction. Increase in viscosity has been explained to be the result of casein micelle disintegration. The properties of acid-set gels prepared from HHP treated milk have been reported by Johnston et al. (1994). Results indicate improved texture (rigidity and resistance to breaking) and syneresis resistance of the gels, measured by drainage or by centrifugation.

In studies conducted by Ancos et al. (2000), the effects of high pressure on the physicochemical, chemical, microbiological and sensory characteristics of stirred low-fat yoghurt; laboratory-made yoghurts were treated at high pressure (100-400 MPa) for 15 min at 20 °C. Pressurized yoghurts exhibited higher viscosity and amino acid contents than did the untreated controls, and the differences were maintained after chilled storage. The application of HHP technology to cheese milk causes differences in cheese composition and ripening in comparison to pasteurised milk cheese. The HHP-treated milk cheeses have higher moisture, salt and total free amino acids contents than raw or pasteurised milk cheeses (Trujillo et al., 1999a,b). In relation to cheese texture and microstructure, Buffa et al. (2001) using uniaxial compression and stress relaxation tests, and confocal laser scanning microscopy showed that cheeses made from raw or HHP treated milk were firmer and less fracturable than cheeses made from pasteurised milk, but differences became less notable toward the end of ripening. Cheeses from pasteurised and HHP-treated milk were less cohesive than from raw milk. Although cheese exhibited a loss of elastic characteristics with ageing, cheeses from HP treated milk were the most elastic initially.

EFFECTS OF HIGH HYDROSTATIC PRESSURE ON PROTEIN SYSTEMS

Proteins are linear polymers of amino acids, for which three or four levels of structural organization can be discerned. The primary structure, which is defined as the amino acid sequence and the location of disulphide bonds (if any), gives a complete description of the covalent bonds of a protein. The secondary structure refers to the way in which the polypeptide chain forms α-helices or β-sheets by intra-or intermolecular hydrogen bonds. The tertiary structure describes how the secondary structure domains fold into a three-dimensional configuration as a consequence of non-covalent interactions between amino acid side chains. The quaternary structure refers to the spatial arrangement of subunits, held together by non-covalent bonds between the polypeptide subunits (multi-meric proteins).

Proteins are delicate structures, maintained by interactions within the protein chain (determined by the amino acid sequence) and by interactions with the surrounding solvent. Changes in environmental conditions such as solvent composition, temperature and pressure can perturb the subtle balance of intermolecular and solvent-protein interactions and can therefore, lead to (complete) unfolding / denaturation of the polypeptide chain. The term ‘denaturation of protein’ indicates the phenomena in which the higher structure of protein is ruptured by environmental changes, while the primary structure is kept without damage (Masson, 1992). The effect of high pressure on proteins has been the subject of many reviews. Pressure treatment can cause a variety of effects on proteins, inducing reversible or irreversible structural modifications leading to protein denaturation, aggregation or gel formation. Structural rearrangements

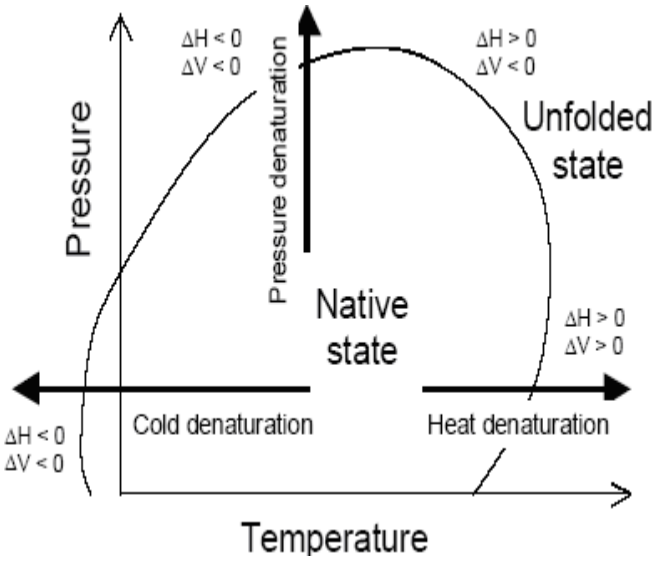

Figure 7: Typical phase transition curve of proteins in the PT-diagram. The relation between heat, cold and pressure-denaturation of proteins is presented by the sign of enthalpy changes (ΔH) and volume changes. (ΔV), Redrawn from Heremans (2002).

taking place in the protein upon pressurization are governed by the principle of Le Chatelier, which states that "processes associated with a volume decrease are encouraged by pressure increases, whereas processes involving a volume increase are inhibited by pressure increases". Gelation by high pressure treatment is due to a decrease in the volume of the protein solution; in contrast, heat denaturation of protein is caused by the violent movement of molecules leading to the destruction of non-covalent bonds. Covalent bonds are unaffected by high pressure, thus the primary structure of proteins remains intact during pressure treatment. Secondary structures of proteins change at very high pressures, which might be explained by the cleavage of hydrogen bonds, which are enhanced at low pressures (Knorr, 1999). These changes depend on the nature and concentration of the protein, as well as on the applied pressure, temperature, treatment time and characteristics of the surrounding environment, such as pH or ionic strength (Rovere, 2001).

Pressure versus temperature effects

Quantitative evaluation of pressure-induced protein denaturation proposed p/T diagrams (Figure 7) which revealed elliptic contours indicating that proteins can yield heat and cold denaturation. One of the practical consequences of this phenomenon is the stabilization against heat denaturation by low pressures. Not only has this been observed in several proteins and enzymes, but it also applies to the effect of pressure on the heat gelation of starch (Thevelein et al., l981; Rubens et al., 1999).

A fundamental difference between pressure and temperature induced protein denaturation is that no change in covalent bonding has been observed in the pressure induced protein denaturation (Masson, 1992). Further, the structure of pressure denatured proteins differs significantly from that of heat denatured proteins. The pressure denatured proteins are relatively compact and retain elements of secondary structure while the heat denatured proteins have the extended, nearly random coil configurations (Ghosh et al., 2001).

Effects of high hydrostatic pressure on food protein

It is well-known that pressure affect the protein and leading to protein denaturation, aggregation or gel formation. The first systematic observation about the denaturation of proteins by high pressure was made by Bridgman (1914) treating egg albumin. He observed that the appearance of the pressure induced coagulum is quite different from that induced by temperature and the ease of pressure induced coagulation increases at low temperatures. However, more systematical studies on the pressure induced denaturation of protein have been conducted after 50 years later using ovalbumin (Suzuki et al., 1960), chymotrypsinogen (Hawley, 1971), or metmyoglobin (Zipp and Kauzmann, 1973). Ovalbumin, the main component of egg white, was denatured under high pressure, as confirmed by the decrease in its α-helical content and DSC endothermic enthalpies (Hayakawa et al., 1992). The results indicated that only conformational changes are induced by pressure treatments because no change in PAGE pattern was observed. Ovalbumin does not form a gel when pressurised for 30 min at ~400 MPa. This relatively high-pressure stability may be due to the presence of four disulfide bonds and strong non-covalent interactions stabilising the three dimensional structure of the protein. Gelation does occur, however, at higher pressures.

Doi et al. (1991) studied the effects of pressure on the stability of network bonds in heat-induced ovalbumin gels. They placed a steel ball on top of a heat-set gel and then pressure-processed. It was found that at pressures 400 MPa, the steel ball penetrated deep into the gels, suggesting that primarily hydrophobic and electrostatic interactions were destabilised in the gel, resulting in the partial melting of the gel. In contrast, cold-set gelatine gels did not melt under pressure treatment at 600 MPa, which suggested that hydrogen bonds were not destabilised by pressure processing. Other researchers demonstrated that ovalbumin gels produced by high pressure processing were more elastic and softer than heat-induced gels although the gels tended to become harder and less adhesive with increasing pressure. Taste and flavor of pressure-induced gels were free of cooked flavor and there was no destruction in vitamins and amino acids (Okamoto et al., 1990; Hayashi et al., 1989). Also, in the study conducted on the effect of pressure at diffe-

rent temperature (10, 25, and 60 °C) and pH (7.6 and 8.8) levels on selected properties of egg white solutions, it is observed that pressure induced an increase in turbidity, surface hydrophobicity, exposed SH content and susceptibility to enzymatic hydrolysis, while it resulted in a decrease in protein solubility, total SH content, denaturation enthalpy and trypsin inhibitory activity. Moreover, it is reported that, the pressure-induced changes in the selected properties were dependent on the pressure and temperature applied and the pH (Plancken et al., 2005).

