HIGH-PRESSURE PROCESSING AS NOVEL TECHNOLOGY IN DAIRY INDUSTRY: A REVIEW

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Abstract

The aim of this review was to summarize available bibliography on the possible applications of high pressure processing in dairy industry, the effect of this non-thermal treatment on bacterial microflora and milk constituents. Traditional thermal treatments applied to milk processing lower nutritional quality because many nutrients are heat labile. To overcome this problem, several non-thermal processing technologies including high hydrostatic pressure (HHP) processing have been developed. Pressures between 400 and 600 MPa inactivate microorganisms including food-borne pathogens; however, high pressure (HP) injured bacteria in milk during storage can recover. All enzymes are inactivated only at pressures of 800 MPa. During HHP the casein micelle size decreases, whey proteins are denaturated, the level of free fatty acids increases. These characteristics indicate that for better understanding and application of HPP in dairy industry research should be done to offer the numerous practical applications to produce microbially safe, minimally processed dairy products with improved performances, and to develop novel dairy products of high nutritional and sensory quality and increased shelf life.

Key words: high pressure, milk, microbial inactivation, functional properties.

Introduction

Nowadays, milk and dairy products are treated at high temperature (70 – 145 °C) to ensure product safety. Heat is by far the most widely used technology utilized to inactivate microbes in foods. Traditional treatments for dairy products include different temperature regimes. The most common milk treatment is pasteurization; it allows decreasing microorganisms counts in the product and ensuring approximately 7 to 20 days shelf-life. Higher thermal treatment temperatures ensure product quality for the longer time period, which is very important for food processing and distribution companies.

Long shelf life of milk is often achieved through ultra-high temperature (UHT) processing for a few seconds at or above 135 °C; however, canning of milk products at 120 °C up to 30 min is still practiced in the dairy industry (Fitria *et al*., 2015). Processing at high temperature lowers the nutritional quality of foods because many nutrients are heat labile. To overcome this problem, several non-thermal processing or `cold processing` techniques including high hydrostatic pressure technology have been developed.

High pressure processing combining high pressure (up to 1000 MPa) and sometimes heating (above 60 °C) has been considered equal to sterilisation, which extends shelf life of foodstuff due to its ability to inactivate bacterial spores at reduced heat and thereby preserving desirable functional properties of foods better than conventional thermal processing (Heinz & Buckow, 2009; Fitria *et al*., 2015).

The use of non-thermal methods for food preservation is due to consumer demands for microbiological safe products without changes in the sensory and nutritional quality of the product. The HHP has emerged as an alternative to traditional thermal processing methods for foods (Muñoz-Cuevas *et al*., 2013). The HHP can be used to process both liquid and solid (water-containing) foods and adds advantages to the foods such as: kills bacteria in the raw food, extends shelf-life, ponders additive free and fresh food, manipulates the texture and enhances desired attributes (digestibility) (Chawla, Patil, & Singh, 2011).

One of the first scientific reports on high pressure applications for food was written by Hite (1899) on shelf-life extension of milk, and HP effect on food-born microorganisms by subjecting milk to a pressure of 650 MPa (Chawla, Patil, & Singh, 2011). Since then the application of HP treatment has been broadened to other food products such as raw and cooked meats, fish and shellfish, fruit and vegetable products, cheeses, salads, dips, grains and grain products, and liquids including juices, sauces, and soups. The range of products now being considered for high pressure treatment continues to grow. At present, 167 industrial installations exist with volumes from 55 to 420 litres and a total annual production volume has increased from 200 000 t in 2009 (Heinz & Buckow, 2009) to 350 000 t in 2012 (Bello *et al*., 2014). Bello *et al*. (2014) reported that HP processed vegetable products account for 28%, meat products for 26%, seafood and fish for 15%, juices and beverages for 14%, and other products for 17% in 2012.

Studies addressing the effect of HP treatment on the quality of dairy products are still limited (Devi *et al*., 2013). It is known that HHP can lead to modifications in the structure of milk components, in particular protein, which may provide interesting possibilities for the development of high value nutritional and functional ingredients (Beresford & Lane, 1999). The development of food ingredients with novel functional properties offers the dairy industry an opportunity to revitalise existing markets and develop new ones.

The aim of this review was to summarize available bibliography on the possible applications of high pressure processing in dairy industry, the effect of this non-thermal treatment on bacterial microflora and milk constituents.

Materials and Methods

Monographic method was used in this study. The review summarizes the available literature on principles of high pressure processing and its application in dairy industry to produce microbially safe, minimally processed dairy products with improved performances, and to develop novel dairy products of high nutritional and sensory quality and increased shelf life. Literature study aimed to cover broad spectrum of published research results on factors affecting microorganisms survival during HPP and impact of HPP treatment on separate milk constituents.

