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Recent Advances in High Pressure Processing of Milk and Milk Products - A review

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ABSTRACT

As the global consumers' demand towards minimally processed fresh-like foods has been continuously increasing, efforts to develop novel food processing technologies have been intensified. Among non-thermal food processing technologies, high pressure processing (HPP) seems to be more advantageous due to its environmentally friendly nature, cost efficiency, suitability for processing foods in any form and its positive impacts on foods' shelf-life as well as providing efficient microbial safety. Microbiological inactivation efficiency of HPP has been well documented but the role of this technology in digestion efficiency of milk compounds is yet to be elucidated in detail. Also, the potential safety hazards and challenges of HPP in foods require more intense studies. This review deals with the recent developments in HPP treatment to milk and milk products.

Keywords: High hydrostatic pressure, Shelf-life, Quality, Dairy, Nutrition

1. Introduction and Technological Background of High Hydrostatic Pressure

Food consumption paradigms have changed dramatically during the last two decades. Today, consumers are keener on consuming safer, healthier and minimally processed fresh-like foods and this demand has become more pronounced during coronavirus disease-2019 pandemic. The efficiency of thermal applications to foods including thermization, pasteurization, ultra-high pasteurization and ultra-high temperature treatments on providing food safety has been known for many decades. Although thermal technologies are widely employed in food processing worldwide, possible adverse effects of heat treatment on some food components have motivated the food community to develop alternative technologies to heating. Efforts have been intensified to adapt some non-thermal food processing technologies from lab/pilot scale to industrial scale, and some non-thermal food processing technologies are now enjoying market success and consumer acceptance. High hydrostatic pressure (HPP), pulsed electric field (PEF), ultrasound, cold plasma, ozonization, irradiation and ultraviolet light are some examples of non-thermal food processing technologies. These technologies are employed in shorter processing time and at lower temperatures which leads to improvement of the nutritional quality of foods as well as sensory quality. Also, the shelf-lives of non-thermally processed foods are improved without impairing the food safety parameters (Mahalik & Nambiar 2010; Alexandre et al. 2012).

HPP has gained popularity in food processing faster than the other non-thermal technologies. About 75% of the scientific papers and patents on non-thermal food processing technologies are directly related with HPP. The jam was the first commercial HPP-processed food introduced into the markets in 1990 followed by retort rice products, cooked hams, and sausages (Yamamoto 2017). HPP is based on the Le Chatelier principle:

"If a dynamic equilibrium is disturbed by changing the conditions, the position of equilibrium moves to counteract the change"

In HPP treatment, isostatic pressure is transmitted to the product through water. Since the water used in HPP is recyclable, it brings about an advantage of reduced energy consumption and environmental protection (Toepfl et al. 2006). The pressure is transmitted uniformly and instantaneously throughout the product, therefore achieving an effect equivalent to pasteurization. A HPP equipment is made up of a high-pressure vessel and its closure, a pressure generator and a material handling mechanism equipped with a temperature control system (Datta & Deeth 1999). The pressure range applied to foods may vary between 300 MPa and 900 MPa depending on the structure and initial microbial load of the product. In practice, the pressure range of 400-600 MPa is sufficient enough to provide microbial safety in many foods (Trujillo 2002). HPP has minimal effect on sensory, nutritional and textural characteristics of the foods (Rendueles et al. 2011; Grundy et al. 2016). The pressure, holding time and temperature applied are the major parameters determining the effectiveness of microbial inactivation.

HPP allows drastically reducing or eliminating the use of preservatives or additives in food. Also, HPP prevents food waste on the retail shelf and in the consumer's refrigerator since it has an extended shelf life (Trujillo et al. 2002). Depending on the processing conditions and product characteristics, the shelf-life of the HPP-treated foods may be extended up to three-fold. HPP may be applied to pre-packed liquid or solid foods. Some high-pressure equipment are suitable for continuous production as well. In the latter case, an aseptic filling may be required depending on the shelf-life expectations and/or safety requirements of the end product. Although HPP has many advantages over traditional heating systems regarding microbial inactivation, it is not effective in the elimination of spores (Pinto et al. 2020). Also, HPP may cause some textural and colour changes in foods under question. Finally, HPP technology is not recommended for the processing of dry products and to ensure microbiological safety, a minimum water activity of 0.8 in foods is required.

