Chapter 4
High Pressure Processing for Food Fermentation

Jincy M. George and Navin K. Rastogi

4.1 Introduction

The consumer demand for minimally processed foods has always been a challenge for food processing sector. Therefore, novel food processing technologies are implemented to meet the challenges with the aim to ensure safe, fresh-like, nutritive foods without the use of heat or addition of food additives including preservatives. The recent scientific research for the food industry has now focused on non-thermal processing techniques, with high pressure processing (HPP) being amongst the few experiencing great potential in commercial settings (Norton & Sun, 2008; Rastogi & Raghavarao, 2007). High pressure processing is a cold pasteurization technique which consists of subjecting food, sealed in flexible and water-resistant packaging, to a high hydrostatic pressure up to 600 MPa (87,000 psi) for few seconds to few minutes. It is the same effect as subjecting the food to an ocean depth of 60 km deep. The technique was named after Blaise Pascal, a French scientist of the seventeenth century whose work included the effects of high pressure processing on fluids. The history of high pressure processing (HPP) dates back to nineteenth century. In 1899 Bert H. Hite at the West Virginia Agricultural Experimental Station examined the effect of pressure on dairy product like milk, meat, fruits and vegetables. Compared to today's high

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J.M. George • N.K. Rastogi (✉)
Academy of Scientific and Innovative Research, Central Food Technological Research Institute, Mysore, Karnataka 570 020, India

Department of Food Engineering, Central Food Technological Research Institute, Mysore, Karnataka 570 020, India
e-mail: nkrastogi@cftri.res.in; nkrastogi@yahoo.com

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pressure processing equipment, the model system (HPP) utilized in the 1890s by Hite was very primitive. Today, with the advancement in techniques like computational stress analysis and new materials, high capacity pressure systems can be manufactured to allow reliable high-pressure treatment of food products at even higher pressures (Hoover, 1993). Experiments into the effects of pressure on microorganisms have been recorded as early as 1884 (Jay, Loessner, & Golden, 2005). In general, high-pressure technology can supplement conventional thermal processing for reducing microbial load, or substitute the use of chemical preservatives (Rastogi, Subramanian, & Raghavarao, 1994). High pressure processing is a natural, environment friendly process with a real alternative to traditional thermal and chemical treatments.

There are two principles applicable to the use of high pressure processing in the food industry. The first is Le Chatelier's Principle, which applies to all physical processes and states that, when a system at equilibrium is disturbed, the system responds in a way that tends to minimize the disturbance (Pauling, 1964). It means with an increase in pressure the volume decreases and vice versa. Second principle is the Isostatic Rule which states that pressure is transmitted instantaneously and uniformly throughout the sample either in direct contact with the pressure medium or hermetically sealed in a flexible packaging material (Olsson, 1995). The pressure applied to food system is transmitted isostatically and instantaneously, irrespective of the shape and size of the food commodity (Smelt, 1998) which offers distinct advantage over conventional thermal processing of large or irregular shaped food products (Kiera et al., 2008).

The fermented food industry is mainly focused on standardizing the properties and extending the shelf life of the product, in order to control the growth of microbes, and to shorten the time-consuming ageing processes required for flavour development. The synergistic effect of high pressure processing and additional hurdles like low pH in case of fermented foods ensures food commodities with better quality attributes and extended shelf life. For example, Bacteriocins work synergistically with HPP inactivating pathogens and increasing their death rate (Galvez, Abriouel, Lucas López, & Omar, 2007). Surviving pressure, cells of the pathogenic bacteria become injured and can be easily inhibited by bacteriocins (Liu et al., 2012). This synergistic action is the basis of the hurdle concept, which implies simultaneous or sequential use of several treatments to achieve product preservation and prolonged shelf life. These treatments include induced changes in aw, pH, temperature and the addition of bacteriocins (Jay et al., 2005). Several reports are available on the use of combination of hurdles with novel techniques like pulse electric field, high pressure processing, irradiation, etc. Hence, an intelligent combination of "hurdles" including non-thermal method of food preservation technique like high pressure processing and competitive microflora may lead to the development of minimally processed foods with added advantage in terms of quality attributes. This approach is described by the phrase "Hurdle technology" (Leistner, 1999; Leistner & Gorris, 1995). The chapter outlines various applications of HPP to extend shelf life of fermented foodproducts.

4.2 Application of HPP for Fermented Food Products

4.2.1 Fermented Meat Products

The meat industry has developed guidelines to control safety of raw meat and other ingredients used in the production of fermented meat products. Some of the measures used to assure the safety of dry fermented sausages involve heating, salting or preservation using vinegar (acetic acid). The use of high temperatures alters the natural characteristics of the product and sensory qualities. Thus, there is a growing need for non-thermal technologies that meet relevant product specifications including improved product safety while maintaining or enhancing sensory characteristics. In HPP, pressure up to 900 MPa is used and its effectiveness on the inactivation of vegetative bacteria in foods has been reported (Aymerich, Picouet, & Monfort, 2008; Considine, Kelly, Fitzgerald, Hill, & Sleator, 2008; Garriga, Grebol, Aymerich, Monfort, & Hugas, 2004; Hugas, Garriga, & Monfort, 2002; Patterson, 2005). Application of HPP in meat processing is widely reported (Considine et al., 2008; Hugas et al., 2002; Patterson, 2005). However, in some cases high-pressure treatment of raw and processed meat can induce lipid oxidation depending on processing parameters of pressure, temperature and time and subsequent storage conditions. Moreover, high pressure can as detrimental as heat treatment in terms of the oxidation level in cold-stored meat products (Simonin, Duranton, & de Lamballerie, 2012). The combination of HPP with biopreservation to enhance bacterial inactivation and reduce the recovery of sublethally injured cells during product storage has been demonstrated in cooked meat products (Aymerich, Jofré, Garriga, & Hugas, 2005; Jofré et al., 2007; Marcos, Jofré, Aymerich, Monfort, & Garriga, 2008).

Shelf-stability of fermented meat products (e.g. sausages) is due to a combination of hurdles, whose interaction inactivates or prevents the growth of undesired microorganisms present in the product (Leistner, 2007). HPP has been reported for various fermented meat products as shown in Table 4.1.

In fermented sausages, high pressure has been proved to be a useful post-process intervention to decrease the levels of several food-borne pathogens (Garriga et al., 2005; Gill & Ramaswamy, 2008; Jofre, Aymerich, & Garriga, 2009; Marcos, Aymerich, Guardia, & Garriga, 2007; Porto-Fett et al., 2010) and has been recognized as a listericidal treatment by FDA and Codex Alimentarius (CAC, 2007; HHS, 2008). Marcos, Aymerich, Garriga, and Arnau (2013) indicated that inactivation of *Enterobacteriaceae* during the whole shelf life of the product could only be prevented with HPP. Pressurization (600 MPa, 5 min) of sliced fermented sausages induced an immediate reduction of *Enterobacteriaceae* counts. Non-pressurized batches showed a 1 log unit reduction of LAB population throughout storage. On the other hand, pressurized batches with or without nisin packed in PVOH films showed reductions of about 2.4 log units at the end of storage. Simon-Sarkadi, Pásztor-Huszár, Dalmadi, and Kiskó (2012) studied the effect of HPP on aerobic plate counts of Hungarian dry fermented sausage during 4 weeks of storage at 8 °C.

