HIGH-PRESSURE PROCESSING AND ITS APPLICATIONS IN THE DAIRY INDUSTRY

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ABSTRACT

High-pressure processing (HPP) is a novel, non-thermal food processing technology. Processing of foods by this method offers an alternative to thermal processing as it is carried out near the ambient temperature, thus, eliminating the adverse effects of heat and keeps the sensory and nutritional attributes of the food fresh like. This paper outlines the salient principles of the high pressure processing, equipment available, microbial inactivation mechanisms, applications of high pressure in the processing of dairy products, effect of pressure treatment on the milk constituents and further research needs for adaptation of the process in the dairy industry.

KEYWORDS

Non-thermal processes, High pressure processing, Pascal's law, Isostatic principle

1. INTRODUCTION

High-pressure processing (HPP) is a non-thermal method of preservation and sterilization of food products, in which a product is subjected to very high pressure, leading to the inactivation of certain microorganisms and enzymes in the food. High-pressure processing is a novel alternative to the thermal processing in the food industry. It allows food to be pasteurized at or near room temperature. High pressure (HP) treated foods have most of the "fresh like" attributes as it enables microorganisms to be destroyed with little or negative effects on flavour components, colours, vitamins and other nutrients, hence, does not hamper the nutritional and organoleptic properties of the product, thus offering considerable advantages as compared to thermal processing.

The terms pascalization, or high hydrostatic pressure (HHP) are same as high-pressure processing [1]. Research on the application of HPP for milk preservation started several decades back [2], which showed that the pressure treatment increased shelf life of milk. Research is still going on to standardize the processing of dairy products by high pressure.

Apart from preservation of the dairy products, this novel process also leads to varied physico-chemical, sensory and functional changes leading to the improvement in quality of treated products. Additionally, high pressure can cause rheological changes in food that may result in beneficial sensory and structural effects. Also, the effects of HPP on the food constituents may be helpful in formulation of novel and value added dairy products. These results, in turn open new arena and offer exciting opportunities for the dairy industry.

In the past few decades, several studies on HPP applications as an alternative dairy processing have been done. This has led to vast number of literature reviews on HPP applications for milk and dairy products. Based on previous results, authors have reviewed the equipment of HPP, microbial inactivation and applications of HPP for treatment of milk and dairy products [3, 4]. The effects of pressure on milk constituents, especially on lipids and proteins and potential of HP-induced modifications of dairy products have been reported elsewhere [5, 6], with a special focus on fermented products, packaging requirements and legal concerns [7]. All these studies converge on one or the other aspects of HPP. The compilation of all the relevant topics together in this subject has been done earlier. But, since the dynamic nature of research, a need has been to review all the previous works on HPP applications in dairy industry with the latest inclusions in the field. Therefore, the purpose of this literature review is to enlighten the recent technological developments and understandings in the field of HPP. This paper highlights the principles of HPP, equipment developed for food processing, microbial inactivation mechanisms by HPP, and applications of HPP on milk and dairy products based on several published data as well as effect of processing on constituents of milk. It suggests that there is an increasing interest of HPP adoption and hence paving of way for its commercialization in dairy industry.

2. PRINCIPLES OF HPP

The HPP is based on two basic principles [8]:

- *Le Chatelier's principle*
- *Isostatic principle (Pascal's Law)*

The Le Chatelier's principle states that 'if a change in conditions is applied on a system in equilibrium, then the system will try to counteract that change and restore the equilibrium'.
The Isostatic principle states that the HPP is volume-independent; therefore, pressure is transmitted instantaneously and uniformly throughout a sample, and pressure is gradients do not exist, so that the size and geometry of the product is irrelevant.

Typically, HPP of food is carried out at 300–600 Mega Pascals (MPa) at the room temperature for 2–30 min.

The HPP is an alternative to thermal processing as it is operated at ambient temperatures, ensuring little or no heat induced sensory changes in its components of food. It is because of the fact that, the smaller organic molecules responsible for colours, flavours, and nutrients (e.g., vitamins) have covalent bonding dominantly or exclusively, which are hardly affected by HPP.
Whereas, the large bio-molecules such as proteins, nucleic acids and polysaccharides that depend on non-covalent bonding (like hydrophobic interactions, hydrogen bonds etc.) to maintain structure and function are most affected due to this.

