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**a Review**

# Application of High Pressure Processing Technology for Dairy Food Preservation - Future Perspective: A Review

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## Abstract

Minimal processing is the subject of major interest for both food preservation and food preparation. At present; thermal processing techniques, pasteurization and sterilization methods are extensively practiced to process milk and milk products, these techniques are well established and validated ensure consumer safety. But there is a need for novel; non-thermal processing technique, so that it can minimize the organoleptically and nutritionally damaged during thermal processing, thereby, maintaining the "freshness" of foods by reducing heat degradation of nutritional and other bioactive components. There are many alternate novel food processing techniques available, amongst them high pressure processing seems a very promising technique for dairy products, as it offers numerous opportunities for developing new shelf stable foods, retaining its natural nutritional value with excellent organoleptic characteristics. In this review, effect of high pressure processing on physico-chemical properties of milk and its constituents are discussed, with focus on fermented milk products. Packaging requirements along with legal and safety concerns also elaborated in brief.

**Keywords:** Milk, novel processing, bioactive components, food preservation.

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## Introduction

These days, in the emerging field of functional foods, minimal processed foods have increased in popularity along with organic foods. With more concern of food safety and health aspect, emergence of, the non-thermal processing technologies are gaining importance in the era of minimally processed foods. One of the promising technology which could serve as an alternative method for food preservation is the application of high pressure processing (HPP). For the first time in history, this technique was proposed by Royer in 1895 to kill bacteria and later by Hite in 1899, explored HPP effects on milk, meat, fruits and vegetables processing. In early 1990s, commercial HPP processed food products; fruit juices, jams, fruit topping and tenderized meats were introduced for the first time in Japanese industry in Tokyo (Mertens, 1995; Thakur, 1998).

HPP is an alternate, non-thermal food processing method, wherein the food is subjected to a very high pressure range from 100 -800 MPa (1MPa = 145.03 Psi or 10 Bar). HPP is also called as high hydrostatic processing (HHP) or ultra high pressure processing (UHHP) or isostatic processing, and it is also considered as a type of cold processing technique, since temperature employed in most of the processing is at ambient range (Farr, 1990). Food preservation using high pressure is a promising technique in food industry, The effect of HPP on microbial safety, quality and sensory characteristics of fruits and vegetables has therefore been widely investigated as an alternative to traditional food processing and preservation methods (Balasubramaniam *et al.*, 2004; Fonberg-Broczek *et al.*, 2005; Yordanov and Angelova, 2010, Mishra, 2011; Chawla *et al.*, 2011). This technique improves food safety by destroying the bacteria that can cause food borne illness and spoilage, but the food remains fresh. This type of foods can be kept for a longer period under better condition. Small molecules, which are the characteristics of flavouring and nutritional components, typically remain unchanged by pressure (Horie *et al.*, 1991). These pressure processed foods have better texture and colour compared to that of heat processed foods. Any food with sufficient moisture can be subjected to HPP. This technique can be used to process both the liquid and solid foods, expect for food materials containing large quantity of air pockets, (e.g. watermelon).

### *Principles of High Pressure Processing*

Hydrostatic pressure is generated by increasing the free energy; this can be achieved by physical compression during pressure treatment in closed system by mechanical volume reduction. Usually HPP accompanied by a moderate increase in temperature, called the adiabatic heating, which depends on the composition of the food product being processed (Balasubramaniam *et al.*, 2004; Hogan *et al.*, 2005). There are three fundamental operational principles underlying HPP, viz; Le-Chatelier's principle, isostatic principle and principle of microscopic ordering. According to the first principle; under pressure, bio-molecules obey the Le-Chatelier principle, i.e. whenever a stress is applied to a system in equilibrium, the system will react so as to counteract the applied stress, reactions that result in reduced volume will be promoted under high pressure, such reactions may result in inactivation of microorganisms or enzymes (Cheftel, 1995; Farkas and Hoover, 2000). The second principle i.e. isostatic principle says that; when food products are compressed by uniform pressure from every direction and then returned to their original shape when the pressure is released (Olsson, 1995). The products are compressed independently of the product size and geometry, because transmission of pressure to the core is not mass/time dependant (Cheftel, 1995; Farkas and Hoover, 2000); hence HPP is also called as isostatic processing technique. The principle of microscopic ordering says that: at constant temperature, an increase in pressure increases the degrees of ordering of molecules of a given substance. Therefore pressure and temperature exert antagonistic forces on molecular structure and chemical reactions (Balny and Masson, 1993).