Effects of pressure on milk proteins have been extensively investigated. β-Lactoglobulin, the main whey protein component is sensitive to high-pressure treatment. Solution studies (Dumay et al., 1994; Galazka et al., 1996) of native β-lactoglobulin have indicated that pressure treatment has a notable effect on the protein's conformational and aggregation properties, which are more extensive at higher concentrations (Olsen et al., 1999). It has been reported that the extended application of high pressure up to 800 MPa has no influence on the surface activity of Calcium-free bovine β-casein (Dickinson et al., 1997). This behavior related to the little ordered secondary and no tertiary structure of Calcium-free. However, in milk itself, where the casein is complexed with colloidal calcium phosphate, the quaternary structure of the casein micelle is substantially affected by high pressure. The gel strength of high-pressure-induced whey protein concentrate gels as a function of pH has been studied by various researchers (Van Camp and Huyghebaert, 1995; Kanno et al., 1997; Walkenstrom and Hermansson, 1997).

Electron microscopy has revealed that heat-induced gels (0.1 MPa for 30 min at 80°C) are stronger and possess a greater number of permanent cross-links between the polypeptide chains. In contrast, high-pressure treatment (400 MPa for 30 min at 20°C) generates fragile gels with a more porous network and fewer intermolecular cross-links (Van Camp and Huyghe-baert, 1995; Dumay et al., 1998). The application of pressure treatments to cheese processing was investigated in order to accelerate ripening period (Messens et al., 2000; Johnston and Darcy, 2000). The rheological parameters and meltability of immature Mozzarella cheese were converged to those of ripened cheese by pressure treatment of 200 MPa due to casein dissociation and internal moisture redistribution. The hardness of cheese increased again above 200 MPa due to the protein denaturation and aggregation.

In Vegetable proteins such as soy, pea, wheat gluten and broad bean that are being widely used in the food industry for the formulation of new food products, high-pressure processing are considered to be a gentler processing operation in comparison to thermal processing.

Matsumoto and Hayashi (1990) and Okamoto et al. (1990) have found that a minimum pressure of 300 MPa for 10 - 30 min is required to induce gelation. It has also been reported that high pressure produces softer gels with a significantly lower elastic modulus than those made by heat treatment. Wheat gluten is often used for its unique cohesive and viscoelastic functional properties, with major application to wheat flours low in gluten. To generate novel textures and products, Apichartsrangkoon et al. (1998) subjected hydrated wheat gluten to pressure heat treatment in the range 200 – 800 MPa at temperatures 20 – 60°C with holding times from 20 – 60 min. The gels formed were very different from heat-processed gluten since they presented a more marked elasticity with high values of moduli of elasticity, which was derived from a 'Young's modulus' that was 2 – 3 times larger than the usual shear modulus (control).

The Effects of combined high-pressure in the range of 300 – 700 MPa, and heat treatment (90°C, 15 min) on the textural properties of soya gels was studied. It is observed when the solutions were pressurised before heat treatment, all the proteins formed self-standing gels (Molina and Ledward, 2003).

In studies conducted by Kato et al. (2000), rice grains (*Oryza sativa* L. Japonica var. Akitakomachi) immersed in distilled water exhibited solubilization and subsequent release of rice allergenic proteins in the range 100 – 400 MPa. In this range of pressure, considerable amount of proteins, 0.2 – 0.5 mg/g of rice, was released with maximum amounts obtained in the pressure range 300 – 400 MPa.

High pressure treatment at different temperatures will induce different effects on meat texture since the weak linkages stabilising the secondary, tertiary and quaternary structures of a protein respond differently to heat and pressure (Galazka and Ledward, 1998). Bouton et al. (1977) found that a pressure of about 100 MPa applied for 2.5 min or longer to post-rigor muscle at 40 – 60°C improved the tenderness of the meat and Beilken et al. (1990) reported that pressure treatment at 150 MPa during treatment at 40 – 80°C prevents the development of the myofibrillar component of toughness, but has little or no effect on the connective tissue component of toughness, other than to raise the temperature at which heat treatment alone produces a decrease in this component.

Fernandez-Martin et al. (1997) found that when pork batters were subjected to combinations of two pressures (200 and 400 MPa) and five heat treatments (10 – 70°C), pressurization may stabilize the proteins against subsequent thermal denaturation. Also, the effects of high pressure (800 MPa) applied at different temperatures (20 – 70°C) for 20 min on beef post-rigor longissimus dorsi texture were studied. Texture profile analysis showed that, when heated at ambient pressure there was the expected increase in hardness with increasing temperature and when pressure was applied at room temperature there was again the expected increase in hardness with increasing pressure. Myosin was relatively easily unfolded by both pressure and temperature and when pressure denatured; a new and modified structure was formed of low thermal stability (Ma and Ledward, 2004).

Most of the studies related to the application of high pressure to sea foods have been conducted on its effects on fish proteins (Angsupanich et al., 1999; Angsupanich and Ledward, 1998; Etienne et al., 2001; Lanier, 1998; Ohshima et al., 1993). The breakdown of ATP-related products is usually catalysed by certain dephospho-rylases inherent in fish muscles. Shoji and Saeki (1989) observed a decrease in IMP level in carp muscle treated at 350 and 500 MPa and subsequent storage at 5. This is due to protein denaturation and deactivation of enzymes (involved in the degradation of ATP and related compounds) during high-pressure treatment.

High pressure treatment of surimi has produced a new gel product with excellent flavour, lustre, density and elasticity, quite different from those treated by heat (Yoshioka and Yamada, 2002). Furthermore, the pressure-induced gels retained the natural qualities (that is, color and flavor) of the raw material without the formation of cooked color and flavor (Okamoto et al., 1990). Pressure-induced surimi gels from marine species like pollack, sardine, skipjack, tuna and squid have been reported to be smoother, more elastic and sensory superior to those produced by heat (Farr, 1990; Thakur and Nelson, 1998; Venugopal et al., 2001).

Effect of high pressure on enzymes

Enzymes are a special class of protein with an active site, formed by the three-dimensional conformation of molecules (Hendrickx et al., 1998). Generally, enzymes characterized by two striking properties their enormous catalytic power and their specificity quite often; the rate of an enzyme-catalyzed reaction is 10^6 to 10^{20} times that of an uncatalyzed one. Enzymes are highly specific, both in the type of reaction catalyzed and the choice of substrate. In view of the specificity of enzymatic reactions, enzymes may be affected by pressure in several ways (Cheftel, 1992):

1) Pressurization at room temperature may bring about reversible or irreversible, partial or complete enzyme inactivation resulting from conformational changes in the protein structure. These changes depend on the type of enzyme, micro-environmental conditions, pressure, temperature and processing time.
2) Enzymatic reactions may be enhanced or inhibited by pressure, depending on the volume change (positive or negative) associated with the reaction. Pressure-induced changes in the catalytic rate may be due to changes in the enzyme-substrate interaction, changes in the reaction mechanisms, or the effect of a particular rate-limiting step on the overall catalytic rate.
3) A macromolecular substrate (protein, starch) may become more sensitive to enzymatic action once it has been unfolded or gelatinized by pressurization.
4) Provided that the cell membrane or the membrane of intracellular organelles is altered, intracellular enzymes may be released from extra-cellular fluids or cell cytoplasm hereby facilitating enzyme-substrate interactions.

Mechanisms of pressure inactivation of enzymes

High pressure can result in both activation and inactivation of enzymes depending on the pressure level and conditions. Usually at low pressure, enzyme activity may be enhanced, and at higher pressure the activity is inhibited (Curl and Janson, 1950). Relatively low pressures of 100 – 200 MPa have been shown to activate monomeric enzymes, whereas higher pressures generally induce enzyme inactivation. Butz et al. (1994a) and Gomes et al. (1996) explained that beside conformational changes, the de-compartmentalization caused by pressure results in enzyme activation. When the food material is in tact, there is compartmentalization between the enzyme and substrates. Application of low pressure in food tissue damages membrane and enzyme leakage leads to enzyme-substrate contact. Sometimes the activity of enzymes is enhanced or curtailed due to pH changes resulting from releasing of some components in the environment by the induced pressure.

The effect of pressure on chemical or biochemical systems is described by the thermodynamical parameter (ΔV) (Equation 1):

$$\left(\frac{\delta \Delta G}{\delta P}\right) = -RT\left(\frac{\delta \ln K}{\delta P}\right) = \Delta V \quad (1)$$

the change of partial molar volume between initial and final state at constant temperature. It is governed by the principle of Le Chatelier, which predicts that application of pressure shifts equilibrium to the state that occupies the smallest volume. Hence, pressure favors reactions accompanied by a volume decrease and vice versa (Heremans, 1982; Gross and Jaenicke, 1994).