2.1. MECHANISM OF INACTIVATION OF MICROORGANISMS

A major function of high-pressure processing of food is destruction of microorganisms. Microbial cell destruction can be attributed to the following detrimental changes that take place when pressure is applied [3]:

- Irreversible structural changes of the membrane proteins and other macromolecules leading to cell membranes destruction
- Disruption of homogeneity of the intermediate layer between the cell wall and the cytoplasmic membrane
- Inactivation of membrane ATPase

- Disruption of nucleic acids and ribosome involved in proteins synthesis

These result into permeabilization of the membranes and concomitant leakage and loss of the contents of the cells and organelles, leading to eventual death of the bacterial cell [8].

Denaturation of the critical enzymes is also responsible for cell destruction.

Yeasts, moulds and most vegetative bacteria are inactivated by pressures between 300 and 600MPa. In general, In general, cells in the exponential phase of growth are less resistant to pressure than cells that are in a stationary phase of growth. Gram-negative bacteria are inactivated at a lower pressure than Gram-positive bacteria, and rod shaped bacteria are more sensitive to pressure than cocci shaped. Spores are more resistant than vegetative cells because of calcium rich dipicolinic acid which protects them from excessive ionization, surviving pressures over 100 MPa [9].

The level of microbial inactivation depends on the pressure applied, duration of treatment, temperature, environment and initial load and types of microorganisms [8].

3. EQUIPMENT

Typically, the HPP equipment has the following necessary components [3, 10]:

- a pressure vessel and its closure
- a pressure generation system
- a temperature control device
- a material handling system

Most pressure vessels for HPP applications are made from a high tensile steel alloy and called ‘monoblocs’ (forged from a single piece of material), able to withstand pressures of 400–600 MPa. For higher pressures, several designs and configurations like “pre-stressed multi-layer or wire-wound vessels” are used [11].

Vessels are sealed by a threaded steel closure, a closure having an interrupted thread, which can be removed more quickly, or by a sealed frame that is positioned over the vessel.

In operation of HPP, the pressure is transmitted in two ways

- Direct compression:

It uses a piston to compress the vessel, in which the pressure transmitting medium resides. The product is introduced in the medium. When compression takes place, the product gets treated

- Indirect compression

After the vessel is evacuated, the pressure transmitting medium is pumped from a reservoir into the pressure vessel using a pressure intensifier until the desired pressure is reached.

Pressure transmitting medium is generally any food-grade fluid. Commonly used medium are simply purified water mixed with a small percentage of soluble oil (for lubrication and anticorrosion), aqueous solution of mono-propylene glycol for high temperature, isopropyl alcohol for low temperature HPP [1].

Slight increase in temperature (3–9°C per 100 MPa, depending on the pressure transmitting medium) takes place due to adiabatic heating [12] and with a corresponding decrease during depressurization.

Temperature is controlled by pumping a heating/ cooling medium through a jacket that surrounds the pressure vessel.

4. APPLICATIONS IN DAIRY INDUSTRY

Research into the application of HPP for milk preservation began long back [2], when it was demonstrated that pressure treatment can extend the shelf life of milk. It was investigated such treatment as an alternative method for milk pasteurization. Several researches have been done on the applications of HPP on milk and milk products.

4.1. FLUID MILK PROCESSING

HP treatment [2] was carried out at 680 MPa for 10 min at room temperature and observed 5–6 log cycle reduction in the number of microorganisms. The effect of HP on milk has been extensively studied [13]. Many researchers have studied inactivation of microorganisms *Listeria monocytogenes, Staphylococcus aureus,* or *Listeria innocua* either naturally present or inoculated in milk [14]; and this offers a promising alternative for the pasteurization of milk [15]. Milk subjected to a pressure of 350 MPa had a shelf life of 25 days at 0°C, 18 days at 5 °C, and 12 days at 10 °C [16]. HP treatment (400 MPa for 15 min or 500 MPa for 3 min) of thermally pasteurized milk increased shelf life by 10 days [17]. Raw milk pressurized at 400 MPa for 30 min at 25°C contained <7 log psychrotrophs/ml after storage for 45 days at 7°C, whereas unpressurized milk contained > 7 log of these bacteria after only 15 days [18].

Further, the combination of high pressure with a bacteriocin (lacticin) was shown as a promising and natural method for increasing the efficiency and safety of HPP of milk. It resulted in a synergistic effect in controlling microbial flora of milk without significantly influencing its cheese-making properties [19]. Other antimicrobial peptides such as lactoferrin and lactoferricin (500μg/ml) in combination with high pressure (155–400 MPa) also resulted in enhanced microbial inactivation [20]. The gram-negative bacteria, in this case, were found to be more sensitive to high pressure, either alone or in combination with nisin, than gram-positive bacteria [21].

