

High hydrostatic pressure technology in dairy processing: a review

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Abstract Consumers demand high quality foods, which are fresh, tasty and nutritious; this has created considerable interest in the development of new food processing techniques. Presently, non-thermal techniques, including high hydrostatic pressure (HHP), are regarded with special interest by the food industry. Pressure ranges between 100 and 1200 MPa have been considered as effective to inactivate microorganisms including food-borne pathogens. HHP also improves rennet or acid coagulation of milk without any detrimental effect on flavour, body and texture and nutrients. Extended shelf-life and a “fresh-like” product presentation emphasize the need to take full account of food safety risks, alongside possible health benefits to consumers. These characteristics offer the dairy industry numerous practical applications to produce microbially safe and minimally processed dairy products with improved characteristics. Thus HHP is a powerful tool to develop novel dairy products of better nutritional and sensory quality, novel texture and increased shelf-life.

Keywords Dairy products · Hydrostatic pressure · Minimally processed · Cold processing

Introduction

In the modern era of health consciousness, consumers are well aware regarding health and consequently about food components. Consumers demand foods which are natural, nutritionally

better, free from chemical preservatives and microbiologically safe with extended shelf-life. Today, many processed foods like juice, milk and canned products are treated at high temperature to kill bacteria. Processing at high temperature lowers the nutritional quality of foods because many nutrients are heat labile. To overcome these problems, several non-thermal processing or “cold processing” techniques including high hydrostatic pressure technology (HHP) have been developed. Temperature employed in most food applications is in the refrigeration to ambient range (Farr 1990). Although, energy optimization and heat recovery have been the focus in the past decades for conventional food processes, their replacement by novel food preservation technologies may provide additional opportunities to reduce energy consumption so as to improve sustainability of food production (Toepfl et al. 2006). These newer processing concepts have gained acceptance owing to their ability to destroy pathogenic microorganisms with minimal heat treatment yielding almost complete retention of nutritional and sensory characteristics of fresh foods without sacrificing shelf-life (Devlieghere et al. 2003). Introducing non-thermal processing prior to drying may provide opportunities to improve the energy efficiency of food processing. High pressure technology is increasingly being used to produce value-added food products.

Hite (1899) was the first to report the effect of HHP on food borne microorganisms by subjecting milk to a pressure of 650 MPa and obtaining a significant reduction in the number of viable microbes. Though this technology was evolved in Japan but number of countries are adopting it and till date variety of food products like jams, fruit juice (tomato juice) (Mohácsi-Farkas et al. 2002), meat, oysters, ham, fruit jellies and pourable salad dressings, salsa and poultry have been processed using this technology (Anon 2006). Nevertheless, interest in HHP derives from its ability to deliver foods with fresh-like taste without added preservatives. In

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high pressure processing, food is subjected to pressures as high as 6000 times the atmospheric pressure usually within the range of 300–700 MPa (Anon 2006) and is effective in killing most of the vegetative bacteria at pressures above 400 MPa. The most attractive feature, which made the process worldwide acceptable, is its uniform processing ability, independent of mass and time. The HHP can be used to process both liquid and solid (water-containing) foods and adds advantages to the foods (Makhal et al. 2003) such as (i) Kills bacteria in the raw food, (ii) Extends shelf-life, (iii) Ponders additive free and fresh food, (iv) Manipulates the texture and (v) Enhances desired attributes (digestibility).

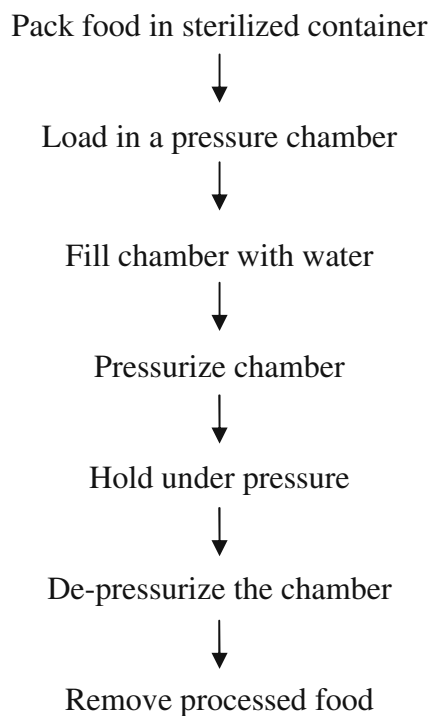
The operating principles behind this technology are as follows:

Le Chatelier's principle: Any phenomenon in equilibrium chemical reaction, phase transition and/or change in molecular configuration is accompanied by decrease in volume, which can be enhanced by pressure (Ramaswamy et al. 1999).

