The Effect of High Pressure Processing on Milk: An Overview

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ABSTRACT

High pressure processing (HPP) is a non-thermal processing technique or cold pasteurization technique used for preservation of different types of food products, due to its lethal effect on pathogenic and spoilage microorganisms, while causing minor effect on food quality and sensorial attributes. Thus, HPP is currently being applied to a variety of food products, especially fruits, vegetables, and meat products, but dairy industry has not implemented the technology yet. This overview presents firstly a brief description of the thermodynamic principles of HPP followed by a description of the high pressure effect on milk components. Generally, HPP treatment of milk in the range of 100–600 Mega Pascal has very significant implications for its constituents depending on pressure level, holding time and temperature. Pressure-induced changes of the milk characteristics includes inactivation of bacteria, disruption of the casein micelles, denaturation of whey proteins, and solubilization of minerals associated with the micelles. In addition, HP treatment of milk at higher temperatures generally increases inactivation of alkaline phosphatase.

Key words: High pressure processing, casein micelles, colloidal calcium phosphate, whey proteins, microorganisms in milk

INTRODUCTION

In this century, the interest and discussion about the health effects of different types of food and the effect of processing has a strong presence in the public and scientific forum. Both the food industry and the consumers are increasingly becoming more health conscious and as a consequence they are ever more concerned about food product components. A consumer typically prefers foods which are natural, with high nutritional value, without chemical preservatives and microbiologically safe (Makhal *et al.*, 2003). Hite (1899) carried out the first study of the effect of high pressure (HP) on food borne microorganisms in milk treated up to 650 Mega Pascal (MPa) and reported a marked decrease in the number of viable microorganisms. Yet, hundred years passed before this food processing technology was introduced in the food industry in Japan in the 1990's, and even later before other countries adopted the technology. Nowadays, HP is used for food products like jams, fruit and vegetable juices, meat (ham, sausages, RTE-products), oysters, fruit jellies, salad dressings, salsa and dips (Mohácsi-Farkas *et al.*, 2002, Anon, 2006). The usage of high pressure for preservation as pressurization in the range of 300-700 MPa is efficient in killing most of the vegetative bacteria (Anon, 2006). The main feature, which made the process popular worldwide, is its uniform processing characteristic, independent of mass and time. The HP can be applied to both liquid and solid foods and may add benefits beyond the extended shelf-life like, retaining flavour and micronutrients, improving water binding and texture and enhance desired features (digestibility) (Makhal *et al.*, 2003). The HP induces several physicochemical changes in milk and the major effects of HP treatment of milk are changes in the mineral balance, whey protein denaturation and the size of casein micelles as highlighted in many reviews (Huppertz *et al.*, 2002, 2006, Lopez-Fandino, 2006, Considine *et al.*, 2007, Devi *et al.*, 2013, Olsen & Orlien, 2016). Thus, a huge scientific knowledge exists, but has not been transformed into practical applications of HP technology in the dairy industry.

The aim of the present overview is to give an overview of the influence of HP processing on the microorganisms, casein micelles, whey proteins, Lipids, enzymes and mineral balance in milk.

Principles of high pressure

There are three principles of HP, namely, Le Chatelier's principle, the isostatic principle and the principle of microscopic ordering. Le Chatelier's principle means that when a force is applied to a system in equilibrium, the system will react to counteract the applied force. The isostatic principle is based on Pascal's principle and means that a pressure change occurring anywhere in a confined incompressible fluid is transmitted throughout the fluid such that the same pressure change occurs everywhere. The principle of microscopic ordering means that at constant temperature, an increase in pressure increases the degrees of ordering molecules of a given substance. For a system in equilibrium the fundamental thermodynamic equation holds:

$$d\Delta G(P,T) = \Delta V dP - \Delta S dT$$

The change in Gibbs free energy is a function of pressure (P) and temperature (T) and is determined by the change in volume (V) and entropy (S).

Together with:

$$\Delta G^{\theta} = -RT \ln K$$

Where K is the equilibrium constant and R is the gas constant.

The following relation governs a system under pressure:

$$\frac{\partial \ln K}{\partial P} = -\frac{\Delta V^{\theta}}{RT}$$

It can be seen that if the change increases with pressure, i.e. K increases, it corresponds to that ΔV^{θ} is negative, thus as $\Delta V^{\theta} < 0 \Rightarrow V_{AB} < V_A + V_B$ the final state (V_{AB}) less than the initial state ($V_B + V_A$). Consequently, the system will be displaced towards the most compact state (Balny & Masson, 1993, Mozhav *et al.*, 1996).

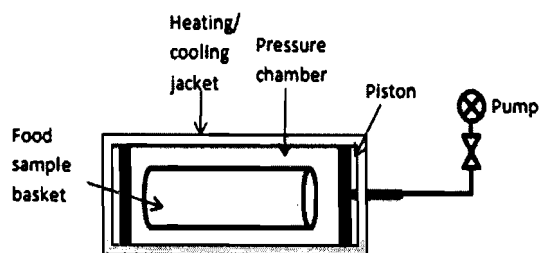As shown in Figure (1), the basic key components of a HP system are the pressure vessel, pressurizing system and supporting units (heating or cooling components)

During HP processing, the food product in the vessel is treated at the given pressure level for desired time and after that the vessel is depressurized. The time needed to achieve the desired pressure in the vessel is determined by the compressibility of the pressure medium and the nature of the food sample, and is typically within minutes to reach 800 MPa. Water is mostly used as the pressure transmitting medium due to its high incompressibility. It is noted, that air in the food material may increase the pressurization time since air is significantly more compressible than water, wherefore, dry food is not suitable for pressurization. The isostatic principle ensures that pressure is transmitted instantaneously and uniformly throughout the product, thereby, no gradient is formed resulting in the advantages that the products gets a uniform treatment and the processing time is reduced considerably in contrast to traditional heating treatment. The HPP is non-thermal treatment although the increase of pressure leads to a small adiabatic rise in temperature typically around 2-3°C per 100 MPa rise in pressure (Balci & Wilbey, 1999, Ohlsson & Bengtsson, 2002).

Effect of high pressure processing on milk

Effect on microorganisms

As already mentioned, Hite (1899) was the first to preserve and treat milk using HPP. He found that the shelf-life of milk could be extended by HP inhibition of microbial activity. The inactivation of microorganisms in milk depends on several factors: the microbial strain, growth temperature, growth phase, composition, pH, water activity of the medium, pressure level, time and temperature of pressure treatment (Balci & Wilbey, 1999, Smelt *et al.*, 2002). The inactivation of microorganisms occurs *via* different mechanisms such as damaging bacterial walls and membranes, blocking protein bacterial synthesis (Landau, 1967), or destruction of ribosomes, inactivation of intracellular enzymes (Balci & Wilbey, 1999), whereas (Hoover *et al.*, 1989, Osumi *et al.*, 1992, Kobori *et al.*, 1995) suggested changing morphology and genetically of yeast cells.