It is known that fat increases the thermal-resistance of microorganisms, but in case of high pressure treatment, fat content in milk (0–5 %) had no significant effect on the destruction [22]. Although Casein and lactose present in milk were the major baro-protective agents to *Escherichia coli* in milk during the treatment.

It was also noted that combination of high pressure with temperature for the processing of milk promoted the formation of few compounds leading to generation of ‘cooked’ milk flavour and sensory acceptance of treated milk was not very high [23].

Milk treated at 400 MPa results in no significant loss of vitamins like B1 and B6 [24].

4.2. CHEESE

HPP has many applications in cheese manufacture. HPP caused casein micelle disruption, whey protein denaturation, increase in milk pH and cheese yield, and reduction in rennet coagulation time, which indicates its significant potential in the cheese-manufacturing [25, 26].

As the heat treatment of milk, in spite of killing harmful pathogens makes the same unsuitable for cheese making leading to increased RCT (rennet coagulation time) and delayed maturing. So, HPP can be good alternative for cheese milk heat treatment.

The HP also reduces RCT and improves cheese yield [27] which can be ascertained from the fact that 15% increase of yield was obtained and 30% decrease in whey proteins in whey in cheese made from HP treated milk, probably due to whey proteins and casein interaction. HP treated milk cheeses have higher moisture, salt and total free amino acids content than raw or pasteurized milk cheeses. High moisture led to pasty and weak texture defects. Such an effect was attributed to the increased water-holding capacity of the milk proteins [28]. High-pressure treatment of cheese curd rather than cheese milk had beneficial effects. Rennet coagulation time was not dependant on the pressure in the lower range (less than 150 MPa), whereas at higher pressures (200–400 MPa) it decreased [29]. HP treatment (400 MPa) of pasteurized milk resulted in decreased rennet coagulation time. At 600 MPa, the rennet coagulation time was found to decrease along with decrease in pH, initial counts of nonstarter lactic acid bacteria, protein and fat content. The treatment increased incorporation of β-lactoglobulin leading to increased yield [30].
Using HPP, cheese ripening process can be greatly accelerated. A Japanese patent [31] reported possible reduction in the ripening time of Cheddar cheese to six months by HP treatment (50MPa and 25°C for three days). HP induces changes in biochemical processes such as glycolysis, lipolysis, and proteolysis during ripening of cheese leading to reduction in ripening time and quality improvement. In cheese manufactured from HP treated milk (at 300 or 400 MPa for 10 minutes), β-casein hydrolysis increased, as well as the level of free amino acids (FAAs). Similar results were reported for HP treated Cheddar cheese (50 MPa for 72 h); however, no textural changes in cheese were observed [32]. The ripening of cheddar cheese due to HPP accelerated the degradation of α_{S1} -casein and accumulation of α_{S1} -1-casein [33]. Levels of free fatty acids in cheese was decreased by HP treatment at 400MPa, indicating slower lipolysis [34]. There are scarce literatures on the effect of HP on the flavour development and volatiles in cheese. Lower levels of butyric acid and acetoin in Gouda cheese after HP treatment between 50 and 400 MPa were reported [35]. Further studies will be necessary to determine the full potential of HP in exploiting cheese ripening.

HP-treated (500 MPa) goats' milk had higher pH and salt content, showed faster maturation, and strong flavours generation [36].

4.3. ICE-CREAM

HPP induces fat crystallization [37], curtails the time required to achieve a desirable solid fat content and thereby reducing the ageing time of ice-cream. HP treatment has an impact on the functional properties of whey proteins. HP treatment of 300 MPa for 15 min enhanced the foaming properties of whey protein concentrate, which when added to low-fat ice cream to improved body and texture of ice-cream, showed an increased overrun and foam stability and hardness the ice cream than ice cream added with untreated whey protein [38].

A team of researchers [39] studied the applications of HP processing in ice-cream manufacture. For this purpose, ice-cream mixes were subjected to pressures of about 200–500MPa for one second to 20 minutes. HPP at 400 or 500MPa for time as low as one second showed increase in the mix viscosity, which, the researchers attributed to the formation of a proteinaceous network of micellar fragments in the mix by reduction in solubility of calcium phosphate on decompression during HPP cycle. Resultant ice-cream showed a strong increase in resistance to meltdown, greatly improved textural characteristics, like mouthfeel and creaminess.

These findings highlight that HP treatment leads to beneficial changes in proteins assists manufacturing of low-fat and stabilizer-free ice creams with improved mouthfeel.

Pressure assisted freezing may be a good area of research for HP treatment of ice-cream.

4.4. YOGURT

Significant structural differences between yogurt made from thermally- or HP-treated milk have been reported [29, 40].