Isostatic principle: The transmittance of pressure is uniform and instantaneous (independent of size and geometry of food) (Ramaswamy et al. 1999).

Operational technology

In high pressure processing, the pressure vessel is filled with a food product and pressure is applied for a desired time following which it is depressurized. A simplified flow-sheet is given below:



The time required to develop pressure in the vessel is influenced by the compressibility of the pressure medium and the nature of the food material. In most cases, water is used as the pressure transmitting medium. Presence of air in the food increases the pressurization time, since air is considerably more compressible than water. The pressure is applied isostatically. Therefore, pressure remains uniform in the product and the entire product undergoes the same treatment (Fig. 1). High pressure is non-thermal in principle, but the pressure increase causes a small adiabatic rise in temperature (Ohlsson and Bengtsson 2002).

The rise in temperature, caused by inner friction, occurs when fluids are compressed to extreme temperatures and can be expressed as

$$\frac{dT}{dp} = \frac{\beta T}{\rho C_p}$$

where, β , ρ and C_p denote the thermal expansivity, the density and the specific heat capacity of the compressed fluid, respectively. The thermo physical properties β , ρ and C_p are pressure-temperature dependent. When these parameters are known, the calculation of the thermal profile during the compression phase is possible (Toepfl et al. 2006). Food is then kept under high pressure for the required process time and upon completion of the exposure in pressurized vessel, depressurization is done quite rapidly. In general, process pressure of 680 MPa results in 15% compression of the liquid treated.

Mechanism of HHP

As stated by Le Chatelier, HHP affects any phenomenon in food systems where volume changes are involved and favours phenomenon, which causes decrease in volume (Anon 2007a). The process affects non-covalent bonds (hydrogen, ionic, hydrophobic bonds) substantially, owing to their sensitivity towards pressure. Compounds with low molecular weight (responsible for nutritional and sensory characteristics) are not affected but high molecular weight components (whose tertiary structure is important for determining its functionality) are sensitive (Carlez et al 1994). Process can be broadly classified into 3 main categories viz., batch, semi continuous and continuous.

Batch operation calls for loading of packed food into the pressure vessel, following which the vessel is sealed and water is pumped into the vessel to displace any air. Upon filling the vessel, pressure relief valve is closed and pressure is allowed to build up within the vessel. Pressure is allowed to remain in contact with the product for a particular time-pressure combination and upon completion, the pressure relief valve is opened to allow

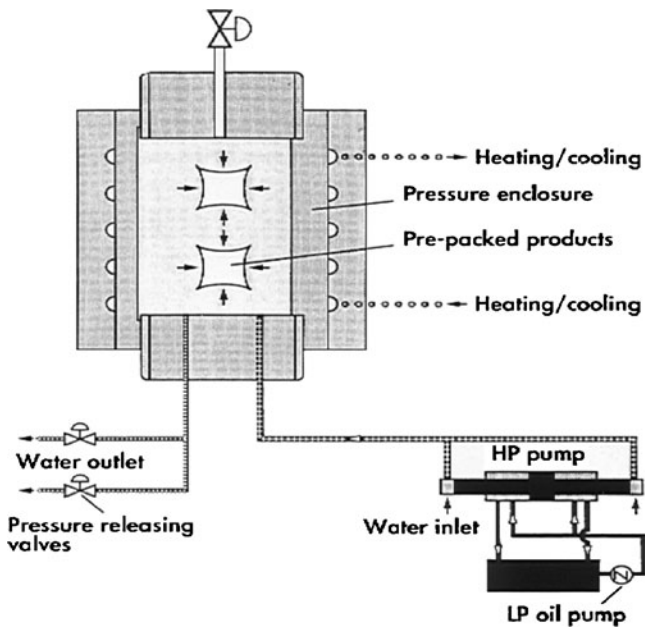


Fig. 1 A typical high-pressure processing system for treating pre-packaged foods (Source: Anon (2008))

the water used for compression to expand and return to atmospheric pressure (decompression). The vessel is opened, the packaged food is removed and is ready for shipment. Advantages include: prevention from cross contamination risk- as packaged food is loaded inside the vessel; no need for clean up between runs and usage of relatively simple equipment as compared to semi continuous systems where free piston is used to increase the compression over the liquid food. Food is introduced via low pressure pump and when the vessel is filled the free piston is displaced. When filled, the inlet port is closed and high pressure process water is introduced behind the free piston to compress the liquid food (Fig. 2). The pressure treatment can be carried out between 3000 and 6000 bars and at temperatures ranging from -20°C to 80°C , according to the equipment used (Anon 2007b).