As seen in Table (1), for a significant reduction (> 4 log units) of naturally present bacteria in raw milk treated at 600 MPa is necessary. Although it is very difficult to compare different species and

Fig. 1. Diagram showing components of high pressure system.

Table 1: High pressure induced inactivation of indigenous bacteria in the raw milk. Inactivation data given correspond to the minimum conditions required to obtain the highest level of inactivation. Adapted from (Huppertz *et al* 2006)

Bacterial group	HP-treatment	Reduction in log CFU/mL	Milk type
Total bacterial count	200 MPa/16h/20°C	3.0	Raw bovine colostrum
Total bacterial count	70MPa/30min/13°C	3.5	Raw milk
Total bacterial count	350 MPa/20min	3.0	Raw whole milk
Total bacterial count	300 MPa/1h/20°C	2.5	Raw milk
Aerobes	400MPa/30min/25°C	1.0	Raw whole milk
Aerobes	600MPa/25min/25°C	7.0	Raw whole milk
Aerobes mesophiles	600MPa/5min/55°C	4.5	Raw milk
psychrotrophs	200MPa/30min/25°C	1.0	Raw whole milk
psychrotrophs	400MPa/30min/25°C	2.5	Raw whole milk

strains, Gram-positive species such as *L. monocytogenes* and *S. aureus* and Gram-negative bacteria such as *E. coli* show higher baroresistant characteristic of the species in raw milk. When raw milk is treated at P ≥ 500 MPa for 5 min at T < 4 °C or T > 55 °C it prolongs the refrigerated shelf life of raw milk to 21 days (Huppertz *et al*. 2006). The level of psychrotrophic bacteria of 7 log cfu/ ml produces a significant amount of proteases and lipases resulting in detectable off-flavours in milk (Stepaniak, 1991). Raw milk treated at 400 MPa for 30 min at 25°C contains less than 7 log/ psychrotrophs/ ml after storage milk for 45 days at 7°C. On the other hand, unpressurized milk contains more than7 log/ ml of the same bacteria after just 15 days. This fact shows a significant role of HPP in extension shelf life of milk (Garcia-Risco *et al*., 1998). When the effect of HPP on bacteria in raw milk is considered, it is needed to take into consideration that many components in food can have the protective role on microbes during HPP (Garcia-Graells *et al*., 1999, Dogan & Erkmen, 2004). Micellar minerals in milk, calcium, citrate, magnesium and phosphate protect microbes from HPP. Calcium and magnesium show stabilizing effect on the cell membrane which is one of the main targets of HPP induced inactivation (Black *et al*., 2007).

Bacterial spores show the highest resistance to HPP and the level of inactivation is influenced by several factors, some of them are different bacterial species, the intensity of treatment and the number of treatment cycles (Farkas & Hoover 2000). It has been reported that in order to achieve HPP-induced inactivation of spores, two treatment steps must be done, the first to germinate spores and the second

to inactivate germinated spores (Sale *et al*., 1970, Heinz & Knorr, 2001).

Effect on casein micelles

Milk proteins are divided into two classes based on their solubility at pH 4.6: whey proteins (the soluble) and caseins (the insoluble) representing 20 and 80% of total milk proteins, respectively. Casein is a phosphoprotein with characteristics different from the other milk proteins and exists as four different types: α_{s1}-, α_{s2}-, β- and κ-casein. It is hydrophobic with relatively high charge, containing a lot of proline and few cystein residues. In milk, the caseins are assembled in casein micelles which are associated colloids that vary in diameters from 50-300 nm, thereby giving milk its white colour. They are hydrated and have around 6% of inorganic materials calculated on the dry matter basis. Inorganic materials are mainly calcium and phosphate, but also some amount of magnesium and citrate. The calcium and phosphate in the micelles are called colloidal calcium phosphate (CCP) and play a crucial role, through electrostatic interactions, in keeping micellar integrity (Holt, 1997, Swaisgood, 2003).

In the first study on HP-induced changes of milk, Hite (1899) concluded that after HP treatment, milk appeared different, "thin and blue or yellow". This is the most striking effect of HP on milk and since then, the casein micelles has been the subject of numerous studies concerning pressure effects due to this pronounced sensitivity to pressure. Schmidt & Buchheim (1970) found that casein micelles in skimmed milk after pressure treatment at 100 MPa for 30 min looked smaller than micelles in untreated milk. In pressurized milk, the structure of casein micelles is provided

by hydrophobic interactions and CCP, the same as in unpressurised milk, but with changes in the micellar sizes, composition and hydration (Huppertz *et al.*, 2006). The HPP of milk causes the solubilization of micellar calcium and the release of calcium from the casein micelles and its transfer to the milk serum (Lopez-Fandino *et al.*, 1998). At the same time, there is transfer of casein from the casein micelles to the serum after HPP (Lopez Fandino *et al.*, 1998, Huppertz *et al.*, 2004e, Anema *et al.*, 2005, Anema, 2008). It has been proved that casein micelles disruption is linked to transfer of calcium phosphate from the casein micelles to the milk serum (Holt, 1997). The other very important factor that contributes to the disruption of casein micelles and reduction of micellar size is pressure-induced hydration of casein micelle structure upon high pressure treatment (Orlien *et al.*, 2006).

The size of casein micelles in milk treated at 100-200 MPa at ambient temperature is comparable to micelles from untreated milk (Needs *et al.*, 2000, Huppertz *et al.*, 2004a, Regnault *et al.*, 2004, Anema *et al.*, 2005). Studies showed that after treatment at 250 MPa for more than 15 min, casein micelles were significantly larger than in untreated milk (Huppertz *et al.*, 2004a, Regnault *et al.*, 2004).

