High Hydrostatic Pressure Treatment for Dairy Applications

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The application of high pressure, rather than heat, to food enables microorganisms to be destroyed without causing significant changes to the color, flavor and nutritional attributes of the food. In this way, food can be preserved in a safe state but still have most of the attributes of a fresh product (Balasubramaniam *et al.*, 2004; Yordanov and Angelova, 2010; Chawla *et al.*, 2011). In addition, high pressure can cause rheological changes in food that may result in beneficial sensory and structural effects. The potential of high-pressure treatment of food was first recognized by Hite in 1899 who investigated such treatment as an alternative method for milk pasteurization. However, it attracted little attention until it was 'rediscovered' in the 1980s. The first food produced commercially by this technology appeared on the market in Japan in 1990; these included yogurt, fruit jellies, salad dressings and fruit sauces.

The process

Typically, high-pressure processing (HPP) of food is performed at 300–600 MPa at room temperature for 2–30 min. During pressurization, an increase in temperature (3–9°C per 100 MPa, depending on the pressure-transmitting fluid) occurs due to adiabatic heating; (Balasubramaniam *et al.*, 2004) a corresponding decrease occurs during depressurization. These changes can be minimized by temperature control of the high-pressure equipment by circulating water. Conversely, this temperature rise can be used to achieve desired effects in the treated foods. During HPP, the pressure is instantaneously and uniformly transmitted in all directions, regardless of the shape or volume (based on Pascal's principle) of the food in question. The large biomolecules such as proteins, nucleic acids and polysaccharides that depend on non-covalent bonding (i.e. hydrophobic interactions, hydrogen bonds) to maintain structure and function are most affected. On the other hand, smaller organic molecules such as those responsible for colors, flavors, and nutrients (e.g., vitamins) in which covalent bonding is the dominant or only type of bonding are hardly affected. Milk treated at 400 MPa results in no significant loss of vitamins B_1 and B_6 (Sierra *et al.*, 2000). The low temperature at which high-pressure treatments are usually performed ensures little or no heat induced changes in these components. However, recent developments in the use of Pressure Assisted Thermal Processing (PATP) and Pressure Assisted Thermal Sterilization (PATS) utilize both heat and pressure and hence cause some heat-induced changes.

Effects of high pressure processing

The use of HPP in food industry has been aimed at increasing the shelf life of the food product. The changes brought about in the food being treated with HPP are discussed herein.

Microbiological effects

Vegetative organisms: A major function of high-pressure processing of food is destruction of microorganisms. When a microbial cell is subjected to high pressure, the following detrimental changes take place:

- Cell membranes are destroyed via irreversible changes to the structure of the membrane macromolecules, particularly proteins
- The homogeneity of the intermediate layer between the cell wall and the cytoplasmic membrane is disrupted

- Membrane ATPase is inactivated
- The nucleic acids and ribosome involved in the synthesis of proteins are disrupted.

The result is permeabilization of the membranes and concomitant leakage of the contents of the cells and organelles, with eventual death of the bacterial cell. In general, Gram-negative bacteria are inactivated at a lower pressure than Gram-positive bacteria, and rod shaped bacteria are more sensitive to pressure than cocci. The pressure sensitivities of yeast are reported to be intermediate between these two bacterial groups. The number of yeasts, moulds, psychrotrophs and coliforms decreased more rapidly with pressure than that of acidic and heat-resistant bacteria and proteolytic microorganisms (Kolakowski *et al.*, 1997). The lower resistance of Gram-negative bacteria compared with Gram-positive bacteria is attributable to their lack of teichoic acid, which strengthens the cell wall of Gram-positive bacteria. Bacteria in the log phase of growth are more sensitive than those in the stationary phase.