Yogurt made from HP treated milk was less susceptible to undesirable syneresis on storage, probably due to changes in gel structure and water-binding capacity of milk proteins [41].

It was reported [42] the making of stirred yogurt from HP treated milk at pressures from 100 to 400MPa and temperatures from 25 to 90°C, and concluded that HPP at higher temperatures would reduce the ultimate viscosity of yogurt.

A possible application of HPP in yogurt manufacture is the treatment following fermentation, to inactivate starter cultures and yeasts and moulds and, thereby, extend the shelf life of the product by prevention of 'post-acidification' [8]. High pressure treatment at 200 to 300 MPa for 10 min at 10 - 20°C showed controlling of 'post- acidification' of yogurt without decreasing the number of viable lactic acid bacteria (LAB) or modifying the yogurt texture. Treatment at higher pressures may destroy LAB.

Potential future research of the use of high-pressure processing (HPP) in yogurt manufacture may involve combining HPP with crosslinking of milk proteins by transglutaminase [43]. Optimization of combination of HPP with heat treatment and/or addition of other ingredients, such as stabilizers or milk powders has also been suggested [8].

An extended shelf- life 'Probiotic yogurt' has been developed using pressure treatment of 350–650 MPa at 10–15°C. HPP (550 MPa) of yogurt maintained desirable sensory characteristics longer than controls during storage for 4 weeks at refrigerated (4°C) or room (20°C) temperature [44, 45].

4.5. CREAM AND BUTTER

When cream was treated at pressure of 600 MPa for up to 2 min, its whipping properties improved and reduced serum loss [46] possibly due to better crystallization of milk fat.

HP treatment of pasteurized cream at 450 MPa at 10 or 25°C did not alter the fat globules size distribution, the pH or its flow behaviour [37]. HP processing of butter or cream may lead to rise in the temperature (8–9°C/100 MPa) [47]. HPP may have a potential application in the physical ripening of dairy cream for butter making.

4.6. OTHER DAIRY PRODUCTS

The use of HPP on treatment of functional dairy products has been studied [48]. They concluded that unfolding of whey proteins under pressure facilitated peptide bonds hydrolysis and reduced allergenicity. They reviewed the use of HP to preserve colostrum. Treatment of colostrum with HP has been shown better retention of biological activity than of heat treatment under certain conditions and ensuring a reasonable shelf life of product [49-51]. The Fonterra Company of New Zealand has patented processes for colostrum preservation using HP treatment [8].

HP treatment (up to 500 MPa) reduced the turbidity of reconstituted skim milk for all combinations of pH (5.5–7.5) and temperature (5–40 °C) due to micelle dissociation [52]. Attempts have been made for optimizing the levels of pressure (200–400 MPa), pressurization time (0–100 min), and coagulation temperature (30–70 °C) for the preparation of chhana (Indian cottage cheese) [53]. There is a great scope for utilizing HPP for the development of probiotic dairy foods with higher viable count.

HP treatment of human milk indicated that with at 400MPa for 5 minutes, helped retaining of all immunoglobulin A (IgA) in milk serum that is susceptible to thermal damage during pasteurization using heat treatment [54].

5. EFFECT OF HPP ON MILK COMPONENTS

High pressure processing of milk has shown to have a significant effect on the milk constituents. Milk constituents undergo various changes, which cause alterations on the quality and functionality of milk [8].

5.1. WATER

Water content of the food gets compressed by about 4 per cent at 100 MPa and 15 per cent at 600 MPa [55]. Depression in freezing point of water was also observed at high pressure to -4°C, -8°C, -22°C at 50, 100 and 210 MPa, respectively [56]. Thus, this technique enables sub-zero temperature food processing without ice crystal formation. It also facilitates rapid thawing of conventional frozen food.

5.2. PROTEINS

Treating skimmed milk under high pressure disrupts casein micelles, which increase with pressure increment [57]. Whey proteins do not hinder HP induced disruption of casein micelles [58]. CCP solubilization is a primary factor inducing micellar disruption on HP treatment [59]. Casein micelle size does change a little at lower pressures (<200 MPa), while higher pressure treatment (250–300 MPa for >15 minutes) can give significant increases in micelle size [29, 60]. HP induces changes in turbidity and lightness of skimmed milk apart from changing casein micelle. The viscosity is increased by about 20 per cent [29]. Caseins show dissociated from the micelle in the following order β-casein>κ-casein > α_{s1}-casein > α_{s2}-casein [29].