Notwithstanding, micelles in milk treated at pressure > 300 MPa were smaller up to 50 % as compared to untreated milk (Needs *et al.*, 2000, Huppertz *et al.*, 2004a, Huppertz *et al.*, 2004b, Huppetz *et al.*, 2004c, Regnault *et al.*, 2004, Anema *et al.*, 2005). It is obvious that HPP at 250 and 300 MPa lead to dissociation of casein micelles but leaving them susceptible to re-association (Orlien *et al.*, 2006). The unstable nature of the casein micelles during and after pressure treatments of 250 and 300 MPa shows disruptive effects between stabilizing and repulsive reactions in the casein micelles at these pressures depending on treatment time. Thus, the affinity of re-association of partly dissociated casein micelles and the caseins influences the broad size distribution of the casein micelles (Huppertz *et al.*, 2004b, Regnault *et al.*, 2004, Anema *et al.*, 2005, Orlien *et al.* 2006). Temperature and pH influence the stability of the casein micelles during HPP by affecting the strength of the hydrophobic and electrostatic interactions. Higher temperature and pH enhance both the hydrophobic and electrostatic interactions in the micelles and, thereby, their barostability (Orlien *et al.*, 2010). Furthermore, higher temperatures favour association at intermediate

pressure treatments and this confirms that re-association can be caused by hydrophobic interactions (Regnault *et al.*, 2004, Anema *et al.*, 2005). Overall, the effects can be summarized in Figure (2) and for detailed description see (Olsen & Orlien, 2016).

Effect of β-Lactoglobulin (β-Lg) and α-Lactoalbumin (α-La)

Whey proteins include β-Lg, α-La, and less significant amounts of serum albumin, immunoglobulins and protease peptones. The β-Lg is quantitatively the dominant whey protein (58 % w/w), α-La is quantitatively the second largest component in whey representing (20 % w/w) of the total whey proteins (Wong *et al.*, 1996). Meanwhile, Johnston *et al.* (1992) found that the amount of non-casein nitrogen decreased in the milk serum with increasing pressure which suggested denaturation and insolubilization/aggregation of whey proteins. The β-Lg was denatured at pressure higher than 200 MPa and α-La at pressure higher than 400 MPa (Huppertz *et al.*, 2002 and references therein). The ß-Lg has two disulphide bond and one free sulphydryl group, while α-La has four disulphide bonds, making β-Lg more sensitive to pressure than α-La explaining the different levels of pressure denaturation (Scollard *et al.*, 2000, Hinrichs & Rademacher, 2004, Huppertz *et al.*, 2004b). According to Ye *et al.* (2004) and Goyal *et al.* (*2013*), at 800 MPa about 90 % of the total ß-Lg and about 50 % of the α-La were denatured.