After an appropriate process hold time, the system is decompressed by releasing the pressure. The treated food liquid material is then transferred to a sterile tank through a sterile discharge port. The treated liquid food is filled aseptically into pre-sterilized containers. Continuous system compresses, the liquid food continuously via provided plug flow hold tube or hold vessel (Anon 2001).

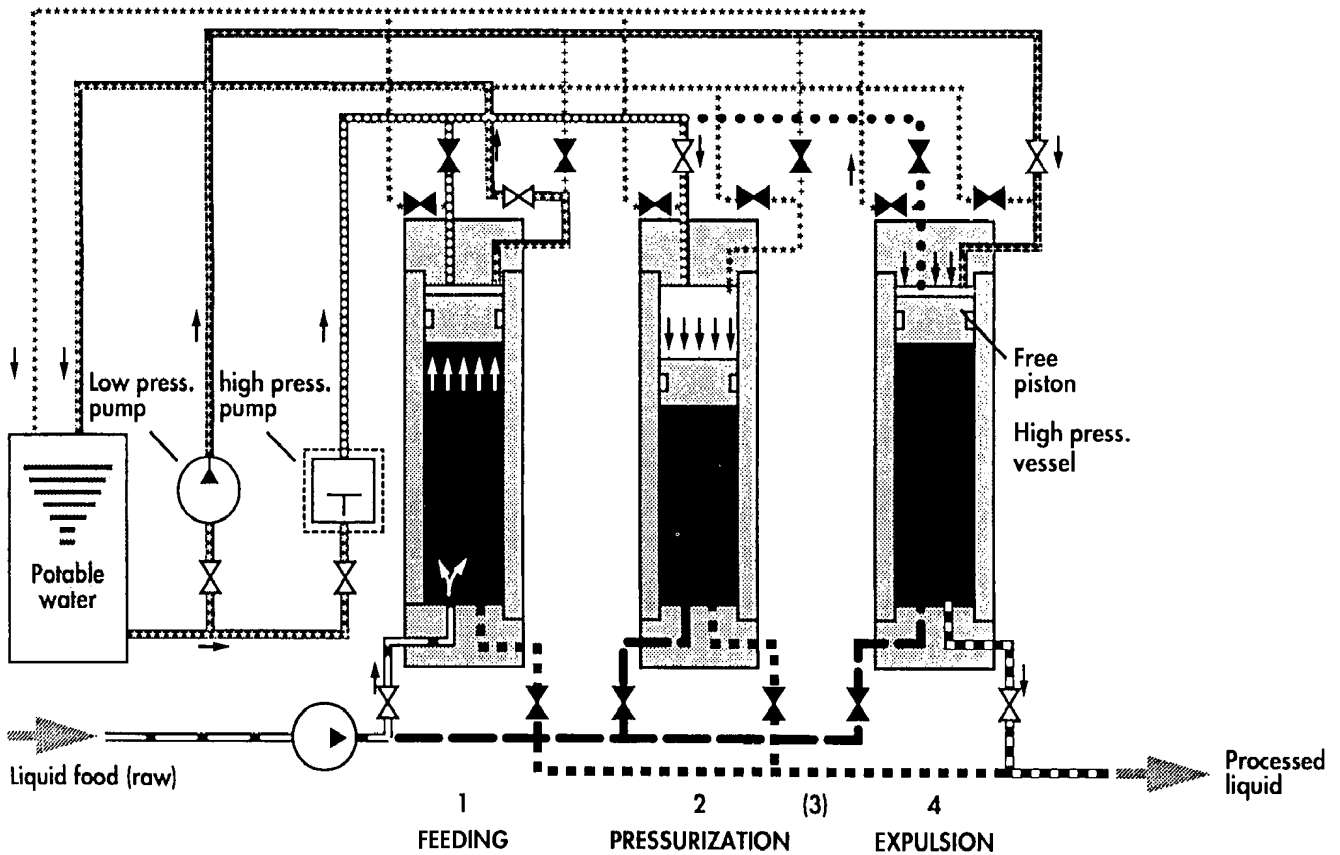


Fig. 2 A multivessel arrangement for semi-continuous high-pressure processing (Source: Anon (2001))

