

Non-Thermal Processing in Dairy: A Review on High Hydrostatic Pressure Effects on Milk Proteins

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Abstract: High Hydrostatic Pressure (HHP) processing is a promising non-thermal food preservation technique that enhances microbial safety and shelf-life while maintaining the nutritional and sensory quality of products. In the dairy industry, HHP significantly influences the structure and functionality of milk proteins. The application of high pressure (typically 100-600 MPa) induces conformational changes, affecting protein solubility, denaturation, and aggregation. Caseins remain relatively stable under pressure, whereas whey proteins like β -lactoglobulin and α -lactalbumin undergo notable structural alterations, which can modify their functional and nutritional properties. These changes may lead to improved digestibility, emulsification, foaming, and even reduced allergenicity. HHP processing thus offers potential advantages in developing value-added and hypoallergenic dairy products. However, optimal pressure conditions must be carefully tailored to preserve desired qualities while minimizing negative impacts. This review explores the mechanisms and implications of HHP on milk proteins, highlighting its relevance in modern dairy processing.

Keywords: Milk Proteins, Casein, Whey Protein, Denaturation, Digestibility

1. Introduction

Food processing encompasses the transformation of raw animal or plant-based materials into consumer-ready products, aiming to minimize undesirable changes in quality. Without such interventions, it would be challenging to preserve food during times of surplus for use in periods of scarcity or to facilitate transportation over long distances (Lund, 2003). Among the key attributes influencing consumer acceptance are sensory characteristics such as texture, flavor, aroma, appearance, and color. These factors shape individual preferences and can significantly affect consumer perception, even among similar products across different brands. As a result, a primary objective of food manufacturers is to innovate and refine processing methods that either preserve or enhance these desirable sensory traits or prevent their degradation during processing. The dairy industry continually seeks innovative processing methods to enhance product safety, extend shelf life, and preserve nutritional and sensory qualities. Traditional thermal treatments, while effective in microbial inactivation, often compromise the functional and nutritional properties of milk proteins. In this context, non-thermal technologies have garnered significant attention, with High Hydrostatic Pressure (HHP) processing emerging as a promising alternative. HHP involves subjecting food products to pressures ranging from 100 to 600 MPa, effectively inactivating pathogenic microorganisms and spoilage enzymes without substantial heat application. This method preserves the sensory and nutritional attributes of dairy products, making it particularly advantageous for milk processing. Milk proteins, primarily caseins and whey proteins, are susceptible to structural modifications under HHP. Casein micelles, which are relatively stable under mild pressures, begin to disintegrate at pressures exceeding 300 MPa, leading to alterations in their functional properties (Goyal *et al.*, 2024). Whey proteins, especially β -lactoglobulin, are more sensitive, undergoing denaturation at lower pressure thresholds, which can impact allergenicity and digestibility. The extent of these protein modifications is influenced by various factors, including pressure level, temperature, pH, and treatment duration. For instance, higher pressures combined with elevated temperatures can enhance protein denaturation, while acidic conditions may promote aggregation (Gharbi *et al.*, 2022). Understanding these parameters is crucial for optimizing HHP treatments to achieve desired functional outcomes in dairy products. Recent studies have also explored the impact of HHP on the bioactive components of milk. HHP treatments have been shown to preserve immunoglobulins and lactoferrin better than thermal pasteurization, maintaining the health-promoting qualities of milk (Koker *et al.*, 2023). Additionally, HHP can enhance the release of bioactive peptides with antioxidant and antihypertensive properties, contributing to the development of functional dairy foods (Landim *et al.*, 2023).

This review aims to provide a comprehensive overview of the effects of High Hydrostatic Pressure processing on milk proteins, highlighting the structural and functional changes induced by HHP and discussing the implications for dairy product quality and functionality.

Milk is a whitish liquid composed of proteins, lipids, lactose, vitamins, and minerals, produced by the mammary glands of mammals to nourish their offspring. In ancient cultures such as Egypt, Iran, and India, milk held significant cultural and religious importance, often associated with reverence for the cow. During the middle ages in Europe, monastic communities, particularly the Benedictines, played a pivotal role in the development of cheese-making techniques. Before the scientific and industrial advancements of the 19th century, fermented milk products like butter and cheese were integral to human diets and food

preservation practices. Milk is characterized as a pale fluid secreted by mammary secretory cells through lactation, a defining trait of mammals. The milk produced is stored in the udder, with colostrum being the initial secretion post-parturition, rich in antibodies and nutrients. The quality of milk is paramount, necessitating proper storage and transportation conditions to maintain its integrity. Milk comprises four distinct physical phases:

- **Gaseous Phase:** Primarily consisting of dissolved gases like carbon dioxide, which are present during milking.
- **Fatty Phase:** Comprising fat globules (2 to 5 μm in diameter) that contain lipids and fat-soluble substances. These globules are enveloped by phospholipid and protein membranes, forming an emulsion within the aqueous phase of milk.
- **Colloidal Phase:** Containing casein micelles associated with calcium and magnesium phosphates and citrates. These micelles are essential for the stability and functional properties of milk, influencing its behavior during processing and digestion.
- **Liquid Phase:** An aqueous solution that includes soluble proteins (whey proteins), lactose, and minerals (electrolytes). The balance between lactose concentration and mineral levels is crucial to maintain milk's isotonicity with blood plasma.