The amount of β-Lg and α-La denaturated at HPP increases with longer holding time, higher temperature and pH of milk (Scollard *et al.*, 2000, Hinrichs & Rademacher, 2004, Huppertz *et al.*, 2004c, Huppertz *et al.*, 2004b). Denaturation of β-Lg decreased when milk was acidified to pH 5.5 or 6.0 before high pressure treatment and increased at pH 7.0 due to enhanced reactivity of its free sulphydryl group at alkaline pH (Arias *et al.*, 2000). According to Anema (2008) prolonged holding time considerably influenced the level of β-Lg denaturation, while α-La was not significantly changed by different holding time. During the store age period within 1–2 days at 20–40°C renaturation of α-La and β-Lg occurs. At lower temperature (5°C) re-association does not take place, because at lower temperature mobility (energy) of atoms is too low to form hydrophobic as well as ionic bonds. In addition, at low temperature strength of hydrophobic interactions is very low (Garcia-Risco *et al.*, 2000, Goyal *et al.*, 2013).
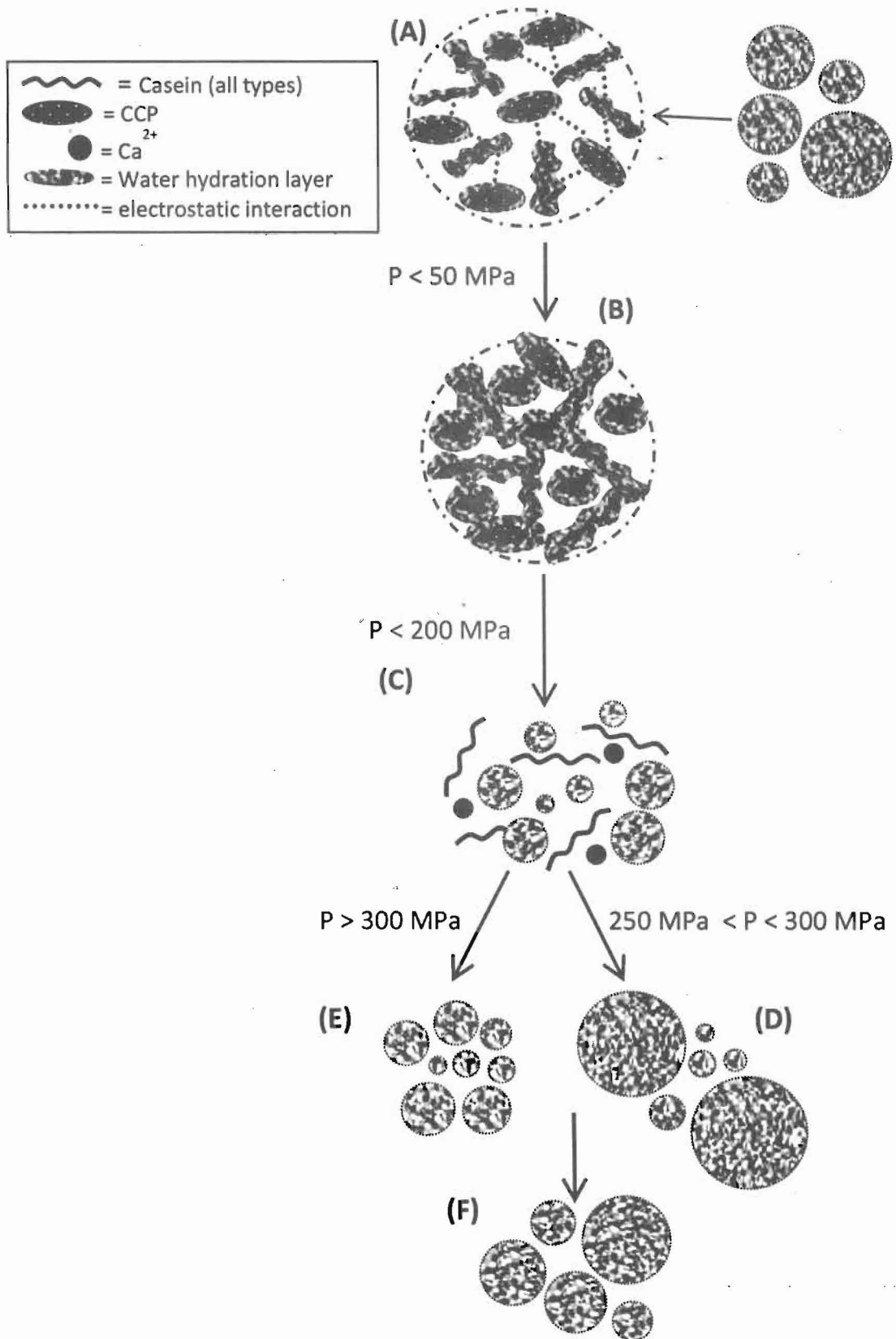
Fig. 2: Pressure-induced dissociation of casein micelles: (A) Native micelle, (B) Hydration of micelle and disruption of hydrophobic interactions, (C) Solubilization of CCP and dissociation into sub-micelles, (D) Re-association, (E) Dissociation, (F) Pressure release (Olsen & Orlien, 2016)
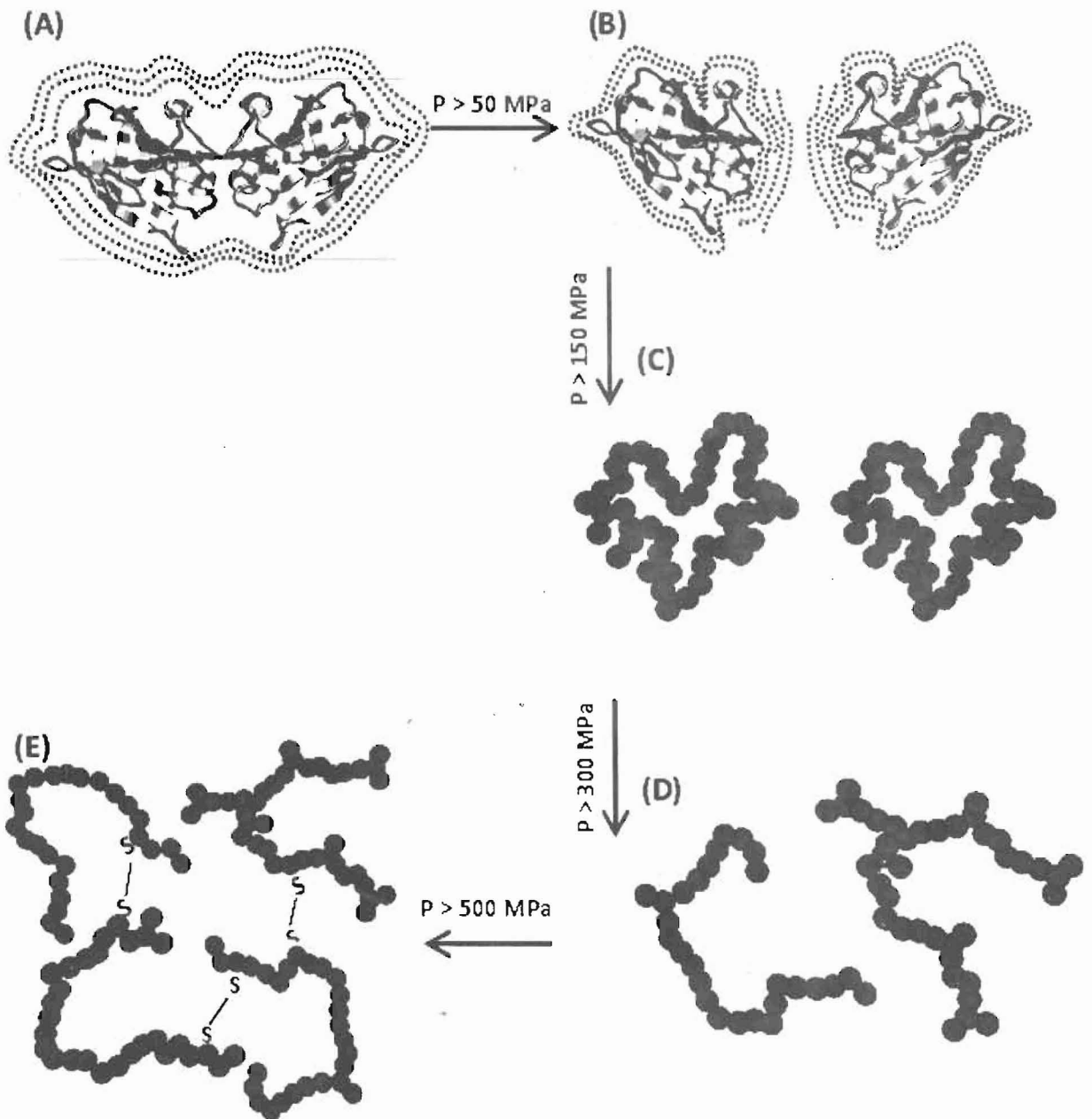
(A)

(B)

P > 50 MPa

P > 150 MPa

(C)

P > 300 MPa

(D)

(E)

P > 500 MPa

Fig. 3: Pressure-induced denaturation and aggregation of β-Lg: (A) Native dimer with water hydration on surface, (B) Monomers, (C) Molten globules and disulphide-bonded dimers, (D) Denaturation and (E) Aggregation or gelation (Olsen & Orlien, 2016).

More is known about the mechanism of β-Lg denaturation due to its higher pressure sensitivity, and for an overview of the process see Fig. (3), while for a more detailed description of the denaturation process see (Olsen & Orlien, 2016).

Effect of HPP on bovine serum albumin, immunoglobulins, lactoferrin and lysozymes

Bovine Serum Albumin (BSA) has 17 disulphide bridges and one free thiol group. It is very resistant to pressure up to 400 MPa due to the large number of disulphide bonds and a very little denaturation occurs above

400 MPa (Lopez-Fandino *et al.*, 1996, Goyal *et al.*, *2013*). Lactoferrin and Lysozymes in milk are resistant to high pressure. Immunoglobulins are more resistant to pressures up to 300 MPa. Immunoglobulins in caprine milk were found to be resistant to pressures up to 300 MPa, although ~ 35 % denaturation occurred after treatment at 500 MPa (Felipe *et al.*, 1997, Goyal *et al.*, *2013*).

Effect of HPP on milk minerals

The minerals in milk present the small fraction of milk (about 8–9 g /L) but play an important role in the structure and stability of the milk system (Holt, 1997).

Minerals consist of the cations such as calcium, magnesium, sodium and potassium and the anions are inorganic phosphate, citrate and chloride, where calcium and phosphate are the major minerals in milk. There is a dynamic equilibrium between CCP and the mineral components in the soluble, serum phase. In the soluble phase, the soluble calcium and the soluble inorganic phosphate are either free ions or associated with other ions (Schmidt, 1982, Holt, 1985, Gaucheron, 2005).

