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High Pressure Processing and Its Impact on Milk Proteins: A Review

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Abstract

High pressure processing (HPP) is a non-thermal or cold pasteurization technique to preserve various food products. High pressure in range of 100–1000 MPa affects size, shape and conformation of various milk proteins. Casein micelles having primary conformation are not affected under low pressure, but disintegrate at high pressure (300 MPa). β-lactoglobulin (β-lg) is one of the most pressure-sensitive proteins and α-lactalbumin (α-la) is one of the most pressure resistant. Bovine serum albumin, lactoferrin and immunoglobulins are not much affected by high hydrostatic pressure (HHP). It has been observed that to achieve the shelf-life of thermally pasteurized milk of 10 days at 10 °C, a pressure treatment of at least 400 MPa for 15 min or 500 MPa for 3 min is required.

Keywords: Casein, immunoglobulins, lactoglobulin, lactalbumin, high hydrostatic pressure

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INTRODUCTION

The application of high pressure, rather than heat, to food enables destruction of microorganisms without causing significant changes to color, flavor and nutritional attributes. In this way, the food can be preserved in a safe state but still has many of the attributes of a fresh product. In addition, high pressure can cause rheological changes in food which result in beneficial sensory and structural effects. The potential of high-pressure treatment of food was first recognized by Hite in 1899 who investigated it as an alternative method for pasteurization of milk [1]. However, it attracted little attention until it was "rediscovered" in the 1980s. When milk is subjected to high pressure (HP), the casein micelles disintegrate into smaller particles [2] with a decrease in milk turbidity and lightness of color, and an increase in viscosity of the milk [3]. The first foods produced commercially by this technology appeared on the market in Japan in 1990. They included yoghurts, fruit jellies, salad dressings and fruit sauces. Several other pressure-treated foods are now manufactured, including pressure-pasteurized milk, but the total amount remains relatively small.

HIGH HYDROSTATIC PRESSURE

According to Pascal's law, pressure acts instantly, isostatically and homogenously, independently of the size and shape of the material. In high-pressure treatment of foods, pressures between 100 and 1,000 MPa are used. This is higher than pressures present in deep sea. In general, the process is batch operated and applied to several liquid and semisolid prepacked foods and food ingredients. High-pressure processing is carried out in three steps as follows: an initial period required for reaching the treatment pressure or come-up time, the time of processing at the desired pressure or holding time, and finally, a short time necessary for releasing the pressure or release time [4].

During high hydrostatic pressure (HHP) treatment, the food packed in a flexible packaging material is put in a high pressure cylindrical vessel where it is surrounded by a non-compressible pressure-transmitting medium, usually water. The transmitting medium is pressurized up to the treatment pressure. This pressure is kept constant from a few minutes to multiples of times for 10 min.

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Purpose of HHP

In the food industry, the main field of application of HHP is food preservation. Food spoilage is very often caused by microorganisms and biochemical processes catalyzed by enzymes. With HHP, a great part of microorganisms can be destroyed and most of the enzymes can be inactivated [5]. Using HHP treatment, undesirable changes and thermal degradation of heat-sensitive food components can be avoided, a major advantage. This treatment is effective at ambient or moderate temperatures. Tests show that this treatment affects only the non-covalent (i.e., hydrogen, ionic and hydrophobic) bonds, and impacts taste, color and nutritional value of foods to a negligible degree. Thermal treatment, on the other hand, changes the covalent bonds and significantly affects the food components. Depending on the kind of food, the effects can be beneficial or undesirable (color, texture, structure, etc.). In general, components with low molecular weight remain intact, while macromolecules (proteins, complex carbohydrates) undergo changes [6].

HHP also affects biochemical reactions. Pressure reduces the size of the molecules and promotes bond formation between side chains [7]. Protein molecules are denatured under high pressure. This is a complex phenomenon: it depends on the structure of the proteins, the extent of the pressure, the temperature and the pH [8]. The effect of HHP on microorganisms depends on the composition of the foodstuffs and the physiological condition of microorganisms.

