



Contents lists available at ScienceDirect

Trends in Food Science & Technology

journal homepage: <http://www.journals.elsevier.com/trends-in-food-science-and-technology>

Novel approaches in improving the quality and safety aspects of processed meat products through high pressure processing technology - A review



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ARTICLE INFO

Article history:

Received 25 September 2015

Received in revised form

1 June 2016

Accepted 3 June 2016

Available online 7 June 2016

Keywords:

Processed meat products

High pressure processing

Multi hurdle approaches

Natural antimicrobials

Natural antioxidants

Active packaging

ABSTRACT

Background: In recent years, there has been growing consumer demand for the minimally processed and chemical additives free Ready-To-Eat (RTE) healthier meat products. On the other hand processed and RTE meat products have been notified as the primary cause for food borne outbreaks in different countries that commonly associated with emerging pathogens such as *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* species.

Scope and approach: High pressure processing (HPP) has been renewed as a best non-thermal intervention for extending the shelf-life and safety of RTE meat products without altering the sensory and nutritional properties. Meat products are complex medium with different physical and chemical compositions that influence the lethality of the microorganisms during HPP. Using high pressure levels (above 600 MPa) for complete sterility of meat products may not be economically feasible more over it may negatively affect the product quality characteristics. The present review aimed to explore the recent research investigations addressed the multi hurdle approaches to increase the effectiveness of HPP at lower processing levels in order to reduce the processing costs and to improve the safety and quality of processed meat products.

Key findings and conclusions: The combination of natural antimicrobials (plant bioactive compounds and bacteriocins) and antioxidants (plant phenolic compounds) as additional hurdles through different mechanisms (active and intelligent packaging) during HPP can definitely be an effective and innovative intervention in ensuring the complete safety of processed meat products. Moreover, the development of low salt meat products with optimum quality attributes can be highly possible through HPP technology.

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1. Introduction

The consequence of globalization with the changes in consumer lifestyle, food patterns and expanding knowledge about the relationship between food and health has brought tremendous transformations in processed food industry within the past few years (Siro, Kopolna, Kopolna, & Lugasi, 2008; Sorenson et al., 2011). Among the foods, meat is one of the important food commodities that provide better and essential nutrition to the humans for years. The presence of high quality proteins, essential amino acids, B-vitamins and dietary fat recognized meat as one of the nutrient dense

food (Biesalski, 2005; Decker & Park, 2010; Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). The highly perishable nature of meat and meat products requires appropriate preservation/processing techniques within minimum time periods in order to arrest it from the microbial and other possible spoilage mechanisms (Hygreeva, Pandey, & Radhakrishna, 2014). Since, the science of food has started to explore the facts about the wellness of being related to consumption of food patterns; especially processed food, the consumers became more concern over the health enhancing properties, convenience, shelf-life properties and safety while selection of processed food from the shelves (Bruhn, 2007; Rubio, Jofré, Aymerich, Guàrdia, & Garriga, 2014; Sentandreu & Sentandreu, 2011). Hence, the increasing consumer demands for the fresh like, nutritious and convenience products triggered both the academic and industrial research towards the invention and application of newer processing technologies called as non-thermal

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processing/mild processing/hurdle techniques (McArdle, Marcos, Kerry, & Mullen, 2011; Raouche, Mauricio-Iglesias, Peyron, Guillard, & Gontard, 2011; Trespalacios & Pla, 2007).