Table 4.1 Synergistic effect of HPP and different hurdles on meat products/effect of HPP on various fermented meat products

Product	Conditions	Results	References
Dry-cured ham	HPP: 600 MPa/5 min/15 °C Nisin: 200 AU/cm^2	Increased inactivation of *L. monocytogenes*	Hereu, Dalgaard, Garriga, Aymerich, and Bover-Cid (2012)
Cooked ham	HPP: 400 MPa/10 min/17 °C 1.4 % potassium lactate and 0.1 % sodium diacetate Enterocin: 2400 AU/g	HPP+lactate diacetate: reduced the levels of *L. monocytogenes* during storage at 1 °C by 2.7 log CFU/g; HPP+enterocin: inactivation of *L. monocytogenes* to 4 MPN/g after three months of storage at 1 °C	Marcos et al. (2008)
Sliced cooked ham	HPP: 400 MPa/10 min/17 °C Enterocin: 2560 AU/g	Shelf-life extension to above 90 days	Liu et al. (2012)
Cured pork carpaccio	HPP: 0, 400 and 600 MPa Freezing temperature (−15 °C vs. −35 °C)	HPP in combination with low freezing temperature can be used successfully to deliver high-quality pork carpaccio with extended shelf life	Realini, Guàrdia, Garriga, Pérez-Juan, and Arnau (2011)
Spanish dry sausages (Salchichon)	HPP: 500 MPa/5 min	High-pressure treatment had no noticeable effect on the physico-chemical and sensory properties of the three samples suggesting that it improves the food safety of salchichon with no detrimental effects on organoleptic properties	Rubio, Martinez, García-Cachán, Rovira, and Jaime (2007a)
Reduced fat and sodium low-acid fermented sausage (fuets)	HPP: 600 MPa for 5 min Probiotic strain: *Lactobacillus rhamnosus* CTC1679	The application of HPP at the end of ripening (day 14) produced an immediate reduction of *L. monocytogenes* to levels <1 log CFU/g	Rubio et al. (2014)
Smoked dry-cured ham	HPP: 600 MPa/5 min	Pressurization caused the elimination of *Salmonella* and *L monocytogenes* in ham after 14 days	Stollewerk, Jofré, Comaposada, Arnau, and Garriga (2012a)
NaCl-free processed dry fermented sausages chorizo acid (pH4.8) low-acid (pH 5.2)	HPP: 600 MPa/5 min/13 °C Fast drying	The HPP treatment assured absence of *Salmonella* and *L monocytogenes* in all samples during refrigerated storage for 91 days	Stollewerk, Jofré, Comaposada, Arnau, and Garriga (2012b)
Dry fermented sausage (chorizo)	QDS (Quick-dry-slice), NaCl-free processing, acidification (4.8 and 5.2), smoking and HPP (600 MPa)	Survival of *Listeria monocytogenes* and *Salmonella* was affected by NaCl-free processing, acidification, smoking and pressurization	Stollewerk, Jofré, and Garriga (2013)

Fermented fish sausage	Fast drying	Pressurization had an important reducing effect on technological microbiota, and eliminated *L monocytogenes, S. enterica*, hydrogen sulphite producing bacteria and *coliforms* immediately after production and during refrigerated storage (4 and 8 °C)	Stollewerk, Jofré, Comaposada, Arnau, and Garriga (2014a)
	HPP: 600 MPa/5 min/13 °C		
Dry-cured ham	NaCl-free processing, acidification, smoking, HPP (600 MPa)	The safest hams were those pressurized, especially AS-S hams, where *L. monocytogenes* was eliminated from 25 g of product immediately after HPP treatment and *S. enterica* after 14 days	Stollewerk, Jofré, Comaposada, Arnau, and Garriga (2014b)
Convenience fermented sausage	HPP: 600 MPa/5 min/12 °C	Combination of HPP with antimicrobial packaging did not produce any extra protection against *L. monocytogenes* compared to antimicrobial packaging alone. The lack of effect of HPP on L. monocytogenes was attributed to a protective effect exerted by the low water activity of the product and its lactate content	Marcos et al. (2013)
	PVOH films containing nisin		
Spanish dry fermented sausage “chorizo”	HPP: 350 MPa/15 min/20 °C	Lower levels of tyramine were observed in chorizo and cooked white sausage “butifarra” treated with HP. High concentrations of agmatine were detected in pressurized chorizo	Ruiz-Capillas, Jiménez Colmenero, Carrascosa, and Muñoz (2007)
Restructured dry-cured hams	Potassium lactate and HPP (600 MPa)	The HPP treatment increased significantly the pH, L*, a* and b* values and the breaking stress, and decreased the water-holding capacity and elasticity (apparent Young’s modulus) of biceps femoris (BF) muscle	Fulladosa, Serra, Gou, and Arnau (2009)
Genoa salami	Combined effect of fermentation, drying and HPP (600 MPa/ 0, 1, 2, 3, 5, or 7 min, 483 MPa/ 0, 5, 7, 10, or 12 min)	After storage for 28 days at 4 °C, *L. monocytogenes* levels decreased by up to an additional 3.0 log CFU/g, whereas an additional decrease of up to about 1.1 and 1.7 log CFU/g was observed in case of *E. coli* O157:H7 and *Salmonella*, respectively	Porto-Fett et al. (2010)
Low-acid fermented sausage	HPP: 600 MPa/5 min, bioprotective cultures (*Enterococcus faecium* CTC8005, *Enterococcus devriesei* CTC8006 and *Enterococcus casseliflavus* CTC8003)	The application of high hydrostatic pressure (HHP) treatment (600 MPa for 5 min) at the end of ripening (day 21) promoted a decrease of 1 log unit in the counts of *S. aureus. E. faecium* CTC8005, which reduced the counts of *L. monocytogenes* ca. 2 log cfu/g immediately after stuffing and in combination with HHP treatment promoted a further reduction of 1 log cfu/g in the pathogen counts	Rubio, Bover-Cid, Martin, Garriga, and Aymerich (2013)

(continued)

Table 4.1 (continued)