Schematic Diagram of Basic Equipment

A typical HHP system consists of four main parts (Figure 1) [9]:
1. A high pressure vessel and its closure
2. A pressure generating system
3. A temperature control device
4. A material handling system

Product (liquid or solid) is packed in poly-ethylene bags. Milk can be packed in PE bags or poly-propylene tubes. This packaged product is put in the vessel, which is filled with non-compressible fluid (water or ethanol); in food processing, potable water is generally used [10]. And then pressure is applied for 2–60 min depending upon the product, desired safety and temperature. About 3 °C temp is increased for each 100 MPa; so to maintain the temperature, temperature controllers (water flow or exchangers) are used.
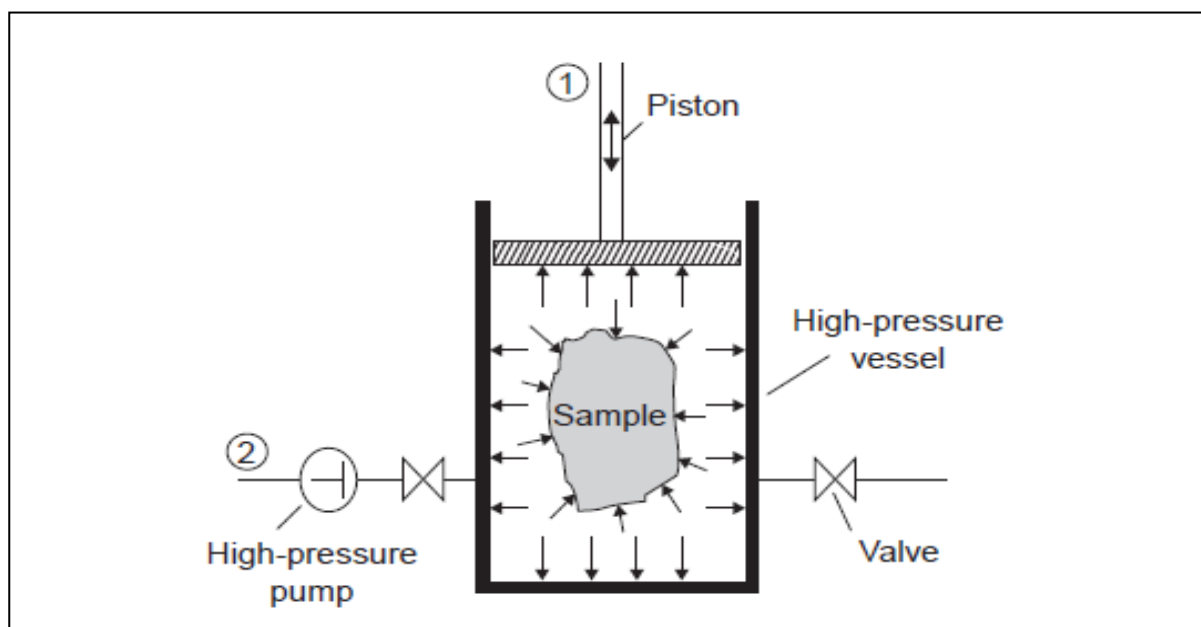

Fig. 1: Schematic Diagram of Basic Equipment Design for HHP of Foods.

Principle of HHP

Pressure and temperature determine many properties of inorganic and organic substances.

In food preservation, thermal processing is common place. If, however, a substance is exposed to increasing pressure, many changes

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will occur, especially at pressures of several hundred MPa [11]. The behavior of biological macromolecules under pressure is important for understanding the effects of HHP on milk. Under pressure, biomolecules obey the "Le-Chatelier" principle, i.e., whenever stress is applied to a system in equilibrium, the system will react so as to counteract the applied stress; thus, reactions that result in reduced volume will be triggered under HHP. Such reactions may result in inactivation of microorganisms or enzymes and in textural changes in foods [12].