Calcium and phosphate equilibrium in milk is significantly affected by the initial temperature, pH and the HPP condition. Increasing milk temperature (30 to 64°C) transfers Ca and P from the serum to the colloidal phase, whereas decreasing temperature (below 30°C) induces solubility of CCP consequently increase Ca and P in serum (Pierre & Brulé 1981, Pouliot *et al.,* 1989). Indeed, the disruptive effect of pressure on hydrophobic and electrostatic interactions that maintain micelle structure must also affect minerals. The effect of pressure on mineral equilibrium includes changes in the balance between colloidal and serum phases and in ionization. However, not many investigations have been reported due to the difficulties in measuring in situ effects of pressure on the mineral balance and ionization. Two studies have shown an increase (53% and 18%) in the concentration of ionic calcium after pressure treatment up to 400 MPa (Lopez-Fandino *et al.,* 1998, Knudsen, 2005). The pressure-induced dissociation of casein micelles described in previous section is accompanied with solubilization of the CCP and thereby mineral release from micelle to serum phase. Thus, immediately after pressurization (100-400 MPa) it was found that the total calcium concentration increased in the serum phase with a maximum at 200 MPa (increase of 51%) (Knudsen, 2005) or 300 MPa (increase of 53%) (Lopez- Fandino *et al.,* 1998). The difference in the increases of ionic and total calcium may be explained by complex formation of the released calcium with released caseins (due to micelle dissociation) or other milk constituents in serum. Moreover, the released calcium may also re-bind to the smaller sub-micelles reversing the balance. The dissociation rate is higher in acidified milk (pH $\leq$ 6) due to the loose colloidal structure rendering the micelles highly sensitive to HP, but it was found that pressurization (up to 400 MPa) only led to small increases in ionic Ca most likely due to rebinding to other compounds (Arias *et al.,* 2000, Orlien *et al.,* 2010). Overall, HP treatment causes solubilization of the colloidal calcium phosphate resulting in increased level of ionic calcium and phosphate and/or complexed-bound calcium and phosphate, although the dissociated calcium phosphate is totally or partly reformed during pressure release and subsequent storage, (Fig. 4).

Effect of HPP on milk enzymes

Generally, the primary structure of enzyme constructed by several covalent interactions remains unaffected upon pressurization. The secondary structure of the enzyme may be affected at higher pressure (above 700 MPa) through irreversible unfolding (Tauscher, 1995). On the other hand, HPP affects the tertiary and quaternary structures of enzymes by modifying the electrostatic, hydrophobic interactions, and the hydrogen bonds (Heremans, 1993). In general, Enzymes can be divided into two groups, according to the effect of HPP thereon, at the first group, enzymes are activated with pressures of 100-500 MPa, an activation that occurs only in monomeric proteins. The second group, includes enzymes that are inactivated when exposed to pressures higher than 500 MPa in combination with relatively high temperatures (Bello *et al.,* 2014). Indigenous enzymes in milk as lipase, xanthine oxidase, lactoperoxidase and γ-glutamyltransferase are resistant to pressures up to 400 MPa at 20-25°C (Lopez-Fandino *et al.,* 1996). Phosphohexoseisomerase was inactivated after pressure treatment at 500 MPa and 20°C for 10 min (Rademacher, & Hinrichs, 2006). Alkaline phosphatase appears quite pressure resistant, with no inactivation in raw milk after treatment at 400 MPa for 60 min at 20°C (Lopez- Fandino *et al.,* 1996), 50% inactivation after 90 min at 500 or 10 min at 600 MPa and 100% inactivation after 8 min at 800 MPa (Rademacher *et al.,* 1998, Rademacher, & Hinrichs, 2006). The effect of HPP on alkaline phosphatase is of interest in milk processing and complete inactivation of alkaline phosphatase occurs only at very high pressures. Also, of interest is the barostability of indigenous milk proteinases, such as plasmin. Treatment of raw milk at 400 MPa reduced plasmin activity, with inactivation being far more significant at elevated temperature, e.g. 60°C. (Garcia-Risco *et al.,* 2000, Scollard, *et al.,* 2000). However, purified plasmin in phosphate buffer, in the presence or absence of sodium caseinate, was almost completely resistant to pressures up to 600 MPa. (Garcia-Risco *et al.,* 2000, Scollard, *et al.,* 2000).
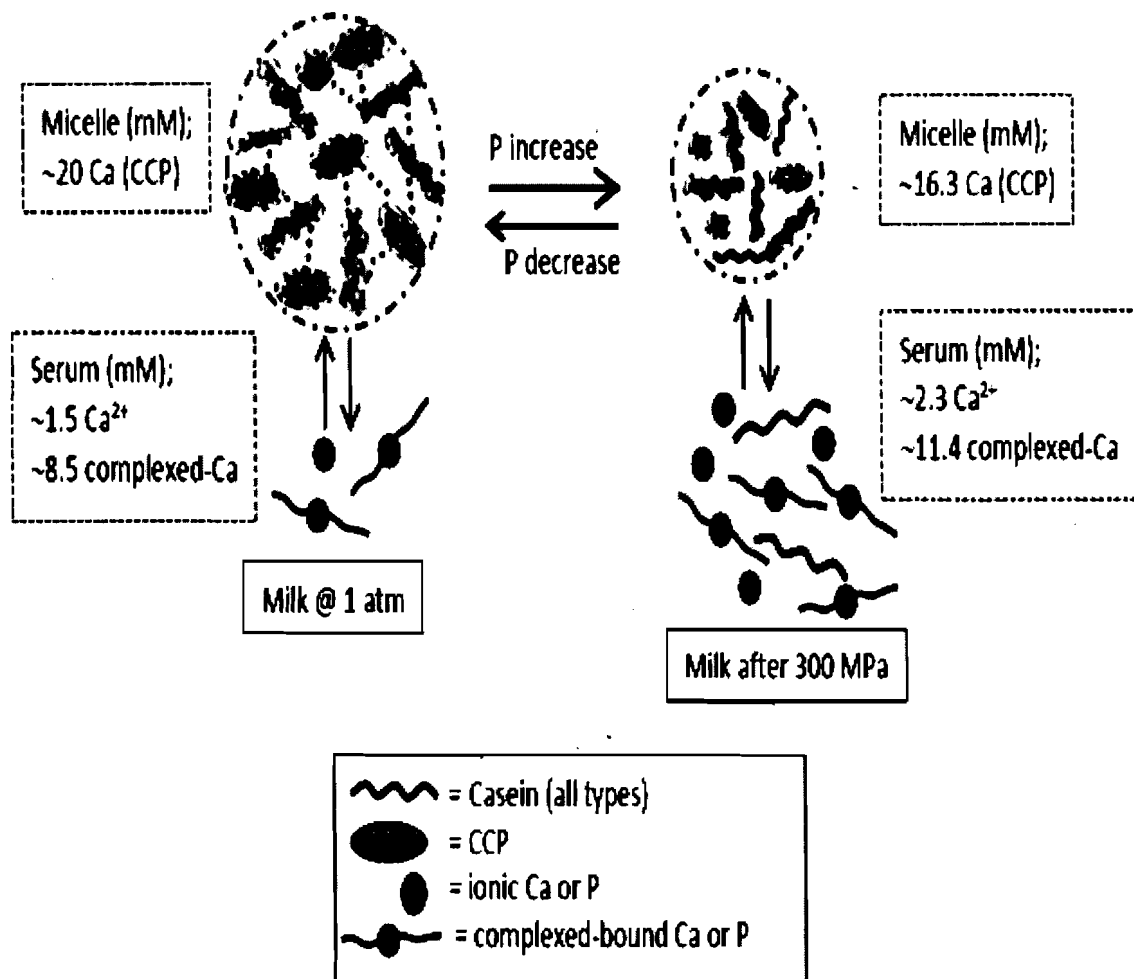
Fig. 4: Effect of pressure on the calcium balance between micelle and serum phases (Knudsen, 2005)