Kinetics of pressure inactivation of enzyme

In general, the decrease of enzyme activity (A) as a function of processing time (t) can be written as seen in Equation 2.

$$\left(\frac{dA}{dt}\right) = -kA^n \quad (2)$$

Often, enzyme inactivation can be described by a first-order kinetic model (Richardson and Hyslop, 1985; Lencki et al., 1992). It is suggested that, in the case of apparent first-order kinetics, one of the disruption formation of different interactions, decomposition of amino acids and aggregation / dissociation reactions in the enzyme inactivation predominates over the others. If

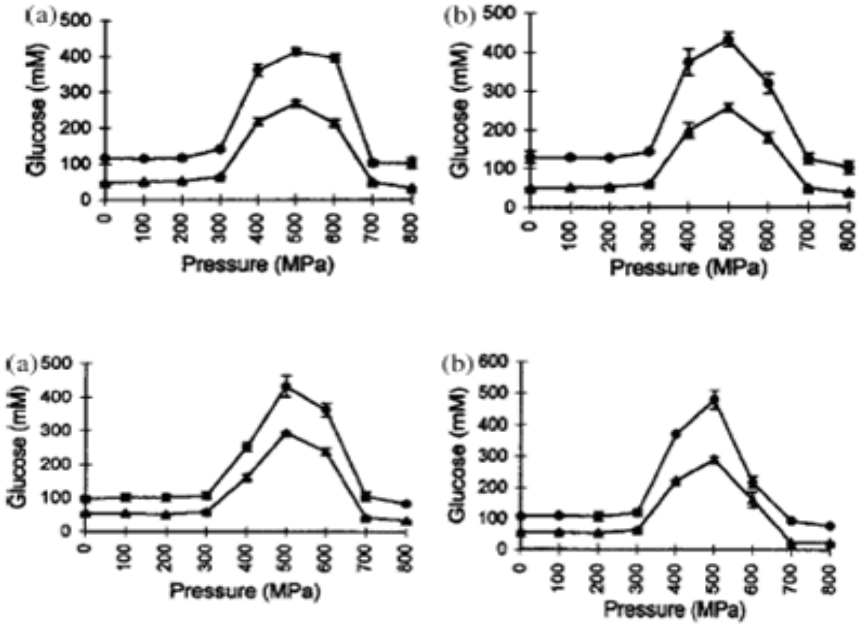

Figure 8. Effect of HHP(100-800 MPa) for (a)10min and (b) 20 min at ambient temperature on amylase activities in wheat flour(•) total soluble carbohydrate,(▲) reducing sugars.(top = barley flour ,bottom = wheat flour)(from Gomes et al.,1998).

several reactions occur more or less at the same rate, complex non first-order kinetics is expected (Lencki et al., 1992). As for pressure-temperature combination effects, a pressure-temperature kinetic data for some enzymes affecting texture, flavor or color of food is exemplified in the following paragraph.

THE EFFECT OF HIGH PRESSURE ON ENZYMES THAT AFFECT FOOD QUALITY

High pressure influence on quality-effecting enzymes specially in fruits and vegetables, have been widely investigated (Butz et al., 1994a; Syderhelm et al., 1996; Cano et al., 1997; Kim et al., 2001). Results from these studies suggest that, pressure-induced changes in catalytic activity of enzymes differ depending on the type of enzyme, the nature of substrates, the temperature and length of processing.

Alpha-amylase

One of the main enzymes present in cereal grains such as wheat and barley are amylases. Amylases, which hydrolyze starch to glucose during fermentation, modify the characteristics of bread dough to obtain bread with volume at the end of the fermentation process. In Studies conducted by Gomes et al. (1998) HHP applied to 25% (w/w) slurries of barley and wheat flours at ambient temperature led to large increases in total soluble carbohydrate and reduced sugar content in samples

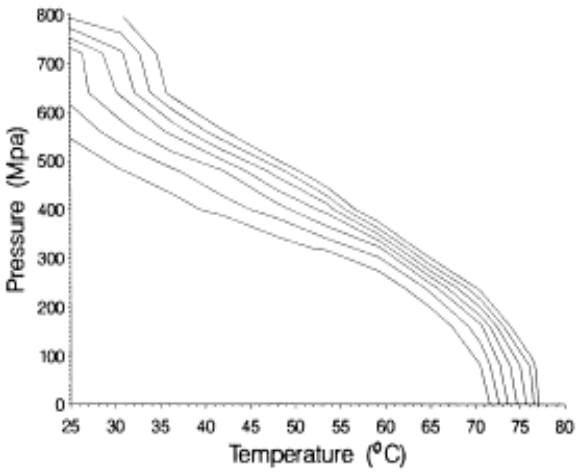

Figure 9. Pressure-temperature kinetic diagram of Bacillus subtilis alpha -amylase (15 mg/ml in 0.01M Tris HCl at pH 8.6), Range: k = 0.01 (lower line) k = 0.07 (upper line).

treated at 400 – 600 MPa for 10 or 20 min (Figure 8). Furthermore on pressure-temperature combination effects, a pressure - temperature kinetic diagram of bacterial alpha – amylase has been exemplified by Ludikhuyze et al. (1997) (Figure 9).

High pressure (HP) inactivation kinetics of commercial amylase in apple juice was evaluated under various test conditions (100 – 400 MPa; 0 – 60 min and 6 – 40°C) using a central composite design of experiments by Riahi et al. (2004). During the pressure hold, as expected, the

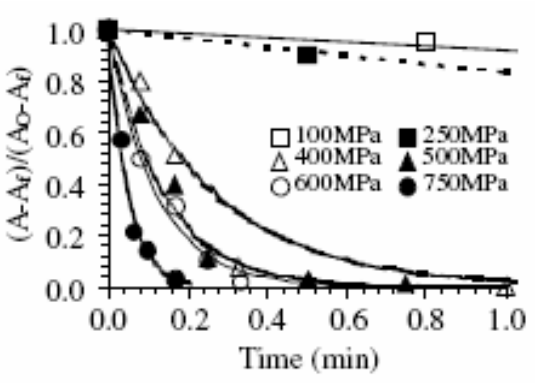

Figure 10. PME inactivation during processing at 60 and various isobaric conditions (100 – 750 MPa).

associated decimal reduction times (D values) decreased with an increase in pressure. Pressure dependency of D values was well described by the conventional death time model and the associated zp values (pressure range to result in a decimal change in D values) at pH 3.0, 3.5 and 4.0 were 518, 646 and 705 MPa, respectively (at 20°C). Almost complete inactivation of the enzyme was possible with the combination of lower pH, higher pressure and higher temperatures.

Pectin methyl esterase

Cloud loss accompanied by gelation of juice concentrates and consistency loss of (tomato) products are a major problem associated with orange juice and tomato quality deterioration. Pectin methyl esterase (PME; EC 3.1.1.11) is responsible for these deteriorations. PME is inactivated by heat in conventional preservation processes, which leads to detrimental effects on flavour, colour and other compounds associated with sensory, nutritional and health related qualities of the product, (Irwe and Olsson, 1994).

High-pressure processing of orange juice can result in a commercial stable product with higher quality (Ogawa et al., 1990). It has been reported that high-pressure treatments of ~ 600 MPa can partially (up to 90%) and irreversibly inactivate orange PME which does not reactivate during storage and transportation (Irwe and Olsson, 1994; Ogawa et al., 1990). Also the inactivation kinetics of endogenous pectin methyl esterase (PME) in freshly squeezed orange juice under high hydrostatic pressure (100 – 800 MPa) combined with moderate temperature (30 – 60°C) was investigated by Polydera. A kinetic model of the pressure inactivation of orange PME (Figure 10) has been proposed by first order kinetics with a residual PME activity (5 – 20%) at all pressure–temperature combinations used (Polydera et al., 2004). Also, the effect of dynamic high pressure homogenization (DHP) alone or in combination with pre-warming on pectin methyl esterase (PME) activity and opalescence stability of orange juice was studied. DHP without heating reduced PME activity by 20%. Warming the juice (50, 10 min) prior to homogenization significantly increased the effectiveness of DHP. It is reported that, the freshness attributes of orange juice treated by warming was improved by DHP treatment (Lacroix et al., 2005).

Moreover, pressure–induced inactivation of PME in white grapefruit (*Citrus paradisi*) at temperatures >58°C in a pressure range of 0.1 – 300 MPa was studied by Guiavarc'h et al. (2005). The obtained results suggest that, a combined high – pressure (low/mild) heat treatment can eliminate up to 80% of the total PME activity, therefore, significantly limiting the cloud-loss defect in juices.

In the both model system and real studies conducted by Balogh et al. (2004) on carrot pectinmethylesterase inactivation was under isothermal and isothermal–isobaric conditions. A first-order kinetic model proposed for carrot pectinmethylesterase under all conditions investigated.