Effects on Bacterial Flora

In the food industry, the main field of application of HHP is food preservation. Food spoilage is very often caused by microorganisms and biochemical processes catalyzed by enzymes. With HHP, a great part of microorganisms can be destroyed and most of the enzymes can be inactivated [5]. HHP also affects the morphology of microorganisms. Survival of microorganisms depends on the extent of pressure, holding time and temperature, composition of the food and the condition and growth phase of microbes [13]. Pressures between 300 and 600 MPa inactivate yeasts, moulds and most of the vegetative bacteria [14]. Bacterial spores can be destroyed substantially only with pressures higher than 1000 MPa. Pressures between 50 and 300 MPa may even stimulate spore germination. Gram-positive are more resistant to HP than gram-negative due to presence of teichoic acid in the former. Teichoic acid is polysaccharide of bacterial cell wall and provides rigidity to cell wall. It is present only in Gram-positive bacteria and is absent in Gram-negative bacteria. It has been found that the HP treatment between 150 and 300 MPa at subzero temperatures could result in a pasteurization effect in foods. This processing is called high pressure cold pasteurization [15].

Advantages of HHP

1. Thermal degradation of heat sensitive foods can be avoided
2. High retention of color, aroma and nutritional value
3. No re-contamination as treatment is given after final packaging of product
4. Environmental friendly as no chemicals are added
5. Positive consumer acceptance
6. Operates at room temperature

Disadvantages of HHP

1. Very expensive – capital cost is very high
2. Non-continuous
3. Additional heat treatment is required to kill spores

Effects of HHP on Milk Proteins

In their native state, proteins are stabilized by covalent bonds (including disulphide bridges) plus electrostatic interactions (ion pairs, polar groups), hydrogen bridges and hydrophobic interactions. Covalent bonds are almost unaffected by HHP, at least at relatively low temperatures (0–40 °C), and so the primary structure of proteins remains intact during HHP treatment [16]. High pressure affects:

1. The quaternary structure (e.g., through hydrophobic interactions)
2. The tertiary structure (hydrophobic and hydrogen bonding)
3. The secondary structure (H- and electrostatic interactions)

In the range of 100 to 300 MPa, these changes are reversible, but pressures > 300 MPa can bring on irreversible denaturation of milk proteins [17]. Very high pressures (> 700 MPa) can induce irreversible denaturation by disrupting the secondary structure of the proteins [18]. Stabilizing hydrogen bonds is enhanced at low pressures and ruptured only at very high pressures. Significant changes to the tertiary structure of proteins, which is maintained chiefly by hydrophobic and ionic interactions, are observed at > 200 MPa [19]. Multimeric proteins, held together by non-covalent bonds, dissociate at relatively low pressures (~ 150 MPa), thereby disrupting quaternary structures. Exposed to pressures above 400 MPa, most of the proteins denature. Sensitivity to pressure or temperature varies with the type of bonds maintaining the structure. Measurements showed that structures with β-sheets are more stable against pressure than those with α-helices. The former is nearly incompressible while the latter can be deformed more easily. Oligomeric proteins dissociate to subunits

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while volume decreases. After dissociation subunits may reaggregate or denature. At pressures above 200 MPa, chains begin to unfold and subunits of dissociated oligomers start reassociating. However, small molecules that have little secondary, tertiary and quaternary structure, such as amino acids, vitamins, flavor and aroma components, remain unaffected [12].

HHP-induced changes in the protein structure have profound effects on a protein's functionality and on its possible food applications [20]. In general, HHP-induced changes in proteins are dependent on additional parameters such as temperature, pH, the solvent used for processing, and the final state of the protein preparation [21].

Sensitivity of Different Bonds to HHP
Hydrobhobic>Electrostatic bonds>Hydrogen bonds > Covalent bonds

Effect on β-Lactoglobulin (Lg) and α-Lactalbumin (La) under HHP
The behavior of whey proteins under HHP is particularly important for milk and dairy products. Johnston *et al.* (1992) were among the first researchers, who investigated the effects of HHP on whey proteins [22]. The authors found that the amount of non-casein nitrogen decreased in milk serum with increasing pressure that suggested denaturation and insolubilization of whey proteins.