II. Shelf Life of Milk

The shelf life is the duration during which food can be stored before it begins to degrade or poses potential safety risks. The quality of the raw materials, manufacturing techniques, packaging methods, storage conditions, and whether the product has been opened all exert effect. Temperature regulation throughout all storage phases is crucial for the majority of food items, both in retail environments and domestic settings. Nearly all dairy products must be refrigerated. Exceptions comprise powdered milk products and items like UHT (Ultra-high temperature) products, which can be stored in the pantry, as well as ice cream, which requires freezer storage. The duration of freshness for each dairy product varies considerably. Pasteurized milk can be preserved for 12-14 days when maintained below 4°C , although yogurt and comparable fermented products can retain their quality for 30-40 days under identical storage circumstances.

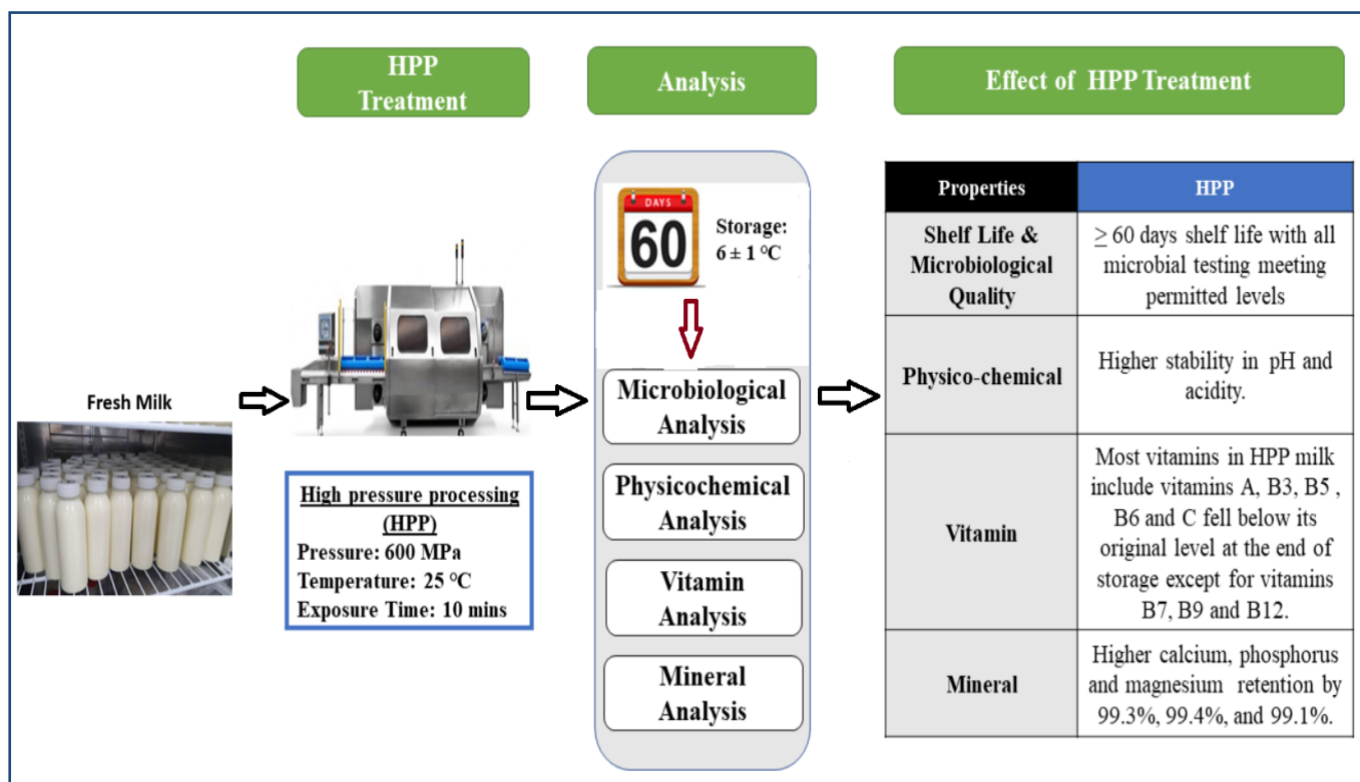


Figure 1. Impact of HPP on the Quality and Shelf Life of Fresh Milk

III. Nutritional Value of Milk

Milk is a nutrient-dense liquid that serves as a primary source of essential nutrients for humans. Its composition provides a balanced array of macronutrients and micronutrients, making it a staple in many diets worldwide.

(a) Macronutrient Composition

A standard 240 mL serving of whole cow's milk contains approximately 8 grams of high-quality protein, 8 grams of fat, and 12 grams of carbohydrates, predominantly in the form of lactose. The proteins in milk are categorized mainly into casein and whey

proteins. Casein constitutes about 80% of the total protein content and is known for its slow digestion rate, which provides a sustained release of amino acids. Whey proteins, making up the remaining 20%, are rapidly digested and rich in branched-chain amino acids, beneficial for muscle synthesis (Healthline, 2019).

(b) Micronutrient Profile: Milk is an excellent source of several vital micronutrients. It provides approximately 300 mg of calcium per 240 mL serving, essential for bone health. Additionally, milk contains significant amounts of phosphorus, potassium, and vitamins such as B12 and riboflavin (Healthline, 2019). Fortified milk products also offer vitamin D, which aids in calcium absorption and bone mineralization.

