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Applications of high pressure technology for milk processing

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ABSTRACT : Consumers growing preference for convenient, fresh-like, healthy, palatable, additive-free, high quality and micro-biologically safe food products creates the need to explore beyond the conventional thermal processing techniques. Consequently, a number of non-thermal approaches to food processing have evolved, of which High Pressure Processing (HPP) technology has proven to be very valuable. Attention in HPP applications on milk and dairy products has recently increased. Studies in this arena have shown that pressures between 300 and 600 MPa are effective to inactivate milk -borne pathogens. In addition to microbial destruction in milk, it has been reported that HPP improves rennet or acid coagulation of milk, ripening of cheese, syneresis and firmness in fermented milk product, ageing of ice cream mix, ripening and fat crystallization of dairy cream, without damaging important natural quality characteristics, such as taste, flavour, vitamins, and nutrients. Present paper highlights the research findings and effects of HPP technology in milk processing.

KEY WORDS : High pressure processing, Milk, Technological changes, Microbial destruction

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INTRODUCTION

Innovation in the dairy industry is crucial in enhancing international competitiveness in a truly global market. Such advancement needs to be critical in meeting consumer demands for 'novel foods' that are not only safe and nutritious, but also natural, economic, convenient, delicious, appetizing and much more. Real challenge is to achieve this within the technical, environmental, nutritional and legal constraints (Datta and Deeth, 1999). The need to meet these objectives necessitates the processors to look beyond the conventional thermal food processing technologies. As a result, numerous non-thermal processing technologies, particularly high pressure (HP), power ultrasonics, and pulsed electric field, hurdle technology, etc., have evolved. Among these technologies, HPP seems the most promising one for food applications. HPP is also referred to as high-hydrostatic pressure processing (HHP) or ultrahigh-pressure processing (UHP) in the literature. Regardless of its nomenclature, the technology has been cited as one of the best innovations in food processing in last 50 years (Dunne, 2005).

HPP of foods is carried out by subjecting foods to 100 - 1000 MPa pressure, temperature during the process may be maintained subzero to more than 100°C and exposure times can be few seconds to over 20 min. The first commercial HP treated product appeared in the market in 1991 in Japan, where HP is now being used for processing products such as jams, sauces, fruit juices, rice cakes and desserts. The prospective application of HP in the food industry has gained recognition in recent years, due to progress in the production of HP equipment which makes the technology more affordable. The non-availability of appropriate equipment hampered early applications of high pressure. However, recent development in equipment design has ensured global acknowledgment of the potential for such a technology in food processing.

The pioneering research into the application of HP to milk dates back to the end of the 19th century (Hite, 1899). In order to execute this new technology in the dairy industry,

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there is a need to comprehend the mechanism and kinetics of pressure – induced degradation, denaturation, inactivation of dairy compounds like nutrients, proteins, micro-organisms, enzymes etc. Beside microbial destruction, the HP effects protein structure and mineral equilibrium, suggesting its different applications in dairy sector. This review highlights the technological and microbial changes that take place in milk on exposure to high pressure.

Milk system :

In general, changes associated with volume reduction are promoted under pressure. The behaviour of biological macro-molecules under pressure is important for understanding the effects of HP on milk. Pressure and temperature determine various properties of inorganic and organic substances. Under pressure, biomolecules follows the Le Chatelier-Braun principle, *i.e.*, whenever a stress is applied to a system in equilibrium, the system will react so as to counteract the applied stress; thus, reactions that result in reduced volume will be promoted under HP. Application of HP on milk results in inhibition and destruction of micro-organism, modifications in physicochemical / technological properties by dissociation of the colloidal calcium phosphate and denaturation of serum protein (Balci and Wilbey, 1999).

Application of HP in inhibition and destruction of microorganism in milk :

Milk plays an important role for human nutrition and is one of the most frequently sold food worldwide. The nutritional composition, high water activity and neutral pH turn milk into an adequate media for microbial development in vegetative and sporulated micro-organisms. Later are thermo-resistant and important for milk deterioration. Heat treatments such as pasteurization, sterilization, ultra high temperature (UHT) are the main treatment applied to microbial stabilization of milk, since they are cheaper and simple. The heat processing of milk affects vitamins (losses around 10 % of folic acid and 15% of B-complex vitamins) and denatures proteins, resulting in the release of sulfured compounds.

Several studies on the inactivation of pathogenic and spoilage micro-organisms in milk (naturally present or inoculated) by HP have demonstrated that it is possible to obtain ‘raw’ milk pressurised at 400–600 MPa with a micro-biological quality comparable to that of pasteurised (72^0C, 15 s) milk depending on the micro-biological quality of milk (Buffa *et al.*, 2001a) but not sterilised milk due to HP resistant spores. Rademacher and Kessler (1997), reported that a shelf life of 10 days at a storage temperature of 10^0C can be achieved by a pressure treatment of 400 MPa for 15 min or 600 MPa for 3 min at 20^0C.