Effect of HPP on milk fat

Relatively few studies have examined the effect of HPP on milk fat, milk fat globules and the milk fat globule membrane (MFGM). Pressures less or equal 400 MPa did not affect the mean diameter or the size distribution of milk fat globules, but higher pressures between 400-800 MPa increased the former and broadened the latter. Since no increase in products of lipolysis was detected, no damage to the MFGM was thought to have occurred under HP (Buchheim *et al.* 1996b). Crystallization of fat can be accelerated, enforced, or initiated because of the shift in the phase transition temperature caused by application of high pressure. The HPP-treated cream had a higher solid fat content than untreated cream, also with a maximum effect at 200 MPa (Buchheim *et al.*, 1996a, b). At pressure up to 200 MPa, the crystallisation and melting temperatures of milk fat were found to increase by 16.3°C and 15.5°C/100 MPa, respectively (Frede & Buchheim, 2000). However, above 350 MPa there is lower extent of milk fat crystallization due to reduced crystal growth because of reduced molecular mobility at higher pressures (Buchheim *et al.*, 1996a, b). As a consequence, high-pressure treatment reduced the ageing time of ice cream mixes and aided the physical ripening of cream for butter making (Buchheim *et al.*, 1996a, b). The fat globule size distribution and flow behaviour of pasteurized liquid cream are not significantly changed by HPP at 450 MPa and 25 °C for 15–30 min or 10 °C for 30 min. Mean diameter of the milk fat globule remains unaffected after high pressure treatment (Buchheim *et al.*, 1996b). Following, high pressure treatment, there is some incorporation of whey proteins into milk fat globule membrane (MFGM) but as there is no increase in lipolysis (Buchheim & Frede, 1996).

CONCLUSION

Milk is a tortuous system of various components, of which the most important are the proteins and the minerals, held together by a complex myriad of equilibria. From this overview it is clear that the milk components are sensitive to pressure. The main effects of HPP of milk being bacterial inactivation, dissociation of micelles in effect releasing minerals and caseins, and β-Lg denaturation. However, each individual effect needs different pressure level to occur and in addition also being dependent on pressure duration and temperature. Surely, much research on the HPP-induced physicochemical changes in milk has been conducted, but more is still needed in order to gain insight and knowledge about this complex system and for the future HPP-applications in the milk industry.

REFERENCES

Anema, S.G.2008. Effect of milk solids concentration on whey protein denaturation, particle size changes and solubilization of casein in high-pressure-treated skim milk. International Dairy Journal, **18**: 228-235.

Anema, S.G., Lowe, E.K. & Stockman, G. **2005.** Particle size changes and casein solubilisation in high pressure-treated skim milk. Food Hydrocolloids Journal, **19**: 257-267.

Anon, **2006.** High pressure processing, fact sheet for food processors. Ohioline.osu.edu/fse-fact/0001 (accessed August 2006).

Arias, M., López-fandiño, R. & Olano, A. **2000.** Influence of pH on the effects of high pressure on milk proteins. Milchwissenschaft, **55**:191-194.

Balci, A. T. & Wilbey, R.A. **1999.** High pressure processing of milk-the first 100 years in the development of a new technology. International Journal of Dairy Technology, **52**:149-155.

Balny, C. & Masson, P. **1993.** Effect of high pressures on proteins. Food reviews international journal, **9**: 611– 628.

Bello, E. F. T., Martínez , G. G., Ceberio, B. F. K., Rodrigo, D. & López, A. M. **2014.** High pressure treatment in foods. Foods, **3**, 476-490, doi: 10.3390/foods3030476

Black, E.P., Huppertz, T., Kelly, A.L. & Fitzgerald, G.F. **2007.** Influence of milk constituents on high pressure induced inactivation of *Listeria innocua* in milk. International Dairy Journal, **17**:104-110.

Buchheim, W. & Frede, E. **1996.** Use of high pressure treatment to influence the crystallisation of emulsified fats. Milchwissenschaft, **117**:228-237.

Buchheim, W., Schrader, K., Morr, C. V., Frede, E., & Sch. utt, M. **1996a.** Effects of high pressure on the protein, lipid and mineral phase of milk: In: Heat Treatments and Alternative Methods (pp. 202–213). Special Issue No 9602. Brussels: International Dairy Federation.

Buchheim, W., Sch. utt, M., & Frede, E. **1996b.** High pressure effects on emulsified fats. In: High Pressure Bioscience and Biotechnology, R. Hayashi, & C. Balny (Eds.) (pp. 331–336). Amsterdam: Elsevier.

Considine, T., Patel, H.A., Anema, S.G., Singh, H. &Creamer, L.K., **2007.** Interactions of milk proteins during heat and high hydrostatic pressure treatments: A Review. Innovative Food Science and Emerging Technologies, **8**: 1-23.

Devi, A.F., Buckow, R., Hemar, Y. & Kasapis, S., **2013.** Structuring dairy systems through high pressuress processing. Journal of Food Engineering, **114**: 106-122.

Dogan, C. & Erkmen, O. **2004.** High-pressure inactivation kinetics of *Listeria monocytogenes* inactivation in broth, milk, peach and orange juices. Journal of Food Engineering, **62**: 47-52.

Farkas, D.F. & Hoover, D.G. **2000.** High pressure processing, kinetics of microbial inactivation for alternative food processing technologies. Journal of Food Science Supplement, **65**: 47-64.

