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Review paper

The application of high pressure processing in decontamination of dry fermented sausages

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A b s t r a ct: High pressure processing (HPP) is a non-thermal postprocessing technology for decontamination of foods with minor impact on their nutritional and sensory characteristics. In the meat industry, HPP is mainly used to increase shelf-life and improve the safety of processed and ready-to-eat meat products. HPP can also be considered as a technique for creation of innovative meat products. In the last decade, numerous scientific studies on HPP application to dry fermented sausages have been published. Many factors, including sausage formulation and processing parameters as well as time, temperature and intensity of treatment, can influence the effectiveness of high pressure in processing these types of products. In general, pressure higher than 400 MPa is necessary to achieve efficacious microbial inactivation. HPP treatment has no detrimental effects on sensory qualities of dry fermented sausages or these effects are minor compared to other decontamination technologies. Also, HPP is environmentally friendly, and hence, wider application of this technology in the food industry is expected in the near future.

Key words: *high pressure processing, dry fermented sausage, meat safety, food quality.*

Introduction

In the last two decades, increased demand for minimally processed and additive– and preservative-free products has highlighted high pressure processing (HPP) as one of the most prominent recent innovations in the food industry. HPP is a non-thermal post-processing technology, mainly used to increase shelf life and to improve food safety. HPP uses a pressure of ≥100 MPa that is transmitted immediately and uniformly through food products using a liquid transmitter, whilst keeping the freshness and nutritive value of the treated products. On the other hand, some negative impacts have also been seen with application of this technology, including changes of quality parameters such as colour, texture and water holding capacity (*Garriga and Aymerich,* 2009*; Simonin et al.,* 2012*; Rastogi,* 2016).

In the meat industry, HPP is mostly applied to ready-to-eat (RTE) meat products (*Mor-Mur and Saldo*, 2012). The main purpose of HPP technology is inactivation of foodborne pathogens and spoilage microbiota, but it can also be used as a technique for creating innovative meat products (*Campus*, 2010; *Simonin et al.*, 2012).

Dry fermented sausages are mainly considered as microbiologically safe products, the safety assurance of which relies on sufficient anti-pathogen effects of multiple antimicrobial factors during the processes of fermentation and drying. However, in cases of initial contamination of the raw materials with higher levels of pathogenic microorganisms and/or insufficient control due to the antimicrobial factors, the safety of these products can become compromised (*Ducic et al.*, 2014; *Ducic and Markov, 2015*). Therefore, the aim of this review is to present updated knowledge dealing with applications and effects of HPP technology on the safety and quality of dry fermented sausages.

The main characteristics and application of HPP technology in the meat industry

In order to construct devices that will meet specific microbiological and nutritional food quality requirements, most companies producing HPP equipment directly cooperate with research centres and food producers. In recent years, the problems with production of pumps that can produce sufficient pressure, and concurrently, with production of large-capacity chambers able to withstand large numbers of production cycles, were successfully resolved. Consequently, due to this development of high

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efficiency HPP machines, processing costs have reduced to acceptable levels (*Bermudez and Canovas*, 2011; *Rastogi*, 2016).

The main parts of an HPP system are a pressure vessel, high pressure pump, closure(s) for sealing the vessel, a device for holding the closure in place while the vessel is under pressure, a system for controlling and monitoring the pressure and optionally, the temperature, as well as a system for transferring food product to and from the pressure vessel (*Campus*, 2010). The volume of pressure vessels indented for the food industry is usually between 35 and 320 litres, while the position of pressure vessels can be either vertical or horizontal. The advantages of horizontal layout include easier filling and emptying, as well as simpler and reliable tracking of treated and non-treated products. If high pressure is used for food treatment concurrently with high temperature, the HPP is usually performed in lower volume vessels containing heaters. In such a vessel, temperatures ranging from 20°C to 150°C can be produced in a short time (*Bermudez and Canovas*, 2011).