Product	Conditions	Results	References
Low-Acid Fermented Sausages (fuet and chorizo)	HPP: 300 MPa/10 min/17 °C	*Salmonella* counts decreased in all studied sausages during ripening at 12 °C and 80 % RH for 27 days. However, the application of HPP as an additional hurdle to the ripening process produced a greater decrease in the *Salmonella* population, showing lower counts (3 MPN/g) in ripened sausages	Marcos, Aymerich, and Garriga (2005)
Norwegian type dry fermented sausages	HPP:600 MPa/10 min 600 MPa/200 s per cycle	The treatment resulted in reduction in *E. coli* by 2.9 $\log_{10}$ CFU/g and 3.3 $\log_{10}$ CFU/g, respectively	Omer et al. (2010)
Fuet	HPP:400 MPa single and combined enterocin AS-48	Drastic 5.5 log cfu/g decrease in *L. monocytogenes*	Ananou et al. (2010)
Fuet and chorizo	HPP:400 MPa /10 min/17 °C starter culture (*Lactobacillus sakei* CTC6626 and *Staphylococcus xylosus* CTC6013)	Sensory analysis showed no differences between lots of chorizo whereas starter fuet was more acid and gummy. High pressure induced an additional reduction of *Enterobacteriaceae* in non-starter sausages. An increase of textural properties was observed after pressurization	Marcos et al. (2007)
Spanish dry fermented sausage “salchichon”	HPP (400 MPa/10 min/12 °C)	Pressure-treated samples showed significantly higher levels of alcohols, aldehydes and alkanes and lower levels of two methyl ketones as compared with control samples	Rivas-Canedo et al. (2009)
Fuets	HPP: 400 MPa Enterocins producing culture: *E. faecium* CTC492	Application of HPP treatment (400 MPa) resulted in reduction of Salmonella less than 1 log CFU/g in all the batches but combination of enterocins and HHP could only reduce the counts of *L. monocytogenes* during storage of the low-acid fermented sausage (fuets) at room temperature and at 7 °C	Jofre, Garriga, and Aymerich (2008)
Spanish fermented sausages (fuet and chorizo)	HPP: 200 MPa/10 min/17 °C Starter culture: *Lactobacillus sakei* (CTC6469 and CTC6626) *Staphylococcus xylosus* (CTC6013 and CTC6169)	HPP prevented *enterobacteria* growth but did not affect Gram-positive bacteria significantly. Subsequently, a strong inhibition of diamine (putrescine and cadaverine) accumulation was observed, but that of tyramine was not affected	Latorre-Moratalla et al. (2007)
Dry-cured ham	**600 MPa /6 min/16** °C	**Absence of *L. monocytogenes* during storage period (120 days)**	**Hereu et al.** (2012)
Sliced beef cured ham	500 MPa/5 min/18 °C	Reduction of *L. monocytogenes* 2 log after 210 days (6 °C)	Rubio, Martínez, García-Cachán, Rovira, and Jaime (2007b)

Salami	483 or 600 MPa/1–12 min	Reduction of *L. L.monocytogenes* numbers by an additional 1.6 to ≥5.0 log CFU g^{-1} when compared to their levels after fermentation and drying; *L. monocytogenes* levels decreased to an additional 3.0 log cfu g^{-1}, during 28 days, under storage at 4 °C	Porto-Fett et al. (2010)
Sliced cooked ham	600 MPa/5 min/10 °C	Reduction of *L. monocytogenes* to levels below 10 cfu g^{-1}	Jofre et al. (2008)
Dry-cured ham	600 MPa/5 min/15 °C	3.5 log inactivation of *L. monocytogenes*	Hereu et al. (2012)
Sliced cooked ham	500 MPa/10 min/25 °C	5 log inactivation of *L. monocytogenes*	Koseki, Mizuno, and Yamamoto (2007)
Turkey breast and ham	600 MPa/3 min/17 °C	3.85–4.35 log reduction in *L. monocytogenes*; during storage remained below detection limit during 154 days	Myers et al. (2013)
Cooked ham	400 MPa/10 min/17 °C	1.9 log reduction *L. monocytogenes* (42 days)	Aymerich et al. (2005)
Dry-cured ham	HPP: 600 MPa/5 min/15 °C Nisin: 200 AU cm^{-2}	Increased inactivation of *L. monocytogenes*	Hereu et al. (2012)
Blood sausages (morcilla de Burgos)	HPP: 600 MPa/10 min	HPP improved the shelf life of morcilla de Burgos to 28 days in comparison with control samples. *Pseudomonas spp.*remained under detection level (b102 CFU/g) after the HPP treatment	Livia, Pásztor-Huszár, Dalmadi, and Kiskó (2012)
Hungarian dry fermented sausages	HPP: 500 MPa/10 min	HPP treatment improved the microbial quality of the sausages and it was effective in the reduction of BA formation during storage (8 °C) for 1 month	Simon-Sarkadi et al. (2012)
Frankfurters	400 MPa, 30 °C, 10 min	Total viable count: 2.16 immediately after processing	Ruiz-Capillas et al. (2007)
Dry-cured ham	Bacteriocin-producing starter cultures and HPP	Shorten drying time	Arnau, Serra, Comaposada, Gou, and Garriga (2007)
Dry-cured fermented ham	400–600 MPa/1.5–20 min	>5 log reduction of *L. innocua* at 600 MPa/20 min, without organoleptic changes	Křepelková and Sovják (2011)

The total viable counts of control samples of semi-dry sausage and extra thick sausage reduced after cold storage, which was followed by regrowth to about 7.5 log CFU/cm^3 within 4 weeks. Treatment with 500 MPa HPP reduced the total viable count by 1–3 log depending on sample type. This difference was more pronounced by the end of storage in case of semi-dry sausage (3 log) and extra thick sausage (5 log). However, significant changes occurred in the organoleptic characteristics of semi-dry sausage. Texture seemed to be the most sensitive to high pressure treatment caused significant changes in firmness of dry and semi-dry sausages, and both samples became softer as a result of pressurization. Smell, firmness and taste of extra thick sausage remained practically unchanged after pressure treatment.

Ananou et al. (2010) demonstrated that in ripened control fuets (a dry-cured sausage), HPP had a low effect on the viability of *Staphylococci* at room or refrigerated storage temperatures. The application of HPP treatment caused an immediate reduction of 1.06 log units in LAB population. However, viable counts recovered to similar values to those of the controls at 18 days storage (at 7 °C or room temperature). Until the end of storage, no significant differences in lactic acid bacteria or *staphylococci* counts were detected between the different types of fuets investigated. However, Rubio, Martín, Aymerich, and Garriga (2014) indicated that the application of high-pressure treatment (600 MPa for 5 min) at the end of ripening (day 14) produced an immediate reduction of *L. monocytogenes* to <1 log CFU/g, which was not detected after 35 days of storage at 4 °C.

Significant changes in volatile profile of high pressure processed fermented meat products have been reported. For example, Rivas-Canedo, Nunez, and Fernández-García (2009) investigated the changes in the volatile profile of Spanish dry fermented sausage "salchichon" when subjected to HPP (400 MPa, 10 min at 12 °C). HPP-treated samples had higher levels of aldehydes (both linear and branched chains), alcohols and some compounds e.g. 1-methoxy-2-propanol, carbon disulfide and benzaldehyde. Similar changes in volatile profile of pork meat have been reported for high-pressure processed samples (Rivas-Canedo, Ojeda, Nuñez, & Fernández-García, 2012).

Changes in biogenic profile of high-pressure processed fermented meat samples have been reported extensively. For example, Latorre-Moratalla et al. (2007) investigated the effects of high hydrostatic pressure (200 MPa) on meat batter just before sausage fermentation and the inoculation of starter culture to improve the safety and quality of traditional Spanish fermented sausages (fuet and chorizo). In batches without starter culture and no HPP treatment biogenic amine accumulation was much lower in fuet compared to chorizo sausages. Tyramine was the only biogenic amine detected in fuet, whereas cadaverine was the major amine in chorizo sausages, which is usually associated with lysine-decarboxylase activity of undesirable Gram-negative bacteria. In spontaneously fermented sausages, the application of HPP resulted in a strong inhibition of diamine accumulation, the levels of putrescine and cadaverine being up to 88 and 98% lower than the non-pressurized batch as shown in Fig. 4.1.