Bacterial spores: HPP at around ambient temperature is limited in its inability to destroy bacterial endospores. Spores are more resistant than vegetative cells because it contains calcium rich dipicolinic acid which protects them from excessive ionization (Timson and Short, 1965; Smelt, 1998). Owing to such limitation, HPP cannot be used for producing sterile products, and all pressure-treated foods have to be kept refrigerated. However, high pressure can stimulate germination of bacterial spores, which enables the resulting vegetative form to be destroyed. Repeated cycling between high and low pressures, 'pulsed' or 'oscillatory' pressurization, and combined high pressure and high temperature, enhances the sporicidal effect. *Clostridium botulinum*, the target organism for sterilization of food products, is used as the marker pathogen for PATS treatment. This is due to its higher resistance to PATP than *Geobacillus stearothermophilus*. The number of *Clostridium botulinum* spores in phosphate buffer was reduced by 5 and 6 log cycles when treated at 827 MPa for 10 min at 40°C and 827 MPa for 20–30 min at 75°C, respectively.

Effect on bacterial flora and keeping quality of milk

Hite (1899) observed 5–6 log cycle reduction in the number of microorganisms when milk was treated at 680 MPa for 10 min at room temperature. Only a small proportion of the bacterial population (mostly sporeformers) could not be inactivated under high-pressure operating conditions. In order to achieve the shelf life of thermally pasteurized milk of 10 days at 10°C, a pressure treatment of minimum 400 MPa for 15 min or 500 MPa for 3 min is required. A reasonable shelf life of milk may be obtained with pressure treatments of 400 or 500 MPa. Some strains of the pathogenic bacteria viz., *L. monocytogenes* and *Staphylococcus aureus* are quite pressure resistant and may not be sufficiently inactivated. Some mutant strains of *Escherichia coli* (i.e. LMM 1010,1020,1030) are particularly bar tolerant being only reduced 2 log cycles at 600 MPa for 30 min. It has been suggested that high-pressure treatment might sub lethally injure a proportion of cells that could then grow slowly at refrigeration temperatures.

Chemical effects

HPP of food differs from heat processing in the chemical effects produced. The pressure induced effects are mostly concerned with secondary and tertiary structural changes in large molecules. Consequently, proteins, including enzymes and polysaccharides in an aqueous environment undergo reversible or irreversible conformational changes resulting in denaturation, dissociation, aggregation or even gelation. By contrast, the heat-induced breaking of covalent bonds in both small and large molecules causes changes to color, flavor and other sensory attributes that are not observed with pressure treatment.

Effect of HPP on milk components

Water: Water content of the food gets compressed by about 4 per cent at 100 MPa and 15 per cent at 600 MPa. Depression in freezing point of water was observed at high pressure to -4°C, -8°C, -22°C at 50, 100 and 210 MPa, respectively (Kalichevesky *et al.*, 1995). Thus, this technique enables sub-zero food processing without ice crystal formation. It also facilitates rapid thawing of conventional frozen foods and pressure shift crystallization. Thereby, cooling to sub-zero temperature in frozen foods by forming very small ice crystals which significantly controls microbial activity helps in improving quality as well extending shelf life of food.

Proteins: Three types of change occur in the casein micelle when pressure is applied at around ambient temperature. Little or no change occurs at 100–200 MPa; at 250MPa the casein micelle size is increased; and at > 300MPa the casein micelle gradually disintegrates until at 500 MPa the particles remaining are about 50 per cent of the size of the original micelle. Thus, skim milk that has been pressure treated at ~500 MPa has a clear, almost transparent appearance and a turbidity of about one-third of that of untreated skim milk. At the same time, the viscosity is increased by about 20 per cent. Individual caseins are dissociated from the micelle; the order of dissociation being β-casein > κ-casein > αs_1-casein > αs_2-casein (Needs *et al.*, 2000). The caseins remain dissociated when the milk is stored at 5°C, but re-associate when the temperature is raised.