(c) Bioactive Components

Beyond its basic nutritional content, milk contains various bioactive compounds, including immunoglobulins, lactoferrin, and growth factors, which contribute to its health-promoting properties. These components play roles in immune function, antimicrobial activity, and cellular growth and differentiation (Lidsen, 2023). Regular consumption of milk has been associated with numerous health benefits. It contributes to bone density maintenance, supports muscle mass preservation, and may aid in weight management due to its protein content and satiety-inducing properties. Moreover, milk consumption has been linked to a reduced risk of cardiovascular diseases, although individual responses may vary (Food & Nutrition Research, 2023). Milk is a complex biological fluid produced by mammals, serving as a primary source of nutrition for their offspring. Its composition varies among species but generally includes water, proteins, fats, carbohydrates, vitamins, and minerals. In bovine milk, approximately 87% is water. The remaining 13% comprises solids, including proteins, fats, lactose, and minerals. Proteins are a significant component of milk, contributing to its nutritional value. In cow's milk, proteins account for about 3.2% of its composition (Harvard T.H. Chan School of Public Health, 2021). These proteins are primarily categorized into two groups: casein and whey proteins. Casein constitutes approximately 80% of the total protein content in cow's milk, while whey proteins make up the remaining 20% (Franzoi *et al.*, 2019).

Table 1. The composition of milk from different mammals in g /100 g of milk.

S.No	Species	Water (g)	Protein (g)	Fat (g)	Lactose (g)	Ash (g)	Energy (kcal)
1.	Cow	87.7	3.4	3.6	4.6	0.7	66
2.	Goat	86.5	3.5	3.8	4.7	0.8	67
3.	Sheep	79.4	6.7	8.6	4.3	1.0	95
4.	Buffalo	83.6	3.9	6.6	5.2	0.8	110
5.	Camel	86.5	4.0	3.1	5.6	0.8	60
6.	Donkey	89.2	1.8	0.6	6.8	0.5	38
7.	Human	87.7	1.1	4.2	7.0	0.2	72
8.	Elephant	67.8	3.1	19.6	8.8	0.7	85

Note: The values are approximate averages and can vary based on factors such as breed, diet, and lactation stage.

(d) Casein Proteins:

Casein proteins are the predominant proteins in milk, forming a gel or clot in the stomach, which makes them highly efficient in nutrient supply. They are categorized into four main types: α s1-casein, α s2-casein, β -casein, and κ -casein. These casein proteins aggregate to form casein micelles, which are essential for the milk's nutritional properties and processing characteristics (Franzoi *et al.*, 2019).

(e) Whey Proteins:

Whey proteins are soluble in milk serum and remain in the liquid portion after curdling. They are rich in essential amino acids and are rapidly digested. Key whey proteins include α -lactalbumin, β -lactoglobulin, immunoglobulins (IgG, IgA, IgM), lactoferrin, and serum albumin. These proteins play various roles in immune defense, nutrient transport, and antimicrobial activity (Healthline, 2022). The balance between casein and whey proteins in milk influences its digestibility and nutritional profile. Casein, being a slow-digesting protein, provides a sustained release of amino acids, making it beneficial for muscle repair and growth. In contrast, whey proteins are rapidly digested, leading to a quick increase in plasma amino acid concentrations, which is advantageous for immediate muscle recovery (Franzoi *et al.*, 2019). Understanding the composition of milk proteins is crucial for various applications in nutrition and food science. The unique properties of casein and whey proteins contribute to the functional and nutritional qualities of milk and dairy products.