With respect to tomato products, PME is an endogenous pectic enzyme found primarily in tomato cell walls that de-esterifies the methyl group of pectin and converts it into low methoxy pectin or pectic acids (Giner et al., 2000). The low methoxy pectin or pectic acids can easily be depolymerized and hydrolyzed by polygalacturonase (PG), resulting in viscosity loss in tomato-based products. To avoid quality losses, partial or complete inactivation of PME together with inactivation of PG during tomato processing are required (Porreta, 1996). Tomato PME seems to be more pressure resistant and its inactivation seems to follow first-order kinetics (Seyderhelm et al., 1996).

Pressure and/or temperature inactivation (at mild temperature, 10 – 64°C, in combination with high-pressure, 0.1 – 800 MPa) of the labile fraction of purified pepper pectin methylesterase (PME) was studied in a model system at pH 5.6. Since an antagonistic effect of pressure and temperature was observed at lower pressures (P < 300 MPa) and high temperatures (> 54°C), it is concluded that, high pressure processing for pepper PME inactivation is only beneficial above 300 MPa (Castro et al., 2005).

Lipoxygenase

Lipoxygenase (LOX, EC 1.13.11.12, linoleate: oxygen 13) oxidoreductase is the enzyme responsible for the production of rancid off-flavours in many vegetables, particularly in leguminosae (Whitaker, 1972; Eskin et al., 1977; Galllard and Chan, 1980; Richrdson and Hyslop, 1985). A relationship between lipoxygenase activity and the occurrence of bitter taste has been found (Baur et al., 1977).

For certain processed vegetables, the inactivation of lipoxygenase by blanching is necessary to prevent off-flavors and thus is necessary to obtain high-quality mini-

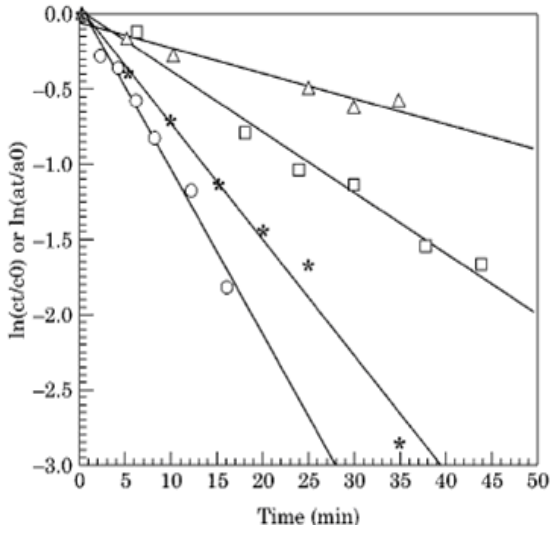

Figure 11. First order kinetics for thermal or pressure denaturation and inactivation of LOX (5 mgrmL in 0.01 mol/L TRIS HCl at pH 9). Denaturation is followed by determining band intensity of the major band in the electropherogram; inactivation is followed by measuring spectrophotometrically the enzyme activity. (*) = denaturation at 64°C; (o) inactivation at 64°C; (Δ) = denaturation at 600 MPa and 20°C; (□) = inactivation at 550 MPa and 20°C.

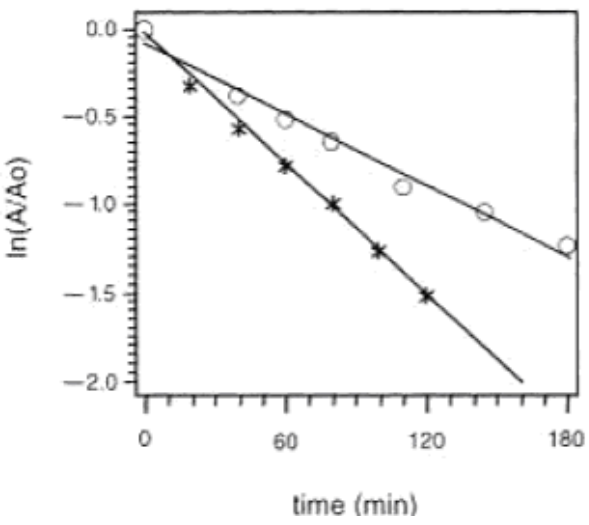

Figure 12. First-order pressure inactivation (850 MPa) of vocado (*) and mushroom (o) PPO at 25°C.

mally processed vegetables. Although heat treatment effectively inactivates lipoxygenase, other proteins (e.g. soy proteins in soybeans) can be simultaneously denatured and lose their functionality and/or solubility. Certain flavours, colours, vitamins and nutrients can also be affected by heat (Borhan and Snyder, 1979; Willlams et al., 1986). In this context, high pressure offers an alternative for the inactivation of enzymes at ambient temperature (Ludikhuyze, 1998; Weemaes et al., 1998).

In crude green bean extract, irreversible lipoxygenase inactivation was reported in the temperature range 55 – 70°C at ambient pressure, whereas at room temperature, pressures around 500 MPa were required to inactivate lipoxygenase. High pressure treatment at 200 MPa and 50 resulted in 10% inactivation, while at least 50%, lipoxygenase inactivation occurred at pressures greater than 500 MPa and thermal treatment between 10 and 30°C (Indrawati et al., 1999). However, in Tris buffer, lipoxygenase activity was significantly inactivated at pH 9.0 and 400 MPa and lost all activity at 600 MPa and all pH values (Tangwongchai et al., 2000).

In soybean products, off-flavor development is highly dependent on the action of lipoxygenase since subsequent decomposition of the resulting hydroperoxides yields especially rancid flavor and beany aroma. Thermal inactivation of enzymes at atmospheric pressure occurs in the temperature range 60 – 70°C. In contrast, pressure – temperature inactivation occurs in the pressure range 50 – 650 MPa at temperatures between 10 and 64°C (Ludikhuyze et al., 1998). Furthermore, the effect of high pressure on the inactivation and denaturation of soybean lipoxygenase is kinetically investigated. (Figure 11). Both thermal and pressure denaturation could be accurately described by a first order kinetic model. In this study, inactivation occur more readily than denaturation, indicating that only minor changes in the tertiary structure are responsible for the loss of enzyme activity (Ludikhuyze et al., 1998).

Polyphenoloxidase

Polyphenoloxidase (PPO) activity (EC 1.14.18.1) causes enzymatic browning of damaged fruits and vegetables, which is believed to be one of the main causes of quality deterioration brown coloration and concomitant changes in appearance and organoleptic properties during post-harvest handling, storage, and processing. Because it represents a major problem in the food industry, inactivation of PPO is highly desirable (Lamhrecht, 1995; ferrar and walker, 1996). Mushroom and potato PPO are very pressure stable, since treatments at ~ 800 – 900 MPa are required for activity reduction (Gomes and Ledward, 1996, Eshtiaghi, et al., 1994, Weemaes et al., 1997).

Grape, strawberry, apricot and apple PPO have shown more sensibility to pressure. Pressure of about 100, 400 and 600 MPa was needed to inactivate PPO in apricot, strawberry and grape respectively (Jolibert et al., 1994; Amati et al., 1996). For several PPO enzymes, it has been reported that, pressure-induced inactivation proceeds faster at lower pH (Jolibert et al., 1994; Weemaes et al., 1997). For example, at room temperature, pressure inactivation of mushroom and avocado PPO at their optimal pH (6.5 and 7, respectively) could be accurately described by a first-order kinetic model (Figure 12). For both mushroom PPO and avocado PPO, the threshold

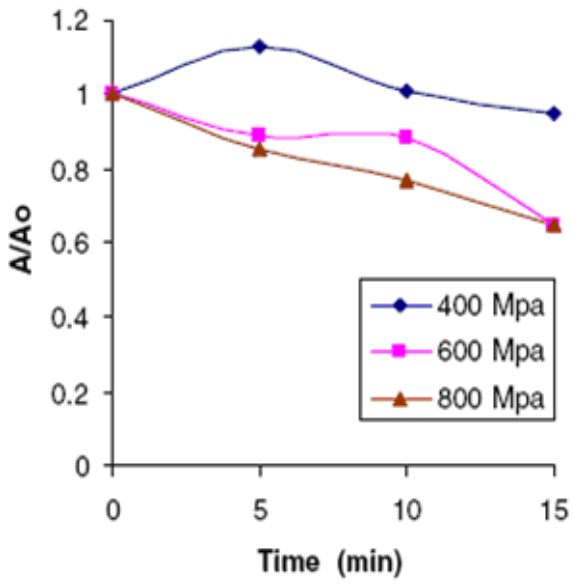

Figure 13. The effect of HPT on the enzyme peroxidase in strawberries.

pressure for inactivation decreased when the pH was lowered below the optimal pH value. In addition to inactivation of PPO at high pressure, pressure-induced activation at low pressure has been reported for apple (Jolibert et al., 1994; Anese et al., 1995; Butz et al., 1994; Asaka et al., 1994) and strawberry PPO (Cano et al., 1997).