### *Components and Working of HPP*

The basic key components of a HP system are a pressure vessel, pressurizing system, and supporting units such as heating or cooling components etc. as shown in Figure 1.

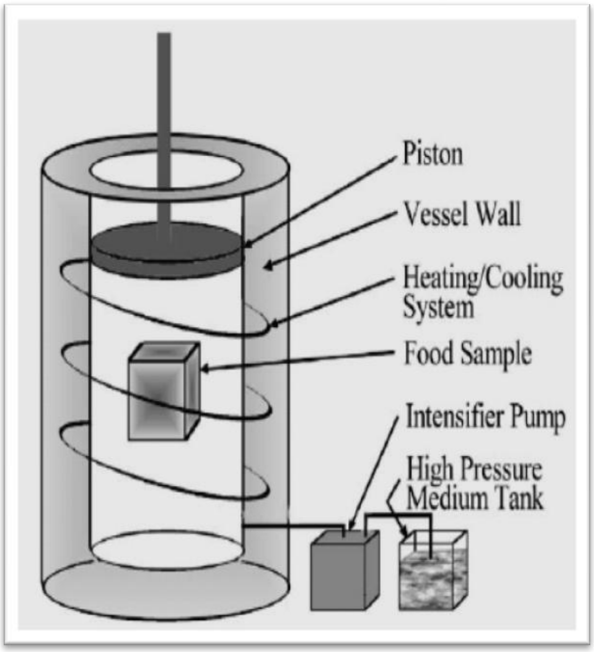


**Fig. 1:** Diagram showing components of HPP.

Once loaded and closed, the vessel is filled with a pressure-transmitting medium. Air is removed from the vessel with an automatic deaeration valve by means of a low-pressure fast-fill-and-drain pump, and high hydrostatic pressure is then generated by direct or indirect compression or by heating the pressure medium (Mertens, 1995). rocessing by HPP is carried out usually in a low compressibility liquid such as water. According to the above principles; the phenomenon of phase transition and chemical changes are accompanied by decrease in volume; favoured by pressure and vice versa. Pressure is instantaneously and uniformly transmitted independent of size and geometry of the food. Resultant pressure regulates most biochemical reactions occurring in foods. In biological systems, the changes that are brought about by HPP on most important volume-decrease reactions includes denaturation of proteins, gelation, hydrophobic reactions, phase changes in lipids (and, therefore, in cell membranes) and increases in the ionization of dissociable molecules due to ‘electrostriction’ (Heremans, 1995).

The high pressure process is characterized by three parameters; temperature (T), pressure (p) and exposure time (t) when compared heat preservation process which is based on only two parameters (T, t). The three parametric HPP offers a broad variability for process design and enhancing the shelf life. In a qualitative approach, process efficiency is assessed in terms of the lethality of the treatment and its structural impact on the food matrix. Evidently, those treatments which are powerful in killing microbes have usually a strong destructive effect on the integrity of the food matrix with severe consequences on quality and consumer acceptance. HPP is used for the preservation and modification of foodstuffs. Thereby, foodstuffs are normally subjected for periods of a few seconds up to several minutes to hydrostatic pressures above 350 MPa. This treatment permits the inactivation of microorganisms and enzymes at low temperatures, whilst valuable low molecular constituents, such as vitamins, colours and flavouring compounds remain largely unaffected and aiming towards retaining the freshness of the processed food.

***Microbial Destruction by High Pressure Processing***

Microbial destruction is the main goal of food processing; beneficial effects of HPP in food are evident only when applied pressures exceed 400 MPa. HPP inactivates most of spoilage and pathogenic bacteria present in milk. Resistance of microorganisms to pressure varies considerably depending on the applied pressure range, temperature and treatment duration, and type of microorganism. The nature of the food is also important, as it may contain substances which protect the microorganism from high pressure. Generally, gram positive bacteria are more resistant to pressure than gram-negative bacteria and yeasts and moulds, due to presence of teichoic acid (a bacterial polysaccharide). On the other side spores are more resistant than vegetative cells because it contains calcium rich dipicolinic acid, as it protect from excessive ionization (Timsson *et al.,* 1965; Smelt, 1998). Heat resistant groups of microorganisms were usually pressure resistant, exponential-phase cells are more pressure sensitive than stationary-phase cells (Dring, 1976). 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). Vegetative bacterial cells are inactivated by pressures between 400 and 600 MPa. The inactivation of virus is supposed to depend on the denaturation of capsid proteins essential for host cell attachment. Figure 2 depicts the damage of cell membranes at high-pressure cycle; compression of cell membrane material as and when pressure is applied and sudden expansion of lipid bi-layer after pressure release, leading to destruction and looses of cell membrane integrity and they therefore cannot reproduce. Once damaged, the cells are unable to control the transport of water and ions across the membranes, leading to collapse of the cells. Lopez-Fandino *et al.,* (1996) reported that, when milk treated at 200 MPa, it resembles to thermization, reduction of aerobic and psychotropic count observed to a great extent. Goat milk processed at 500 MPa for 15 minutes has been stated to be as efficient as pasteurized milk (Buffa *et al.,* 2001).