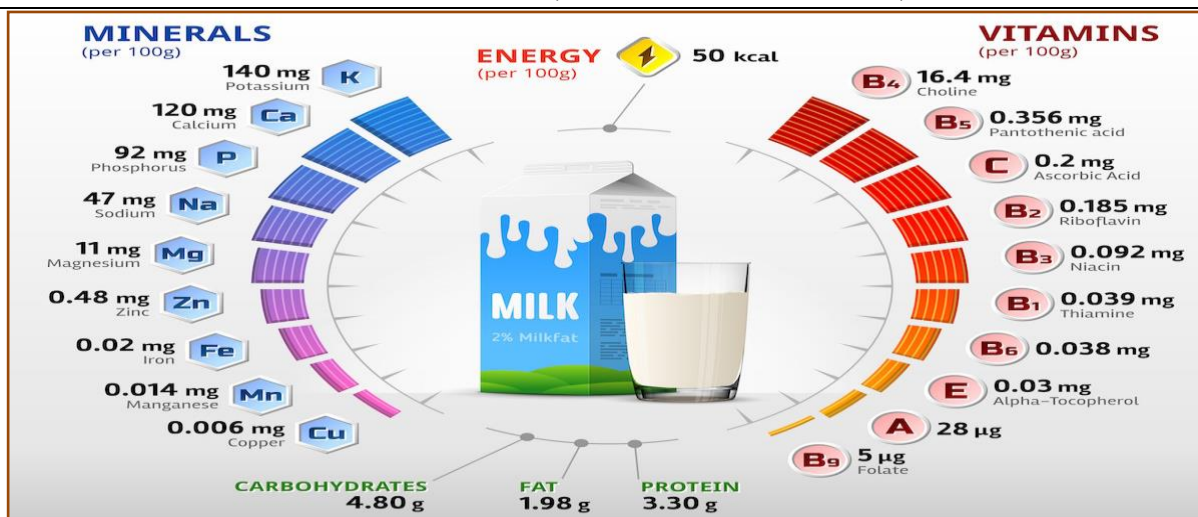


Figure 2. The nutritional aspects of the milk

IV. Global Overview of Milk Production

Global milk production has experienced significant growth over the past few decades, driven by increasing demand for dairy products and advancements in dairy farming practices. In 2024, world milk production is projected to reach approximately 981 million tonnes, marking a 1.5% increase from 2023. This growth is primarily attributed to expansions in Asia, particularly in countries like India and China. India remains the world's largest milk producer, contributing about 22% to global production. Other major producers include the United States, Pakistan, China, and Brazil. However, despite these increases, global milk production in 2023 was 4 million tonnes below the five-year average, highlighting ongoing challenges in meeting the rising demand. The dairy sector faces various challenges, including climate change impacts, disease outbreaks, and economic pressures. For instance, in 2024, milk production declined in several key regions, including the United States, New Zealand, and parts of Europe, due to factors such as high feed costs, labor shortages, and adverse weather conditions. Looking ahead, global milk production is expected to return to growth in 2025, with an estimated increase of 0.6% year-on-year. This recovery is anticipated across most major producing regions, although Australia's production remains highly weather-dependent.

Table 2. The milk production statistics of the top-producing countries (FAO, 2024)

S.No.	Country	Milk Production (Metric Tonnes)
1	India	213,779,230
2	United States	102,747,320
3	Pakistan	62,557,950
4	China	39,914,930
5	Brazil	35,944,056
6	Russia	33,188,890
7	Germany	32,977,956
8	France	25,028,850
9	United Kingdom	21,563,492
10	New Zealand	21,051,000

Note: The data presented above is sourced from the Food and Agriculture Organization (FAO) of the United Nations, which provides comprehensive statistics on global agricultural production.

V. Commercial Applications of Milk Proteins

Milk proteins, primarily casein and whey, are utilized across various industries due to their unique functional and nutritional properties. Their applications span the food industry, pharmaceuticals, cosmetics, packaging, and more.

• Food Industry

In dairy products the casein is fundamental in cheese-making, where it coagulates to form the curd, providing structure and texture to the cheese (Tetra Pak, n.d.). Whey proteins, on the other hand, are incorporated into yogurts and beverages to enhance nutritional value and improve texture (Sunkesula, 2021). Whey proteins are crucial in infant formula formulations due to their high digestibility and amino acid profile, closely resembling human milk (Yalcin, 2006). Milk protein concentrates are used to improve the texture, mouthfeel, and shelf-life of baked goods and confections, owing to their water-binding and emulsifying properties (Ejeahalaka *et al.*, 2020).

• Pharmaceutical and Nutraceutical Applications

Whey proteins release bioactive peptides during digestion, which exhibit antihypertensive, antioxidant, and antimicrobial activities, making them valuable in functional foods and nutraceuticals (Sultan *et al.*, 2014). Casein micelles have been explored as carriers for bioactive compounds and drugs, offering controlled release and protection of sensitive ingredients (Fraj *et al.*, 2021).

• Packaging and Edible Films

Casein-based films are developed as biodegradable packaging materials due to their excellent barrier properties against oxygen and aromas, although their water sensitivity remains a challenge. Whey protein-based edible films are used to coat fruits, vegetables, and cheeses, extending shelf-life and providing a medium for incorporating antimicrobial agents (Bahram *et al.*, 2014).