Peroxidase

In vegetables, peroxidase (POD) (EC 1.11.1.7) induces negative flavor changes during storage, Moreover, POD is often used as an indicator for evaluating the efficiency of blanching processes because, it is the most heat-stable enzyme in vegetables. Consequently, its inactivation indicates thermal inactivation of all other vegetable enzymes (Richardson and Hyslop, 1985; Quaglia et al., 1996).

POD is also very pressure resistant, and pressure inactivation proceeds only when POD is subjected to very high pressures. For POD from horseradish, combinations of 800 – 900 MPa with temperatures in the range of 55 – 70°C are required to induce any significant inactivation. For POD extracted from carrots (pH 6 – 7) irreversible and complete loss of enzyme activity can only be achieved at 9 kbar (1 min). In the study conducted by Garcia-Palazon et al. (2004), the effects of high hydrostatic pressure on the strawberry the maximum POD inactivation (35%) was achieved after an HPT at either 600 MPa or 800 MPa for 15 min (Figure 13). This result was in good agreement with previous work by (Cano et al., 1997) in strawberries where an inactivation of 25% was achieved after an HPT of 15 min at 230 MPa. The effect of high pressure on strawberry is presented as a function of the ratio A/A0 over pressurizing time. No POD activity was detected in red raspberries, either fresh or after the HPT.

In case of lactoperoxidase, the major peroxidase in milk, the barotolerance at temperatures ranging from 10 – 30°C was strongly dependent on the surrounding medium. Pressure inactivation was much more pronounced in Tris buffer pH 7 than in milk. In Tris buffer, initial activity was reduced by 70% at 600 MPa and 25 for 2 min (Seyderhelm et al., 1996).

Beta-glucosidases

Beta-glucosidases (β-glucoside glucohydrolase, EC 3.2.1.21) catalyse the hydrolysis of aryl and alkyl β-D-glucosides. In plants, β-glucosidases are involved in different key metabolic events, the release of flavour volatiles being the most important in terms of flavour bioformation in fruits (Hostel, 1981). The effect of HHP on the activity of β-glucosidase in crude strawberry extracts is also reported in high pressure ranging from 200 – 800 MPa by Zabetakis et al. (2000). The enzyme β-glucosidase was found to be activated when pressures of 200 and 400 MPa were applied but inactivated considerably in the 600 and 800 MPa treatments. In the article by Garcia-Palazon et al. (2004), the effects of high pressure on the β-Glucosidases activity of strawberry and red raspberries are presented as a function of the ratio A/A0 over pressurizing time (Figure 14).

Proteolytic and catheptic enzymes

Proteolytic and catheptic enzymes cause changes in texture, and shelf life, and promote spoilage (Ashie et al., 1996; Hansen et al., 1996; Ashie and Simpson, 1996). Lipolytic enzymes cause the release of free fatty acids (Ohshima et al., 1992), which could affect the sensory properties of fish (Lovern, 1962), cause bleaching of carotenoid pigments, in the skin and flesh (Tsukuda, 1970) and are known to promote protein denaturation. Proteolytic degradation is helpful in the tenderization of mammalian meats. High pressure technology can be used to reduce the ageing process. High pressure (1000 to 2000 atm) treatment of meat enhances the endogenous proteolytic activity that take place during meat conditioning by the release of proteases from lysosomes and by denaturation of the tissue protein. Ohmori et al. (1991), Matsumoto (1979), Sikorski et al. (1976) and Ohshima et al. (1992) demonstrated a decrease in free fatty acid content and inhibition of enzymatic degradation of phospholipids in cod muscle treated at ~405 MPa for more than 15 min. It was shown that fish enzymes such as cathepsin C, colla genase, chmotrypsin and trypsin-like enzymes were more susceptible to high pressure at 100 – 300 MPa (Ashie

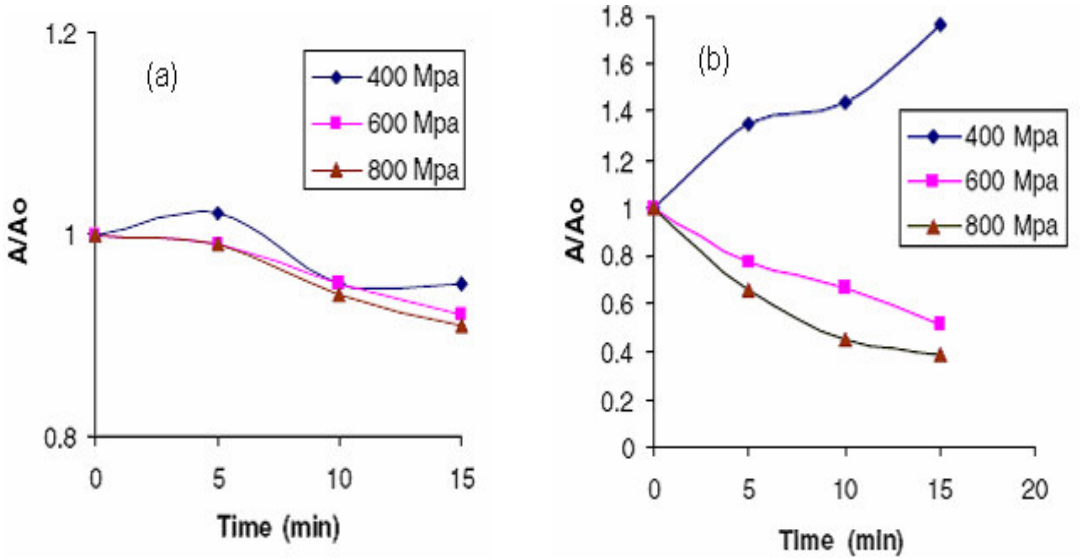

Figure 14. The effect of HPT on the enzyme Beta-glucosidase in red raspberries (a) the effect of HPT on the enzyme Beta-glucosidase in strawberries (b).

and Simpson, 1996; Simpson, 1998) and decreased autolytic activity was found at pressures over 200 MPa in octopus (Hurtado et al., 2001).

High pressure can be applied to inactivate enzymes inherent to salmon muscle, and a good quality product with longer shelf life can be developed.

EFFECTS OF HIGH PRESSURE ON STARCHES

Starch granules are semi crystalline particles mainly composed of linear amylase and highly branched amylopectin, which are high molecular mass polymers. Starch from different botanical sources contain from 15 – 30% of amylose, although, there are some mutant wheat varieties which can produce from 1.2 – 39.5% of amylose (Bocharnikova et al., 2003) or corn varieties with 100% of amylopectin ('waxy' starch) and up to 75% of amylose ('amylose extender') (Gidley and Bociek, 1985). Recently, many studies have focused on the structure of starch granule in respect of a better understanding of the mechanism of starch gelatinisation, retrogradation and stability of starch gels as well (Błaszczak et al., 2001; Rubens and Heremans, 2000).

Starch are utilized in many food products to increase viscosity or to form gels. Because starch is insoluble in water, a mixture of starch with water forms a suspension. Starch granules in suspension swell with heat and the viscosity of the suspension increases depending on starch concentration. Thermal processing changes the physicochemical properties of starch; such as increased water solubility and viscoelastic behavior (Fennema, 1996; Rao, 1999; Jobling, 2004). Heat induced changes in starch are well examined and characterized. More detailed analysis by small angle X-ray scatterings and wide–angle X-ray scatterings reveals that, the amorphous growth rings undergo hydration during gelation (Jenkins et al., 1994; Jacobes et al., 1998). It is well known that, starch of different botanical origins vary also in their size and shape and show different physical and chemical properties including gelatinization temperature (e.g. 52 – 66ºC for wheat and 62 – 67ºC for corn) and viscosity after heat treatment (Roos, 1995).

High hydrostatic pressure (HHP) has been shown to affect starch polymers causing gelatinization of them (Douzals et al., 1996; Douzals et al., 1998). High pressure induces hydration of the amorphous phase followed by a distortion of the crystalline reign leading to destruction of the granular structure. Microscopic observation show a different degree of swelling for some pressure-treated starches than for temperature-treated ones (Stute et al., 1996). It is assumed that, difference in gelatinization mechanism may cause these effects. Douzals et al. (1998) found that, wheat starch granules swelled as a result of HHP treatment. Studies on 16% (w/w) wheat starch showed that starch gelatinization by pressure as evaluated by differential scanning calorimetry (DSC) starts at 300 MPa and starch is completely gelatinized at 600 – 700 MPa, at 25°C (Douzals et al., 1996; Douzals et al., 1998; Zuo et al., 1999; Rubens and Heremans, 2000).