Due to the compression of matter volume at high pressure, the processing increases food temperature by 5°C to 15°C or about 3°C with each 100 MPa increase, depending on food composition, the rate of pressure increase and the shape and fullness of the vessel. After decompression, the temperature of food returns to the initial temperature. Similarly, pressure reversibly decreases food pH; sometimes a change of more than one unit is manifested, along with reduction of protein stability (*Cheftel*, 1997; *Campus*, 2010; *Mújica-Paz et al*., 2011). Prior to application of HPP on solid food, like fermented dry sausages, the food is vacuum packaged in plastic materials that are able to conform to the treated product's compression of at least 19% of the original volume. Packaging materials are selected on the basis of their integrity and insulating capacity, so they are not distorted by the application of high pressure, and if migration of molecules from packaging into the food product occurs, this must be within the permissible limits (*Juliano et al*., 2010). Modified atmosphere packaging is also used in some circumstances instead of vacuum packaging, but in that case, due to additional gas compression, application of HPP takes longer which, as a consequence, increases the production costs (*Mújica-Paz et al*., 2011).

Mode of HPP action on microorganisms

The effects of HPP on microorganisms in/on meat and meat products are dependent on many characteristics of microorganism and food product. HPP inactivation of microorganisms is caused by various changes that occur in the cell membrane, the cell wall, ribosomes and enzymes. Damage of cell membrane is the main cause of cell death, due to disturbances of permeability, osmotic pressure and transport systems (*Patterson*, 2005; *Campus*, 2010; *Simonin et al.*, 2012; *FDA*, 2014). Additionally, high pressure directly leads to denaturation and agglomeration of proteins and subsequent inactivation of the enzymes (*Bajovic et al.,* 2012).

Single- or multi-cell parasites are severely affected as a consequence of their complex structure, even at a lower pressure ranging from 200 to 300 MPa (*Yuste et al.,* 2001; *Simonin et al*., 2012). Moulds and yeasts exhibit moderate HPP resistance, with the exception of certain ascospores of heat resistant moulds (*Neosartorya*, *Talaromyces*, *Byssochlamys*), which are able to withstand pressures higher than 600 MPa (*Chapman et al.,* 2007; *Smelt*, 1998).

The vegetative forms of bacteria are more resistant than moulds and yeasts. Due to the thicker and stronger cell wall, Gram positive bacteria are more resistant than the Gram negatives; also, cocci are more resistant than rods (*Murchie et al*., 2005; *Patterson*, 2005). Bacterial cells in the exponential phase are more sensitive to pressure compared to the cells in the stationary phase (*McClements et al*., 2001; *Manas and Mackey*, 2004; *Hayman et al.,* 2007). Spores cannot be destroyed by application of high pressure alone, as treatment intensity at usual processing temperatures is inadequate. Furthermore, pressure-assisted thermal processing (PATP) is a method used for food sterilization that combines high pressure (>600 MPa) and temperatures above 60°C. The advantages of PATP include a lower processing temperature and/or shorter exposure of the product to high temperature, compared to conventional sterilization (*Cheftel*, 1995; *Bermúdez-Aguirre and Canovas*, 2011; *Mújica-Paz et al., 2011*; *Simonin et al*., 2012). Another strategy combines high pressure and temperature (<100°C) – the aim of this treatment is to enable germination of bacterial spores first, so the resultant vegetative bacteria are sensitive to the high pressure (*Rendueles et al*., 2010). This method is still not used commercially, and the reasons include high variability of spore germination under the HPP, as well as the economic aspects of the process (*Ahn et al*., 2007; *Wilson et al*., 2008; *Tores et al*., 2010).

Viruses exhibit diverse resistance to HPP, but can be inactivated by high pressure. Prions are destroyed only by using extremely high pressure (≥700 MPa) concurrently with high temperature (≥60°C) (*Campus*, 2010). *Heindl et al.* (2008) found that after application of 800 MPa (5 min, 80°C), infectivity of prions significantly decreased.