Application of HHP in dairy industry

Effect of HHP on casein HHP greatly influences the physico-chemical and technological properties of milk. When subjected to HHP, the casein micelles get disintegrated into smaller particles, which in turn are accompanied by an increase in casein and calcium phosphate levels in the serum phase of milk with a decrease in both non-casein nitrogen and serum nitrogen fractions (Law et al. 1998). Pressure above 3000 atmosphere tends towards irreversible denaturation as compared to reversible denaturation within range of 1000–3000 atmospheres (Jaenicke 1981). HHP promoted extensive disruption of casein micelles in the 250–310 MPa range. However, addition of whey protein to casein isolates protected the micelles from high pressure induced disruption (Harte et al. 2007).

Pressurization of milk causes conformational changes in milk proteins. With the application of HHP the size and number of casein micelles increases (as casein micelle dissociates into submicelles due to weakening of hydrophobic and electrostatic interactions between sub micelle and further aggregation of sub micelle to big cluster) as spherical particles change to form chains or clusters of sub-micelles (Huppertz et al. 2006). This reduces the rennet coagulation time. Similar results were reported by Sivanandan et al. (2008) along with decrease in milk turbidity and lightness (Johnston et al. 1992).

Effect of HHP on whey proteins High pressure treatment also enhances pepsin hydrolysis of β -lactoglobulin (β -LG) at 400 MPa, reduction in antigenicity and Immunoglobulin E (IgE) binding of β -LG, which further opens the possibility of obtaining hypoallergenic hydrolysates of β -LG (Chicon et al. 2008). A pressure treatment of 500 MPa at 25°C denatures lactoglobulin. Denaturation of immunoglobulins and lactalbumins occurs only at the highest pressures, particularly at temperature above 50°C, which gives an idea of preservation of colostrum immunoglobulins which otherwise gets damaged during heat treatment (Felipe et al. 1997).

Liu et al. (2005b) studied the effect of HHP on hydrophobicity of whey protein concentrate and recorded that HHP treatment of whey protein concentrate yields increase in the number of binding sites which leads to certain modifications in proteins that enhance hydrophobicity and shows promising results for improving functional properties of foods. Similar observations for improved hardness, surface hydrophobicity, solubility, gelation and emulsifying properties were recorded in whey proteins functionality (Lee et al. 2006a).

Effect of HHP on fat Hydrostatic pressure up to 500 MPa catalyses modifications in size and distribution of milk fat

globules of ewes' milk. HHP treatments at 25 and 50°C showed a tendency to increase the number of small globules in the range 1–2 μ m whereas at 4°C the tendency gets reversed (Gervilla et al. 2001). However, no damage to the milk fat globule membrane occurred. These modifications in distribution of milk fat globules could be due to aggregation and disintegration of fat globule membrane. Also, HHP-treated milk showed advantage of stability against creaming off, when done at 25 and 50°C, but at 4°C reverse phenomenon was observed.

Studies carried out by Gervilla et al. (2001) on free fatty acids (FFA) content (lipolysis of milk fat) in ewes' milk showed that HHP treatments of 100–500 MPa at 4, 25 and 50°C did not increase FFA content. Even some treatments at 50°C showed lower FFA content than fresh raw milk. Thus, this phenomenon is of great interest to avoid production of off flavours, which otherwise develop because of lipolytic rancidity in milk.

Effect of HHP on milk sugar Lactose in milk and milk products may isomerise to lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds have been observed after pressurization (100–400 MPa for 10–60 min at 25°C) suggesting no Maillard or lactose isomerization reaction occurs in milk after pressure treatment (Lopez-Fandino et al. 1996).

Effect of HHP on colour Micelle disintegration induced by HHP treatment also affects milk colour. A study was carried out by Harte et al. (2003) to observe the series of changes during combined treatment of thermal and HHP in yogurt manufacture. They observed that milk subjected to HHP treatment and thermal treatment followed by HHP, loses its white colour and turns yellowish. This might be due to reduction in size of casein micelles (Needs et al. 2000). Whereas milk when first subjected to HHP followed by thermal treatment regained its whitish colour and this may be attributed to reversible nature of casein micelles (or reaggregation of disrupted micelles) towards HHP treatment when applied in the range of 300–676 MPa followed by thermal treatment. Similar observations were recorded by Gervilla et al. (2001) when ewes' milk was HHP treated.