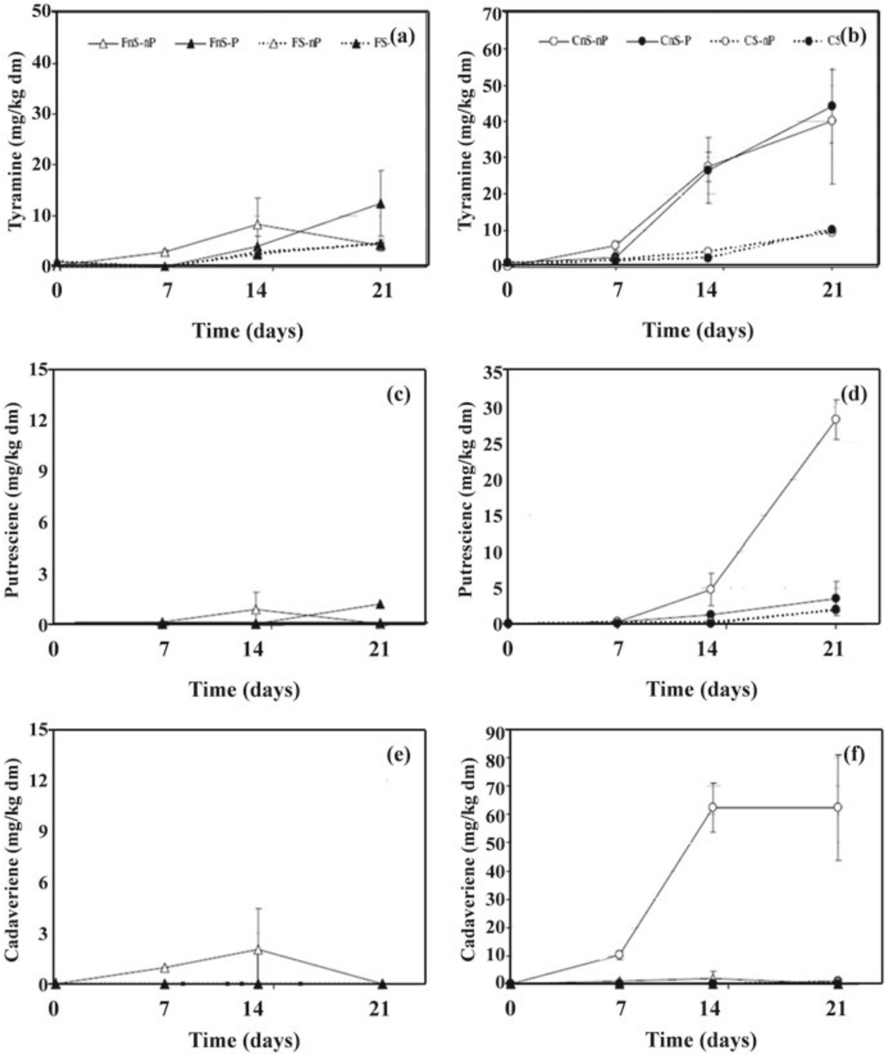

Fig. 4.1 Variation in biogenic amine contents during the manufacture of fuet (**a**, **c**, **e**, *left column*) and chorizo (**b**, **d**, **f**, *right column*) through spontaneously (nS) and starter (S) mediated fermentation, without (nP) and with (P) high hydrostatic pressure treatment (From Latorre-Moratalla, M.L., Bover-Cid, S., Aymerich, T., Marcos, B., Vidal-Carou, M.C., Garriga, M. 2007. *Meat Science*, 75: 460–469. With permission)

4.2.2 Fermented Dairy Products

The dairy industry is focusing on standardizing the properties and extending the shelf life of the milk products. Today, there has been a strong demand in the consumption of probiotic bacteria using food products, including probiotic dairy products due to increasing consumer awareness about the impact of food on health.

Fermented milk is the most popular and most consumed probiotic food carrier throughout the world. Several studies about the development of probiotic fermented milks (Almeida et al., 2008; Kearney et al., 2009; Oliveira, Perego, Converti, & Oliveira, 2009) and probiotic fermented beverages (Castro, Cunha, Barreto, Amboni, & Prudêncio, 2009; Zoellner et al., 2009) have been reported. Pasteurization of milk for the destruction of pathogenic microorganisms and to kill the natural microflora has been traditionally carried out by heat treatment. The potential of HPP treatment as an alternative method to heat pasteurization of milk was proposed almost a century ago (Hite, 1899) and has been investigated for a range of dairy products Apart from pasteurization effects, HPP provides new opportunities for homogenization effects. Table 4.2 summarizes various studies highlighting the application of HPP in

Table 4.2 Synergistic effects of HPP and different hurdles on dairy products

Product	Conditions	Results	References
Raw-milk cheese	300 MPa/10 min/10 °C 500 MPa/5 min/10 °C Bacteriocin:10^6 CFU/mL	*E.coli* O157:H7 counts to levels below 2 log units in 60-day-old cheeses	Rodriguez, Arques, Nunez, Gaya, and Medina (2005)
Milk	250–350 MPa/5 min/20 °C Nisin: 0–500 IU/mL	Treatment at 400 MPa for 5 min with 500 IU/ml nisin, or at 250 MPa for 5 min with 500 IU/mL nisin, gave *a*>8 log reduction of *E. coli* and *P. fluorescens*, respectively	Black, Kelly, and Fitzgerald (2005)
Yoghurt	Supplemented Probiotic bacteriae *Lactobacillus Acidophilus* and *Bifidobacterium* spp., using HP processing	Cultures maintained populations of 10^6–10^7 log CFU/g during a 4 week storage at 4 °C	Jakowska, Wisniewska, and Reps (2005)
Probiotic Fermented milk	HPH: (60 MPA, one homogenized stage alone or combined with 90 °C/30 min heat treatment) *Probiotic bacteria:L. paracasei* and *L. acidophilus*	Survival of cultures 7.0 log CFU/g	
Blackberry yoghurt	HPP: 550 MPa/10 min/4 °C	The treatment with HPP inactivated yeasts and moulds and no growth was observed following prolonged storage at 25 °C	Walker et al. (2005)
Probiotic low-fat yoghurt	HPP:676 MPa/5 min Thermal treatment:85 °C/30 min	The combined HPP and heat treatment of milk before fermentation, and after inoculation of 0.1 % (for both cultures), resulted in a creamy and thick consistency texture which does not require the addition of stabilizers	Penna et al. (2007)

(continued)

Table 4.2 (continued)