The effect of HPP technology on microorganisms additionally depends on the product characteristics, such as water activity (a_w), pH, salt content and the composition of the raw material. To achieve appropriate antimicrobial effects on products with lower a_w, especially if a_w is lower than 0.9, these products need to be exposed to higher pressure. Microorganisms injured by HPP are usually more sensitive to low a_w, so HPP on low a_w products is followed by inhibition of microbial recovery during storage (*Considine et al.,* 2008; *Simonin et al.*, 2012). HPP's antimicrobial effects increase with declining pH (*Garriga and Aymerich*, 2009). However, the composition of more complex food products (i.e. contain proteins, lipids, sugars, vitamins and some cations) reduces the antimicrobial effects of HPP (*Hauben et al*., 1998; *Rubio et al*., 2007; *Considine et al*., 2008; *Mor-Mur and Escriu*, 2009; *Campus*, 2010). Furthermore, HPP works synergistically with, or is supported by, some antimicrobial substances (e.g. bacteriocins, nitrites, organic acids), modified atmosphere packaging (containing CO_2 or vacuum packed), as well as by low temperatures during food storage (*Garriga and Aymerich*, 2009; *Jofré et al*., 2010; *Bajovic et al*., 2012; *Rodríguez-Calleja et al*., 2012). Considering that, the results of investigating HPP antimicrobial effects in buffers and synthetic media cannot be always extrapolated (*Ananou et al*., 2010; *Campus*, 2010).

HPP effects on microbiological and sensorial quality of dry fermented sausages

In the meat and meat product industry, pressure of 400–600 MPa for 3 to 7 minutes, mostly at room temperature, is applied for inactivation of pathogens and spoilage microbiota (*Bajovic et al*., 2012). The most important foodborne pathogens associated with dry fermented sausages are nontyphoidal *Salmonella* spp., *Listeria monocytogenes* and pathogenic *Escherichia coli* (*Ducic et al*., 2016). Numerous published studies on HPP effectiveness on microbiological and sensorial quality of dry fermented sausages in production of dry fermented sausages are outlined below.

Omer et al. (2010) investigated HPP application on inactivation of enterohaemorrhagic *E. coli* (*E. coli* O103:25), inoculated at a level of 6.8 log CFU g^{-1} in two types of Norwegian fermented dry sausages (morr and salama). Pressure of 600 MPa applied in three cycles lasting for 200 seconds (6 minutes in total) at an initial temperature of 12°C, led to 3 logs reduction of the inoculated pathogen. In an earlier study, *Gill and Ramaswamy* (2008), applying the same pressure (600 MPa) on two types of semi-dry fermented sausages (All Beef and Hungarian salami; a_w 0.927 to 0.968; pH 4.8 to 6.3), found more than 4 logs reduction of *E. coli* O157, while statistical differences in reductions were not found in treatments that lasted for 3, 6 or 9 minutes. Significantly, Gill and Ramaswamy also investigated sausages after four weeks of storage at 15°C. In All Beef salami, the number of *E. coli* O157 increased during storage, while in Hungarian salami, which had lower pH and a_w, the pathogen numbers remained at the same level. In both of these studies, high pressure did not cause any significant changes in the treated products sensorial characteristics.

A comprehensive study performed by *Porto-Fett et al*. (2010) investigated effects of high pressure (483 MPa during 1, 2, 3, 4 and 5 minutes at 20°C as well as 600 MPa during 5, 7, 10 and 12 minutes at 20°C) applied to Genoa salami inoculated with several *L. monocytogenes*, *E. coli* O157:H7 and *S.* Typhimurium strains. The initial level of each pathogen strain-cocktail was approximately 7 logs per gram of sausage batter. After fermentation and drying, sausages were HPP treated, stored at 4°C and monitored for levels of inoculated pathogens for 28 days. *S.* Typhimurium and *E. coli* O157:H7 were completely eliminated in the majority of the samples. After the application of HPP (600 MPa during 5 minutes), reduction of *L. monocytogenes* ranged from 1.6 to 6 logs, depending on fermentation and drying conditions. In the same conditions of pressurization (600 MPa, 5min), *L. monocytogenes* was under the detection limit (≤ 1 log) in some samples immediately after the treatment, although it was detected during the storage phase. This study confirmed earlier findings that *L. monocytogenes* is more resistant to high pressure than *E. coli* or *Salmonella*, as well as that the direct antimicrobial impact of high pressure decreases with lower a_w of food product.