Contrary to thermal treatments, where covalent as well as non-covalent bonds are affected, HHP treatment at room and mild temperatures disrupts relatively weak chemical bonds (hydrogen bonds, hydrophobic bonds, ionic bonds) only. Thus, small molecules such as vitamins, amino acids, simple sugars and flavour compounds remain unaffected by HHP treatment. HHP treatment of milk at 400 MPa (@ 2.5 MPa/sec for 30 min at 25°C) results in non-significant loss of vitamin B1 and B6 (Sierra et al. 2000). Garcia-Risco et al. (2000) found that HHP treatments at 400 MPa for 15 min at 40–60°C reduces the proteolytic activity and at 25–60°C

improves the organoleptic properties of milk, suggesting that these combined treatments could be used to produce milk of good sensory properties with an increased shelf-life.

Effect of HHP on milk flavour components Liu et al. (2005a) studied the effect of HHP on flavour binding properties using whey protein concentrate and observed that treatment with 600 MPa at 50°C resulted in an increase in number of binding sites of WPC from 0.23 to 0.39 per molecule of protein for heptanone and from 0.21 to 0.40 for octanone.

Cheese production from pressure treated milk Milk pasteurization (heating at 72–74°C for 15 s or equivalent treatments) destroys pathogenic and almost all spoilage microorganisms and it is the most important heat treatment applied to cheese milk to provide acceptable safety and quality. However, milk pasteurization is known for its adverse effects with respect to many sensory characteristics of cheese, leading to alterations in texture and often delayed maturation (Grappin and Beuviel 1997). Thus the technology can be used to increase microbiological safety and quality of milk to produce high quality cheeses. As mentioned, HHP processing of milk at room temperature causes several protein modifications such as whey protein denaturation and micelle fragmentation and also alters mineral equilibrium. It has been observed that denaturation of whey proteins is due to applied pressure and results in interaction between denatured whey protein and casein, which in turn increases the retention of former within casein matrix of cheese. Thus, these changes result in modifying the technological parameters of milk to make cheese, improving the rennet coagulation properties and yield of cheese (Trujillo et al. 1999, San Martin-Gonzalez et al. 2004, Zamora et al. 2007).

Study conducted on Caciotta cheese showed an increase in yield by 3% over heat treated milk along with improved water binding capacity of proteins, higher retention of curd and also induced a significant modification in the volatile compound profiling of cheese and assisted in accelerated ripening process (Lanciotti et al. 2006, Stewart et al. 2006). Lopez-Pedemonte et al. (2006) evaluated the combined effect of ultrahigh pressure homogenization (UHPH) followed by HHP treatment on *Staphylococcus aureus* and found complete inactivation after 15 days storage of cheese made from UHPH and HHP treated milk. Similar observations were recorded in soft chesses made from pasteurized cow milk inoculated with 2 strains of *Staphylococcus aureus* CECT 4013 or ATCC 13565 (Lopez-Pedemonte et al. 2007a) and in case of *Listeria monocytogenes* and *Yersinia enterocolytica* in cheese by Lopez-Pedemonte et al. (2007b) and de Lamo-Castellvi et al. (2005). Microbiological quality of cheeses made from HHP-treated milk (500 MPa for

15 min at 20°C) is comparable to pasteurized milk (72°C for 15 s) cheeses (Buffa et al. 2001). However, the application of pressure to cheese milk causes differences in cheese composition and ripening in comparison to pasteurized milk cheese. The HHP-treated milk cheeses retain higher moisture, salt and total free amino acids contents than raw or pasteurized milk cheeses. On the other hand, cheeses made from HHP-treated milk showed a similar level of lipolysis as in cheeses made from raw milk, whereas the level of lipolysis in cheese made from pasteurized milk was lower and this behaviour was explained by heat-sensitive but partial pressure-resistant characteristics of the indigenous milk lipase. Also pressure treated cheese shows more viscoelastic texture and poses less resistance to flow (Messens et al. 1999).

In a study conducted by Lee et al. (2006b), low fat processed cheese food prepared from ultra high pressure treated whey protein resulted in acceptable firmness and meltability. However, the texture was undesirable because of sandy or grainy texture. This could be due to unfolding of whey protein during ultra high pressure treatment which contributed in rough protein matrix as revealed in the microstructure studies.