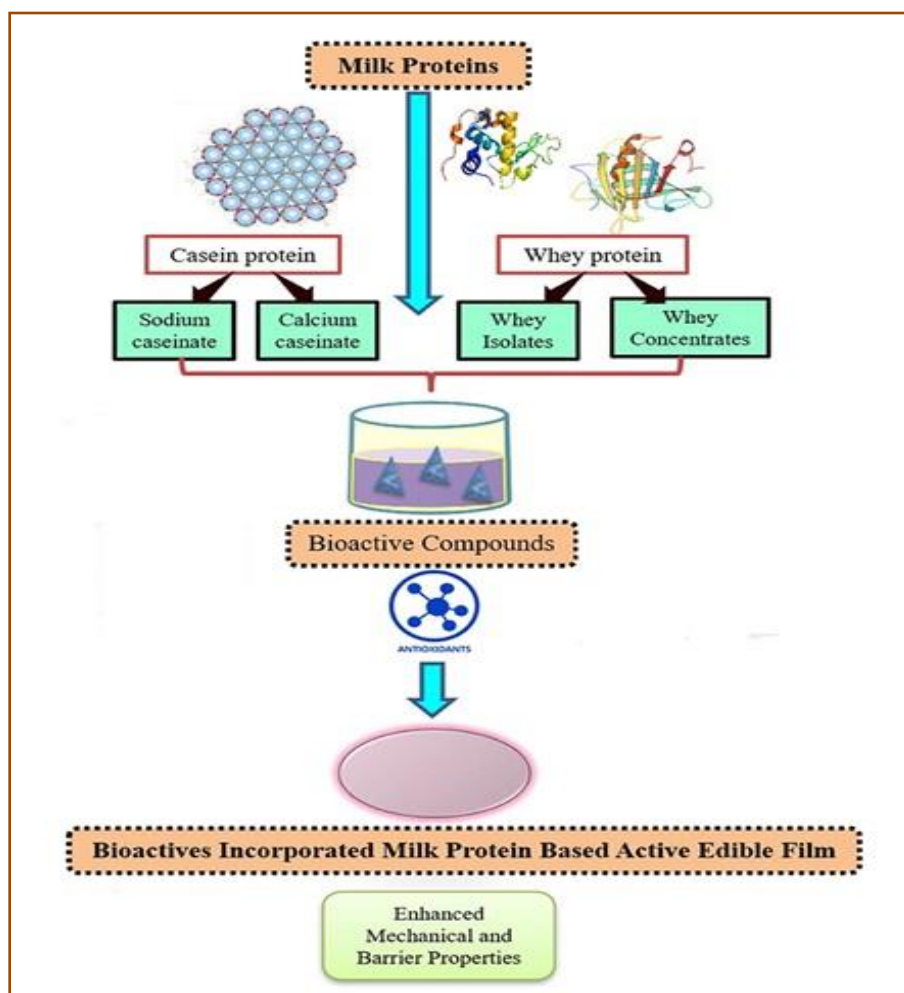


Figure 3. The Application of Milk Protein (Chaudhary *et al.*, 2022)

VI. High-Pressure Processing: Principles and Technologies

High-Pressure Processing (HPP), also known as high hydrostatic pressure (HHP) or pascalization, is a non-thermal food preservation method that inactivates pathogenic and spoilage microorganisms by applying high levels of hydrostatic pressure, typically between 100 and 600 MPa, to food products. This technique effectively extends shelf life and ensures food safety while

maintaining the sensory and nutritional qualities of the food. HPP operates on the principle that when food is subjected to high pressure, the pressure is transmitted uniformly and instantaneously throughout the product, regardless of its size and geometry. This uniform application of pressure leads to the inactivation of microorganisms and enzymes that can cause spoilage, without significantly affecting the covalent bonds responsible for the food's nutritional and sensory attributes (Balasubramaniam *et al.*, 2015). The process involves placing pre-packaged food products into a high-pressure chamber filled with a pressure-transmitting fluid, usually water. The chamber is then pressurized to the desired level for a specific duration, typically ranging from a few seconds to several minutes, depending on the product and desired outcome. HPP is widely applied in the food industry for products such as juices, meats, seafood, dairy, and ready-to-eat meals. The technology is particularly beneficial for preserving the freshness and extending the shelf life of products without the need for chemical preservatives or high-temperature treatments. Additionally, HPP has been explored for applications like thawing, freezing, and extraction of bioactive compounds, showcasing its versatility in food processing

• HPP on Milk Protein

Among the several components of milk, milk proteins have garnered the greatest attention regarding studies on HP-induced alterations. Given that caseins and whey proteins are distinctly different kinds of proteins, they will be addressed separately in this section. These zones have been meticulously evaluated recently by Huppertz *et al.*, (2006). The individual caseins possess several notable characteristics. They are diminutive, pliable molecules exhibiting elevated surface hydrophobicity. They are phosphoproteins containing naturally occurring phosphorus esterified to serine residues. The number of phosphate groups varies from 1 in κ -casein to 13 in α s2-casein. α s2- and κ -casein each possess two cysteine residues per mole. Among the prominent whey proteins, β -lg comprises two cystine residues (intramolecular disulfide bonds) and one cysteine residue per mole; Ia contains four intramolecular disulfide linkages (S-S) and lacks free sulfhydryl groups (SH). Neither β -lactoglobulin nor α -lactalbumin have phosphoserine accumulations. At the pH of milk, the local state of β -lg mostly occurs as a dimer stabilized by hydrophobic interactions among monomers, with the free sulfhydryl group sequestered at the monomer-monomer interface. Casein micelles are structured protein aggregates, varying in size between 50 and 500 nm. A model of micelle structure, as described by User (2020), suggests that the association of caseins is governed by a balance of attractive hydrophobic interactions and electrostatic repulsion. At the typical pH of milk and temperatures between 20°C and 30°C, the negative charge on phosphoserine accumulations is neutralized by colloidal calcium phosphate, which cross-links the protein and allows the hydrophobic regions of adjacent particles to interact, thereby further consolidating the micelle.