ß-Lg is most sensitive to HHP. ß-Lg has only two disulphide linkages and one free –SH group [8, 23–26]. So it is less rigid as compared to α-La which has four disulphide bonds. Treatment of raw milk at up to 100 MPa does not denature ß-Lg [23, 27] and remains in native monomer form. At pressure > 100 MPa, unfolding of ß-Lg starts and free – SH group is exposed; which may interact with k- casein or other unfolded ß-Lg molecules [23, 28]. It results in increase in size of casein micelles and a small extent of aggregation among ß-Lg molecules. Application of higher pressures results in considerable denaturation of ß-Lg, reaching 70–80% after treatment at 400 MPa [23, 27, 29–31]. Relatively, little further denaturation of ß-Lg occurs at 400–800 MPa [23]. During storage, renaturation occurs within 1–2 days at 20–40 °C. 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. And thus at low temperature, strength of hydrophobic interactions is very low. A synergistic effect of temperature on denaturation of ß-Lg has been reported. About 100% denaturation was observed at 300 MPa at 60 °C or at 400 MPa at 40 °C [29, 31]. Between 100 and 450 MPa, it gets unfolded and forms dimmers via disulphide linkages. This conversion is reversible during storage. Between 450 and 800 MPa, it forms polymers via disulphide bonds and this is irreversible. It has been reported that no denaturation of ß-Lg at pressures ≤ 100 MPa was observed, but the extent of denaturation increased with increasing pressure, with an abrupt and large increase between 300 and 400 MPa [32]. About 90% of the total ß-Lg was denatured at 800 MPa. The level of α-La denaturation was much lower than that for β-Lg; only about 10% of the α-LA was denatured at 600 MPa, but about 50% of the α-LA was denatured at 800 MPa (Figure 2). The extent of HHP-induced denaturation of α-La and β-Lg increases with increasing holding time, temperature, and pH of milk [30, 33].
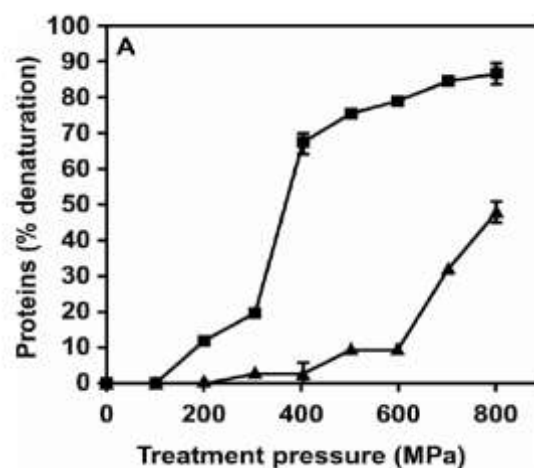

Fig. 2: *Denaturation of β- Lg (■) and α- La (▲) in Milks that Were Treated at High Pressures from 100 to 800 MPa for 30 min* [32].

α-La is one of the main components of whey protein, and its role in lactose biosynthesis is well documented. The concentration of α-La in cow milk varies from 1.2 to 1.5 g/L, and it is the second largest component by concentration (20%) in the whey protein fraction after β-Lg. α-Lactalbumin has four

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intramolecular disulfide bonds with no free thiol groups and shows the best characterized molten globule (MG) state, which is highly stable and is therefore a favorite model for studying the folding of proteins [34]. α-La is more resistant to denaturation under pressure as it has 4 disulphide linkages (β-Lg has 2). Denaturation of α-La starts only at > 400 MPa. Under HHP of 400–800 MPa, no transformation of monomers into disulphide-bonded aggregates was observed [35], because it has no free –SH group [23]. As α-La has no free thiol groups in the molecule and only a negligible fraction of the protein form aggregates even at pressures as high as 1000 MPa, so thiol-induced oligomerization of this protein at HHP can only be achieved by the addition of low-molecular-weight reducing agents such as cysteine, 2-mercaptoethanol, or dithiothreitol [36]. However, at ~1000 MPa, small aggregates of α-La were reported due to bonding between Cys 6- Cys 120, which was more sensitive to cleavage due to its environment [37]. The extent of HHP-induced denaturation of α-La and β-Lg increases with increasing holding time, temperature, and pH of milk [24, 27]. In HHP-treated whole milk, some α-La and β-Lg are also found associated with the milk fat globule membrane [32].