Results and Discussion

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 a closed system by the mechanical volume reduction. HP processing is usually accompanied by a moderate increase in temperature (adiabatic heating) which depends on the composition of the food product being processed (Knorr, 2002; Knorr, Heinz, & Buckow, 2006; Naik *et al*., 2013).

The operating principles behind high pressure technology are as follows:

Le Chatelier's principle: whenever 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 (Carlez *et al*., 1994).

Isostatic principle: when food products are compressed by uniform pressure from every direction and then returned to their original shape when the pressure is released. The products are compressed independently of the product size and geometry, because transmission of pressure to the core is not mass/time dependant (Carlez *et al*., 1994).

The high pressure process is characterised 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 processing parameters allow great flexibility in the design of the process (Heinz & Buckow, 2009; Naik *et al*., 2013).

Application of high pressure in dairy industry

HP technology can be used to increase the microbiological safety and quality of milk to produce high quality cheeses. In relationship to the structure formation of the cheese matrix, the applications of HP treatment can be classified into: improvement of rennet coagulation, assistance of curd formation, enhanced salting and/or ripening, and improvement of the microstructure/texture of cheese or fresh cheese (Devi *et al*., 2013). High hydrostatic pressure may modify parameters controlling proteolysis during cheese ripening and has already been found useful to shorten the maturation period (Butz *et al*., 2000; Iwanczak & Wisniewska, 2005). The HP-treated cheese have higher moisture, salt and total free amino acids contents than raw or pasteurised milk cheeses (Trujillo *et al*., 2002).

Two strategies have been used to improve yoghurt quality and preservation by means of HP: yoghurt making from HP-treated milk and pressurisation of yoghurt to inactivate microbiota (Trujillo *et al*., 2002).The application of the high pressure in preliminary treatment of milk used for yoghurt production improved firmness of the curd and limited its syneresis (Jankowska,Wiśniewska, & Reps, 2005). HP processing of milk before fermentation has been successfully used (Udabage *et al*., 2010) to manufacture low fat set-type yogurt (12% total solids) with a creamy consistency, requiring no addition of polysaccharides. The application of HHP to milk for yoghurt preparation could be an alternative to the use of food additives, which can affect the taste, flavour, aroma, and mouth feel of yoghurt (Sfakianakis & Tzia, 2014).

In order to produce low-fat ice cream with similar textural properties to full-fat samples, whey protein is often incorporated to the ice cream mix. Partially denatured whey protein is expected to give better foaming properties, which hinder excessive growth of ice crystals and hold air bubbles during the freezing process (Devi *et al*., 2013). Ice cream from pressure treated mixes showed a slower melting rate and improved sensory properties compared to the control ice cream, possibly because of the formation of pressure induced protein gels (Huppertz *et al*., 2011).

Effect of high pressure on bacterial flora in milk

A major function of high pressure processing of food is the destruction of microorganisms. HP inactivates most of spoilage and pathogenic bacteria present in milk. Most of the reported bacteria are inactivated in milk after treatment at 400 – 600 MPa (Shigehisa *et al*., 1991; Patterson, 2005; Rodriguez *et al*., 2005; Okpala, Piggott, & Schaschke, 2009; Rivalain, Roquain, & Demazeau, 2010; Udabage *et al*., 2010; Gustavo, Espejo, & Hern, 2014; Meirelles *et al*., 2014; Pedras *et al*., 2014; Pedras, Tribst, & Cristianini, 2014; Sfakianakis & Tzia, 2014). The mechanisms of microbial inactivation by HP are not fully understood yet, but are thought to act in several ways. The viability of vegetative microorganisms may be affected by inducing structural changes at the cell membrane or by the inactivation of enzyme systems which are responsible for the control of the metabolic actions (Knorr & Heinz, 2001; Heinz & Buckow, 2009) and ribosome disintegration (Farkas & Hoover, 2001).

Survival of microorganisms depends on the extent of pressure, holding time and temperature, composition of the food and the condition and growth phase of microorganisms (Goyal *et al*., 2013). Pressures up to 150 MPa are not able to ensure more than 2 decimal reduction of different microorganisms, while pressure at 200, 300 and 400 MPa are required to reach high level of microbial inactivation (Pedras *et al*., 2012). Temperature and HP can cause considerable microbial inactivation when applied alone, but it has been observed that these two treatments combined can confer dramatically improved inactivation levels, particularly with regard to bacterial spores (Considine *et al*., 2008). The type of substrate and composition of the food can have a dramatic effect on the response of microorganisms during pressure treatment. Certain food constituents, like proteins, carbohydrates, lipids and vitamins, can have a protective effect on microbial inactivation (Erkmen, 2011). Water activity (a_w) of the food is also an important parameter determining the effectiveness of HP treatment (Bulut, 2012). The environment around the microorganism can significantly influence HP inactivation, e.g. low pH in a suspending medium can render pathogens more sensitive to the effects of HP treatment (Datta & Deeth, 1999; Alpas & Bozoglu, 2002).