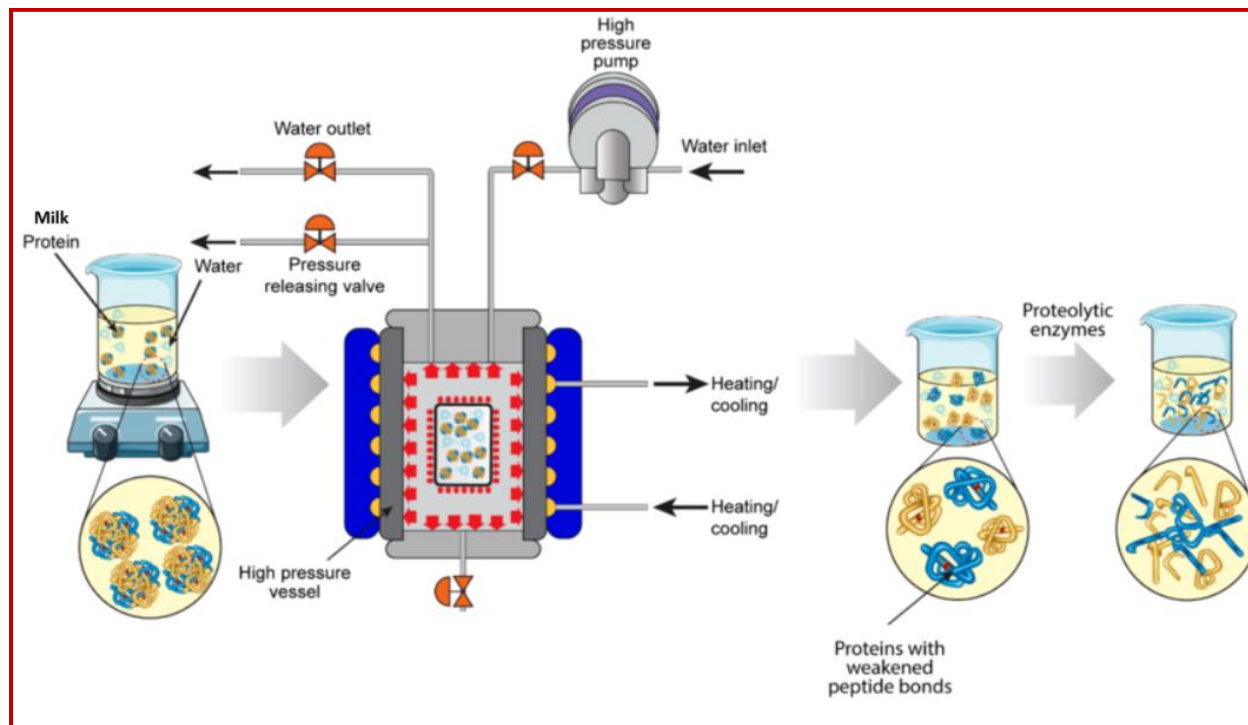


Figure 4. The effect of HPP on milk protein (Farahnaky *et al.*, 2022).

A notable outcome of this arrangement is that κ -casein will typically be concentrated toward the surface of the micelle. Due to κ -casein's singular hydrophobic region and its inability to form colloidal calcium phosphate bridges, it serves as an inhibitor to promote polymerization. Whey proteins, constituting approximately 20 % of the total milk protein in bovine milk, are globular proteins. The principal proteins in this category include α -lactalbumin (α -la), β -lactoglobulin (β -lg), bovine serum albumin

(BSA), and immunoglobulins A (IgA), G (IgG), and M (IgM). Their globular structure renders whey proteins, unlike caseins, susceptible to heat and pressure-induced denaturation. β -lactoglobulin is minimally stable under pressure, typically unfolding at pressures exceeding 100 MPa, whereas α -lactalbumin exhibits significantly greater stability, with denaturation generally observed only at pressures surpassing 400 MPa (Lopez-Fandino, 2006; Considine *et al.*, 2007). The unfolding process exposes the reactive free sulfhydryl groups of β -lactoglobulin, enabling them to undergo irreversible sulfhydryl-disulfide exchange reactions with: (1) other β -lactoglobulin molecules; (2) whey proteins distinct from β -lactoglobulin; (3) cysteine-containing caseins; (4) proteins associated with the milk fat globule membrane; and (5) other cysteine-containing milk proteins, such as plasmin. The extent of these interactions depends on the availability of disulfide clusters in the proteins. The high-pressure-induced denaturation of whey proteins depends on a variety of factors. In skim milk, the majority of HP-denatured whey protein associates with casein micelles (Huppertz *et al.*, 2004), however in whole milk, a significant proportion of denatured whey protein also interacts with the milk fat globule membrane (Huppertz, 2010).

VII. Multifaceted Impacts on Milk Protein Resulting From High- Pressure Processing

High-Pressure Processing (HPP) is a promising non-thermal technology applied in dairy processing for microbial inactivation while retaining the nutritional and sensory qualities of milk. However, HPP induces complex structural and functional changes in milk proteins that can influence product quality, digestibility, and allergenicity.

• Structural Modifications of Milk Proteins

Milk proteins primarily include caseins and whey proteins. Caseins form micellar structures and are relatively pressure-stable, whereas whey proteins such as β -lactoglobulin (β -LG) and α -lactalbumin (α -LA) are more pressure-sensitive (Messens *et al.*, 1997). At pressures above 400 MPa, whey proteins undergo partial unfolding due to the disruption of non-covalent interactions (Schmidt *et al.*, 2015), exposing internal hydrophobic residues. Such unfolding allows formation of new disulfide bonds or hydrophobic interactions between protein molecules, leading to aggregation (Lopez-Fandiño *et al.*, 2007). This denaturation is pressure- and time-dependent and varies with factors like pH and temperature.

• Protein-Protein Interactions and Aggregation

High pressure induces complex formation between unfolded whey proteins and casein micelles, especially at pH values close to the isoelectric point (Anema, 2008). β -Lactoglobulin can bind to κ -casein on the micelle surface, altering micelle size and stability (Ye *et al.*, 2004). These changes can impact the physicochemical properties of milk and its processing behavior.