Effect on Bovine Serum Albumin, Immunoglobulins, Lactoferrin and Lysozyme

Bovine Serum Albumin (BSA) is a single polypeptide of 582 amino acids, having 17 disulphide bridges and one free thiol group, Cys 34. Structure of BSA protein is composed of 76% helix, 10% turns and 23% extended chain and no beta (ß) sheets. It is very resistant to pressure up to 400 MPa [27], probably due to large number of disulphide linkages and a very little denaturation occurs above 400 MPa. Immunoglobulins are more resistant to pressures up to 300 MPa. Immunoglobulins in caprine milk were resistant to pressures up to 300 MPa, although ~ 35% denaturation occurred after treatment at 500 MPa [38]. L_f and lysozyme in milk are resistant to HP. Few data are available on HP-induced denaturation of other whey proteins (Table 1).

Table 1: Susceptibility to High Pressure of Whey and Other Milk Proteins.

Proteins	Susceptibility to HP induced denaturation	Susceptibility to heat induced denaturation
ß-lactglobulin	+++++	++
Immunoglobulins	+++	+++++
α-lactalbumin	++	+
BSA	++	+++

Effect of HHP on Caseins

Casein micelles are influenced considerably by HHP treatment. In one of the first studies, electron microscopy was used to examine the size of casein micelles after HHP treatment [39]. Since then, several methods have been used to detect changes in casein micelles during or following pressurization, such as transmission electron microscopy, laser granulometry, photon correlation spectroscopy, and turbidimetry. Casein micelle size is affected only slightly by HP treatment at pressures ≤ 200 MPa at 20 °C [37, 40, 41] due to having very little secondary and tertiary structures. Casein's native form is of primary structure having covalent bonds (peptide and disulphide bonds). Because HHP does not affect the covalent bonds, so it does not affect the casein micelles under < 300 MPa.

Basically under high pressure, defragmentation of casein micelles occurs, in which micelles are broken up into more soluble components like α_{s1}, α_{s2}, ß- and k-caseins. Defragmentation occurs due to following reasons:

- Solublization of colloidal calcium phosphate (CCP) [29, 42–44]; which is responsible for crosslinking caseins and neutralizing the negatively charged phosphoserine groups [45], so stability of micelles is disrupted.
- Disruption of hydrophobic and electrostatic interactions in casein micelle structure [16, 46], which are responsible

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for binding individual caseins within the casein micelles [45].

Effect of HHP on Casein Micelle Size

Effect of HHP on casein micelle size is shown in Table 2. Increase in size at 250 MPa is due to interaction of denatured β-Lg with k-casein micelle [23, 28, 37]. At > 300 MPa, reduction in size by 50% is due to fragmentation of casein micelles [25, 33, 40, 41, 47]. When raw skim bovine milk was treated with HP at 100–600 MPa for 30 min, considerable increase in the levels of αs$_1$- and β-caseins in the soluble phase of milk were observed [48]. Levels of both αs$_1$- and β-caseins in the soluble phase increased with pressure up to 250 MPa, to ~ 12 or 18% of total αs$_1$- or β-casein in milk, respectively. After treatment at 400 or 600 MPa, levels of these caseins in the soluble phase of milk were slightly lower than those in milk treated at 250 MPa, but remained considerably higher than those in untreated milk or milk treated at 100 MPa. Consistent reports regarding soublization of αs$_1$- and β-caseins were given by various workers [29, 30]. On storage at 20 °C for 24 or 48 h, levels of soluble β-casein decreased in milk treated at 100–600 MPa; It could be due to the reformation of micellar particles from fragments of HP-disrupted casein micelles [28, 37]. Again, it could be due to the reformation of hydrophobic bonds [28]. Micellar caseins may re-associate under prolonged pressurization at 200–300 MPa, because hydrophobic bonds are favored over hydrophobic solvation. Re-association does not take place at higher pressure [48].