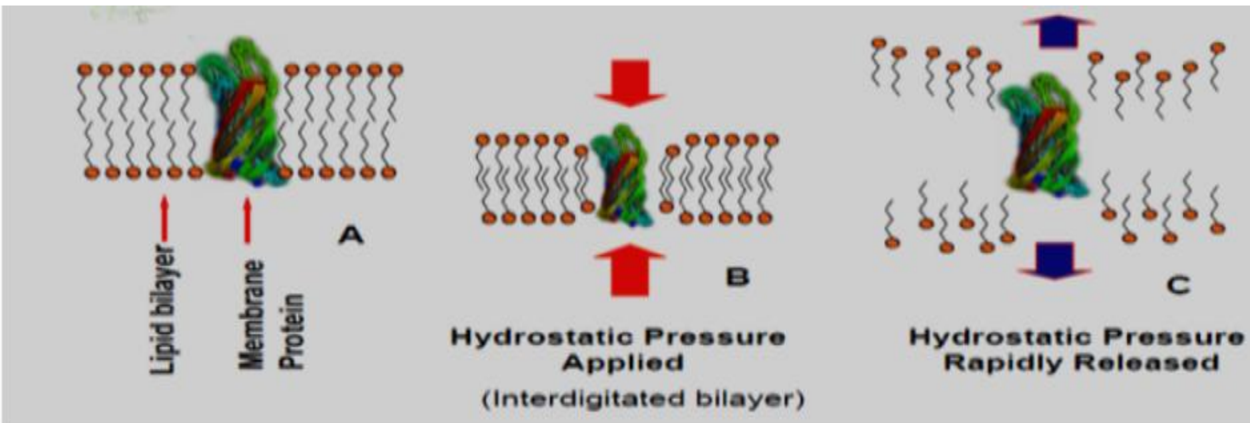


**Fig. 2:** Effect of high pressure on cell membrane materials.

***Impact on Physico-Chemical Properties of Milk***

White colour of milk is due to scattering of light particles by fat globules and casein micelles. Hunter Luminance value (L-value) of milk, generally read as a measure of whiteness (Harate *et al.,* 2003), was reported to reduced by HPP treatment, due to disintegration of casein micelles, thus leading to decreases in the turbidity of milk. Treatment of milk at 200 MPa shows slight effect on L-value; while at 250-450 MPa significant decreased in the L-value was observed. When skim milk treated at 600 MPa for 30 min, L value decreased from 78 to 42 and skim milk becomes almost translucent or semi-transparent (Desobry-Banon *et al.,* 1994).

Water content of the food gets compressed by about 4% at 100 MPa and 15% at 600 MPa. Depression in freezing point of water observed at high pressure to -4°C to -8°C or -22°C at 50, 100 or 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, helping in improving quality as well extending shelf life of food.

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 was observed. The increase in the content of diffusible calcium has been reported following HPP. In case of previous 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.

HPP reduces the time period required to induce fat crystallization and this is due the fact that solid/liquid transition temperature of milk fat shifts to high value at high pressure. It has been reported that crystallization temperature of milk fat increased by 16.3°C at100 MPa and melting temperature also increased by 15.5°C. Hence, high pressure treated cream has higher solid fat content than untreated cream, ageing time of ice cream also drastically reduced. Surprisingly, milk fat globule membrane (MFGM) is not destroyed by high pressure treatment of milk even up to 800 MPa. 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 MFGM but as there is no increase in lipolysis, the membrane is not damaged (Buchheim and Frede, 1996).

Milk Proteins, in native state are stabilized by covalent bonds (peptide and disulphide bonds), electrostatic interactions, hydrophobic interactions and hydrogen bridges. Covalent bonds are almost unaffected by HPP and hence primary structure of proteins remains intact during high pressure treatment. Sensitivity of different bonds to HHP is in the order of Hydrobhobic > Electrostatic bonds > Hydrogen bonds > Covalent bonds.