Shelf-life extension of meat products is a complex phenomenon that majorly depends on the successive control of oxidation and microbial spoilage processes under room and refrigerated storage conditions (Fung, 2010; Karabagias, Badeka, & Kontominas, 2011). The detrimental effect of high temperature processing on the nutritional and sensory properties and the possible health risks associated with synthetic antioxidants and antimicrobials present in meat products has opened new doors in food research for the development of minimally processed/hurdle processed foods/natural antioxidants and antimicrobials added food products (Campus, 2010; Lahucky, Nuernberg, Kovac, Bucko, & Nuernberg, 2010). From the past few years, both the research organizations and social media have been actively working to promote the consumer awareness about the newer food processing technologies and associated benefits related to their health and convenience aspects. In this regard, some recent reports have acquired positive responses from the consumer level, and the consumers too could realize and ready to accept the foods that are being processed by novel processing techniques (Sorenson et al., 2011). Consequently, ensuring the quality and safety of Ready To Eat (RTE) meat products while meeting the consumer demands for minimally treated, chemical additives free is been a great challenge to the meat industry (Bover-Cid, Belletti, Garriga, & Aymerich, 2012). In such products, the risk of emerging pathogen *Listeria monocytogenes* is primarily concerned due to that, the organism can able to grow in extreme conditions (low temperature, low pH and low water activity) (Juck, Neetoo, Beswick, & Chen, 2012). Recent research reports have indicated that *Listeria monocytogenes* has been recorded with high notification rates in occurrence of listeriosis in some countries including United States, Finland, Denmark and Spain with highest rates reported in Finland (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2014). In this regard, RTE meat products are being reported to be the primary source for the occurrence of this organism in Europe. Even though, high heat treatment eliminates *L. monocytogenes* in RTE meat products, surplus chances are there for recontamination during handling, slicing and repacking operations (Lowder, Waite-Cusic, & DeWitt, 2014; Hereu, Bover-Cid, Garriga, & Aymerich, 2012a).

High pressure processing (HPP) or high hydrostatic pressure processing (HHPP) is a novel non thermal preservation/processing technique. It is being used to extend the shelf life quality of fresh and processed food products without altering/minimal impact on the sensory and nutritional properties (Marcos, Aymerich, Garriga, & Arnau, 2013). As the HPP technique works under the isostatic condition, it involves in uniform distribution of pressure throughout food material with irrespective of size and shape. Due to uniform pressure distribution, the shape and dimensions of the food material remains same in the container/packet (de Alba, Bravo, & Medina, 2012; Han et al., 2011). Probably, high pressure (HP) research on meat and meat products is been progressing with promising results and renewed as one of the best non thermal processing techniques to improve the technological and microbial quality of meat and meat products. (Simonin, Duranton, & de Lamballerie, 2012, Fig. 1). After the first reports of Hite (1899) on effect of HPP on food preservation model systems, it leads to the use of high pressure treatment as non thermal processing technology. HPP was tested with various food products to extend their shelf life at ambient and refrigerated storage conditions. The research works of Macfarlane (1973) and Macfarlane and Morton (1978) revealed the important aspect related to the improvement in meat tenderness with HP treatments and moreover continued research studies of Cheftel and Culioli (1997); Cheah and Ledward (1996) indicated

the changes associated with several quality characteristics of meat products (Jung, Ghoul, & de Lamballerie-Anton, 2003). Nowadays with the advances in food engineering the commercial setups of high pressure processing units could reach the pressure levels up to 1000 MPa and being used for pasteurization and commercial sterilization of different food products (Rendueles et al., 2011). Currently research scientists and meat technologists are showing considerable interest in application of high pressure processing as non thermal preservation and decontamination/pasteurization/sterilization technology for extending the shelf life and safety of commercial processed RTE meat products (cooked and cured) (Scheinberg, Svoboda, & Cutter, 2014). The high level risks associated with the processed meat products is the recontamination of spoilage and other emerging pathogens during handling, packing and slicing. (Sun & Holley, 2010). Recent studies have shown that treatment of meat products at around 400–600 MPa could be viable alternative intervention to thermal processing that could ensure the microbial safety of packed RTE meat products (Fulladosa, Serra, Gou, & Arnau, 2009). On the other hand food safety regulatory agencies such as United States Food and Drug Administration (USFDA), Food Safety and Inspection Service (FSIS) have been permitted the HPP as an acceptable method for elimination of *L. monocytogenes* in processed meat products (de Alba et al., 2012).