Non-thermal food processing technologies are accepted as novel technologies in many countries. Some countries mandate risk analysis of the products manufactured using one or more of the non-thermal food processing technologies. European Union (EU) accepts HPP technology as a 'novel' food processing technology (EC 258/97). As in the standard applications of the EU, the regulations are in the form of 'roof regulation' and the applicability of novel technologies including the implementation details are determined by country regulations. At this point, a position paper published by French authorities in 2010 on HPP technology includes the statement that applications performed at room temperature and around 5 minutes up to 600 MPa pressure are harmless (Jung & Tonello-Samson 2018). This means no risk analysis is required for such foods as long as the conditions stated above are strictly followed. According to the EU framework agreement (European Community Treaty), products produced in any community member country have the right of free circulation within the EU. Today, according to "Regulation of the European Parliament and of the Council on novel foods, amending Regulation (EU) No 1169/2011 of the European Parliament and of the Council and Repealing Regulation (EC) No 258/97 of the European Parliament and of the Council and Commission Regulation (EC) No 1852/2001", application of HPP in food processing is allowed. By now, no chemical and/or microbiological risk that is directly associated with HPP application has been reported. While the number of industrial-scale HPP equipment was 2 in 1994, this figure has reached just over 600 today. In the dairy industry, HPP is being currently used to process cheese milk (Pastoret brand Spanish cheese) or packaged cheese after production (Mu brand Cheddar sticks and Duetto Hot Pepperoni + cheese bars, United Kingdom). In Mexico and Lebanon, two companies are using HPP at the industrial scale to extend the shelf-life of vacuum-packed cheeses. In Australia, cold-pressed milk equivalent to heat-pasteurized milk is being produced commercially (Made by Cow®, Australia). In New Zealand, a bovine colostrum product branded as Col+® is being manufactured by directly processing colostrum with HPP. The last two examples are devoid of any form of heat treatment and approved as safe by country authorities.

2. HHP Treatment of Milk

2.1. Effects of HPP on microbial inactivation in milk

Milk and dairy products are suitable mediums for the growth and survivability of a range of contaminant microorganisms. The degree of contamination and the processing temperature in different milk processing stages significantly determine the diversity of the microbial community in milk (Dash et al. 2022).

Some spoilage and pathogenic microorganisms including Shiga toxin-producing *Escherichia coli, Salmonella* spp., *Listeria monocytogenes, Campylobacter* spp., and *Yersinia* spp. which are known to cause food-borne infections, intoxications and toxicoinfections in humans, are frequently associated with milk and milk products (Dhanashekar et al. 2012; EFSA 2016; Melini et al. 2017; Lee et al. 2019). Therefore, it is important to take every possible measure to avoid such microbiological safety hazards in industrial food processing. The application of HPP generally damages microbial cell walls and membranes, inactivates enzymes, degrades chromosome DNA, and unfolds and/or dissociates proteins, causing gelation, especially, at higher pressures (i.e., >600 MPa) (Chiozzi et al. 2022; Yang et al. 2012).

It is known that bacterial cells, yeasts and molds are more sensitive to pressure than spores. While pressure treatment at 400-600 MPa at the ambient temperatures inactivate the former microbial groups, much higher pressures and/or longer treatment time are required for the elimination of spores. Specifically, pressure conditions higher than 1200 MPa are considered capable of inactivating spores with relative success; but this very high pressure is not preferred by the food industry since gelation or textural weaknesses in the products are likely to occur. In general, Gram-positive bacteria (*i.e.*, *Listeria monocytogenes*, *Staphylococcus aureus*) are more resistant to high pressure than Gram-negative bacteria (*i.e., Pseudomonas, Salmonella* spp., *Yersinia enterocolitica*, *Vibrio parahaemolyticus*). This is due to the presence of teichoic acid in the peptidoglycan structure of gram-positive cell wall, giving cell wall a more rigid structure (Katsaros et al. 2016).