The resistance of micro-organisms to pressure in food depends on HPP conditions (pressure, time, temperature, cycles, etc.), food constituents and the properties and the physiological state of the micro-organism. The bacterial spores are always more resistant than vegetative cells and they can survive at pressure of 1000 MPa. Bacterial spores, however, can often be stimulated to germinate by pressures between 50–300 MPa. Germinated spores can then be killed by heat or mild pressure treatments. Gram-positive micro-organisms tend to be more resistant to pressure than gram-negative micro-organisms. However, a considerable variation in pressure resistance within strains of the same species has been demonstrated in both gram-positive and gram-negative micro-organisms. Gram-positive micro-organisms need the application of 500–600 MPa at 25^0C during 10 min to achieve inactivation, while gram-negative micro-organisms are inactivated with treatments of 300–400 MPa at 25^0C during 10 min. Vegetative forms of yeasts and moulds are the most pressure sensitive (Smelt, 1998).

McClements *et al.* (2001) studied the effect of variation in pressure resistance on growth stage (exponential and stationary phase) with respect to growth temperature (8 and 30^0 C) between two strains of *Listeria monocytogenes*, *Bacillus cereus*, and *Pseudomonas fluorescens*. Exponential-phase cells were significantly less resistant to pressure than stationary-phase cells for all of the three species studied (P < 0.05). Growth temperature was found to have a significant effect at the two growth stages studied. Exponential cells grown at 8^0 C were more resistant than those grown at 30^0 C, but for stationary-phase cells the reverse was true. *B. cereus* stationary-phase cells grown at 30^0 C were the most pressure resistant studied. *L. monocytogenes* showed the most sublethal damage compared to *B. cereus* and *P. fluorescens*. *B. cereus* spores were more resistant to pressure than vegetative cells. Pressure treatment at 400 MPa for 25 min at 30^0 C gave a 0.45-log inactivation. Pressure treatment at 8^0 C induced significantly less spore germination than at 30^0 C.

Studies conducted by Gervilla *et al.* (1997) on Ewe’s milk containing 6 per cent fat inoculated with *Listeria innocua* 910 CECT at a concentration of 10^7 CFU/ml shown that low-temperature (2^0 C) pressurizations produced higher *Listeria innocua* inactivation than treatments at room temperatures (25^0 C). Pressures between 450 and 500 MPa for 10 to 15 min were needed to achieve reductions of 7 to 8 log units. The kinetics of destruction of *L. innocua* were first order with *D*-values of 3.12 min at 2^0 C and 400 MPa and 4 min at 25^0 C and 400 MPa.

Application of HP in modification of physico-chemical properties of milk :

HP treatment significantly affects many constituents of milk. The structure of casein micelles is disrupted and the whey proteins, α-lactalbumin and β-lactoglobulin, are denatured, with the former being more resistant to pressure than the latter. Pressure-induced shifts in the mineral balance in milk also occur and moderately high pressures (100–400 MPa) induce the crystallisation of milk fat. However, milk enzymes seem to be quite resistant to pressure. As a result of pressure-induced effects on individual milk constituents, many physico-chemical properties of milk are affected which are discussed below.

Appearance and colour :

The earliest and most prominent difference between heat and pressure treatment of skim milk is the appearance of the milk directly after treatment. On heat treatment the skim milk becomes whiter. On storage, the milk remains white regardless of the storage conditions. In contrast, on pressure treatment skim milk becomes translucent or semi-transparent with a slightly yellow hue. On storage at refrigerated conditions (about 5^0C), it will retain the semi-transparent appearance for long periods (several days). On the contrary, if the milk is held at room temperature, it becomes progressively more turbid, but does not return to the original appearance of the untreated milk. However, on heating pressure-treated milk at elevated temperatures (70^0C) causes it to return to an appearance similar to that of the original untreated skim milk (Gaucheron *et al.*,1997).

Johnston *et al.* (1992a) proposed that the decrease in *L** value could have been mainly due to disintegration of casein micelles by pressure into small fragments and also Gervilla *et al.* (2001) observed the same, he reported a decrease of *L** (lightness) and an increase of greenness (-*a**) and yellowness (+*b**) when ewe's milk was HP treated. Needs *et al.* (2000) observed that when skim milk treated at 600 MPa for 15 min significant changes in *L**, *b** and *a** values, that could be also perceived visually. Warming of samples from 4 to 43^0C derived back colour values of HP milk towards the values of untreated milk, although not to the same initial point. Treatment at 200 MPa at 20^0C had only a slight effect on the L-values, but treatment at 250–450 MPa significantly decreased the L-value of pasteurised or reconstituted skim milk. Treatment > 450MPa had little further effect on the L-values. Mussa and Ramaswamy (1997) found that cow's milk shows the low sensitivity of milk colour to pressure.

pH of milk :