Garriga et al. (2005) examined the effect of 400 MPa at 17°C during 10 minutes on microbiological and sensorial properties of two Spanish mildly fermented sausages (fuet and chorizo). As a result of the applied pressure/time, the authors stated that safety and shelf life were improved, i.e. the number of *Enterobacteriaceae* was reduced, *L. monocytogenes* was unable to grow, while inoculated *S.* Typhimurium was not detected.

Marcos et al. (2007) conducted a study using the same type of sausages but without inoculation of pathogens; beside antimicrobial effects, the HPP (400 MPa, 17°C, 10 minutes) effect on sensorial properties was assessed as well. In fuet and chorizo, HPP reduced the *Enterobacteriaceae* level by 1 and 3.8 log CFU g^{-1}, respectively. The number of *Enterococci* decreased by 2 log CFU g^{-1} in chorizo sausages only, confirming that sensitivity of the enterococcal population to HPP is variable and is influenced by the numbers and species composition of each product. By using a texture profile analysis (TPA) method for the HPP-treated sausages, it was found that these products had higher cohesiveness, chewiness and springiness compared with untreated products. Regarding sensorial properties, the only difference noticed by the sensory panel was a slight decrease in colour intensity manifested in HPP-treated sausage. Furthermore, HPP did not cause higher levels of lipid oxidation (measured as thiobarbituric acid reactive substances (TBAR) values).

Marcos et al. (2013) investigated the antilisterial effect of combined use of high pressure and antimicrobial packaging on fermented dry sausages that were produced without NaCl and stored for 90 days at 12°C (considered as the worst case scenario of storage conditions in consumers' refrigerators). Sliced products were prepared using accelerated drying (Quick Dry Slice process – QDS®) and then inoculated on the surface with a three-strain cocktail of *L. monocytogenes* (the concentration of was $5x10^5$ CFU g^{-1}). Pressure of 600 MPa (5 min, 12°C) did not reduce pathogen levels, and this was, according to the authors, a consequence of low a_w of the treated products (a_w <0.9). High pressure treatment used in conjunction with nisin led to greater *Listeria* reduction (an additional 0.5 logs, so 1.9 logs compared to 1.4 logs) than was obtained by using nisin only.

Similar research that was conducted by *Stollewerk et al*. (2012) pointed out that the QDS process in combination with high pressure (600 MPa, 5 min, 13°C) provides safe fermented dry sausages even after 91 days of storage under refrigeration. This study investigated sausages that, during production, were inoculated with *L. monocytogenes* (30 CFU g^{-1}) and *Salmonella* spp. (15 CFU g^{-1}); these concentrations of pathogens were chosen to be in line with the level of "common" contamination of fermented dry sausages on the market found in other studies.

Jofre et al. (2009) investigated the sensitivity of *L. monocytogenes*, *Salmonella* and *Staphylococcus aureus* strains to high pressure and enterocins A and B in Spanish fermented dry sausages (fuet). Each of these pathogens was inoculated as a strain-cocktail with concentration of 2.7 log CFU per gram of sausage batter. After the drying process, sausages were subjected to 400 MPa pressure (17°C, 10 min) and stored for 20 days at 20°C and thereafter, at 7°C. In HPP-treated fuet, the reduction of *Salmonella* occurred earlier compared to untreated controls, whereby at the end of storage time, the level of this pathogen was < 1 log CFU g^{-1} in each sausage. Adding enterocins disabled *L. monocytogenes* growth, whilst in control sausages, the level of this pathogen increased by 5 log CFU g^{-1} during the production process. Notably, in sausages with no enterocins added and stored at refrigeration temperatures, high pressure led to a long term inhibitory effect; in other words, *L. monocytogenes* reduction of 5 log CFU g^{-1} occurred no sooner than in the last week of storage. The level of *S. aureus* during sausage production increased by about 3 log CFU g^{-1} and remained at that level during the entire storage time.