Microorganisms in lag phase are more sensitive to HP than those in stationary phase (Bello *et al*., 2014). This behaviour could be explained by the fact that in the lag phase the microorganism is in the process of cellular division and the membrane is more sensitive to environmental stresses. Pressures between 300 and 600 MPa inactivate yeasts, moulds and most of the vegetative bacteria. In general, yeasts and moulds can be inactivated at 200 – 400 MPa, but when they are in the spore or ascospore state or in a food with a very high concentration of sugar, the pressure needed to inactivate them could be close to 600 MPa (Bello *et al*., 2014). Spores are more resistant than vegetative cells and can survive at pressure of 1000 MPa (Zhang & Mittal, 2008; Reineke, Mathys, & Knorr, 2011). Pressures between 50 and 300 MPa may even stimulate spore germination. Germination can be markedly increased (to 95 – 99%) when spores are treated in the presence of L-alanine. Gram-positive microorganisms are more resistant to pressure than Gram-negative (Patterson, 2005), 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 (Alpas & Bozoglu, 2002) with the same time-temperature combination. It has been suggested that the cell membrane structure is more complex in Gram-negative bacteria, making it more susceptible to environmental changes caused by pressure (Shigehisa *et al*., 1991).

However, some researchers have demonstrated recovery of HP-injured bacteria in milk during further storage (Bozoglu, Alpas, & Kaletunç, 2004; Patel *et al*., 2006; Bulut, 2012; Muñoz-Cuevas *et al*., 2013). The recovery phenomenon during storage is a critical issue from the viewpoint of food safety. Research on the effect of high pressure on milk showed that HP-injured cells could be restored within 1–15 days, indicating the potential for bacterial recovery on these food products (Bozoglu, Alpas, & Kaletunç, 2004). Koseki, Mizuno, & Yamamoto (2008) investigated the effect of mild-heat treatment (30–50 °C) following HP treatment (550 MPa) on the inhibition of recovery of *Listeria monocytogenes* in milk. The results of this study would contribute to safer production of high-pressure-processed food by controlling bacterial recovery.

The impact of high pressure treatment on constituents in milk

HP treatment affects many milk constituents, such as the proteins and the fat fraction. In contrast, small compounds such as vitamins, amino acids, simple sugars and flavour compounds remain unaffected by HHP treatment (Chawla, Patil, & Singh, 2011).

Effect of HP on casein and whey proteins. A large number of factors, e.g., temperature, time, micelle concentration, pH, additives and pre-treatment of casein micelles affect the disruption of casein micelles and reformation of casein particles under pressure. HP produces casein micelles disintegration into smaller diameter particles, with a decrease of turbidity and lightness and an increase of viscosity of the milk. Solubilisation of colloidal calcium phosphate leads to disruption of casein micelles with increasing pressure and time (Huppertz *et al*., 2006) and in milk, micelle disruption is complete at 400 MPa. At 250 and 300 MPa reformation of casein particles from disrupted micelles occurs, but this process does not occur at lower or higher pressures (Harte *et al*., 2003). Casein micelle disruption decreases with increasing temperature (Gebhardt, Doster, & Kulozik, 2005; Orlien, Boserup, & Olsen, 2010). Addition of whey protein to casein isolates protected the micelles from high pressure induced disruption (Chawla, Patil, & Singh, 2011).

During pressure treatment, the whey proteins are denatured under conditions where hydrophobic interactions are reduced and the solubility of calcium phosphate is increased, so more calcium phosphate is moved to the serum phase (Datta & Deeth, 1999; Anema, 2008; Baier, Schmitt, & Knorr, 2015). A pressure treatment of 500 MPa at 25 °C denatures lactoglobulins (Chicón *et al*., 2006). Denaturation of immunoglobulins and lactalbumins occurs only at the highest pressure, particularly at temperature above 50 °C, which gives an idea of preservation of colostrum immunoglobulins which otherwise gets damaged during heat treatment (Chawla, Patil, & Singh, 2011).

Effect of HP on milk lipids. During milk processing, the membrane of milk fat globules (MFG) is altered, making the action of lipase to triglycerides possible and increasing the levels of the free fatty acids (FFA) in milk. This process (lipolysis) is a good index on the damage of the MFG membrane. When raw milk was pressurized at 200 MPa at 4 °C for 10 or 20 min, the short-chain FFA did not change, while treatment for 30 min increased the FFA content slightly (Kim *et al*., 2008). Studies carried out by Gervilla, Ferragut, & Guamis (2001) on free fatty acids (FFA) content (lipolysis of milk fat) in ewe's milk have showed that HP treatments between 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. The difference between the results of the two studies could be explained by the difference in the applied pressure level.