Frede, E., & Buchheim, W. **2000.** The influence of high pressure upon the phase transition behaviour of milk-fat and milk-fat fractions. Milchwissenschaft, **55**: 683–686.

Felipe, X., Capellas, M. & Law, A.J.R. **1997.** Comparison of the effects of high- pressure treatments and heat pasteurization on the whey proteins in goat's milk. Journal of Agricultural and Food Chemistry, **45**: 627–631.

Garcia-Graells, C., Masschalck, B. & Michiels, C.W. **1999.** Inactivation of *Escherichia coli*

in milk by high hydrostatic pressure treatment in combination with antimicrobial peptides. Journal of Food Protection, **62**: 1248-1254.

Garcia-Risco M.R., Cortes, E., Carrascosa A.V. & Lopez-Fandino, R. **1998**. Microbiological and chemical changes in high pressure-treated milk during refrigerated storage. Journal of Food Protection, **61**: 735-737.

Garcia-Risco, M.R., Olano, A. & Ramos, M. **2000**. Micellar changes Induced by High Pressure. influence in the proteolytic activity and organoleptic properties of milk. Journal of Dairy Science, **83**: 2184-2189.

Gaucheron, F. **2005**. The minerals of milk. Reproduction Nutrition Development, **45**: 473-483. (http://www.edpsciences.org/rnd or http://dx.doi.org/10.1051/rnd:2005030).

Goyal, A., Sharma, V., Upadhyay, N., Sihag, M. & Kaushik, R. **2013**. High pressure processing and its impact on milk proteins: A review. Research and Reviews: Journal of Dairy Science and Technology, **2**: 1-9.

Heinz, V. & Knorr, D. **2001**. Effects of high pressure on spores. In: Ultra High Pressure Treatments of Foods, M.E.G Hendrickx &D. Knorr (Eds) New York, London: Kluwer Academic/Plenum pp. 77-113.

Hite, B. H. **1899**. The Effect of Pressure in the Preservation of Milk, A Preliminary Report. West Virginia Agricultural experiment station.

Holt, C. **1985**. The Milk Salts: Their Secretion, Concentrations and Physical Chemistry, In: Developments in Dairy Chemistry – 3. Lactose and Minor Constituents, Fox, P.F. (Ed.). Elsevier Applied Science Publishers, London, England, pp. 143–181.

Heremans K. **1993**. The behaviour of proteins under pressure. In: High-Pressure Chemistry, Biochemistry and Materials Science. Winter R, Jonas J, (Eds). New York: Springer. p 443–69.

Holt, C. **1997**.The milk salts and their interaction with casein. In: Advanced Dairy Chemistry. Vol. 3: Lactose, Water, Salts and Vitamins, Fox, P.F. (Ed). Chapman and Hall, Londres, pp. 233–254.

Hoover, D.G., Metrick, C., Papineau, A.M., Farkas, D. F. & Knorr, D. **1989**. Biological effects

of high hydrostatic pressure on food microorganisms. Food Technology, **43**: 99-107.

Hinrichs, J. & Rademacher, B. **2004**. Kinetics of combined thermal and pressure-induced whey protein denaturation in bovine skim milk. International Dairy Journal, **14**: 315–323.

Huppertz, T., Fox, P.F. & Kelly A. L. **2004a**. High pressure-induced denaturation of α-lactalbumin and β-lactoglobuline in bovine milk and whey: a possible mechanism. Journal of Dairy Research, **71**: 489-495.

Huppertz, T., Fox, P.F. & Kelly, A. L. **2004b**. High pressure treatment of bovine milk: effects on casein micelles and whey proteins. Journal of Dairy Research, **71**: 97-106.

Huppertz, T., Fox, P.F. & Kelly, A.L. **2004c**. High pressure treatment of bovine milk: effects on casein micelles and whey proteins. Journal of Dairy Research, **71**: 97-106.

Huppertz, T., Fox, P.F. & Kelly, A.L. **2004d**. Dissociation of caseins in high pressure-treated bovine milk. International Dairy Journal, **14**: 675-680.

Huppertz, T., Fox, P.F., Kruif, K.G. & Kelly, A.L. **2006**. High pressure induced changes in bovine milk proteins: A review. Biochimica et Biophysica Acta, **1764**: 593-598.

Huppertz, T., Kelly, A. L. & Fox, P.F. **2002**. Effects of high pressure on constituents and properties of milk. International Dairy Journal, **12**: 561-572.

Huppertz, T., Grosman, S. Fox, P.F. & Kelly, A.L. **2004e**. Heat and ethanol stabilities of high pressure-treated bovine milk. International Dairy Journal, **14**:125-133.

Johnston, D.E., Austin, B.A. & Murphy, R.J. **1992**. Effects of High Hydrostatic Pressure on Milk. Milchwissenschaft, **8**: 206-209.

Kobori, H., Sato, M., Tameike, A., Hamada, K., Shimada, S. & Osumi, M. **1995**. Ultrastructural effects of pressure stress to the nucleus in *Saccharomycess cerevisiae*: a study by immune electron microscopy using frozen thin sections. FEMS Microbiology Letters, **132**: 253-258.

Knudsen, J.C. (2005). High Pressure Effects on Milk Proteins Ph D. Thesis. The Royal Veterinary and Agricultural University, Denmark.

Landau, J. **1967**. Induction, transcription and translation in *Escherichia coli*: a hydrostatic pressure study. Biochimica et Biophysica Acta, **149**: 506-512.

Lopez-Fandino, R. **2006**. High pressure-induced changes in milk proteins and possible applications in dairy technology. International Dairy Journal, **16**: 1119–1131.

Lopez-Fandino, R., Carrascosa, A. V. & Olano, A. **1996**. The effects of high pressure on whey protein denaturation and cheese- making properties of raw milk. Journal of Dairy Science, **79**: 929–936.

Lopez-Fandino, R., De la Fuente, M. A., Ramos, M. & Olano, A. **1998**. Distribution of minerals and proteins between the soluble and colloidal phases of pressurized milks from different species. Journal of Dairy Research, **65**: 69–78.

Makhal, S., Vashishtha, B., Mandal, S. & Kanawjia, S.K. **2003**. High hydrostatic pressure in food preservation: Philosophy and development. Indian Food Industry, **22**: 38–45.

Mohácsi-Farkas, C., Kisko, G., Meszaros, L. & Farkas, J. **2002**. Pasteurization of tomato juice by high hydrostatic pressure treatment or by its combination with essential oils. Acta Alimentaria, **31**: 243–252.