The degree of resistance of milk associated pathogenic and/or spoilage microorganisms against high pressure is affected by multiple factors such as species and strains, bacterial shape, inoculum level, physiological state of bacteria, milk composition, processing pressure, holding time, temperature, and growth phase (Zagorska et al. 2021; Serna-Hernandez et al. 2021).

HPP treatment of donkey milk at 400 MPa for 180 s resulted in <10 colony forming unit/mL of *Pseudomonas* spp., *Enterobacteriaceae*, and *Bacillus cereus* after 30-day storage at 4 °C (Giacometti et al. 2016). HPP application to goat's milk at 600 MPa for 7 minutes at 15 °C did not cause an increase in coliforms, *B. cereus*, yeast and molds during 22 days of storage at 8 °C (Tan et al. 2020). HPP treatment of milk at 345 MPa for 5 min at 50 °C was reported to result in 8 log reduction of *E. coli* and *L. monocytogenes*; however, the reduction in *S. aureus* counts was rather limited (5.33 log) under the same conditions (Alpas et al. 2000).

A summary of the recent studies on the pressure resistance of microorganisms commonly found in different milk categories is given in Table 1. On the other hand, potential impacts of HPP treatment on microorganisms that are less frequently associated with milk such as *Staphylococcus aureus*, *Coxiella burnetii*, and *Mycobacterium*, have been subjected to limited number of scientific evaluations. In a recent project run by Özer et al. (2022), the effectiveness of HPP treatment at 200, 400 or 600 MPa for 5 or 10 min on inactivation of *Mycobacterium tuberculosis*, *Eschericia coli* O157:H7 and spore-forming *Bacillus* spp. in bovine colostrum microfiltrate was investigated. Results demonstrated that except for the treatment at 200 MPa for 5 or 10 min, all other pressure conditions totally inactivated the target pathogens. On the other hand, HPP at 200 MPa for 5 or 10 min failed to inactivate *E. coli* O157:H7 and *M. tuberculosis*. Recently, Yang et al. (2020) showed that multi-cycle HPP treatment (2 x 2.5 min at 600 MPa) resulted in higher level of microbial eradication with satisfactory level of preservation of milk quality than single-cycle treatment (1 x 5 min at 600 MPa). Extra care must be taken in the eradication of pathogenic microorganisms by HPP as their toxins may withstand HPP conditions applied. For example, the number of *Aeromonas hydrophila* AH191 may well be reduced by at least 9 orders of magnitude after HPP treatment at 250 MPa for 30 min at 25 °C, but their toxins may remain unaltered, as concluded by Durães-Carvalho et al. (2012).

2.2. Effect of HPP on milk compounds

Due to the high water activity (i.e., >0.9 a_w), most dairy products including raw or processed milk and fermented dairy products are classified as highly vulnerable foods with a short shelf-life. The HPP successfully extend the shelf-life of milk and milk products without impairing the physical and/or organoleptical characteristics of end product to a large extent (Wang et al. 2016). However, milk components, including fat, casein, whey proteins, enzymes, and minerals can be affected by HHP treatments (Ravash et al. 2022). The method and conditions of processing or the type of milk affect the changes in milk fat. Huppertz et al. (2011) reported that high pressures treatments of cow's milk at 100-600 MPa at 20 ºC for 60 min do not influence fat globules. Kiełczewska et al. (2020) demonstrated that HPP treatment of caprine milk at 200-500 MPa for 10 min at 20 °C did not affect the particle size during storage, the colour values, and the overall fatty acids (FAs) profile, but the ratio of branched chain FAs increased. A decrease in FAs upon high pressure treatment to ewe's milk was reported by Gervilla et al. (2001). Milk fat globule membrane size in the range of 1-2 μm tended to increase but those in the range of 2-10 μm decreased after being pressurized at 100-500 MPa at 25 or 50 °C.