Product	Conditions	Results	References
Milk	HPP:345 MPa Bacteriocin (5000 AU/mL sample)	Combination of bacteriocin and HPP treatment resulted in more than 8 log cycle reduction in cell population of *S. aureus* and *L. monocytogenes* was achieved in milk samples stored at 25 °C upto 30 days	Alpas and Bozoglu (2000)
Yoghurt	HPP: 400–600 MPa	Yoghurt processed at a pressure of 550 MPa maintained its beneficial sensorial characteristics longer than the non-pressurized yoghurt, during storage for 4 weeks at refrigerating (4 °C) and room (20 °C) temperatures. The pressure prevented the post-acidification in the yoghurt	Jakowska et al. (2005)
Yoghurt	HPH: 300-750 bar	HPH milk treatment enhanced the viability of *S. thermophiles* and *L. delbrueckii spp. bulgaricus* during refrigerated storage of yoghurt and favoured the growth of the former, compared to that of the latter, thus reducing the risk of post-acidification	Lanciotti, Vannini, Pittia, and Guerzoni (2004)
Goat's milk cheese	HPP: 50 MPa/72 h, 400 MPa/5 min and 400 MPa/5 min followed by 50 MPa/72 h all at 14 °C	By measuring proteolysis indexes, 400 MPa treatments were found to accelerate ripening (14 days in contrast to 28 days conventionally) due to enhanced enzyme activity from inoculated starter culture. pH was higher in 400 MPa treatments compared to the other treatments	Saldo, Sendra, and Guamis (2000)
Goat's milk cheese ripening	HPP: 500 MPa at 20 °C for 15 min	Organic acid levels of cheese made with pressure-treated goat's milk rose gradually for 60 days	Buffa et al. (2001)
Curd formation and firming	HPP: 100–800 MPa/0–60 min	High-pressure treatment up to 250 MPa did not result in any change in curd yield but the moisture content decreased by 5 % in comparison with untreated milk	Huppertz, Fox, and Kelly (2004)

(continued)

Table 4.2 (continued)

Product	Conditions	Results	References
Gorgonzola cheese rinds	400–700 MPa/1–15 min	High-pressure treatment was effective in reducing of *L. monocytogenes* on Gorgonzola cheese rinds without significantly changing its sensory properties	Carminati et al. (2004)
Fermented milk	HPP: 300 and 600 MPa/5 min and pH (4.30, 5.20 and 6.50)	The rate of proteolysis in milk samples at pH values of 5.20 and 6.50 during storage was significantly reduced by treatment at 600 MPa. Treatment at 600 MPa also reduced the viable counts of both Candida yeast species to below the detection limit (1 CFU mL^1) at all pH levels for the entire storage period	Daryaei, Coventry, Versteeg, and Sherkat (2010)
Goat milk cheese	HPP: 400 MPa or 600 MPa/7 min)	Treatments at 600 MPa at the three stages of cheese maturation decreased the counts of undesirable microorganisms in mature Ibores cheese (day 60), such as psychrotrophic bacteria, *Enterobacteriaceae* and *Listeria* spp. Mature cheeses (day 60) pressurized at the beginning of ripening showed a higher variation of texture profile analysis. In the sensory analysis, cheeses treated at day 1 showed a significant change of appearance, odour and texture	Delgadoa, González-Crespoa, Cava, and Ramírez (2012)
Starter-free fresh cheese	500 MPa (5 min,16 °C)	The results showed that pressurized cheeses presented a shelf life of about 19–21 days when stored at 4 °C, whereas control cheese became unsuitable for consumption on day 7–8. On the other hand, cheese treated at 500 MPa was firmer and more yellow than the untreated one	Evert-Arriagada et al. (2014)

(continued)

Table 4.2 (continued)

Product	Conditions	Results	References
Fresh cheese	HPP: 300 and 400 MPa for 5 min at 6 °C	Cheeses treated at 300 and 400 MPa, stored at 4 °C, presented a shelf life of 14 and 21 days, respectively, compared to untreated control cheese, which presented a shelf life of 7 days. On the other hand, HP treatments modified the texture (more firm) and colour (more yellow) compared to control cheeses	Evert-Arriagada et al. (2014)
Rennet-coagulated soft cheese	HPP: 291 MPa/29 min	Fat content increased apparently as moisture decreased significantly after HP treatment of above 100 MPa. Increased pressures reduced lipid oxidation but increased yellowness although the latter showed more effect over redness in the HP-treated fresh cheese. Also, increased pressures increased hardness and decreased acidity and adhesiveness in HP-treated fresh cheese	Okpala et al. (2010)
Stirred yoghurt from reconstituted skim milk	HPP treated at 100, 250 or 400 MPa, at 25, 70 or 90 °C, for 10 min prior to starter culture inoculation	HP treatment of skim milk at 25 °C, before or after heat treatment, gave stirred yoghurts of similar viscosities to that made from conventionally heat-treated milk. Lower viscosities were obtained when stirred yoghurts were made with milk HP-treated at elevated temperatures	Udabage et al. (2010)
Reconstituted cow milk	HPP: 200 and 600 MPa, 0 and 25 min, and heat treatment 15 °C and 65 °C, respectively	Particle size distribution of the heat-assisted high-pressure treated milk showed that the number of fat globules below 1×10^{-6} m increased as pressure and pressurization time increased	Sahu and Mallikarjunan (2012)
Hispánico cheese	HPP: 200 and 300 MPa	The use of ewe raw-milk curd pressurized at 200 and 300 MPa, stored frozen and thawed for Hispánico cheese manufacture had increased cheese yield because of the lower dry matter content	Alonso, Picon, Gaya, Fernández-García, and Nuñez (2012)

(continued)

fermented dairy products. Patrignani et al. (2007) evaluated the effects of various factors like milkfat content, non-fat milk solids content, and high-pressure homogenization on rate of fermentation of the probiotic strain *Lactobacillus paracasei* BFE 5264 inoculated in milk. Loss of *Lactobacillus paracasei* strain during refrigerated storage, texture parameters, volatile compounds and sensorial properties of the coagula obtained were investigated. Patrignani et al. (2007) observed significant effect of independent variables on the measured attributes of fermented milks. The coagulation times were significantly affected by pressure and added milk fat, and the rheological parameters such as firmness, viscosity index and consistency of the fermented milk increased with the pressure applied to the milk. Rodriguez-Alcala et al. (2015) indicated that neutral and polar lipids remained stable in the pressure treated sample (250–900 MPa).

Consumer demand for shelf-stable yoghurt is difficult due to problem of storing live microflora at ambient conditions. The use of heat treatments for extension of shelf life of yoghurt has resulted in syneresis and a decrease in viscosity during storage. The application of hydrostatic pressure directly to yoghurts has been proposed as an alternative to the use of additives, which can adversely affect the yoghurt taste, flavour, aroma and mouthfeel (De Ancos, Cano, & Gomez, 2000). Penna, Rao-Gurram, and Barbosa-Cánovas (2007) investigated the effect of HPP on microstructure of low-fat probiotic yoghurt. Microstructure of HPP (C and D) processed yoghurt has more interconnected clusters of densely aggregated protein of reduced particle size, on the other hand heat-treated (A and B) milk yoghurt had fewer interconnected chains of irregularly shaped casein micelles (Fig. 4.2).