• Functional Property Alterations

- **Solubility:** HPP can increase solubility of whey proteins initially due to unfolding, but higher pressures (>500 MPa) may cause irreversible aggregation and precipitation (Malaysian *et al.*, 2011).
- **Emulsifying and Foaming Properties:** Unfolding of proteins exposes hydrophobic and charged regions, enhancing emulsifying properties (Huppertz *et al.*, 2004). However, excessive aggregation may impair foaming ability (Heremans, 2002).
- **Gelation:** HPP can induce cold gelation in whey proteins, enabling gel formation without heating, which is beneficial for heat-sensitive formulations (Chawla *et al.*, 2011).
- **Enzymatic Hydrolysis and Digestibility:** HPP-treated milk proteins may exhibit increased susceptibility to enzymatic hydrolysis due to exposure of cleavage sites (Perez-Baena *et al.*, 2009). This can enhance the production of bioactive peptides with antihypertensive or antioxidant properties (Ozturk *et al.*, 2010). Thus, HPP may improve protein digestibility and nutritional quality.
- **Allergenicity and Bioactivity:** Structural changes from HPP can mask or destroy epitopes responsible for allergenic reactions, especially in β -LG, a major milk allergen (Chicon *et al.*, 2008). While this may reduce allergenicity, complete elimination is not always achieved. Conversely, the release of bioactive peptides through pressure-induced hydrolysis can provide functional benefits such as immunomodulation (Korhonen, 2009).
- **Technological Implications in Dairy Products:** In dairy processing, HPP can improve texture, viscosity, and water-holding capacity in yogurt and cheese due to protein modifications (Dumay *et al.*, 2013). Additionally, pressure-treated milk has shown promise in developing hypoallergenic infant formulas and functional beverages (San Martín-González *et al.*, 2007).

VIII. Effect of High-Pressure Processing on Bovine Serum Albumin, Immunoglobulin, Lactoferrin BSA

High-pressure processing (HPP) significantly influences the structural integrity, biological activity, and functionality of minor milk proteins, particularly Bovine Serum Albumin (BSA), Immunoglobulins (Igs), and Lactoferrin (LF). These proteins, though present in smaller quantities than caseins and whey proteins, are critically important for immune function, iron-binding, and transport activity in dairy systems.

(a) Bovine Serum Albumin (BSA)

Bovine Serum Albumin is a globular protein with a molecular weight of approximately 66 kDa and is known for its ligand-binding capacity. It has a compact α -helical structure stabilized by 17 disulfide bridges (Harmsen & De Jong, 2009). Under HPP conditions (≥ 300 MPa), BSA unfolds due to disruption of non-covalent interactions, leading to partial denaturation. This results in increased surface hydrophobicity and subsequent aggregation via exposed thiol groups (Considine *et al.*, 2008). However, BSA shows relatively greater pressure resistance than β -lactoglobulin, requiring pressures closer to 500–600 MPa for significant denaturation (Torrezan *et al.*, 2007). At 400–500 MPa, BSA maintains some structural integrity but loses tertiary conformation (Kato *et al.*, 2000). Denaturation is more pronounced at neutral to alkaline pH compared to acidic environments. The altered BSA structure may reduce its bioavailability or binding function, although it may enhance emulsification in certain dairy formulations.

(b) Immunoglobulins (Igs)

Immunoglobulins (particularly IgG) are important immune components in bovine milk, contributing to passive immunity in infants and calves. These are large, multi-subunit proteins (~150 kDa) sensitive to structural disruption. Immunoglobulins are highly pressure-labile, especially in the range of 300–400 MPa. Pressure induces dissociation of the light and heavy chains, along with conformational unfolding, resulting in loss of antigen-binding activity (Kume & Hayakawa, 1999). At 200–300 MPa, IgG retains partial activity. At >400 MPa, irreversible denaturation occurs, with significant loss of immunoreactivity (Ganju *et al.*, 2009). HPP may reduce the immune-protective function of milk when applied at high pressures, limiting its use in infant formula unless carefully optimized.

(c) Lactoferrin (LF)

Lactoferrin is an iron-binding glycoprotein (~80 kDa) with antimicrobial, antioxidant, and immunomodulatory properties (Pierce *et al.*, 2009). Its tertiary structure is stabilized by iron coordination and glycosylation patterns. Lactoferrin is relatively pressure-resistant compared to immunoglobulins and retains much of its structure up to 400 MPa (Wan *et al.*, 2009). However, higher pressures (>500 MPa) result in unfolding of lobes and potential aggregation. At 300 MPa, LF shows minimal change in iron-binding activity. At 500–600 MPa, iron-saturation is affected, and minor aggregation is observed (Chicon *et al.*, 2008). Additionally, partial unfolding may enhance antimicrobial properties by exposing cationic regions that interact with bacterial membranes (Wakabayashi *et al.*, 2006). Lactoferrin functionality can be preserved under moderate pressure, supporting its use in functional foods.