HHP may cause modifications in milk proteins. Depending on the HHP conditions, casein micelles may be disintegrated into smaller sub-micelles which are further re-associated (Anema et al. 2005a; Huppertz et al. 2004ab; Orlien 2021; Cadesky et al. 2017). While pressures between 100-200 MPa have limited (if any) modifications in casein micelles, pressures around 250 MPa led to the aggregation of casein micelles and increased the average size of micelles (approximately 25%). Pressures above 400 MPa resulted in breakdown in hydrophobic interactions and decreased the average size of micelles (up to 50%) (Bravo et al. 2015). Whey proteins, especially β-lactoglobulin, may undergo a pressure-driven denaturation (Anema et al. 2005b; Lopez-Fandiño et al. 1996). Whey proteins

Table 1. Recent studies of applications of high-pressure processing in different milk categories

Dairy products	*Pressure*	*Exposure time*	*Temperature*	*Effect*	*Reference*
Raw milk	600 MPa	5 min	18 °C	5 log reductions for *E. coli*, *Salmonella* and *L. monocytogenes*	Stratakos et al. (2019)
Cow and goat milks	450 MPa	7 min	15 °C	Increased shelf life (up to 22 days to 8 ◦C), with no increase in *Bacillus cereus*, mesophilic aerobic spores, coliform, yeast and mold	Tan et al. (2020)
Raw milk	300 MPa	30 min	25 °C	Inactivation in *Salmonella* spp., *E. coli*, *Shigella*, and *Staphylococcus aureus*	Yang et al. (2012)
Raw whole milk	600 MPa	5 min	40 °C	3 log reductions for total bacterial count and *E. coli*	Liu et al. (2020)
UHT whole milk	500 MPa	10 min	25 °C	6.20 log reductions for *L. monocytogenes*	Misiou et al. (2018)
Reconstituted milk powder	500-600 MPa	3-5 min	25 °C	Inactivation of *Escherichia coli*, *Pseudomonas fluorescens* and *Enterobacter aerogenes* Increases in the levels of microorganism-derived lipopolysaccharides	Machado et al. (2019)
Human milk	593.96 MPa	233 s	25 °C	6-7 log reductions for *Bacillus cereus, Bacillus aureus*	Rocha-Pimienta et al. (2020)

UHT: Ultra-high temperature treatments

may undergo pressure-driven denaturation of which level is affected by time, temperature and pH of the milk and milk products being pressurized (Huppertz et al. 2004ab; Hinrichs & Rademacher 2004; Arias et al. 2000). Under relatively harsh the processing conditions (i.e., at >300 MPa for >30 min), β-LG irreversibly unfolds which leads to increase in hydrophobicity and hence protein aggregation (Pittia et al. 1996). As the hydrophobic groups buried inside the globular structure of whey proteins become unmasked, the hydrophobicity of the whey proteins increases (Lim et al. 2008). In general, secondary structure of whey proteins is relatively more stable against HPP than the tertiary and quaternary structures (Velez-Ruiz et al. 1998). It has been shown that changes in solubility of whey protein isolate (WPI) is dependent on HPP conditions (Kanno et al. 1998). While no change was observed in solubility of WPI at 400MPa for 10 min, a clear decrease in solubility after HPP at 690 MPa for 5 to 30 min was evident (Lee et al. 2006). Any change in protein leads to a variety of functional characteristics which in turn contribute to the improvement of the organoleptic properties of HPP-treated dairy products (Ravash et al. 2022).

Compared to thermal pasteurization, HPP treatment produces little or no damage on various classes of immunoglobulins (Ig) present in dairy products, which contribute positively to human health (Huang et al. 2020). The HPP treatment of human colostrum at 200 MPa for 2.5, 15 and 30 minutes at 8 °C resulted in insignificant changes in IgA, IgM and IgG (Sousa et al. 2014). It was recently reported that immunoglobulin concentration in human milk remained unchanged after HPP processing at 400 MPa for 5 min and at 593.96 MPa for 233 s (Rocha-Pimienta et al. 2020).