Mozhaev, V.V., Heremans, K., Frank, J., Masson, P. & Balny, C. **1996**. High pressure effects on protein structure and function. Proteins: Structure, Function & Genetics, **24**: 81-91.

Needs, E. C., Stenning, R. A., Gill, A. L., Ferragut, V. & Rich, G. T. **2000**. High-pressure treatment of milk: Effects on casein micelle structure on enzymic coagulation. Journal of Dairy Research, **67**: 31– 42.

Ohlsson, T. & Bengtsson, N. **2002**. Minimal processing technologies in the food industry. Woodhead Pub l Ltd, Cambridge.

Olsen, K. & Orlien, V. **2016**. High pressure processing for modification of food biopolymers. In: Innovative Food Processing Techniques. Knoerzer, K., Juliano, P., and Smithers, G. (Eds). Cambridge, Woodhead Publishing Limited.

Orlien, V., Boserup, L. & Olsen, K. **2010**. Casein micelle dissociation in skim milk during high pressure treatment: Effect of pressure, pH and temperature. Journal of Dairy Science, **93**:12-18.

Orlien, V., Knudsen, J.C., Colon, M. & Skibsted, L.H. **2006**. Dynamics of casein micelles in skim milk during and after high pressure treatment. Food Chemistry, **98**: 513-521.

Osumi, M., Yamada, N., Sato, M., Kobori, H., Shimada, S. & Hayashi, R. **1992**. Pressure effects on yeast cells ultrastructure: Change in the ultrastructure cytoskeleton of the dimorphic yeast *Candida tropicalis*. In: High Pressure Bioscience & Biotechnology. Hayashi, C.B.R. (Ed) Springer Publishing Company, pp. 9-18.

Pierre, A. & Brulé, G. **1981**. Mineral and protein equilibria between the colloidal and soluble phases of milk at low temperature. Journal of Dairy Research, **48**:417– 428.

Pouliot, Y., Boulet, M. & Paquin, P. **1989**. Observations on the heat-induced salt balance changes in milk. 1. Effect of heating time between 4°C and 90°C. Journal of Dairy Research, **56**: 185–192.

Rademacher, B. & Hinrichs, J. **2006**. Effects of high pressure treatment on indigenous enzymes in bovine milk: Reaction kinetics, inactivation and potential application. International Dairy Journal, **16**: 655–661.

Rademacher, B., Pfeiffer, B., & Kessler, H. G. **1998**. Inactivation of microorganisms and enzymes in pressure-treated raw milk. In High Pressure Food Science, Bioscience and Chemistry, N. S. Isaacs (Ed.), (pp. 145–151). Cambridge: The Royal Society of Chemistry.

Regnault, S., Thiebaud, M., Dumay, E. & Cheftel, J.C. **2004**. Pressurization of raw skim milk and of a dispersion of phosphocaseinate at 9°C or 20°C: Effects on casein micelle size distribution. International Dairy Journal, **14**: 55-68.

Sale, A. J. H., Gould, G.W. & Hamilton, W. A. **1970**. Inactivation of bacterial spores by high pressure. Journal of General Microbiology, **60**: 323-334.

Schmidt, D.G. **1982**. Association of Caseins and Casein Micelle Structure. In: Developments in Dairy Chemistry, Fox PF (Ed), El-Sevier, London, pp. 61–86.

Schmidt, D.G. & Buchheim, W. **1970**. Electronen Mikroscopische Untersuhung der Fein

Strucktur von Casein Micellen in Kuhmilch, Milchwissenschaft. pp: 596-600.

Scollard, P. G., Beresford, T.P., Needs E.C., Murphy, P.M. & Kelly, A.L. **2000**. Plasmin activity, β-Lactoglobuline denaturation and proteolysis in high pressure treated milk. International Dairy Journal, **10**: 835-841.

Smelt, J.P., Hellemons, J. C. & Patterson, M. **2002**. Effects of high pressure on vegetative microorganisms. In: Ultra High Pressure Treatments of Foods, Hendrickx, M.E.G. (Ed). Springer Publishing Company, pp: 55-76.

Stepaniak, L. **1991**. Factors affecting quality and possibilities of predicting shelf-life pasteurized and ultra- high temperature heated milk. Italian Journal of Food Science, **3**: 11-26.

Swaisgood, H.E. **2003**. Chemistry of the caseins. In: Advanced dairy chemistry -1: proteins. Fox, P.F.& Mc Sweeney, P.L.H. (Eds.), Part A. New York: Kluwer Academic/Plenum Publishers.

Tauscher, B. **1995**. Pasteurization of food by hydrostatic high pressure: Chemical aspects. Z Lebensm Unters Forsch, **200**:3–13.

Wong, D. W. S., Camirand, W. M. & Pavlath, A. E. **1996**. Structures and functionalities of milk proteins. Critical Reviews in Food Science & Nutrition, **36**: 807-844.

Ye, A., Anema, S. G. & Singh, H. **2004**. High pressure induced interactions between milk fat globule membrane proteins and skim milk proteins in whole milk. Journal of Dairy Science, **87**: 4013- 4022.

تأثير المعاملة بالضغط العالى على اللبن: نظرة شاملة

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المعاملة بالضغط العالى هى تقنية المعالجة غير الحرارية او البسترة الباردة لحفظ انواع مختلفة من المنتجات الغذائية وذلك نتيجة التأثير المميت للكائنات الحية الدقيقة المرضية والمسببة للتلف، فى حين ان لها تأثيرات طفيفة على جودة الغذاء و صفاته الحسية. ويجرى حاليا تطبيق هذه التقنية على مجموعة واسعة من المنتجات الغذائية وخاصة الفواكه ، الخضروات ومنتجات اللحوم على الرغم من انها لم تطبق فى صناعة الالبان حتى اليوم.

وهذه المقالة تعرض أولاً وصفاً موجزاً لمبادئ الديناميكا الحرارية للمعالجة بالضغط العالى وكذا وصفاً لتأثير الضغط العالى على مكونات اللبن. وبصفة عامة، فان معاملة اللبن بالضغط العالى فى مدى مابين ١٠٠ – ٦٠٠ ميجا بسكال ، لة اثار كبيرة جداً على مكوناته تبعاً لمستوى الضغط، ، زمن التعرض و درجة الحرارة. تتضمن التغيرات الناجمة عن الضغط العالى للبن تثبيط البكتيريا و اضطراب جسيمات الكازين و دنترة بروتينات الشرش و اذابة الأملاح المرتبطة بالجسيمات. بالاضافة إلى أن المعاملة بالضغط العالى على درجات حرارة عالية تزيد بشكل عام من تثبيط إنزيم الفوسفاتيز القاعدى.