Effect of HHP on Hydration of Casein

Hydration of micelles is increased due to mainly two reasons. First is due to interaction of denatured β-Lg, with k-casein, net negative charge on casein/or casein components increases; and thus hydration or solubility increases. And the second is due to disruption of casein micelles into α$_{s2}$, α$_{s1}$, ß and k-caseins, which are more soluble in water as compared to whole casein micelle.

Order of Dissociation of Casein Variants in Bovine Milk under HHP

$$k > ß > \alpha_{s1} > \alpha_{s2} \qquad [3]$$

Order of dissociation depends upon the phosphate content of casein variants. More the phosphate residues, more is the stability or less is the dissociation. The numbers of phosphate residues in casein components are

$$k = 1, ß = 5, \alpha_{s1} = 8, \alpha_{s2} = 11.$$

CONCLUSIONS

Under HHP, β-Lg is unfolded and forms dimers, polymers and aggregates with k-casein depending upon the extent of pressure and temperature. Soublization of colloidal calcium phosphate occurs under HP resulting fragmentation of casein micelles. Disruption of hydrophobic, ionic and H-bonds results in the conversion of 2, 3 and 4 structures into primary structure; which could be reversible or irreversible depending upon the intensity of pressure.

Table 2: Effect of HHP on Casein Micelle Size [25].

Pressure (MPa)	Temperature	Effects on casein micelles	Change during storage
≤ 200 MPa	20–40 °C	No effect	-
250 MPa	40 °C	~ 30% increase in size of micelle	Reversible
> 300 MPa	40 °C	~ 50% decrease in size of micelle	Irreversible

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REFERENCES

1. Hite BH. The Effect of High Pressure in the Preservation of Milk. *West Virginia Agricultural Experimental Station Bulletin* 1899; 58:15–35p.
2. Trujillo AJ, Capellas M, Saldo J, et al. Applications of High-Hydrostatic Pressure on Milk and Dairy Products: A Review. *Innov. Food Sci. Emerging Technol* 2002; 3:295–307p.
3. Huppertz T, Kelly AL, Fox PF. Effects of High Pressure on Constituents and Properties of Milk. *Int. Dairy J* 2002; 12:561–572p.
4. Hogan E, Kelly AL, Sun DW. High-Pressure Processing of Foods: An Overview. In: Sun DW (Ed.). *Emerging Technologies for Food Processing.* Elsevier Academic Press, San Diego; 2005; 1–31p.
5. Mertens B, Deplace G. Engineering Aspects of High Pressure Technology in the Food Industry. *Food Technol* 1993; 47(6):164–169p.
6. Datta N, Deeth HC. High Pressure Processing of Milk and Dairy Products. *The Australian J. Dairy Technol* 1999; 54:41–48p.
7. Hoover DG, Metrick C, Papineau AM, et al. Biological Effects of High Hydrostatic Pressure on Food Microorganism. *Food Technol* 1989; 43(3):99–107p.
8. Hinrichs J, Rademacher B. Kinetics of Combined Thermal and Pressure-Induced Whey Protein Denaturation in Bovine Skim Milk. *Int. Dairy J* 2004*;* 14: 315–323p.
9. Mertens B. Hydrostatic Pressure Treatment of Food: Equipment and Processing. In: Gould GW (Ed.). New Methods of Food Preservation. Glasgow: Blackie Academic & Professional; 1995; 135–158p.
10. Myllymaki O. High Pressure Food Processors. In: Ohlsson T. (Ed.). High Pressure Processing of Food and Food Components—A Literature Survey and Bibliography. *Goeteborg: sik.* 1996; 29–46p.
11. Buchheim W, Prokopek D. Treatment of Milk and Other Foods by High Hydrostatic Pressure. *Eur. Dairy Mag* 1992; 4(4):4–7p.
12. Balci AT, Wilbey RA. High Pressure Processing of Milk—the first 100 Years in the Development of a New Technology. *Int. J. Dairy Technol* 1999; 52:149–155p.
13. Patterson MF, Quinn M, Simpson R, et al. Sensitivity of Vegetative Pathogens to High Hydrostatic Pressure Treatment in Phosphate-Buffered Saline and Foods. *J. Food Prot* 1995; 61(4):432–436.
14. Toepfl S, Mathys A, Heinz V, et al. Review: Potential of High Hydrostatic Pressure and Pulsed Electric Fields for Energy Efficient and Environmentally Friendly Food Processing. *Food Reviews Int* 2006; 22:405–423p.
15. Luscher C, Sunderhoff JK, Knorr D. Pasteurisation of Meat in Frozen Conditions by High Pressure. In: Proceedings of Innovations in Traditional Foods, Valencia, Spain. *Intrafood* 2005; 2:1379–1382p.
16. Mozhaev VV, Heremans K, Frank J, et al. Exploiting the Effects of High Hydrostatic Pressure in Biotechnological Applications. *Trends Biotechnol* 1994; 12:493–501p.
17. Rastogi NK, Raghavarao SMS, Balasubramaniam VM, et al. Opportunities and Challenges in High Pressure Processing of Foods. *Crit. Rev. Food Sci. Nutr* 2007; 47:69–112p.
18. Balny C, Masson P. Effect of High Pressures on Proteins. *Food Rev. Int* 1993; 9:611–628p.
19. Hendrickx M, Ludikhuyze L, Van den broek I, et al. Effects of High Pressure on Enzymes related to Food Quality. *Trends Food Sci. Technol* 1998; 9:197–203p.
20. Kresic GV, Lelas Z, Herceg-Rezek A. Effects of High Pressure on Functionality of Whey Protein Concentrate and Whey Protein Isolate. *Lait* 2006; 86:303–315.
21. Nabhan MA, Girardot JM, Campagna S, et al. Isolation and Characterization of Copolymers of β-lactoglobulin, α-lactalbumin, κ-casein and αs1-casein Generated by Pressurization and Thermal Treatment of Raw Milk. *J. Dairy Sci* 2004; 87:3614–3622p.
22. Johnston DE, Austin BA, Murphy RJ. Effects of High Hydrostatic Pressure on Milk. *Milchwissenschaft* 1992; 8:206–209p.