Effects of HPP on milk enzymes show a dependency on HPP conditions. This is an important feature because HPP can be used to control enzymatic activities in dairy products such as mature cheeses, i.e., activation or deactivation of proteolytic and lipolytic enzymes. For example, high pressure application at 400MPa in processing of bovine milk (Munir et al. 2020) and at 200-300 MPa in ewe milk (Alonso et al. 2012) stimulated the proteolysis during cheese maturation (Munir et al. 2020; Alonso et al. 2012).

3. HPP-mediated Chemical and Physicochemical Modifications in Milk and Milk Products

3.1. Effects on milk pH

As a result of increase in the concentration of ionized calcium in milk serum caused by HHP-driven solubilization of micellar calcium phosphate, changes the pH of milk (Huppertz et al. 2004a, 2002; Liepa et al. 2016). The composition of milk-more specifically the buffering capacity of casein micelles- also influences the changes in the pH levels of milk subjected to HPP (Iturmendi et al. 2020). Fat may have a protective role on HPP-mediated casein micelles' dissociation, thereby diminishing the variation in the milk's pH (Yang et al. 2020; Iturmendi et al. 2020).

3.1.1. Effects on emulsion stability

Emulsion stability is related with the changes in physical parameters of macromolecules including size distribution, flocculation and droplet arrangement over time. Emulsion destabilization is often linked with the changes in physical appearance of a food product, development of undesirable flavors and rapid degradation of nutrients. These changes are due to the exposure of the fat fraction to oxidation and other chemical reactions triggered by emulsion destabilization. HPP affects native milk proteins including ones located on the milk fat globule membrane; hence these modifications affect the emulsion stability of milk. Milder pressure applications can improve emulsion stability *via* exposure to hydrophobic groups *via* the mechanisms explained above. Exposure of hydrophobic groups yields reductions in droplet size as well as modifications in molecular flexibility of milk proteins. This eventually leads to more evenly distribution of polar and non-polar amino acid residues and improves the emulsifying properties. In addition, protein denaturation generates the formation of low-molecular-weight components, and these seem to promote emulsion stability. However, higher pressure treatments, i.e., >400 MPa, seem to adversely affect the emulsification capacity of native milk proteins which leads to decrease in solubility of whey proteins (Gharibzahedi et al. 2019).

3.1.2. Effects on viscosity

While unconcentrated whole and skimmed milk show generally a Newtonian fluid characteristic, concentrated milk display a pseudoplasticity with a shear-thinning flow behavior. Milk viscosity is affected by HPP at varying levels depending on the HPP pressure applied and the time of exposure (Janahar et al. 2021; Serna-Hernandez et al. 2021). Another key factor is the increase in casein micelle hydration caused by HPP-triggered partial disintegration of casein micelles (Zhang et al. 2020).