In a similar study, *Rubio et al.* (2013) found that 600 MPa (5 min, 15°C) applied at the end of fuet's production process (21 days) reduced the number of *Enterobacteriaceae* by at least 0.3 logs (i.e. from 1.3 log CFU g^{-1} to < 1 log CFU g^{-1}). Furthermore, by applying HPP, the levels of *L. monocytogenes* and *S. aureus* (each pathogen inoculated at a concentration of 3.5 log CFU g^{-1}) were reduced by about 1 log CFU g^{-1}. The HPP effect manifested immediately after the treatment or after 7 days of storage at 14°C. On the other hand, the number of useful bacteria (lactic acid bacteria, coagulase negative cocci) did not change significantly after exposure to HPP.

Rubio et al. (2007) investigated HPP effects (500 MPa, 5 min, 18°C) on microbiological, sensorial and physicochemical properties of Spanish dry fermented sausages, salchichon, which were produced from the meat and back fat of pigs fed on control diet or with food enriched in oleic or linoleic fatty acids. After the HPP application, sliced sausages were stored for 210 days at 6°C and during that time were assessed on several occasions for instrumental colour measurement, sensorial properties, as well as numbers of the main groups of microbiota. The sensory properties of HPP-treated versus control sausages were similar, as was colour (lightness, redness, yellowness). Immediately after HPP application, the level of aerobic psychrotrophs and intestinal anaerobes was reduced by up to 2 log CFU g^{-1}, and the level of lactic acid bacteria was reduced by about 1 log CFU g^{-1}. However, during storage, a significant reduction of these microbial groups was observed in control sausages and thus, after 210 days, the difference between HPP treated and untreated sausages was lower than at the beginning of storage. This study also found HPP reduced *Pseudomonas* spp. (by 3.5 log CFU g^{-1}) and moulds and yeasts (by >1.5 log CFU g^{-1}).

The possibility of processing fermented dry sausages with high pressure applied at the beginning of the production process was investigated in the study conducted by *Marcos et al.* (2005). Sausage batter was inoculated with various strains of *Salmonella* and *L. monocytogenes*, while the concentrations of both pathogens were roughly 6×10^2 per gram of stuffing. One day after production (stuffing into casings), sausages were exposed to 300 MPa (17°C, 10 min) and then subjected to the ripening process. As a result of the cumulative effects of high pressure and the ripening process, the number of *Salmonella* was about 1.5 logs lower in HPP-treated sausages than in control (non-HPP treated) sausages, in which the reduction, as a consequence of ripening only, was 0.5 logs. In contrast, *L. monocytogenes* numbers decreased by ~1 log after HPP, but after ripening, numbers increased to ~ 2 logs, while in control sausages at the end, *L. monocytogenes* was at a very low level or had completely disappeared. According to the authors, the reason for this phenomenon was the HPP-induced, temporary decrease in numbers of lactic acid bacteria; this slowed the pH drop and reduced the production of antilisterial factors and consequently enabled recovery of sub-lethally damaged *L. monocytogenes*. Furthermore, in HPP treated sausages, a "whitening" effect of pressure (increase in brightness (*L* *) was observed, and the authors explained this as a consequence of globin denaturation and/or haeme displacement or release.