Cheese is one of the most versatile dairy products available and consumed in various forms. Application of HPP has shown to improve shelf life and safety profiles of cheese while inducing desirable changes in techno-functional and organoleptic properties. Evert-Arriagada, Hernández-Herrero, Guamis, and Trujillo (2014) demonstrated the commercial application of HPP to increase the starter-free fresh cheese shelf life. High-pressure treatment of cheese did not affect panellists' preference for treated cheese over the non-treated cheese. Moreover, the preference mean score (6.5) for the pressurized cheeses stored during 22 days and for the freshly made cheese was almost the same. Bermúdez-Aguirre and Barbosa-Cánovas (2012) studied fortification of mozzarella cheese using selected sources of omega-3 and nonthermal approaches (PEF and HPP). The growth of coliforms was delayed using thermal pasteurization and pressurization of milk; however, PEF did not delay the growth of coliforms, showing counts around 2.8 log after 8 days of storage. The mesophiles counts of cheeses processed with PEF and thermal treated milk were close to 7 log, while the pressurized milk showed a lower microbial growth of 5.8 log at the end of storage of 12 days. Similar profiles for psychrophiles were observed during storage, with HPP being the most effective treatment for milk processing before cheesemaking. Lopez-Pedemonte, Roig-Sagués, Lamo, Gervilla, and Guamis (2007) demonstrated reductions in *Staphylococcus aureus* counts on HPP treatments (300, 400 and 500 MPa at 5 °C and 20 °C). While reductions obtained for HPP treatments at 5 °C differed significantly between 300, 400 and 500 MPa in both *Staphylococcus aureus* strains, for 20 °C HPP treatments differences only became

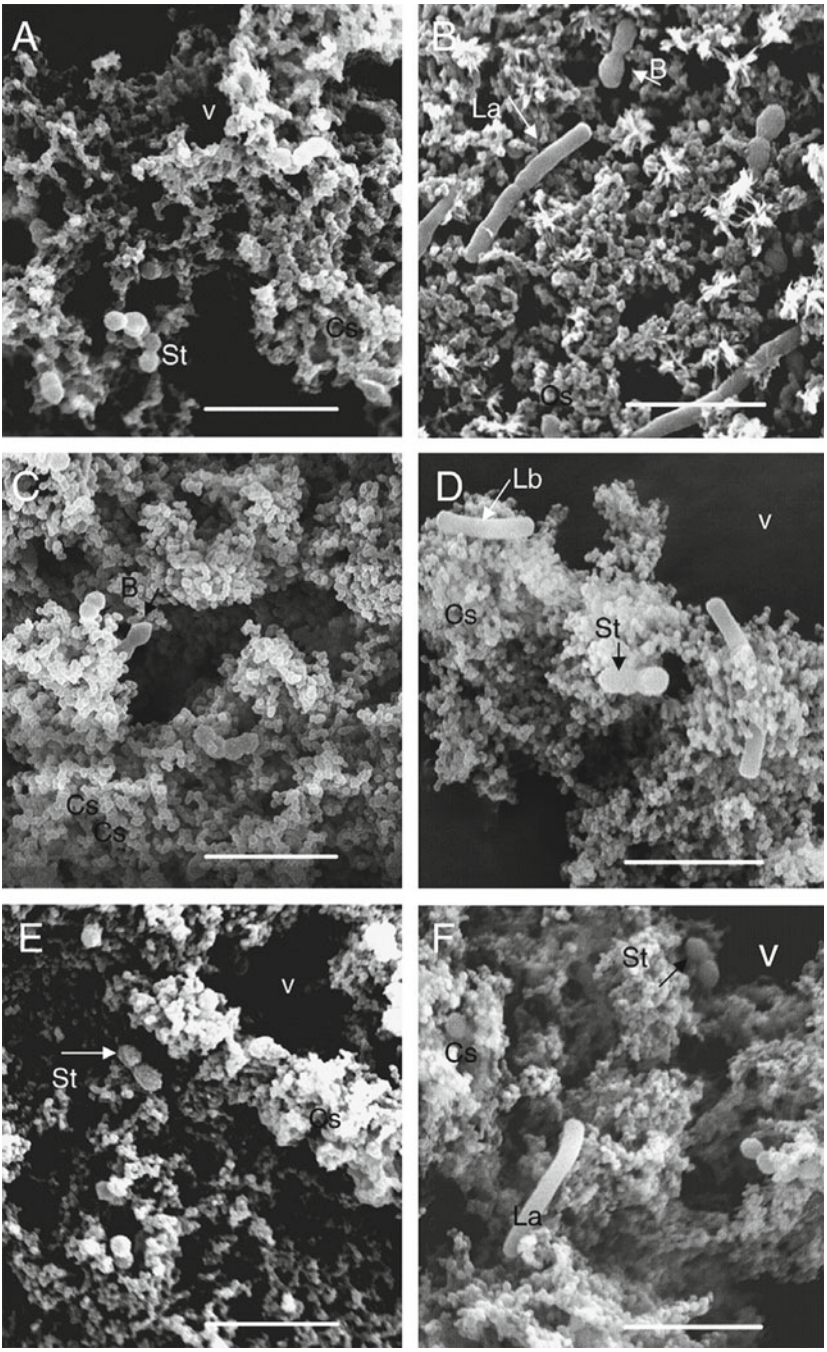

Fig. 4.2 Scanning electron microscopy micrographs of yoghurt fermented with starters YO MIX 236 (**a**, **c**, **e**) and DPL ABY 611 (**b**, **d**, **f**) with different treatments: (**a**, **b**) heat, (**c**, **d**) HPP, (**e**, **f**) heat + HPP. St, *Streptococcus thermophilus*; Lb, *Lactobacillus delbrueckii ssp. bulgaricus;* La, *Lactobacillus acidophilus*; B, *Bifidobacterium longum*; v, void space; cs, casein (From Penna, A.L.B., Subbarao-Gurram, Barbosa-Canovas, G.V. 2007. *Food Research International*.40: 510–519. With permission)

significant after applying 500 MPa pressure. Similarly, Lopez-Pedemonte, Roig-Sagues, Lamo, Hernandez-Herrero, and Guamis (2007) indicated that counts of control samples for both strains of *L. monocytogenes* did not significantly differ during storage. In contrast, counts of all treated samples diminished with storage time.

Serrano, Velazquez, Lopetcharat, Ramirez, and Torres (2005) indicated that short and moderate hydrostatic pressure (MHP) treatments accelerated the shredability of Cheddar cheese. Both MHP (345 MPa for 3 and 7 min) and higher pressure (483 MPa for 3 and 7 min) treatments applied to 1 day milled curd cheddar cheese induced a microstructure resembling that of ripened cheese as shown in Fig. 4.3. The reduction in the amount of crumbles as well as increase in desirable physical properties such as surface smoothness and shred mean length and uniformity in pressure-treated samples was reported. Further, the pH of the control cheese samples increased during storage. During storage, pH fluctuated and the pH difference on day 27 was lower than initial pH difference (day 0) but remained higher for pressure-treated samples with 483 MPa>345 MPa>control. Dhakal et al. (2014) demonstrated that in case of high pressure treatment (450 MPa and 600 MPa) of