Stolt et al. (2001) found that, under pressure barley starch formed pastes with creamy texture similarly to wheat, corn, tapioca and pea starch. They also reported that, the properties of starch pastes and gels obtained under high pressure differed from those of the heat-gelatinised ones. They explained that phenomenon, pointing out the role of the stabilizing effect of amylose under high pressure. Waxy maize starch disintegrated under high pressure, whereas amylo-maize starch did not

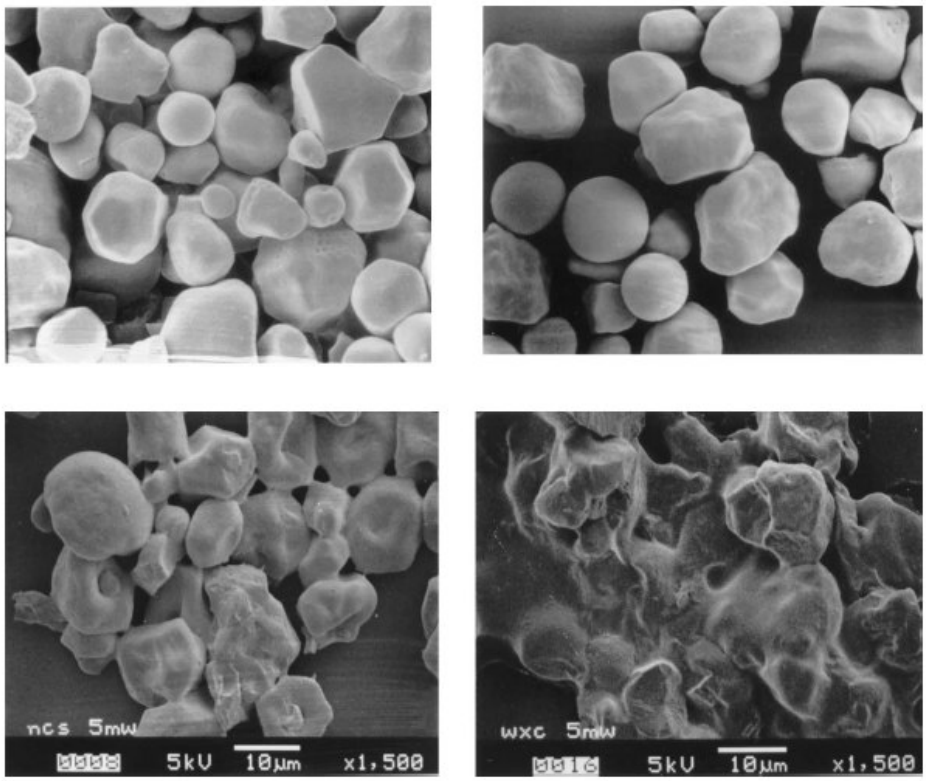

Figure 15. The SEM of normal maize starch (left) and waxy maize starch (right): control (top), pressurized at 690 MPa in 1/1water/starch (w/w) ratio for 5 min (bottom) (Katopo et al., 2002).

change its granular structure even at 900 MPa (Figure 15). High pressure was also found to affect rheological properties of barley starch. However, starch granules remained intact and no leaching of amylose was found (Stolt et al., 2001). Effect of high pressure on the structure of Potato starch–water suspension (10%) subjected to high pressure treatment at 600 MPa for 2 and 3 min was investigated by Błaszczak et al. (2005).

The F.t-i.r. analysis of starch preparations showed that high pressure significantly affected the intensity of bands corresponding to the amorphous and more ordered part of starch structure. The DSC analysis showed a decrease in gelatinization temperatures upon high pressure treatment. It is reported that, the inner part of the granule was almost completely filled with gel-like network, with empty spaces growing in diameter towards the centre of the granule (Figure 16).

Gelatinized starch recrystallizes during storage, affecting the texture and shelf life of food products. This phenomenon is known as retrogradation. Retrogradation contributes to the quality defects in foods such as loss of viscosity and precipitation in soups and sauces. Jouppila et al. (1998) reported that water content, storage temperature, and the temperature difference between storage temperature and glass transition temperature were important factors in retrogradation of thermally treated corn starch. The Avrami equation was shown to describe the starch crystallization behavior based on x-ray diffraction (Jouppila et al., 1998) and DSC data (Jouppila and Roos, 1997).

Douzals et al. (1998) reported that, recrystallization of the HHP gelatinized wheat starch (30% dry matter) during storage reached to an asymptotic value after 6 days, and the extent of retrogradation was higher for starch gelatinized by heat than starch gelatinized by pressure at 600 MPa. Since retrogradation depends on the botanical source of the starch, temperature, and starch concentrations, it is important to explore the impact of HHP processing on retrogradation characteristics of starch from different botanical origin. Native starch produces weak bodied, cohesive, rubbery pastes when cooked and undesirable gels when the pastes are cooled. It has been known that starch, pasted and dried without excessive retrogradation, can be re-dissolved in cold water. Such starches, called pregelatinized starch or instant starch, are precooked starch. Pregelatinization is one of the methods used to modify the native starch in order to improve several characteristics including increased solubility (Fennema, 1996; Walter, 1998). Also, it is important to characterize the potential of HHP processing to modify the unctional properties of starch. For successful application of high pressure processed starch to produce commercial food products, it is important to study the impact of HHP on modification of

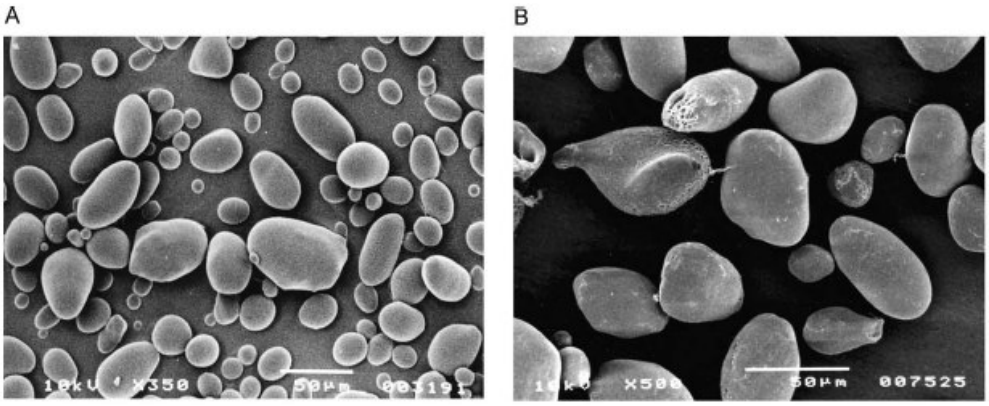

Figure 16. SEM microstructure of potato starch: native (A); treated with high pressure at 600 MPa for 2 min (B) (Błaszczak et al., 2005).

physical properties of starch relevant to food processing and storage. Having knowledge about the physical properties of HHP treated starch, it is essential to optimize the processing protocols so as to improve the physical stability and textural characteristics of food products. King and Kaletunc (2005) evaluated the effect of HHP processing on the crystallization kinetics of wheat and corn starches and its effect on rheological characteristics during storage. In this context the corn and wheat starch gelatinized on the order of 600 - 700 MPa, at 25°C and stored at 23°C exhibited characteristics of strong gels for a longer period of time than that of the starches stored at 4ºC. The data collected in this research showed that HHP and thermal processing have advantageous effects for industrial use compared to native corn and wheat starch.