Cheese ripening acceleration Cheese ripening, though is the last step in cheese making, deserves a special attention and importance in cheese making owing to its expensiveness and relation with the final quality of the product. Thus, accelerated ripening is highly desirable. Most of the work in this field has been done using elevation of ripening temperature, addition of cheese slurries or exogenous enzymes or by the use of adjunct starters, either as such or in modified form. The potential use of HHP to accelerate cheese ripening was first elucidated in a patent by Yokoyama et al. (1992). The authors reported a decrease in free amino acids, compared with untreated cheese, when pressure exceeded 300 MPa. However, the method of cheddar manufacturing was substantially different from conventional cheddar manufacturing. In particular, starter addition to the milk was at least 10 fold higher than conventional inoculation rates. In certain cheese varieties such as Mozzarella and Gouda, increased rate of proteolysis on exposure to pressure treatment of 400–600 MPa for 5–15 min was observed (San Martin-Gonzalez et al. 2004). Cheese prepared from ewes' milk showed similar (enhanced proteolysis) trend when treated with HHP (Juan et al. 2006).

Many pressure conditions have been tested for accelerating cheese ripening which involves 'high' HHP treatments (400–600 MPa) short times (5–15 min) or an initial 'high' HHP treatment (400–600 MPa) short times (5–15 min) followed by a 'low' HHP treatment (50 MPa) long time (72 h) for different cheese varieties. The enhancement effect is assumed to be caused by the release of starter

enzymes. Saldo et al. (2001) evaluated the effect of HHP treatment (50 MPa for 72 h) on ‘Garrotxa’ goat cheese and found that HHP induce proteolysis along with improved solute diffusion, water holding capacity and salt distribution.

Yoghurt and ice-cream Yoghurt, a popular dairy product, suffers from common defects of syneresis and low viscosity. Quality of yoghurt can be improved in terms of its preservation and rheological properties by pressure treatment. Skim milk treated with combined treatments of high hydrostatic pressure (400–500 MPa) and thermal treatment (85°C for 30 min) shows increased yield stress, resistance to normal penetration, elastic modulus and reduced syneresis (Harte et al. 2003). Acid gels obtained from high-pressure homogenized milk showed a linear decrease in whey holding capacity and retention of more than 20% whey after centrifugation for 25 min (Hernandez and Harte 2008). Similarly, Needs et al. (2000) recorded lower values of fracture stress in set yoghurts made from pressure treated milk (60 MPa for 15 min) compared to heat treated milk. Yoghurt prepared from milk that was ultra high pressure homogenized at 200 and 300 MPa at 30 and 40°C considering modifications induced in the fat fraction that could delay the lipid oxidation and lower the degree of lipolysis, resulted in expected results (Serra et al. 2008).

Reps et al. (1999) found that HHP treatment of 400 MPa completely inactivated *Lactobacillus bulgaricus* but *Streptococcus thermophilus* was more resistant towards pressure, with resistance varying from strain to strain and giving an idea that shelf-life of yogurt can be enhanced by HHP treatment. Penna et al. (2006) carried out a study involving combined treatment of HHP (676 MPa for 5 min) and heat (85°C for 30 min) for low fat yoghurt, using different probiotic starter cultures and reported yogurt gel with higher consistency index value along with acceptable rheological and textural properties. Another study on low-fat yoghurt prepared using similar HHP and thermal treatment conditions, resulted in dense aggregated protein structure with smooth surface, compact gel with improved gel texture and improved viscosity as compared to fewer interconnected chains in untreated yoghurt (Penna et al. 2007).

HHP treatment induces fat crystallization, shortens the time required to achieve a desirable solid fat content and thereby reduces the ageing time of ice-cream mix and also enhances the physical ripening of cream for butter making (Buchheim and Frede 1996). Pressurization treatment improves whipping ability of cream when treated for 2 min at 600 MPa and is possibly due to better crystallization properties of milk fat (Eberhard et al. 1999). Excessive or lesser than the desired treatment does not cause appreciable change in properties of cream. Study with modified whey protein concentrate added at a

concentration of less than 10% in ice-cream mix exhibited enhanced overrun and foam stability, confirming the effect of HHP on foaming properties of whey proteins in a complex system (Lim et al. 2007).