In case of whey proteins the amount of non-casein nitrogen in milk serum decreases with increasing pressure, suggesting denaturation and insolubilisation. Among all whey proteins β-lactoglobulin (β-Lg) has got maximum susceptibility to pressure induced denaturation followed by immunoglobulin and then α-lactalbumin (α -La). The difference in the stability of α-La and β-Lg is may be due to the more rigid molecular structure of former, also partially by the number of intra-molecular disulfide (-S-S-) bonds and lack of free sulfahydryl (-SH) in the α-La. Better emulsion, foaming and textural properties have observed (Johnston *et al.,* 1992) from milk protein after high pressure treatment and may find its application as techno-functional ingredients in different foods.

Application of high pressure has been shown to destabilize casein micelles on reconstituted skim milk. Size distribution of spherical casein micelles decrease from 200 to 120 nm; maximum changes have been reported to occur between 150-400MPa at 20°C. High pressure treatment increases the transfer of individual caseins from the colloidal to the soluble phase. Dissociation of the caseins was reported in the order $\kappa > \beta > \alpha s1 > \alpha s2$. This order largely corresponds to the serine phosphate content of the caseins, indicating that caseins which are more tightly bound casein dissociated to a lesser extent (Needs *et al.,* 2000). This strategy may find application in the manufacture of casein species enriched ingredients or individual casein isolates for improved biofunctionality.

Most of the technologically important milk enzymes loose their activity only after 300 MPa. Alkaline phosphatase (ALP), an index enzyme for pasteurization in milk, appears to be quite resistant to pressure. No inactivation of ALP in milk has been reported after treatment up to 400 MPa for 60 min. and complete inactivation of ALP has been observed only after treatment of milk at 800 MPa for 8 min. The other two indigenous milk enzymes proposed as indicators of heating of milk around pasteurization temperature viz., lactoperoxidase and γ-glutamyltranspeptidase are also quite resistant to pressures up to 400 MPa at 20-25°C. Plasmin activity reduces at 300 MPa; hence rate of proteolysis is lower than that of untreated milk.

Other bioactive molecules such as vitamins, amino acids, simple sugars and flavour compounds remain unaffected by the HPP. Milk treated at 400 MPa results in no significant loss of vitamins $B_1$ and $B_6$ (Sierra *et al.,* 2000). The effect of high pressure treatment of milk on trace bioactive molecules like Growth factors, Oligosaccharides, CLA and sphingolipids has not been studied.

### *Significance in Product Manufacturing*

Since, HPP has influence on milk components particularly proteins, it is bound to effect technological properties of milk during manufacture of various milk products. Salient findings and potential applications of HPP in dairy industry are summarized in Table 1.

#### *Yoghurt Making Properties*

Firmness of yoghurt made from high pressure treated milk shown to increase with increasing pressure, this may be linked to the fact that disruption of casein micelles, resulting in a greater effective area for surface interaction. Yoghurt prepared from low fat milk and exposed to 300 MPa/10 min prevents after-acidification (developed acidity after packing) and significantly improved shelf life. Acidification of yoghurt milk with glucono-δ-lactone (GDL) at 200 MPa for 20 min resulted in fine coagulum, homogeneous gel than that of heat treated milk (Harate *et al.,* 2003; Tanaka and Hatanaka, 1992).

*Rennet Coagulation Time*

Rennet coagulation time (RCT), is the time at which the milk coagulum becomes firm enough for cutting after rennet addition. RCT reported to reduced markedly when pressure exposure at 200 MPa; the decreased RCT is related to a reduction in casein micelle size, leading to increased specific surface area and increased probability of inter-particle collision (Needs *et al.*, 2000; Arias *et al.*, 2000). However, at high pressure (400 MPa), RCT again increases as denatured whey proteins are incorporated into the gel and their presences interferes with secondary aggregation phase; thereby, reduce the overall rate of coagulation.

*Cheese Making Properties*

Milk treated at 300-400 MPa significantly increase wet curd yield by up to 20% and reduces both the loss of protein in whey and the volume of whey. The effect is due to the denaturation of β-Lg and thus its incorporation in the curd. This leads to high cheese yield to the extent of 7%. Quick maturation and stronger flavour development has also been reported when treated at 400-600MPa/5-15 min cycle (Huppertz *et al.*, 2002; Arias *et al.*, 2000). This will help in accelerating cheese ripening process and provides better opportunity for improving cheese prepared from low fat milk. The cheese curd obtained from milk treated by HP gives dense network of fine strands thereby having a great potential for the design of new products due to the creation of modified textures, tastes and functional properties.