4. Effect of HPP on Digestion Profiles of Milk Compounds

Although the digestion efficiency of milk components such as proteins and lipid have been well documented (Kopf-Bolanz et al. 2014; Lorieau et al. 2018; Mat et al. 2016; Mulet-Cabero et al. 2019; He et al. 2015), the impact of HPP on digestion profiles of milk components has been subjected to the limited number of scientific studies so far. The effect of HPP on *in vitro* digestion efficiency of β-lactoglobulin (Maynard et al. 1998; Chicón et al. 2008a), α-casein (Hu et al. 2017), whole milk (Liu et al. 2020) and whey protein isolate (Chicón et al. 2008a) were investigated. The level of tryptic hydrolysis of β-lactoglobulin B increased with increasing pressure and the highest tryptic hydrolysis was obtained at 300 MPa treatment (Stapelfeldt et al. 1996). The peptide profiles of β-lactoglobulin and whey protein isolate treated with HPP in the range of 100-800 MPa and at 400 MPa, respectively, were not affected by the pressure treatment (Maynard et al. 1998; Chicón et al. 2008a). HPP treatment at 200 MPa for 5 min resulted in the highest pepsin digestibility of α-casein (Hu et al. 2017) and gastric digestion profiles of whole milk remained unchanged after 600 MPa for 5 min (Liu et al. 2020). As the duration of HPP extends, the digestion efficiency of α-casein decreases (Hu et al. 2017). More recently, Aalaei et al. (2021) investigated the *in vitro* static gastric digestion profiles of milk proteins subjected to HPP at 400 MPa for 15 min, 600 MPa for 5 or 15 min. The authors used two different static digestion models simulating adult and elderly people's gastric system. Overall, digestion of proteins subjected to HHP at 600 MPa for 5 or 15 min was slower in the elderly model than the adult model evidenced by the high concentration of long chain peptides in the former. Interestingly, HPP at 400 MPa for 15 min improved the protein hydrolysis in the elderly model and yielded more or less similar peptide profiles to the adult model. Increasing pressurizing time at 600 MPa did not cause any further increase in the digestion efficiency of proteins. The majority of the peptides yielded upon *in vitro* simulated gastric digestion of whey proteins had a length of 16-20 amino acids, indicating high digestion efficiency in whey proteins. In general, caseins, α-lactalbumin and bovine serum albumin are resistant to HHP at 400-600 MPa (Liu et al. 2020; Yang et al. 2020; Lopez-Fandiño et al. 1996). Above 600 MPa, an interaction between caseins and β-lactoglobulin occurs *via* thiol-disulphide bonds (Bogahawaththa et al. 2018). This eventually slows down the digestion of milk proteins. Regarding milk protein digestion efficiency, 400 MPa seems to be suitable at which microbiological safety is also ensured (Özer et al. 2022). Digestion efficiency of α-casein subjected to HHP at 200 MPa for 5 min decreased by 36-43 % when pressure conditions were set to 600 MPa for 15 min due to casein aggregation (Hu et al. 2017). Similarly, digestion of κ-casein was reduced by half after being processed at 600 MPa (17%) compared with 400 MPa treatment (36%), due possibly to its aggregation with β-lactoglobulin at 600 MPa (Bogahawaththa et al. 2018). In general, although major whey proteins are denatured by HPP, their digestibility does not change at a significant level. Vilela et al. (2006) found that digestibility of single-cycle or triple-cycle pressure-treated WPI with pepsin was higher than untreated WPI after 30 min of digestion (51% and 68% vs 30.9% reduction in WPI, respectively). These findings were further supported by Iskandar et al. (2015) who showed that after 30 min of pancreatic digestion, the degree of pressure-treated WPI hydrolysis reached 95% but the degree of hydrolysis of untreated native WPI was 83%. Most recently, Zhang et al. (2022) demonstrated that the digestion profile and level of retention of nutrients of donor human milk were similar to raw milk but not the samples treated with holder pasteurization at 62.5 °C for 30 min. Protection of lactoferrin in donor human milk by HPP was also higher than those thermally treated (Pitino et al. 2019; Sergius-Ronot et al. 2022). In contrast, a decrease in lactoferrin at very high-pressure applications (550 to 800 MPa) was evident in bovine milk (Bravo et al. 2015). Upon HPP treatment to human donor milk at 350 MPa at 38 °C, the levels of metabolic hormones including insulin, nesfatin-1, cortisol and leptin remained unchanged, glucagon-like peptide 1 level increased and apelin and adiponectin levels decreased (Marousez et al. 2022). However, classical holder pasteurization (62 °C for 30 min) caused dramatic decreases in those metabolic hormones except for adiponectin which remained unchanged.

HPP also effectively reduces the allergenicity of milk proteins depending on the pressurizing conditions (Beran et al. 2009; Huang et al. 2014). In an extensive study, Kleber et al. (2007) demonstrated that the allergenicity of β-lactoglobulin- one of the major allergenic proteins in milk- increased as the pressure and treatment time extended from 200 MPa to 600 MPa and from 0 min to 30 min at <25 °C, respectively. However, when the treatment temperature was increased above 25 °C, the allergenicity of β-lactoglobulin decreased but still higher than the untreated samples. This may be due to the pressure-driven unfolding of whey proteins resulting in the generation of new epitopes which are buried in three-dimensional (3D) structure of folded native proteins (Mills & Mackie 2008). On the other hand, there is no clear correlation between the degree of protein denaturation and the allergenicity, indicating the complexity of food ingredients in food matrices. In most cases, the combination of HPP and enzymatic treatment (i.e., trypsin or chymotrypsin) is applied to reduce allergenicity of proteins (Chicón et al. 2008b).