Effect of HHP on microorganisms Milk, being a perishable commodity, is usually heat-treated with specific time temperature combination, to provide acceptable safety and shelf-life. Though heat treatment extends the shelf-life but simultaneously destroys its natural nutrients. Hence, to preserve its nutritional value, HHP treatment as an alternative can be advocated. The resistance of microorganisms to pressure is variable depending upon processing conditions (pressure, time, temperature and cycles), food constituents, its properties and the physiological state of the microorganism (Smelt 1998). Cells at their exponential growing stage are more sensitive to pressure than in the stationary phase. The bacterial spores are more resistant than vegetative cells and they can survive at pressure of 1000 MPa (Cheftel 1992). However, it has been found that the pressurization along with mild heat treatment triggers spores to germinate and after that they lose their resistance to pressure and heat and get killed (Gould and Sale 1970; Knorr 1995; Gould 2000). Gram-positive microorganisms are more resistant to pressure than Gram-negative, e.g. Gram-positive organisms need an application of 500–600 MPa at 25°C for 10 min to achieve inactivation while Gram-negative organisms can be inactivated with 300–400 MPa with the same time- temperature combination.

Vegetative forms of yeasts and moulds are most pressure sensitive compared to their spores (Smelt 1998) and results in 99.99% of reduced activity of psychrotrophics, lactococci and total bacterial count (Pereda et al. 2007) along-with reduction in yeast and mould growth (Daryaei et al. 2006). Vachon et al. (2002) revealed that dynamic high pressure treatment inactivates 3 major food pathogens (*Listeria monocytogenes* LSD 105-1, *Escherichia coli* O157:H7 ATCC 35150 and *Salmonella enteritidis* ATCC 13047) present in milk. High pressure applied in the range of 300–500 MPa for 15 min resulted in inability of lactococci to acidify milk, though the peptidolytic activity remained constant even after treatment. Thus, forming the basis for elaborating technological guidelines of the production process of an enzymatic preparation capable of intensifying proteolytic transformations in dairy products (Krasowska et al. 2005).

Many studies have proved that HHP treatment at 400–600 MPa gives raw milk the same quality as that of pasteurized milk. However HHP does not give the quality of sterilized milk (due to presence of pressure resistant spores). HHP is equally effective in destroying pathogenic and spoilage microorganisms. For example, to achieve a shelf-life of 10 days at a storage temperature of 10°C, a

pressure treatment of 400 MPa for 15 min or 600 MPa for 3 min at 20°C is necessary (Rademacher and Kessler 1997). In order to get quality comparable that of sterilized milk, combined treatment of HHP along with heat is an important consideration in this regard.

Advantages of HHP

HHP treatment provides innumerable advantages over conventional processes being rapid and providing uniform distribution of pressure throughout the sample irrespective of size and shape, which helps to produce less thermal degradation. The process suitably handles both particulate and pumpable foods with less process time dependence (as cycle takes no longer than 6 min compared to traditional sterilization processing, which takes an hour or more and thus unveils the opportunities for new process/ product development). Pressure also accelerates traditional thermal inactivation kinetics of microorganisms and eliminates the risk of various food borne pathogens such as *Escherichia coli*, *Salmonella* and *Listeria* inside the packaged food products without additives.

Compared to thermal process, food may not undergo significant physical and chemical changes as HHP does not affect the flavour, nutritional value or texture properties of the product. Acidic foods are particularly good candidates for high pressure processing. A shelf-life extension of 2–3 folds over non pasteurized counterpart has been reported by various workers along with improved food safety. These advantages have led to high consumer acceptability of HHP treated products like glucamole, juices, oysters, ham, fruit jellies and jams, pourable salad dressings, salsa, poultry and rice products, which are available in supermarkets.

One of the limitations of this process is that food must contain water, as the entire process is based upon compression. Protein rich foods get deteriorated in appearance as pressure-induced denaturation is visually evident. Structurally fragile foods such as strawberries are prone to pressure disintegration. The process is also not suited for enzyme and spore inactivation. Hence, low acidic shelf-stable products such as soups are not yet commercially available because of the limitations in killing spores with HHP.

Epilogue

HHP products are becoming choice of a modern consumer in terms of health and safety aspects. Being one of the emerging technologies in developing countries, high pressure technology offers the food technologists an opportu-

nity to develop novel products with enhanced shelf-life and higher safety with better sensory and nutritional aspects. Being applicable to a wide range of products, this technology offers food processors to manufacture minimally processed shelf stable products. Also these non-thermal technologies provide a potential to reduce energy requirements in food processing industries.

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