HP treatment of milk at lower pressure (up to 100 MPa) showed no denaturation of β-lactoglobulin but at higher pressures (≥400 MPa) 90% denaturation of β-lactoglobulin was observed [61]. Whereas, in whey pressures up to 300 MPa gave significant denaturation in β-lactoglobulin, while, further pressure increase decreased β-lactoglobulin [62]. Denaturation of α-lactalbumin only happens at ≥400MPa, reaching about 70% after 30 minutes treatment at 800 MPa [63]. Milk solids concentration has relatively little effect on the denaturation of whey proteins due to HP treatment [60]. Other whey proteins like bovine serum albumin showed no denaturation in HP treated milk (at 100–400 MPa) [61]. The immunoglobulins also show stability to HPP; about 90% of immunoglobulin G (IgG) in colostrum remained in native state after HP treatment (500 MPa for five minutes) [51].

5.3. ENZYMES

Milk enzymes vary in their sensitivity to high pressure. Lipoprotein lipase, xanthine oxidase and lactoperoxidase showed resistant up to pressures up to 400 MPa [7]. Phosphohexose isomerase, γ-glutamyltransferase and alkaline phosphatase in milk are partially inactivated at pressures

exceeding 350, 400 and 600 MPa respectively and are completely inactivated at pressures of 550, 630 and 800 MPa respectively [64].

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 unlike thermal pasteurization.

HP treatment also has influences on the proteolysis in milk. The treatment of milk at lower pressure (up to about 300 MPa) has insignificant effect on plasmin activity, but, at higher pressures (30 minutes at 600 MPa) up to about 75% inactivation is observed, [65]. Hence, proteolysis was increased during the storage for low pressure treatment, whereas after 500 MPa, the proteolysis during storage of milk was less than that observed in raw milk.

The combination of HP treatment (300–600 MPa, 40–60°C) and homogenization resulted in inactivation of protease activity in milk, which extended its shelf life [66].

5.4. FAT

HP treatment of milk affects the fat in milk in many ways. Treatment at 100–600MPa at <40°C does not affect fat globule size in milk [67, 68] or cream [37]. However, the treatment of cream at 800MPa for 10 minutes increased fat globule size in cream [69].

Association of β-lactoglobulin (at >100 MPa), α-lactalbumin (at ≥700 MPa) and κ-casein (at >500MPa) with the fat globule membrane take place in HP treated milk [68].

HP treatment (400 MPa for 4h at 45°C) of the high melting fraction of milk fat also significantly suppresses thermal deterioration [70].

Treatment at 100–250 MPa may promote the cold agglutination of milk fat globules, which may lead to clusters of fat globules during cold storage leading to faster creaming. However, at >400 MPa, reduced cold agglutination, and hence reduced creaming occurs as a result of HP induced denaturation of immunoglobulins [8].

Critical threshold pressure between 300 and 500 MPa can trigger oxidation in lipids [71].

5.5. MILK SUGAR

Pressurization of milk (200-400 MPa for 10-60 min at 25°C) lead to no changes in the lactose, suggesting no Maillard reaction and isomerization occurs due to pressure treatment [61].

5.6. MINERAL

Mineral balance of milk gets affected at high pressure and effect is on both the distribution between colloidal and soluble phase as well as on the ionization. The increase in the content of soluble calcium has been reported by 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 [72].

Solubilization of CCP increases with increasing pressure up to about 400 MPa [73]. The concentration of ionic calcium in milk has been observed to slightly increase, immediately after HP treatment [74].

6. CONCLUSIONS

The HPP is a 'novel' and non-thermal technology has the potential for use as an alternative to thermal processing. Flavour and aroma components contributing to the sensory quality, and nutritional quality remained unaffected by pressure treatment. Several researches have been done on HP treatment on milk and milk products. These have provided a detailed understanding about the complex changes that take place in milk under high pressure like the dissociation of caseins micelles from the colloidal to the soluble phase, influence turbidity of milk etc. However, it cannot be denied that the dairy industry has been comparatively slow to adopt HP processing, as compared to product meat and sea-foods, jams, juices. Principal hindrances to adoption is the high cost of equipment and low throughput of HPP equipment, meaning high value, perishable materials are treated. Recent instances of commercialization of HPP in dairy industry can include HP treated Yogurt and Cheese spread. The varied physico-chemical and sensory properties obtained using the HPP offer exciting opportunities for the dairy industry. New opportunities in HPP may include exploring HP induced changes in milk giving functional ingredients, preservation of colostrum and human milk by HPP treatment, which may be of interest to the entrepreneurs. More research is required for optimization of the process in the future to fully establish it in the dairy industry.

ACKNOWLEDGEMENTS

The authors would like to thank all the researchers who have previously done the scientific work in the field. This work did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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