The sensitivity of whey proteins to denaturation by pressure is in the order: Lactoferrin > β-lactoglobulin > immunoglobulin > bovine serum albumin > α-lactalbumin. Denaturation of β-lactoglobulin commences at ~150 MPa and increases with increasing pressure and temperature. Almost complete denaturation of whey proteins occured at 750 MPa at 30°C for 30 min or 450 MPa at 60°C for 15 min. The denatured β-lactoglobulin mostly becomes associated with the casein micelle; some of them attaches to the milk fat globule membrane in whole milk. The attachments are via disulfide bonds resulting from sulfhydryl-disulfide interactions. Among whey proteins, α-lactalbumin is the most resistant to denaturation by pressure. It is denatured only at pressures > 400 MPa, becoming 70 per cent denatured at 800 MPa. Immunoglobulins have increased resistance to denaturation. Better emulsion, foaming and textural properties have been observed from milk protein after high pressure treatment and may find application as techno-functional ingredients in different foods (Johnston *et al.*, 1992).

Enzymes: Milk enzymes vary in their sensitivity to high pressure. Lipase, xanthine oxidase and lactoperoxidase are resistant to pressures up to 400 MPa. Phosphohexose isomerase, γ-glutamyl transferase and alkaline phosphatase in milk are partially inactivated at pressures exceeding 350, 400 and 600 MPa respectively; they are completely inactivated at pressures of 550, 630 and 800 MPa respectively. The effect on alkaline phosphatase is of interest in milk processing. Since complete inactivation of alkaline phosphatase occurs only at very high pressures, this specific enzyme is not an appropriate indicator of effective 'pasteurization' by high-pressure treatment.

Fat: Crystallization of fat can be accelerated, enforced, or initiated because of the shift in the phase transition temperature caused by application of high pressure. As a consequence, high-pressure treatment reduced the ageing time of ice cream mixes and aided the physical ripening of cream for butter making. The fat globule size distribution and flow behavior of pasteurized liquid cream are not significantly modified by HPP at 450 MPa and 25°C for 15–30 min or 10°C for 30 min. Mean diameter of the milk fat globule remains unaffected after high pressure treatment. Following, high pressure treatment, there is some incorporation of whey proteins into milk fat globule membrane (MFGM) but as there is no increase in lipolysis (Buchheim and Frede, 1996).

Mineral*:* Mineral balance of milk gets affected at high pressure and effect is both on the distribution between colloidal and diffusible phase as well as on the ionization. The increase in the content of diffusible calcium has been reported following HPP. In case of previously heated milk, HPP treatment solubilizes both native and heat precipitated colloidal calcium phosphate (CCP) which leads to slight increase in pH (Johnston *et al.,* 1992). In general pH of milk increases following high pressure treatment and this change in pH is reversible.

Effects on dairy products and processes

Yogurt: Pressure treatment at 200 - 300MPa for 10 min at 10 - 20°C can be used to control 'post- acidification' of yogurt without decreasing the number of viable lactic acid bacteria (LAB) or modifying the yogurt texture. Treatment at higher pressures destroyed LAB. When exceeding

400 MPa for 15 min, *Lactobacillus delbrueckii* subsp. *bulgaricus* was inactivated, whereas *Streptococcus thermophilus* was more resistant but it lost its acidifying capacity. An extended shelf-life 'Probiotic yogurt' has been developed using pressure treatment of 350–650 MPa at 10–15°C. The process inactivated spoilage microorganisms such as yeasts and molds but not specially selected pressure-resistant probiotics, extending the shelf life of yogurt to 90 days.

Acid-set gels*:* Acid-set gels made from milk treated at high pressure (600 MPa) for 15 min had improved mechanical properties (gel rigidity and gel breaking strength). An increase in 8-9 fold in gel rigidity was noted with increased resistance to syneresis. Such effect was attributed to a pressure-induced increase in cross-linking sites and improved gel structure due to increased numbers of network strands.