5. HPP Treatment of Dairy Products

5.1. Impact of HPP on physical stability of whey-based beverage formulations

Fermented whey-based beverages, a promising way to valorize by-products of dairy manufacturing, are often associated with limited shelf-life due to the post-acidification occurring during storage. Time-dependent sedimentation as a result of heat-induced denaturation of whey proteins is another major challenge for whey-based fermented and non-fermented beverages. Severe thermal treatments applied above 70 °C result in protein denaturation, accompanied by loss of aqueous solubility and foaming properties (Kester & Richardson 1984; Pittia et al. 1996). Pega et al. (2018) investigated the effects of HPP at 200 MPa for 10 min or 400 MPa for 1 min on the properties of a fermented beverage manufactured from sweet whey using the starter lactic acid bacteria *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*. The authors concluded that the flavor and texture of beverages treated by HPP were maintained up to 45 days post-processing with no changes in chromatic parameters. Sampedro et al. (2009) studied the effects of heat treatment, PEF and HPP processing on pectin methyl esterase (PME) activity and levels of volatile compounds in an orange juice - milk mix beverage. The conditions for inactivation of PME at >90% were as follows: thermal treatment at 85 °C for 1 min, PEF treatment at 25 kV/cm at 65 °C or HPP treatment at 650 MPa at 50 °C. After HPP treatment, the average losses of volatile compounds were between 14.2% and 7.5% at 30 °C, 22.9%, and 42.3% at 50 °C.

Barba et al. (2012) developed an orange juice-milk beverage using HPP at 100-400 MPa for 2 to 9 min. The authors demonstrated that the loss of ascorbic acid in the beverages was <10%, soon after HPP treatment and high-pressure treatment time had no significant effect on ascorbic acid losses. Similar results were reported by Bull et al. (2004) who showed that the ascorbic acid concentration of fruit and vegetable juices was not influenced significantly by HPP at mild temperatures. On the contrary, the colour changes in the HPP-treated samples were more remarkable as the pressure and treatment time increased (Barba et al. 2012).

HPP was demonstrated to better protect the antioxidant capacity of whey-based sweet lime beverages compared with thermal treatment (45.8% vs 76.7%). Sensory parameters of the same beverage remained unchanged during storage as well (Bansal et al. 2019).

5.2. Cheese

HPP has been demonstrated to affect the enzymatic coagulation time, acceleration of ripening, increasing of yield, and modifications to physicochemical and sensory properties of cheese (Chawla et al. 2011; San Martín-González et al. 2006; Naik et al. 2013; López-Pedemonte et al. 2007; Huppertz et al. 2002; Lopez-Fandiño et al. 1996; Nuñez et al. 2020; Chopde et al. 2014; Martínez-Rodríguez et al. 2012; Costabel et al. 2016). Early studies showed that HPP treatment to milk resulted in increased yield in Cheddar (Drake et al. 1997) and semi-hard goat cheese (Trujillo et al. 1999) without impairing the cheese flavor compared to those made from pasteurized milk. There is no clear consensus on the effects of HPP on cheese quality and processing time. For example, Delgado et al. (2011) demonstrated that the original cheese flavor was not maintained when HPP was applied to cheese made from raw milk at the early stages of maturation. However, this does not mean the development of inferior quality in the end product. Escobedo-Avellaneda et al. (2021) failed to detect any impact of HPP treatment on the coagulation time of milk in Oaxaca cheese production (a pasta-filata type variety). As discussed above, HPP triggers interactions between β-lactoglobulin and κ-casein, leading to retarding caseinomacropeptide release by chymosin. Milder pressure treatments, i.e., ≤200 MPa cause a reduction in rennet coagulation time of milk. This situation is possibly associated with the increase in the surface area of the casein micelles due to the decrease in the size of the casein micelle with the effect of high pressure, and thus the expansion of the area for the action of chymosin (San Martín-González et al. 2006; Naik et al. 2013). Accelerating cheese ripening without impairing the quality characteristics of cheese is highly desirable by cheesemakers. HPP has been proved to have positive effects on accelerating cheese ripening without altering the quality and sensorial attributes (Chopde et al. 2014; San Martín-González et al. 2006). Cheese-ripening involves a series of complex biochemical reactions mainly regulated by milk enzymes, and proteolysis is the most important biochemical event largely determining the flavor and texture changes in cheese (San Martín-González et al. 2006). The high pressure alters the bacterial cell wall as discussed earlier and modifies the casein matrix, making it more susceptible to proteolytic enzymes activities. Also, HPP-triggered bacterial lysis results in release of bacterial enzymes at higher rates. In addition, the shifts in pH levels and modification of water distribution provide better conditions for enzymatic activity (Chopde et al. 2014; Martínez-Rodríguez et al. 2012). Acceleration of goat's milk cheese ripening by HPP at 400 MPa was also reported by Saldo et al (2000), but the HPP-treated cheeses had crumbly body and bitter flavor defects compared to the untreated cheeses.