***Advantages of HPP***

Main benefits of HPP in dairy food processing include inactivation of microorganisms, structural modification of proteins and depression of freezing point of water. These could be used advantageously in several segments of food industry including sea food, meat and dairy industry. Some of the advantages of HPP are listed below:

Retains natural antimicrobial systems without changing the sensory and nutritional quality of foods and extend shelf life up to 2-3 folds.

Major advantages as significant reduction of heating, this will minimize thermal degradation of food components.

Inactivation of microorganisms, spores and enzymes.

High retention of flavor, colour and nutritional value.

Pressure is transmitted uniformly and instant so that food product retains its shape.

Potential for the design of new products due to the creation of new textures, tastes and functional properties.

Clean technology, flexible system for number of products and operation.

Process time is less dependence of product shape and size.

Reduced requirement of chemical additives, and Increased bioavailability.

***Disadvantages of HPP***

Food must contain water, as the whole phenomenon is based on compression.

Some enzymes are very pressure resistant.

May not inactivate spores.

Structurally fragile foods needs special attention, and

High installation cost.

**Table 1:** Salient findings and potential application in dairy industry .

| Conditions | Product and Salient results | References |
|---|---|---|
| 200-250 MPa | The combination of high pressure with bacteriocin such as lacticin resulted in synergistic effect in controlling microbial flora of milk without significantly influencing the cheese making properties. | Morgan *et al.*, (2000). |
| 100-600 MPa/ 0-30 min at 20°C | Rennet coagulation time of heated milk decreased with increasing pressure and treatment time. The strength of the pressure treated coagulum from heated milk was considerably greater and the yield of cheese curd also found 15% greater than that from unheated /unpressurized milk; the protein content of the whey was 30% lower. | Huppertz *et al.*, (2002) |

| | | |
|---|---|---|
| 200-400 MPa | Decrease in the rennet coagulation time. | Needs *et al.,* (2000) |
| 200-400 MPa | Pressure treatment of cheese milk increased the yield of low-fat cheese by improving protein and moisture retention. Pressurization of pasteurized milk improved its coagulation properties. Cheese made from pressurized and pasteurized milk showed increased protein and moisture retention as well as improved coagulation properties. The protein degradation and development of texture and flavor was also rapid and the product had lower hardness and cohesiveness and higher sensory scores. | Molina *et al.,* (2000) |
| 200-500 MPa/ 60 min, 20◦C | Periodic oscillation of pressure was very effective for the destruction of pathogen such as Listeria monocytogenes, Escherichia coli and Salmonella enteritidis. | Vachon *et al.,* (2002) |
| 250-500 MPa / 5 min at 20°C. | Combining high pressure and Nisin for a greater inactivation of gram-positive bacteria than when either was applied individually. The gram-negative bacteria were found to be more sensitive to high pressure, either alone or in combination with Nisin than gram-positive bacteria. Such a combination of hurdles may allow lower pressures and shorter treatment times to be used without compromising the product safety. | Black *et al.,* (2005) |
| 300-500 MPa | Denaturation of β-Lg, decrease in plasmin activity and enhancement of proteolysis were observed possibly due to the increase in the availability of substrate bonds to plasmin which is facilitated by the miceller structure. | Scollard *et al.,* (2000) |
| 300-600 MPa | Increase in cheese yield by high-pressure treatment of cheese milk due to denaturation of whey proteins and increased moisture retention was also found. Higher moisture content of cheese made from high-pressure treated milk due to the fact that casein molecules and fat globules may not aggregate closely and may allow moisture to be trapped or held in cheese. | Drake *et al.,* (1997) |
| 345-483 MPa /3 and 7 min | High-pressure treatment accelerates shredability of Cheddar cheese. Shreds from unripe milled curd Cheddar cheese could be produced with high visual acceptability and improved tactile handling using high pressure. | Serrano *et al.,* (2005) |
| 400 MPa /3 min | HPP of milk was shown as a gentle process than conventional procedures for extending the shelf-life of milk, no significant variation in the content of $B_1$and $B_6$ vitamins was observed | Sierra *et al.,* (2000) |
| 400 MPa/15 min or 500 MPa/3 min | Treatment of thermally pasteurized milk resulted in increased shelf life by 10 days. | Rademacher and Kessler (1997) |
| 50 MPa / 3 days at 25°C | Accelerating the ripening of commercial cheddar cheese by high-pressure treatment due to the degradation of αS1-Casein | O'Reilly *et al.,* (2000) |
| 50-500 MPa /20-200 min | Ripening of Gouda cheese was accelerated by the application of high pressure. | Messens *et al.,* (2000) |
| 680MPa/ 10 min, room temperature | 5-6 log cycle reduction in microorganism and combined effect of pressure and temperature (67-71°C), further resulted in increased shelf life. | Hite *et al.,* (1914) |