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23. Scollard PG, Beresford TP, Needs EC, et al. Plasmin Activity, β-lactoglobulin Denaturation and Proteolysis in High Pressure Treated Milk. *Int. Dairy J* 2000; 10:835–841p.

24. Huppertz T, Fox PF, Kelly AL. Dissociation of Caseins in High Pressure-Treated Bovine Milk. *Int. Dairy J* 2004; 14:675–680p.

25. Huppertz T, Fox PF, Kelly AL. Dissociation of Caseins in High Pressure-Treated Bovine Milk. *Int. Dairy J* 2004b; 14:75−680.

26. Zobrist MR, Huppertz T, Uniacke T, et al. High Pressure-Induced Changes in Rennet-Coagulation Properties of Bovine Milk. *Int. Dairy J* 2005; 15:655–662p.

27. Lopez-Fandino R, Carrascosa AV and Olano A. The Effects of High Pressure on Whey Protein Denaturation and Cheese-Making Properties of Raw Milk. *J. Dairy Sci* 1996; 79:929–936p.

28. Needs EC, Capellas M, Bland AP, et al. Comparison of Heat and Pressure Treatments of Skim Milk, Fortified with Whey Protein Concentrate, for Set Yoghurt Preparation: Effects on Milk Proteins and Gel Structure. *J. Dairy Res* 2000a; 67:329–348p.

29. Lopez-Fandino R, De la Fuente MA, Ramos M, et al. Distribution of Minerals and Proteins between the Soluble and Colloidal Phases of Pressurized Milks from Different Species. *J. Dairy Res* 1998; 65: 69–78.

30. Arias M, Lopez-Fandino R, Olano A. Influence of pH on the Effects of High Pressure on Milk Proteins. *Milchwissenschaft* 2000; 55:191–194p.