PRESSURE EFFECTS ON WATER

Since water is one of the main components of foods, the influence of pressure on water must be considered. Foods containing much water and little gas exhibit compressibility similar to that of water (Venugopal et al., 2001). Adiabatic compression of water increases the temperature ~3°C/100 MPa (Figure 17, Knorr, 1999). Self ionization of water is also promoted by HP, lowering the pH. Phase transition of water can be performed under pressure. At ~1,000 MPa water freezes at room temperature, whereas the freezing point can be lowered to –21°C at 210 MPa. The behavior of water under pressure offers potential applications in food processing, such as pressure-shift freezing and fast thawing, non-frozen storage under pressure at sub-zero temperature, and formation of different ice polymorphs (Venugopal et al., 2001). The freezing point of water decreases with increasing pressure; a release of pressure helps form rapid and uniform nucleation, growing smaller ice crystals in the food and causing minimum damage, helping to increase the freezing rates compared to a conventional

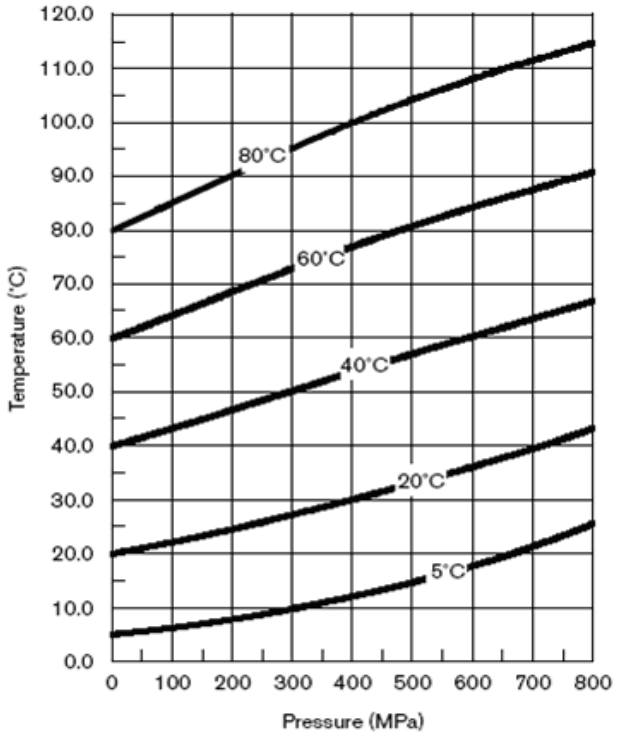

Figure 17. Adiabatic heating during compression of water at different initial temperatures (Knorr, 1999).

freezing process (Venugopal et al., 2001). This allows gentle processing of foods or food constituents (starter cultures) with minimal structural damage.

Application of high pressure in freezing and thawing

Principles

Freezing is one of the most successful methods for long term preservation of the natural quality attributes of

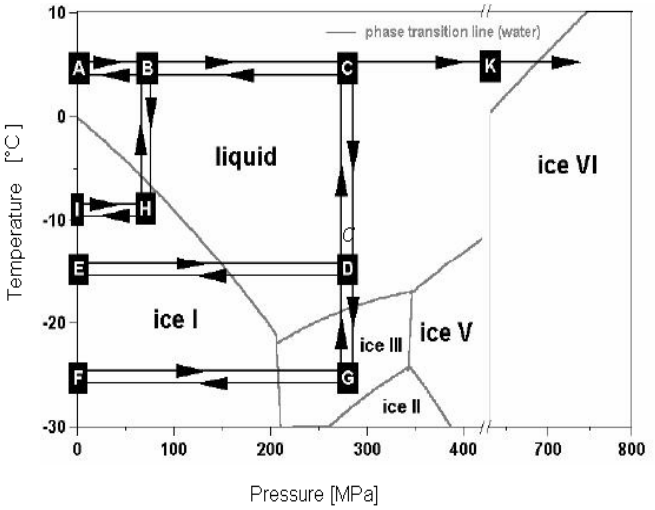

Figure 18. Possibilities and definitions of high pressure processing on phase transitions of water modified according to Knorr et al. (1998). 1: subzero storage without freezing (A, B, C, D, C, B, A); 2: pressure assisted freezing (A, B, H, I); 3: pressure assisted thawing (I, H, B, A); 4: pressure shift freezing (A, B, C, D, E); 5: pressure induced thawing (E, D, C, B, A); 6: pressure assisted freezing to ice III (A, B, C, D, G) and subsequent transformation to ice I (G, F); 7: solid-solid (ice I/ice III) transformation (F, G) and subsequent pressure assisted thawing of ice III (G, D, C, B, A); 8: freezing above 0°C (A, B, C, K, ice VI). Redrawn from Schlüter (2003).

perishable foods. Because of the reduction of chemical and biological processes of food as a result of reduction of available liquid water as ice, combined with subzero temperatures, it is widely established that freezing of food provides a safe and convenient way of shelf life extension without negative effects on the nutritional quality. In addition, the subsequent thawing of the frozen food is a process assuming no meager importance. The ice crystals that form can also cause considerable drip loss and textural, color and organoleptic modifications after thawing. However, parameters like product geometry, the flow rate of the surrounding fluid, the thermophysical properties, as well as the temperature difference between the product and the environment have significant influences on the rate of phase transition. Since freezing and subsequent thawing of foods cause undesirable changes in their texture and sensory properties, studies of the effects of freezing and thawing rates on food quality have led to development of the combination of slow and rapid freezing and thawing in order to creating uniform crystals of ice. Recent investigations to improve freezing and/or thawing processes of foods showed an increasing interest in the use of high hydrostatic pressure to support phase transitions (Cheftel et al., 2000; Denys et al., 2002; Cheftel et al., 2002; Li and Sun, 2002).

The superiority of high pressure in freezing and thawing is due to the effects of high pressure on ice-water transformation related to food technology. The main principle of pressure supported phase transition in food with high water content can be seen on the phase diagram of water. The phase diagram of water shows that the melting temperature of water decreases with pressure, down to -21°C at 210 MPa (the triple point liquid/ice I/ice III) whiles the opposite effect is observed above this pressure. This phenomenon allows the achievement of rapid freezing and thawing of foods that mainly contain water and preservation of foodstuffs at subzero temperatures in the liquid state.

Besides a depression of the freezing-point, a reduced enthalpy of crystallization can be observed, thereby accelerating phase transition processes (Kalichevsky et al., 1995). According to the phase diagram of water, different types of high-pressure freezing and thawing processes can be distinguished in terms of the way in which the phase transition occurs: high-pressure assisted freezing (phase transition under constant pressure) high-pressure shift freezing (phase transition due to a pressure release) and high-pressure induced freezing (phase transition initiated by a pressure increase and continued at constant pressure). In the pressure assisted thawing process, where the phase transition is obtained by heating at constant pressure and, the pressure induced thawing process, where the phase change is induced by pressurization. Subzero storage without freezing (this term implies already clearly that the process has no ice crystal formation associated). The processing steps based on a terminology introduced by Knorr et al. (1998) are shown in Figure 18.

The effects of high pressure on the freezing and thawing of foods

With respect to the food quality parameters, the advantages of pressure-assisted and pressure shift freezing have been widely reported and prevention of food damages was shown (Sanz et al., 1997; Otero et al., 1998; Levy et al., 1999; Teramoto and Fuchigami, 2000; Chevalier et al., 2001). Most of these reports focus on the advantageous effects of pressure-shift freezing on texture and structure of product as a result of the formation of smaller ice crystal. Kanda et al. (1992) in their studies compared the quality of pressure-shift frozen and air-blast frozen tofu. They observed that tofu frozen by pressure release resulted in better structure than air-blast and have no drip loss. Also its taste and texture were the same as before freezing. It is reported that pressure–shift freezing of tofu produced fine ice crystal to result in lower structural damage (Kanda et al., 1992; Kanda and Aoki, 1993).

Koch et al. (1996) compared the quality changes on potato subjected to pressure shift samples with air-blast frozen samples. They observed that pressure-shift freezing of potato cubes resulted in less damage of the cell structure, less drip loss, and less enzymatic browning than conventionally frozen cubes. Also, the effects of different processing steps on the cell membranes, texture,

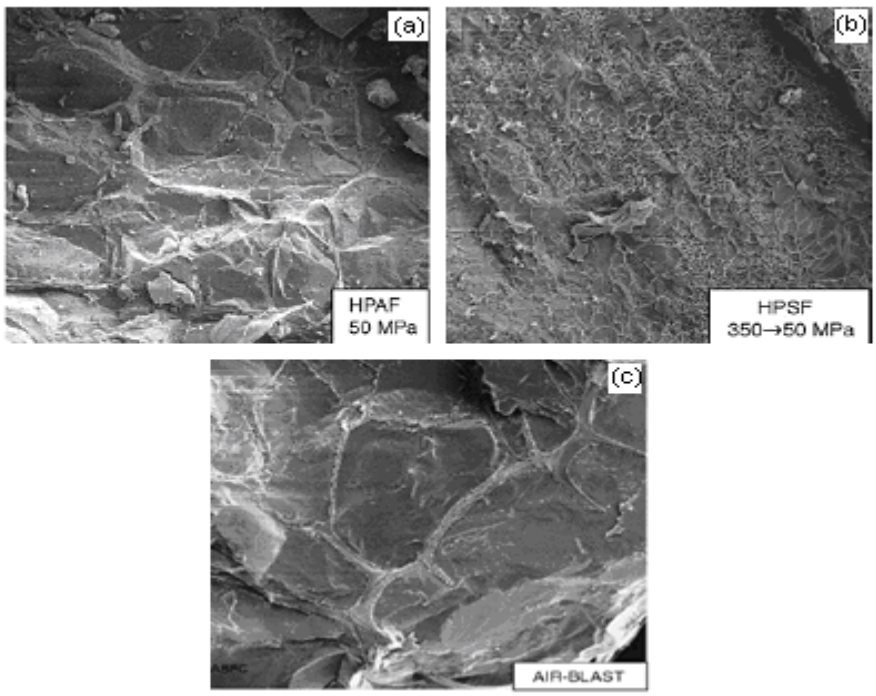

Figure 19. Micrographs of high-pressure frozen gelatin samples. (a) High-pressure assisted freezing under 50 MPa. (b) High-pressure shift freezing from 350 MPa/-17°C to 50 MPa. (c) an air-blast frozen gelatin sample (air temperature: -40°C, air speed: 5.5 m/s).

color and visual appearance of potato tissue were investigated by Luscher et al. (2005). Besides pressure-shift freezing, the processes of freezing to ice III and ice V, as well as storage at -27°C and 250 MPa up to 24 h (metastable liquid state of water) of potato samples were examined. The direction of solid–solid phase transitions (phase transition of ice I – ice III or phase transition of ice III – ice I) influenced the result of high pressure, low temperature processing significantly.