### *Packaging Requirements of HPP*

Packaging technology for HPP involves different considerations, based on whether a product is processed in-container or packaged after processing. For batch in-container process flexible or partially rigid packaging is best suited. On the other hand, fluid products require continuous or semi-continuous systems, which are aseptically packaged after pressure treatment. The effectiveness of HPP is greatly influenced by the physical and mechanical properties of the packaging material. The packaging material must be able to withstand the operating pressures, have good sealing properties and the ability to prevent quality deterioration during the application of pressure. At least one interface of the package should be flexible enough to transmit the pressure. Thus, rigid metal, glass or plastic containers cannot be used (Rastogi *et al.,* 2007). The most common packaging materials used for high pressure processed food are polypropylene (PP), polyester tubes, polyethylene (PE) pouches, and nylon cast polypropylene pouches. Plastic packaging materials are the best suited for HPP packaging application, because of reversible response to compression, flexibility and resiliency. The headspace must be also minimized while sealing the package in order to ensure efficient utilization of the package as well as space within the pressure vessel. Packaging materials for high pressure processing must be flexible to withstand a 15% increase in volume followed by a return to original size, without losing physical integrity, sealing or barrier properties. The headspace must be minimized as much as possible (Lambert, 2000) in order to control the deformation of packaging materials and ensure efficient use of the package and space in the pressure vessel. Sufficient headspace also minimizes the time taken to reach the target pressure. Film barrier properties and structural characteristics of polymer based packaging material were unaffected when subjected to pressures of 400 MPa for 30 min at 25°C (Nachamansion, 1995).

### *Legal and Safety Concerns of HPP Foods*

HPP treated food items falls under the category of novel food as per the definition of 'Novel Food' followed in European Union (EU) countries (Regulation (EC) No 258/97). In EU countries, introducing novel foods to the market, food companies need to get an approval that those products are in compliance with the food law. Food safety issues, the achievable extension of shelf-life and the legislative situation need to be inspected. The ‘‘Novel Foods Regulation’’ defines novel food as a food, that does not have a significant history of consumption within the EU before the 15th of May, 1997 (Heinz and Buckow, 2010) and such foods are subject to a pre-market safety assessment. Further, the legislations defines Novel foods as follows: foods and food ingredients to which has been applied a production process not currently used, where that process gives rise to significant changes in the composition or structure of the foods or food ingredients which affect their nutritional value, metabolism or level of undesirable substances.

If a food falls under the definition of novel food, the person responsible for placing it on the market has to apply for an authorization. High pressure treated foodstuffs have been marketed in Japan since 1990, in Europe and United States since 1996. Information relating to the adverse effects of high pressure on toxins, allergens, and nutrients are rare. There are no published reports available on health and safety issues of HPP foods. For the developing nations, where there is no such regulations established, for them supportive data on validation may be required, and to be sorted out before the marketing of high pressure treated foods. Further, markers/indicators of the effectiveness of HPP treatment needs to be worked out before the enforcement of legal requirements for such processed dairy foods.

## Conclusion

The main effects of high pressure treatment in milk appears to include 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 and nutritional quality remain unaffected. This 'novel' non-thermal technology has the potential to use in development of a whole new generation of value added functional or neutraceuticals foods. Physico-chemical and sensory properties obtained from this technology offer exciting opportunities for dairy industry. Careful process design, intensive kinetic and nutritional evaluations for process development and monitoring are the focus area; high safety margins, superior quality and reasonable costs are the driving forces. However, further research is required to evaluate the full commercial potential of high pressure treatment of milk through complete understanding of the effects of pressure. Studies have indicated that HPP treatment has no effect on the health promoting attributes in orange juice and tomato puree and the same is expected in case of milk also. New opportunities in preservation of colostrum and human milk by HPP treatment may be of interest to the entrepreneurs as this treatment is likely not to affect many bioactive components in such products.

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