Table 3. High-Pressure Sensitivity and Functional Responses of Minor Milk Proteins

Protein	Pressure Sensitivity	Functional Impact	Reference Highlights
BSA	Moderate (400–500 MPa)	Partial unfolding and aggregation	Considine <i>et al.</i> , 2008; Kato <i>et al.</i> , 2000
Immunoglobulins	High (>300 MPa)	Loss of immunoreactivity	Kume & Hayakawa, 1999; Ganju <i>et al.</i> , 2009
Lactoferrin	Low–Moderate (≥ 500 MPa)	Retains function at moderate pressure	Wan <i>et al.</i> , 2009; Wakabayashi <i>et al.</i> , 2006

IX. Impact of HPP on Caseins

Caseins are the major proteins in bovine milk, accounting for approximately 80% of the total protein content. They exist primarily as colloidal casein micelles composed of α s1-, α s2-, β -, and κ -caseins, stabilized by calcium phosphate nanoclusters and hydrophobic interactions (Fox & Brodtkorb, 2008). These micelles are relatively resistant to heat, but they undergo specific structural and functional changes under high-pressure processing (HPP).

• Pressure-Induced Micellar Disruption

HPP affects the integrity of casein micelles by altering calcium phosphate bridges and disrupting non-covalent interactions. At pressures above 200–300 MPa, partial micellar dissociation occurs due to the solubilization of colloidal calcium phosphate (CCP), resulting in reduced micelle size and increased hydration (Schmidt *et al.*, 2015). Further pressure (>400 MPa) can lead to extensive micellar fragmentation (Huppertz *et al.*, 2004).

• Effects on Individual Casein Types

κ -casein, located on the micellar surface, is highly sensitive to pressure and may detach, exposing the micellar core and reducing stability (Anema & Li, 2003). β -casein can be released from the micelle into the serum phase under pressure due to weakened hydrophobic interactions (Huppertz *et al.*, 2006). This release increases at low temperatures. α s1- and α s2-caseins are less affected directly but may undergo conformational changes depending on the pressure level and ionic environment.

- **Consequences for Functional Properties**

Casein micelles are dynamic colloidal assemblies in bovine milk, typically ranging from 50 to 500 nm in diameter, stabilized by colloidal calcium phosphate (CCP) and hydrophobic interactions. High-pressure processing (HPP) significantly influences the size and structural organization of these micelles due to mechanical compression and physicochemical alterations.

- **Pressure-Induced Reduction in Micelle Size**

Recent studies have demonstrated that HPP can lead to a notable decrease in casein micelle size. Xu *et al.* (2023) reported that ultra-high-pressure jet processing (UHPJ) at pressures between 100–300 MPa resulted in a reduction of average casein micelle size from approximately 180 nm to 167.47 nm. This size reduction is attributed to the disruption of micellar structures under pressure. Similarly, Hemar *et al.* (2020) observed a 42.5% decrease in the size of reformed casein micelles when subjected to pressures exceeding 500 MPa for 15 minutes.

- **Role of Colloidal Calcium Phosphate (CCP)**

CCP plays a crucial role in micelle architecture by cross-linking phosphoserine residues in α - and β -caseins. Under pressure the CCP is progressively solubilized, especially above 250 MPa. This weakens the internal cohesion of micelles and triggers micellar shrinkage or collapse (Anema & Li, 2003). The reduction in calcium-mediated interactions is considered a primary driver of size reduction and micelle destabilization.

- **Structural Alterations Observed via Microscopy**

High-pressure treatments not only reduce micelle size but also induce structural changes. Scanning Electron Microscopy (SEM) analyses have revealed that HPP at 600 MPa for 2 minutes leads to agglomeration, deformation, and fragmentation of casein micelles. These structural changes are indicative of the micelles' response to high-pressure conditions. SEM studies confirm that HPP treatments result in morphological changes in casein micelles. A study by Wang *et al.* (2022) revealed that treatment at 600 MPa for 2 minutes caused deformation, fragmentation, and partial agglomeration of micelles indicates the signs of micellar disassembly due to pressure-induced denaturation.

- **Influence of Processing Parameters**

The impact of HPP on micelle dimension is modulated by several processing variables are the pressure level and duration: Increasing pressure and holding time leads to more extensive micellar fragmentation (Xu *et al.*, 2023). The temperature lower processing temperatures (4–20 °C) enhance β -casein dissociation from the micelle surface (Huppertz *et al.*, 2006). The milk pH and ionic composition: Acidic pH or altered mineral balance reduces micellar stability and enhances the effects of pressure (Corredig *et al.*, 2004).

- **Functional Implications**

The pressure-induced modifications in casein micelle size and structure have practical implications. Enhanced Emulsifying Properties: Smaller micelle sizes increase the surface area, potentially improving emulsification in dairy products. Altered Gelation Behavior: Structural changes can influence the gelation properties of milk, affecting the texture of products like yogurt and cheese. Micelle size reduction and structural changes have key functional consequences. Increased surface area improves emulsifying properties, while altered micellar integrity can enhance cold-set gelation and rennet coagulation behavior (Chawla *et al.*, 2011). These modifications are beneficial in the production of yogurt, cheese, and high-protein dairy beverages.

Conclusion

High Hydrostatic Pressure (HHP) is an emerging non-thermal technology with significant potential in dairy processing. It effectively modifies milk protein structures without compromising nutritional value or sensory quality. HHP enhances functional properties like solubility, emulsification, and gelation, while also improving microbial safety and shelf life. This technique offers a promising alternative to thermal treatments, preserving the natural characteristics of milk and meeting consumer demand for minimally processed, high-quality dairy products.

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