The HPP-treated cheeses when compared to traditionally processed cheeses have smaller and uneven compartments in their matrices; however, during the aging process, the differences are almost non-existent, and the final products are relatively similar (Nuñez et al. 2020). Additionally, the interior color of the cheeses is modified by HPP treatment. HPP treatment causes a loss of brightness and an increase in yellowness in the cheeses. Readers are recommended to refer to Nuñez et al. (2020) for an extensive review on the effects of HPP on cheese characteristics.

5.3. Yogurt & fermented milks

The effect of HPP treatment on acid-type dairy gels has been subjected in many scientific reports up to now (Ferragut et al. 2000; Harte et al. 2002; Lanciotti et al. 2004; Vieira et al. 2019; Walsh-O'Grady et al. 2001). In general, within the pressure range of 300-700 MPa, the rheological properties of yogurt-type gels improve which eliminates the necessity for stabilizers and contributes to clean label productions (Loveday et al. 2013). Formation of yogurt matrix relies mainly on the interactions between denatured whey proteins and caseins *via* thiol-disulfide bonds. During the fermentation, the pH is reduced by the action of yogurt starter bacteria to the isoelectric point of caseins and a 3D gel matrix consisting of interacted milk proteins is formed (Soukoulis et al. 2007; Harte et al. 2003). The viscosity of yogurt milk increases immediately after HPP treatment and remains almost stable for a period of 60 days under cold storage conditions. Yogurt samples made from milk treated with high pressure treatment at 676 MPa for 30 min had similar rheological and water holding properties to gels made from heat treated milk at 85 °C for 35 min, with very different microstructural characteristics (Harte et al. 2002). This feature stemmed from the reaggregation of disrupted micellar fragments by high pressure during fermentation process. On the other hand, when skim milk was supplemented with whey protein hydrolysate or concentrate (whey protein concentrate-80) prior to HPP treatment, the resulting yogurt had inferior gel properties (Sakkas et al. 2019).

HPP reduces syneresis, a common defect in yogurt and causes thicker and smoother body in yogurt. In addition, in yogurt treated with HPP, the color characteristics -specifically to the L* and b* values- are altered, and the yield stress and the water holding capacity of yogurt matrix are increased. Regarding lipolysis and proteolysis, no clear differences between HPP-treated and thermally treated yogurt samples were reported (Walker et al. 2006; Harte et al. 2003).

Kefir made from HPP-treated whole milk had lower elastic and viscous characteristics, and lightness and color intensity than untreated control and HPP-treated kefir made from skim milk (Renes et al. 2020).

6. Conclusion

Although the advantageous of HPP of foods to food processors, consumers and environment are beyond doubt, more scientific evidence is required to be confident about its chemical safety. Majority of the studies, so far, have focused on the microbiological safety of the food processed by HPP. However, chemical interactions triggered by HPP are yet to be evaluated more deeply. In many countries, food regulations directly related with non-thermal food processing technologies are lacking. This eventually limits the level of industrialization of HPP and similar non-thermal food processing technologies. Also, the effects of HPP on digestion efficiency of milk compounds deserve more attention.

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