Effect of HP on lactose. Lactose in milk and milk products may isomerise in lactulose by heating and then degrade to form acids and other sugars. No changes in these compounds are observed after pressurisation (100 – 400 MPa for 10 – 60 min at 25 °C), suggesting that no Maillard reaction or lactose isomerisation occur in milk during pressure treatment (López-Fandiño, 2006; Chawla, Patil, & Singh, 2011).

Effect of HP on minerals. HP does not affect minerals as such, but may affect the food matrix resulting in improved bioavailability and health benefits (Barba *et al*., 2015). HP treatment increases the level of ionised calcium in milk, as well as the level of total calcium in the serum phase of milk. HP-induced shifts in the mineral balance of milk result in an increase in milk pH, by around 0.1 unit. The shifts in salts and the increase in milk pH are rapidly reversible after HP treatment, particularly when the milk is stored at a temperature above 10 °C (Huppertz, Kelly, & Fox, 2002). An increasing concentration of Ca, P and Mg in serum upon increasing pressure to

Table 1

Impact of high pressure processing on milk quality parameters

Parameter	Treatment condition	Major findings	References
Bacterial flora	300 MPa/10 min/10 °C	inactivation of *E.coli* O157:H7	Rodriguez *et al*., 2005
	500 – 600 MPa/10 min/25 °C 300 – 400 MPa	inactivation of Gram-positive organisms inactivation of Gram-negative organisms	Alpas & Bozoglu, 2002
	200 – 400 Mpa close to 600 MPa	inactivation of yeasts and moulds inactivation of yeasts and moulds (in the spore or ascospore state) and most of vegetative bacteria	Bello *et al*., 2014
	50 – 300 MPa	stimulatation of spore germination	Gould & Sale, 1970
Casein and whey protein	400 MPa	casein micelle disruption	Huppertz *et al*., 2006
	500 MPa/ 25 °C	denaturation of lactoglobulin	Chawla, Patil, & Singh, 2011
Lipids	200 MPa/30 min/4 °C	increase in the FFA content	Kim *et al*., 2008
Lactose	100 – 400 MPa/ 10 – 60 min/25 °C	no Maillard reaction observed	Chawla, Patil, & Singh, 2011
Vitamins	400 MPa/30 min/ 25 °C	insignificant loss of vitamin B_1 and B_6	Sierra *et al*., 2000
Enzymes	400 MPa	lipase, xanthine, oxidase, lactoperoxidase are resistant	Naik *et al*., 2013
	550, 630 and 800 MPa	phosphohexose isomerase, γ-glutamil transferase anad alkaline phosphatase are completely inactivate respectively	Sakharam, Prajapati, & Jana, 2011

400 MPa was also reported (López-Fandiño, 2006; Barba *et al*., 2015) for bovine, caprine, and ovine milk immediately after HP treatment.

Effect of HP on vitamins in milk. Studies carried out by Sierra *et al*. (2000) did not find any losses either of B group vitamins in pressurised milk. HP treatment of milk at 400 MPa (2.5 MPa/sec for 30 min at 25 °C) results in non-significant loss of vitamin B_1 and $B_{6.}$ However, any information on the behaviour of some vitamins during storage of pressurised milk is still lacking.

Effect of HP on enzymes in milk. Milk enzymes vary in their sensitivity to high pressure. Lipase, xanthine oxidase, and lactoperoxidase are resistant to pressures up to 400 MPa (Naik *et al*., 2013). Phosphohexose isomerase, γ-glutamyl transferase, and alkaline phosphatase (ALP) in milk are partially inactivated at pressures exceeding 350, 400 and 600 MPa respectively; they are completely inactivated at pressures of 550, 630 and 800 MPa respectively (Sakharam, Prajapati, & Jana, 2011). No inactivation of alkaline phosphatase (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 (Naik *et al*., 2013).

Summary about HPP impact on milk and dairy product quality of various studies is given in the Table 1.

Conclusions

High pressure processing is currently of great interest and perspective in food research and industry, as a possible alternative to thermal processing. Literature study revealed that high hydrostatic pressure processing can be used in the dairy industry to increase the microbiological safety as well to modify functional properties of foods. However, it can affect a wide range of constituents in milk and dairy products: increasing level of the free fatty acids, decrease casein micelle size, denaturating whey proteins in milk. For better understanding and application of HP processing in dairy sector, research should be done to offer the dairy industry numerous practical applications to produce microbially safe, minimally processed dairy products with improved performances and to develop novel dairy products of high nutritional and sensory quality and increased shelf life.

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