HP treatment alters the distribution of minerals, chiefly calcium and phosphate and the level of ionized minerals. HP treatment raises the concentration of ionic calcium in milk. As a result of increases in the concentration of phosphate in the milk serum, increases in milk pH occur (Zobrist *et al.*, 2005). Johnston *et al.* (1992b) reported no significant effect of pressure up to 600MPa on the pH of skim bovine milk, several succeeding studies have observed increases, of varying magnitude, in the pH of HP treated caprine milk (De la Fuente *et al.*, 1999) or raw (Buchheim *et al.*, 1996a), pasteurised or UHT treated (Schrader and Buchheim,1998) bovine milk. Processing at higher pressures or lower temperatures increased the degree of the pH-shift, which was less in raw or pasteurised milk than in UHT treated milk and may thus be related to dissolution of colloidal calcium phosphate. Increase in pH is rapidly reversible on subsequent storage at 20^0C, but virtually irreversible at 5^0C (Zobrist *et al.*, 2005).

Particle size changes :

When skim milk is subjected to a HP treatment at about 300 MPa, a substantial decrease in the size of the particles was found, with the average size decreasing from about 200 to 100 nm, regardless of the temperature at pressurization. However, in pressure range of 200 and 300 MPa, pH of the milk, temperature, pressure and duration of the pressure treatment affects an increase in particle size (Anema *et al.*, 2005a). Early reports suggested that the denaturation of the whey proteins may be responsible for this aggregation phenomenon (Huppertz *et al.*, 2004a) but successive studies carried on whey protein- depleted systems point out that the aggregation is owed entirely to the casein micelles (Anema *et al.*, 2005b). Pre-heat treated milk at 90^0C/10 min shown particle size changes similar to those in unheated milk over the entire pressure range (Huppertz *et al.*, 2004c), demonstrating that the pre-denaturation of the whey proteins has little effect on the aggregation or disaggregation phenomenon in pressure-treated milk. This change in particle size was accompanied by a considerable increase in the level of serum phase casein, and the changes in size and serum phase casein appeared to be correlated. The aggregation of the casein micelles was more pronounced as the temperature at pressurization was increased and the duration of pressure treatment was increased (Anema *et al.*, 2005a). Gervilla *et al.* (2001) conducted study on ewe's milk and reported that high pressure up to 500 MPa produces some modifications on size and distribution of milk fat globules of ewe's milk. HP treatments at 25 and 50^0C showed a tendency to increase the number of small globules in the range 1–2 mm, whereas at 4^0C the tendency was the reverse. Gaucheron *et al.* (1997) reported that Pressure treatment of about 250 MPa and at temperatures above 20^0C, two distinct populations of sizes were observed, with the formation of particles larger and smaller than the original casein micelles. When the pH of the milk was reduced from the natural pH (6.7) to 6.5, the particle size decreased as the pressure increased, with a marked decrease in size above about 200 MPa (Huppertz *et al.*, 2004a).

Renneting properties of milk :

HP can be applied at several stages during cheese making but here only the work related to rennet coagulation will be discussed. Desorby-Banon *et al.* (1994) reported that HP treatment of <150 MPa had no effect on the RCT (rennet coagulation time). However, RCT was reduced markedly after treatment at 200 - 600 MPa. In general, rennetting properties of milk subjected to HP treatment of 200 MPa for 30 min are improved, whereas pressures above 300 MPa increase the rennet coagulation time of milk (Buffa *et al.*, 2001b). The pressure-induced disruption/disintegration of the casein micelles appears to reduce the RCT and the cutting time, whereas the denaturation of the whey proteins increases the RCT and the cutting time, in a similar fashion to that for the heated milk. These results have been interpreted as two opposing phenomena. Temperature of treatment and pH of the milk has considerable effect on the RCT of HP-treated milk. Treatment at 50-60^0 C (200 MPa) delayed rennet coagulation of milk (Lo´pez Fandino *et al.*, 1998a). Arias *et al.* (2000) reported acidification of milk (pH-5.5) before HP treatment decreased its RCT whereas alkalinisation (pH-7.0) had the opposite effect.

Destruction of volatile compounds :

HP processing of milk at room and mild temperatures only disorders moderately weak chemical bonds such as hydrogen bonds, hydrophobic bonds, ionic bonds. Thus, small molecules such as vitamins, amino acids, simple sugars and flavor compounds remain unchanged by the HP treatment (Cheftel *et al.*, 1992). Sierra *et al.* (2000) reported that HP treatment of milk at 400 MPa results in no significant loss of vitamins B1 and B6. Garcia *et al.* (2000) found that HP treatments at 400 MPa for 15 min at 40-60^0 C reduce the proteolytic activity and at 25-60^0 C maintain or improve 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.

Conclusion :

From the comprehensive knowledge of the mechanism and kinetics of pressure – induced degradation, denaturations, inactivation of dairy compounds like nutrients, proteins, micro-organisms, enzymes etc., this technology can be effectively applied in rennet or acid coagulation of milk, ripening of cheese, syneresis and firmness in fermented milk product, ageing of ice cream mix, ripening and fat crystallization of dairy cream and many other dairy products.

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