Latorre-Moratalla et al. (2007) also treated sausages before fermentation, but they applied lower pressure (200 MPa, 17°C, 10 min). As a result, *Enterobacteriaceae* growth during fermentation and drying was absent or it was present to a lesser extent compared with control sausages; HPP also led to reduction of some biogenic amines (like putrescine and cadaverine) in treated sausages. This HPP application did not reduce technologically useful microorganisms (*Lactobacillus* spp., coagulase negative cocci); moreover, their numbers were greater (by up to 1 log CFU g^{-1}) in finished, HPP-treated sausages. The authors stated that the pH, a_w, proteolysis and product colour were not different from the usual values. This study also found that the pressure of 200 MPa did not reduce tyramine levels, although this flaw can be superseded by adding decarboxylase negative starter cultures to the sausage batter.

Omer et al. (2015) stated that if trimmings (frozen or chilled) intended for production of morr sausages or salami are exposed to 600 MPa (6 min, 12°C), a better hygienic status of final sausages is achieved. However, the final products, in that case, were of diminished quality regarding colour, smell, taste and texture as well as of lower total sensorial acceptability. Nevertheless, after six weeks of storage, the difference in sensorial properties was less expressed, especially if frozen trimmings were treated. Also, sausages produced from HPP raw meat had lower a_w and weight compared to those made from untreated meat.

The effect of HPP on reduction of biogenic amines in fermented dry sausages was explored by *Ruiz-Capillas et al*. (2007). Sliced chorizo sausage (3 mm thick slices) was exposed to 350 MPa (15min, 20°C) and then stored for 160 days at 2°C. HPP application induced slightly reduced numbers (0.5 to 1 log CFU g^{-1}) of aerobic mesophiles and lactic acid bacteria during storage. Additionally, HPP resulted in significantly less tyramine, putrescine and cadaverine, which was explained to be a consequence of lactic acid bacteria reduction.

Bolumar et al. (2015) investigated the possibility of producing fermented sausages that would contain lean meat treated by high pressure (600 MPa, 5 min, 20°C) instead of pig back fat. The sensory panel found that the sausages with HPP-treated lean meat added instead of 35% of fatty tissue had better taste, while the smell, texture and colour (instrumentally measured) were not statistically different compared to the control sausage. Furthermore, there was no difference in the extent of lipid oxidation (TBAR value, peroxide value), or in the numbers of aerobic mesophiles or *Enterobacteriaceae.*

The study conducted by *Alfaia et al*. (2015) examined HPP application on Portuguese fermented sausage (chouriço) with different combinations of pressure (202 to 600 MPa) and time (154 to 1800 seconds). It was found that 400 MPa for 154 to 960 seconds reduced spoilage microbiota (*Enterobacteriaceae* and *Pseudomonas* spp.) and fungi to below the limit of detection (<1 log CFU g^{-1}), while technologically useful microbiota (lactic acid bacteria, coagulase negative cocci, *Enterococcus*) were reduced by 1 to 1.5 log CFU g^{-1}. This treatment also enhanced colour, cohesion and firmness of sausages. Neither of the pressure/time combinations of treatments led to increased lipid oxidation (TBAR values).

Conclusion

HPP is a non-thermal technology that improves the safety of meat and meat products and extents their shelf life. Pressures of at least 400 MPa successfully inactivate microorganisms. For fermented dry sausages, HPP is applied, primarily, as an additional decontamination step on packaged products. HPP has no detrimental effects on sensorial qualities of dry fermented sausages. Moreover, improved texture through increased cohesiveness, firmness and chewiness is observed, while negative effects on colour are rare and/or less expressed and are mainly related to applying HPP treatment at the beginning of the process. Due to the development of highly efficient HPP machines, processing costs have been reduced to acceptable levels, and this has led to wider application of this technology in the food industry. As an environmentally friendly and waste-free technology, HPP has a high acceptance among consumers. HPP encourages development of innovative fermented meat products and it is expected to be more widely applied in the future.

Conflict of interest. The authors declare that they have no conflicts of interest.

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