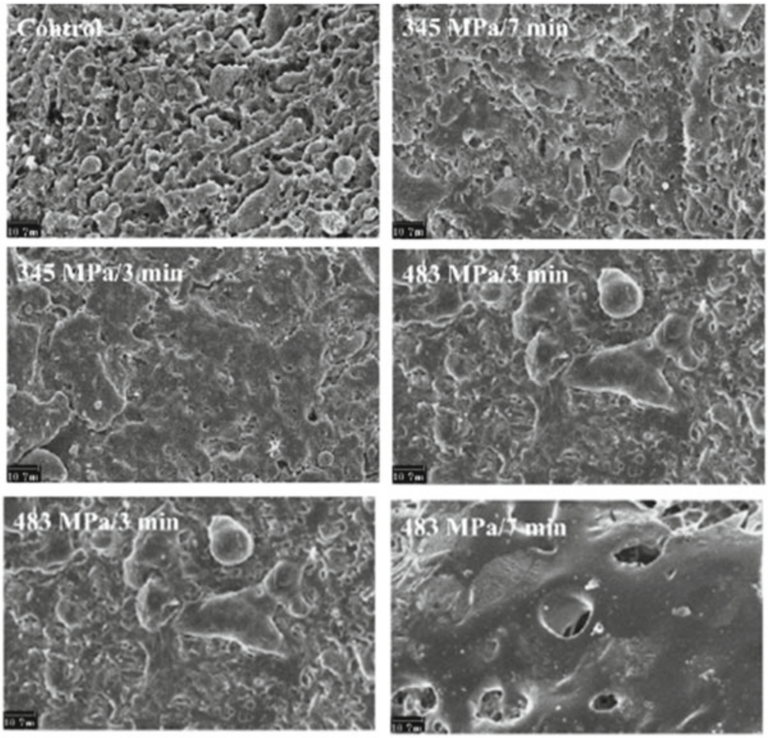

Fig. 4.3 Scanning electron microscope micrographs of control (**a**) and pressure-treated (**b**–**f**) milled curd cheddar cheese (From Serrano, J., Velazquez, G., Lopetcharat, K., Ramirez, J.A., Torres, J.A. 2005. *J. Food. Sci*. 70 (4): 286–293. With permission)

almond milk, the amandin content, which is the major almond allergen could no longer be detected, whereas, thermally processed samples did not show significant reductions unless the samples were treated at a temperature higher than 85 °C.

4.2.3 Alcoholic Beverages

Currently a range of food products such as fruit juices, sea foods and meat products can be found on market shelves all around the world (Matser, Krebbers, van den Berg, & Bartels, 2004). However, few data have been reported about the use of HPP for beer and wine. Although, rice wine (nigori sake) is one of the earliest HHP-treated commercial products that appeared on the Japanese market (Suzuki, 2002), no HHP-treated alcoholic beverage such as beer and wine is introduced to date.

Wine cannot be treated with heat since its characteristics such as flavour, taste and colour are very sensitive to temperature (Mermelstein, 1998). Therefore, a common practice is the addition of sulphur dioxide (SO_2) to wine to reduce the microbial population of the grape must and to preserve the final product for long period of time (Ribéreau-Gayon, Dubourdieu, Donèche, & Lonvaud, 2006). SO_2 acts as an antimicrobial agent and antioxidant in wine (Amerine, Berg, & Cruess, 1967; Romano & Suzzi, 1993). However, SO_2 may have negative effects on human health (Romano & Suzzi, 1993). Therefore, wine industry is challenged to meet consumers' demands of reducing the levels of SO_2 used in wine production (Du Toit & Pretorius, 2000). Tabilo-Munizaga et al. (2014) studied the effects of high hydrostatic pressure (HPP 400–500 MPa for 3–10 min) on the protein structure and thermal stability of Sauvignon blanc wine. It was observed that higher thermal stability and major structural changes observed in wine proteins were obtained by 450 MPa pressure for 3 and 5 min. With this pressure–time combination, the structural conformations achieved by the wine proteins could provide higher thermal stability and thus delay haze formation in wine during 60 days storage. Santos et al. (2013) studied the effect of high-pressure treatments on the physico-chemical properties of a sulphur dioxide-free red wine. The wine pressurized at 500 MPa presented more scents of cooked fruit and spicy aroma. The untreated wines presented less perceived fruity and floral aroma and had a more pronounced metallic and leather aroma than the other wines. Comparing the taste assessment of the different wine samples, the pressurized wines presented a similar taste assessment than the wine with SO_2. The untreated wines showed a higher acidity and lower balance. In terms of colour, the pressurized wines presented higher values of brown and limpidity and lower values of violet than unpressurized wines. After 12 months of storage, pressurized wines showed a better sensorial assessment, with the pressure treatments imparting aged-like characteristics to the wines. The results demonstrated that HPP can influence long-term red wine physico-chemical and sensorial characteristics. HPP results in an increase of condensation reactions of phenolic compound. The compounds formed with higher degree of polymerization become insoluble in wine along storage. Mok et al. (2006) studied the pasteurization of low-alcohol red wine (ethanol 9 %, pH 3.27, acidity 0.068 %, total sugar 0.85 %) using HPP (100–350 MPa for 0–30 min at 25 °C). Corrales, Butz, and Tauscher (2008) applied HPP to wine from the dornfelder grape variety. A decrease in the concentration of malvidin-3-*O*-glucoside in pressurized (600 MPa at 70 °C for 1 h) samples was detected. Wherein, wine subjected to pressure treatment (600 MPa, 70 °C for 10 min) exhibited no significant differences in anthocyanin composition and antioxidant activity.

The aerobic bacteria decreased below the detection limit after 20 min pressurization at 300 MPa and 10 min pressurization at 350 MPa as well as initial yeast count (5.46 log) decreased to 2.46 and 1.15 log after HPP treatment at 300 MPa for 10 and 20 min, respectively. Puig, Vilavella, Daoudi, Guamis, and Minguez (2003) investigated the microbiological and biochemical stabilization of wines by use of HPP. A white wine (with 40–50 mg L^{-1} of total SO_2) and a red wine (with 80–90 mg L^{-1} of total SO_2) were used. Two yeasts: *S. cerevisiae* and *Brettanomyces bruxellensis* (107 CFU L^{-1} of each wine), two lactic acid bacteria: *L. plantarum* and *Oenococcus oeni* (109 CFU L^{-1} of each wine), and two acetic acid bacteria: *A. aceti* and *A. pasteurianus* (109 CFU L^{-1} of each wine) were inoculated into wines. HPP treatments were done at 400 or 500 MPa for 5 or 15 min with 4 °C or 20 °C of temperature. HPP treatments resulted 6 log reduction for yeasts and 8 log reduction for bacteria.

Commercially, beer is pasteurized to guarantee microbiological stability during its shelf life (Zufall & Wackerbauer, 2000). However, heat can cause protein denaturation, promoting the formation of new tannin-protein complexes, with consequent turbidity enhancement (Stewart, 2006). In addition, heat processing promotes the Maillard reaction, resulting in an alteration of beer colour to reddish (Castellari, Arfelli, Riponi, Carpi, & Amati, 2000) and the formation of undesirable flavours (Stewart, 2006). These are related to oxidation and staling, with the development of off-flavours (long chain aldehydes) (Zufall & Wackerbauer, 2000). HPP may be used to increase the shelf life of special quality beers without altering the original characteristics of the untreated product and without heat treatments or filtration. Thus, HPP could be an alternative to the conventional pasteurization of beer. The first trial of HPP on brewing process of beer was carried out by Fischer, Schöberl, Russ, and Meyer-Pittroff (1998). Fischer et al. (1998) concluded that HPP treatment (300–700 MPa, 5 min) of bright lager beer samples packed in polyethylene naphthalate bottles did not significantly change the colour, foam durability and the spectrum of flavour materials. In contrary, Perez-Lamela, Reed, and Simal-Gándara (2004) indicated that HPP treatment (300–600 MPa, 20 min) of beer resulted in an increase in the foaming and haze characteristics of the beer. Gänzle, Ulmer, and Vogel (2001) investigated the effect of ethanol and hop extract on inactivation of *L. plantarum* in model beer system during and after HPP treatment. Addition of ethanol and hop extract accelerated HHP inactivation of *L. plantarum* in model beer. Buckow, Heinz, and Knorr (2005) investigated the combined effect of HPP (0.1–900 MPa) and temperature (30–75 °C) on the activity of β-glucanase from barley malt. Thermostability of β-glucanase was found to be highest at 400 MPa. Wherein, at temperatures above 55 °C and ambient pressure, as well as at (900 MPa, 40 °C), the inactivation of β-glucanase was higher resulting in complete loss of enzyme activity in less than 30 min.