Cheese: High-pressure treatment of milk at ≤ 300MPa decreased the rennet coagulation time (RCT) and increased the curd firming rate, curd firmness and curd yield during cheese manufacture. At such pressures, the casein micelle was largely intact or increased slightly in size, and the extent of denaturation of β-lactoglobulin was modest. At higher pressures (> 300 MPa), the RCT remained unaffected or increased when compared to that of untreated milk. The effect of high pressure appeared to be the result of two mechanisms with opposite effects (i) disintegration of the casein micelle and (ii) denaturation of β-lactoglobulin with resultant association with the casein micelles. The quality of Cheddar cheese manufactured from high-pressure treated (31-min cycle at 586 MPa) milk was not significantly different in sensory quality to that made from high-temperature, short-time (HTST) pasteurized milk. However, the pressurized milk cheese had higher moisture content, which led to pasty and weak texture defects. Such an effect was attributed to the increased water-holding capacity of the milk proteins (Drake *et al.,* 1997). High-pressure treatment of cheese curd rather than cheese milk had beneficial effects.

Cream, Butter and Ice-cream: The cream (30 and 43 per cent fat) was subjected to HPP of 100 to 150 MPa at 23°C for pasteurization and then studied for freeze fracture and transmission electron microscopy (Buchheim and Nour, 1992). It was observed that pasteurization induced fat crystallization within the small emulsion droplets mainly at the globule periphery. Fat crystallization increased with the length of pressure treatment and was maximal after processing at 300-500 Mpa. Moreover, the crystallization proceeded during further storage, after pressure release. Two potential applications of HPP were fast ageing of ice cream mix and physical ripening of dairy cream for butter making. Whipping properties improved when cream was treated at pressure of 600 MPa for up to 2 min (Eberhard *et al*., 1999) probably due to better crystallization of milk fat. When treatment conditions exceeded the optimum condition, an excessive denaturation of whey protein occurred which resulted in longer whipping time and destabilization of whipped cream.

Dumay *et al.* (1996) studied the effect of HPP on pasteurized and UHT sterilized dairy creams (35 per cent fat) at 450 MPa at 25°C for 15 or 30 min or at 10 or 40°C for 30 min. In case of pasteurized cream, pressurization at 450 MPa at 10 or 25°C did not modify its fat globules size distribution or its flow behavior or the pH. No further acidification was observed during storage of cream for 8 days at 4°C. In contrast, HPP carried out at 40°C induced surface changes in fat globule; changes being partly reversible with storage time. Sterilized cream was more sensitive than pasteurized cream to aggregation phenomenon. A limiting effect of high-pressure application on fat-rich product could be the induction of autoxidation. Oleic acid was not affected by HPP, but autoxidation of linoleic acid was increased by pressure exceeding 350MPa.

Conclusion

The main effects of high pressure treatment in milk appeared to involve dissociation of caseins micelles from the colloidal to the soluble phase, resulting in reduced turbidity of milk, decreased RCT, increased pH and reduction in whiteness. Flavour and aroma components contributing to the sensory quality, and nutritional quality remained unaffected by pressure treatment. The effect of HPP on lactose has not been studied so far. This 'novel' non-thermal technology has the potential for use in the development of a whole new generation of value-added foods with enhanced functionality. The varied physico-chemical and sensory properties obtained when harnessing such novel technology offer exciting opportunities for the dairy industry. New opportunities in preservation of colostrum and human milk by HPP treatment may be of interest to the entrepreneurs.

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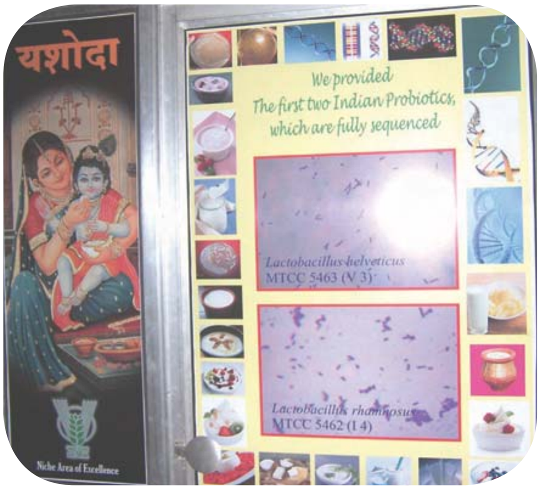

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