31. Garcia-Risco MR, Olano A, Ramos M, et al. Micellar Changes Induced by High Pressure. Influence in the Proteolytic Activity and Organoleptic Properties of Milk. *J. Dairy Sci* 2000; 83:2184–2189p.

32. Ye A, Anema SG, Singh H. High-Pressure–Induced Interactions between Milk Fat Globule Membrane Proteins and Skim Milk Proteins in Whole Milk. *J. Dairy Sci* 2004; 87:4013–4022p.

33. Gaucheron F, Famelart MH, Mariette F, et al. Combined Effects of Temperature and High Pressure Treatments on Physicochemical Characteristics of Skim Milk. *Food Chem* 1997; 59:439–447p.

34. Lopez-Rodiles JO, Arroyo-Maya IJ, Jaramillo-Flores ME, et al. Effects of High Hydrostatic Pressure on the Structure of Bovine α-lactalbumin. *J. Dairy Sci* 2010; 93:1420–1428p.

35. Patel HA, Singh H, Anema SG, et al. Effects of Heat and High Hydrostatic Pressure Treatments on the Aggregation of Whey Proteins in Whey Protein Concentrate Solutions. *Food New Zealand* 2004; 4:29−35p.

36. Jegouic M, Grinberg VY, Guingant A, et al. Thiol-Induced Oligomerization of α-lactalbumin at High Pressure. *J. Protein Chem* 1996; 15:501−509p.

37. Huppertz T, Fox PF, Kelly AL. High Pressure Treatment of Bovine Milk: Effects on Casein Micelles and Whey Proteins. *J. Dairy Res* 2004a; 71:97−106p.

38. Felipe X, Capellas M, Law AJR. Comparison of the Effects of High-Pressure Treatments and Heat Pasteurization on the Whey Proteins in Goat's Milk. *J. Agric. Food Chem* 1997; 45:627–631.

39. Schmidt DG, Buchheim W. Elektronenmikroskopische Untersuchung der Feinstruktur von Caseinmicellen in Kuhmilch. *Milchwissenschaft* 1970; 25:596–600p.

40. Desobry-Banon S, Richard F, Hardy J. Study of Acid and Rennet Coagulation of High Pressurized Milk. *J. Dairy Sci* 1994; 77:3267–3274p.

41. Needs EC, Stenning RA, Gill AL, et al. High-Pressure Treatment of Milk:Effects on Casein Micelle Structure and on Enzymic Coagulation. *J. Dairy Res* 2000b; 67:31–42p.

42. Buchheim W, Schrader K, Morr CV, et al. Effects of High Pressure on the Protein, Lipid and Mineral Phase of Milk. In: *Heat Treatments and Alternative Methods.* Special Issue No 9602. Brussels: Int. Dairy Fed 1996; 202–213p.

43. Schrader K, Buchheim W, Morr CV. High Pressure Effects on the Colloidal Calcium Phosphate and the Structural Integrity of Micellar Casein in Milk. Part 1. High Pressure Dissolution of Colloidal Calcium Phosphate in Heated Milk Systems. *Nahrung* 1997; 41:133–138p.

44. De la Fuente MA, Olano A, Casal V, et al. Effects of High Pressure and Heat Treatment on the Mineral Balance of

_____

_____

Goats' Milk. *J. Dairy Res* 1999; 66:65–72p.

45. Horne DS. Casein Interactions: Casting Light on the Black Boxes, the Structure of Dairy Products. *Int. Dairy J* 1998; 8:171–177p.

46. Mozhaev VV, Heremans K, Frank J, et al. High Pressure Effects on Protein Structure and Function. *Proteins: Structure, Function and Genetics* 1996; 24:81–91p.

47. Huppertz T, Grosman S, Fox PF, et al. Heat and Alcohol Stabilities of High Pressure-Treated Bovine Milk. *Int. Dairy J* 2004c; 14:125–133p.

48. Huppertz T, Fox PF, Kruif KG, et al. High Pressure Induced Changes in Bovine Milk Proteins: A Review. *Biochimica Et Biophysica Acta* 2006; 1764:593–598p.