Effect of HPP on quality parameters of lager beer was studied by Buzrul, Alpas, and Bozoglu (2005a). Unpasteurized lager beer samples from a commercial brewery were treated either by HPP (200–350 MPa, 3 and 5 min, 20 °C) or by conventional heat pasteurization (60 °C for 15 min). The colour, protein sensitivity and chill haze values increased as the pressure and pressurization time increased. Change in bitterness was higher in conventional heat pasteurization, but pressures up to 300 MPa had no significant effect on bitterness. In another study Buzrul, Alpas, and Bozoglu (2005b) investigated the effects of HPP on shelf life of lager beer. Filtered bright lager beer samples were either treated with HPP (350 MPa, 3 and 5 min, 20 °C) or by conventional heat pasteurization (60 °C for 15 min). However, HPP-treated samples had lower bitterness and protein sensitivity and higher chill haze values than the heat pasteurized samples at the end of the storage period. Fischer et al. (2006) examined the effect of pH (4.0, 5.0, 6.0 and 6.5) and HPP treatment (300 MPa, 20 °C for various holding times up to 120 min) on *L. plantarum* (moderately hop tolerant) in model beer system. It was observed that inactivation was more effective at lower pH values, where up to 5 log reductions could be achieved.

4.2.4 Bakery Products

Different scientific reports described the effect of HPP on specific cereal components properties or model systems, namely starch and gluten (Apichartsrangkoon et al., 1998; Gomes et al., 1998; Kieffer et al., 2007). HPP induced starch gelatinization, following different mechanism than thermally induced gelatinization (Gomes et al., 1998). HPP treatment provokes swelling of starch but keeping granule integrity; as a consequence HPP-treated starches modify their microstructure and rheological properties in a different way than thermally treated ones (Gomes et al., 1998; Stolt et al., 2000).

Scanning electron microscopy was used to determine the effect of the HPP on dough microstructure. Scanning electron micrographs of wheat doughs treated at 50, 150 and 250 MPa for 4 min are showed in Fig. 4.4. Untreated wheat dough was characterized by having a continuous structure with the intact starch granules embedded in the matrix structure of proteins and soluble solutes. Dough treated at pressure of 50 and 150 MPa showed well-defined starch granules with diverse size, and the surrounding structures (mainly of protein nature) were progressively reduced, being confined in the case of 150 MPa to agglomerates of starch granules. Drastic changes were observed in dough treated at 250 MPa where starch granules as individual structures disappeared adopting a discontinuous film-like organization similar to what happened after swelling and gelatinization (Barcenas, Altamirano-Fortoul, & Rosell, 2010). Barcenas et al. (2010) reported that the treatment of wheat dough with HPP induced a rapid reduction of the microbial population but sufficient mould and yeast survival, for ensuring bread dough fermentation at moderate pressure conditions (50–250 MPa, for 2 min at 20 °C). This study suggests that high hydrostatic processing in the range 50–200 MPa could be an alternative technique for obtaining novel textured cereal based products.

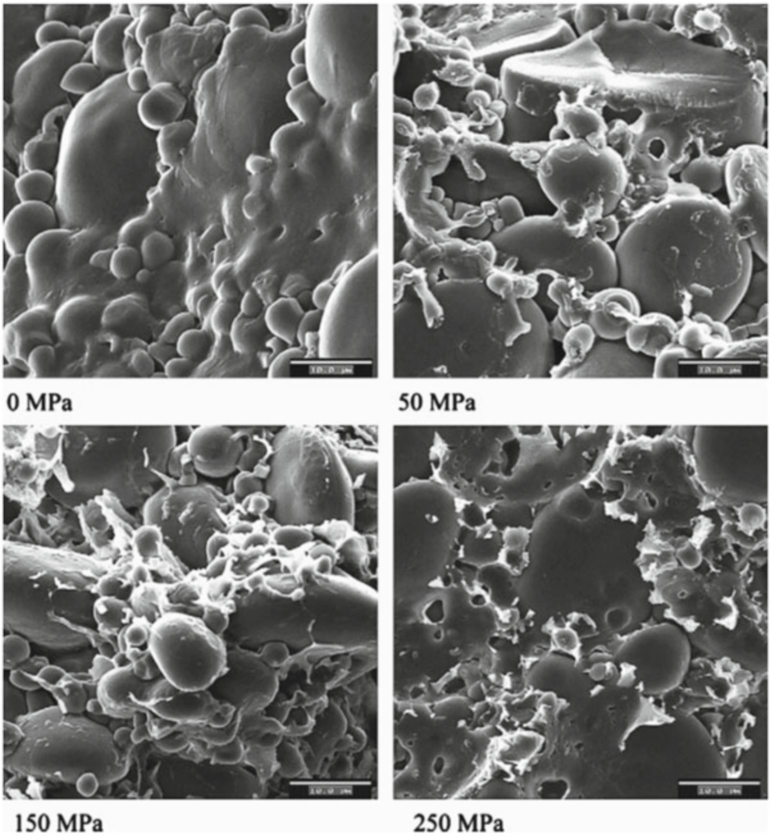

Fig. 4.4 Scanning electron micrographs of wheat dough exposed to varying pressure levels (0–250 MPa/4 min) (From Barcenas, M.E., Altamirano-Fortoul, R., Rosell, C.M. 2010. *LWT—Food Science and Technology*.43: 12–19. With permission)

4.3 Concluding Remarks and Future Outlook

HPP has attracted considerable interest in food industry due to its promising effects in food preservation. Consumers require safe and natural products without added preservatives. Consuming fermented foods such as probiotic drink (kefir, kombucha), sauerkraut, kimchi, as well as fermented meat products helps in maintaining proper balance of gut bacteria and digestive enzymes. Synergistic effect of HPP in combination with multiple hurdles such as low pH, a_w and acidic environment in fermented foods helps in achieving an increased rate of inactivation of spoilage microbes and endogenous enzymes. The hygienic quality of raw materials may be improved by decreasing microbial load through pasteurization, in the dairy industry. However, conventional heat treatments in the case of fermented meat products cause detrimental changes. Hence, alternative mild preservation techniques like high pressure processing show promising opportunities. However, there are certain technological limitations. For example, bacterial spores are highly pressure resistant, since pressures exceeding 1200 MPa may be needed for their inactivation (Knorr, 1995). Sterilization of low-acid foods (pH > 4.6) requires combination of high pressure and other forms of relatively mild treatments like heating (Rastogi & Raghavarao, 2007). Nonetheless, HPP has promising applications to satisfy consumer demand for high-quality food products. HPP enables extended shelf life and safety of fermented meat and dairy products with improved sensory and organoleptic characteristics. Hence, HPP can be considered as an innovative technique that can be employed by the food industry to meet the raising consumer demands for safe and nutritious fermented food products.

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