Fernandez et al. (2005) compared the effects of high-pressure shift freezing and high-pressure assisted freezing on the microstructure of gelatin gel samples (10% gelatin, w/w). Results clearly showed that, high-pressure shift freezing is the more advantageous method. The super cooling attained after the expansion and the consequent instantaneous freezing of water, together with the temperature drop in the pressure medium, induced short phase transition times and a homogeneous distribution of small ice crystals throughout the sample. The mean phase transition time in air-blast experiments with large samples was 108 min. The resulting ice crystals were large and polygonal shaped, with a mean equivalent diameter of 202 µm, ranging from 65 – 311 µm. Ice crystals in high pressure assisted frozen samples at 50 MPa (Figure 19a) had a mean equivalent diameter of 346 µm, ranging from 82 – 442 µm. On the other hand, high pressure shift frozen samples after expansion to 50 MPa (Figure 19b) presented small ice crystals with a mean equivalent diameter of 5.6 µm. The variability was low, and ice crystal diameters ranged from 1.1 – 38.8 µm. The mean phase transition time was 163 min.

Pressure assisted freezing may be of special interest to avoid coarse ice crystallization and obtain a smooth texture in various types of ice creams (including low fat) or sherbets. Unilever Company has patented combinations of HP processing and freezing for improved consistency and smoothness, and slower melting of ice creams (Keenan et al., 1998).

Histological studies on pork samples were conducted by Martino et al. (1998). They compared the size and location of ice crystals in large pieces of pork muscle frozen by pressure-shift freezing to those obtained from pork frozen by air-blast and cryogenic fluid (nitrogen liquid) freezing. Classical methods induced thermal gradient and therefore, a non-uniform ice crystal distribution that caused structural damage while in the pressure shift frozen samples, small-sized ice crystals were observed in the center and the surface of treated samples.

Pressure-shift freezing (200 MPa, -18°C) of Norway lobsters was compared with air-blast freezing (-30°C). Pressure shift freezing induced a significant increase in the toughness of the Norway lobster tails compared to the air-blast frozen samples. These changes of texture were attributed to myosin or actin aggregation induced by high pressure. (Chevalier et al., 2000).

Fuchigami et al. (1997a, 1997b) reported that improvements in texture and histological damage are achieved in

pressure-shift frozen carrots. Otero et al. (1998) compared the damage to the microstructure of egg plants frozen by conventional air freezing and by pressure-shift freezing. Pressure-shift frozen samples had the appearance of fresh samples, and no differences between centre and surface cell structure were observed (indicating uniform nucleation).

Otero et al. (2000) confirmed the beneficial effects of pressure-shift freezing on whole peaches and mangoes as compared to air-blast frozen samples. The authors reported that the cell damage at sample centre was much less in pressure-shift frozen samples than in air blast frozen, evidenced from scanning electron microscopic analysis. This beneficial effect might result from the formation of smaller ice crystals due to enhanced super cooling and homogeneous nucleation during pressure release. Besides other materials, the textural and microstructure studies of tissue Chinese cabbage (Fuchigami et al., 1998) have been carried out. In all cases studied, the beneficial effects of pressure-shift freezing in comparison with convention freezing were confirmed. A frozen product can be forced to the liquid area in the phase diagram by applying high pressure.

Research on high pressure-assisted thawing of frozen fish and meat has shown the possibilities of significantly reducing the thawing time (Deuchi and Hayashi, 1992; Murakami et al., 1994; Zhao et al., 1998; Massaux et al., 1999a) as well as reduction in drip loss after thawing (Murakami et al., 1992; Okamoto and Suzuki, 2001) and subsequent cooking (Massaux et al., 1999b; Chevalier et al., 1999; Rouillé et al., 2002). Chevalier et al. (1999) in their studies on whitting filets observed lower drip loss when higher pressurization rates of thawing were applied at given pressure.

Also pressure assisted thawing is connected with colour and texture changes which tend to be a nature of the product. Murakani et al. (1992) observed a decrease of thawing time and drip for frozen tuna muscle that was thawed at high pressure with respect to samples thawed under atmospheric pressure. Total drip volume decreased when increasing thawing pressure while no changes was observed in the microbial population. Also depending on the pressure level applied, they observed a colour changes in samples when subjected to pressure. However Deuchi and Hayashi (1992) reported that, frozen beef at -20^{o}C was thawed at 50 MPa without changing natural color and with minimum drip volume. But higher pressure levels induced protein denaturation resulting in whitening of meat.

Eshtiaghi and Knorr investigated pressure-assisted thawing (600 MPa, 15 min, 50^{o}C) of frozen strawberries as a pretreatment thermal processing. After the treatment, they observed increase in sugar uptake and improvement of the microbial quality by 2 logarithmic cycle reduction (Eshtiaghi and Knorr, 1996).

When pork meat was thawed by high pressure the drip decreased and the water-holding capacity of the meat improved (Okamoto and Suzuki, 2001). Therefore, reducing the drip loss and lowering the processing time can be seen as major advantages of high pressure-assisted thawing. While almost no changes in colour or penetration force of a pressure-thawed (210 MPa) beef product was observed (Zhao et al., 1998); discoloration and toughening of a pork sample occurred and increased with an increasing working pressure (Massaux et al., 1999a). Therefore, it was concluded that the freezing-thawing process under a pressure of 100 MPa seems to be an advantageous treatment for pork because there is no exudate, and only a slight discoloration and toughening of meat (Massaux et al., 1999b). Furthermore, meat softening was found to be induced during high pressure treatment. At 200 MPa unfavourable changes were provoked by high pressure thawing of pork meat (Okamoto and Suzuki, 2001). When frozen tuna back muscle was thawed under various hydrostatic pressures at various temperatures, the colour of thawed samples was changed (Murakami et al., 1992).

Carp muscles treated by high pressure in the range of 100 – 300 MPa lost their transparency, together with an increase of the L-values and an increase of pressurisation. The different possibilities of processing food products in the high-pressure (subzero) low-temperature (HPLT) range described by Bennet et al. (2004) include ice phase transitions that should affect the viability of unwanted microorganisms when present in treated products. A recent study from Luscher et al. (2004) indicated that inactivation of the gram positive bacterium *Listeria innocua* is more effective after it had undergone Ice I –III solid–solid phase transition. In the study conducted by Shen et al. (2005) high pressure inactivation processes, especially at subzero temperatures, were performed on *Bacillus subtilis* vegetative cells at various pressure, temperature and time combinations. For treatments of cells between 250 and 350 MPa at -25^{o}C, a double effect of extracellular solid–solid (Ice I–III) phase transition and possible intracelluar solid–liquid phase transition is suggested to be the key in mediating the observed drop in viability (Shen et al., 2005).

CONCLUSIONS

The following items are the main conclusions in the use of high pressure drawn from the review:

The use of high pressure for food preservation has the potential to address the requirements of consumers to prefer "minimally preserved" foods. High pressure processing has the potential to develop into a preservation technique that is applied on a large scale in the food industry, in particular for products where retention of flavors and nutrients is desired.

The use of high pressure as new emerging technology to inactivate microorganisms without the application of heat, or with the use of less heat than would be otherwise necessary, is attractive from the point of view of product quality. Two facts limit its usefulness at the present time.

First, although it allow killing of vegetative micro-organisms, it fail until now, when applied alone, to destroy spores and some food enzymes. Bacterial spores remain the organisms most tolerant to high pressure so that, sterilization (as opposed to pasteurization) is not yet possible. Second, the kinetics of inactivation that results from high pressure treatment in some case is different from that resulting from heating, so that a careful new approach, e.g. to product safety, will be needed if application of the technique continues to be promoted.

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