In HPP applied pressure, temperature and time are the critical factors that determine the lethality of microorganisms in particular food matrix (Bajovic, Bloumar, & Heinz, 2012). The nature of the food product such as low water activity, high fat, high protein and high solute concentration have been identified as important factors that can increase the barotolerance of microorganism and reduce the extent of bacterial inactivation and further leads to recovery of sublethally damaged cells during storage period (Rendueles et al., 2011; Szerman et al., 2011). Recent research investigations explored novel hurdle strategies to improve the process lethality rates with combination of high pressure with potent hurdles (Ananou et al., 2010). The hurdles such as; *Incorporation of natural antimicrobials and antioxidants either direct addition/through active packaging; use of high temperatures; low temperatures and modified atmosphere packaging*. Application of novel multi hurdle strategies during HPP of meat products may bring down the processing costs and moreover it allows using of moderate pressure levels in order to maintain the better quality characteristics in terms of texture and appearance.

Sodium chloride/salt is a most essential and functional ingredient in meat products that gives desired flavor, taste, color and texture (Ruusunen & Puolanne, 2005). It also importantly contributes for water holding capacity (WHC) and fat binding capacities which are believed to be important technological quality aspects that they further determine the cook loss and final composition of the product. (Weiss, Gibis, Schuh, & Salminen, 2010). Processed meat products are one of the promising sources for high levels of dietary sodium due to its functional properties as well as preservative effects (Grossi, Søltoft-Jensen, Knudsen, Christensen, & Orlien, 2012). The increasing consumer awareness about the intake of sodium chloride and associated health risks related to hypertension and other possible degenerative diseases has led to the development of low salt meat products with possible potential strategies (Desmond, 2006). The most extensively used strategies to reduce the sodium levels in meat products are replacing with other salts and substitutes like potassium chloride, sodium lactate, potassium lactate etc., (Fulladosa et al., 2009; Fulladosa, Sala, Gou, Garriga, & Arnau, 2012). In the mean time, incorporation of these substitutes may negatively affect the sensory and other quality characteristics. Most recent studies have evaluated the favorable effects of HP treatment during the development of low salt meat

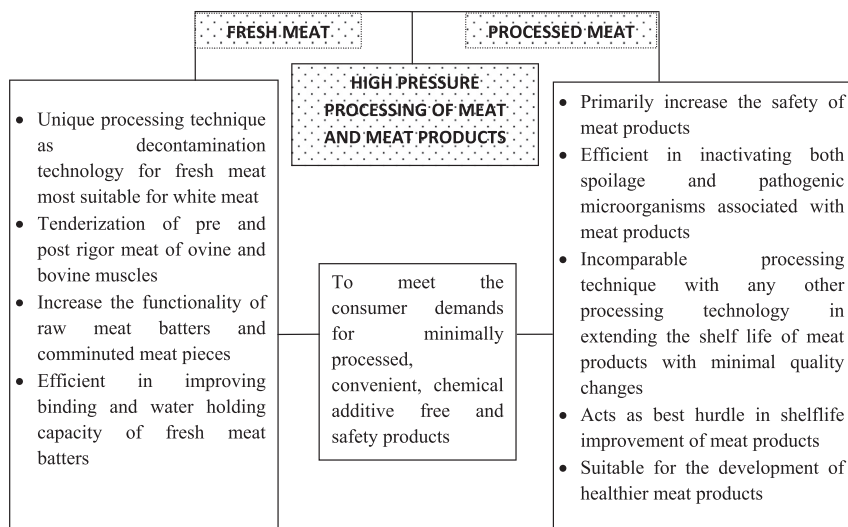


Fig. 1. Important functions of high pressure processing in development of high quality fresh and processed meat products.

products due to the possibilities in improving the muscle protein functionality characteristics under HP conditions. In very recent studies, the application of HPP seems to improve the technological quality characteristics such as cook yield, water holding capacity and textural attributes of low salt meat products (O'Flynn, Cruz-Romero, Troy, Mullen, & Kerry, 2014; Sikes, Tobin, & Tume, 2009; Tintchev et al., 2013; Yang, Han, Bai, et al., 2015; Yang, Han, Wang, et al., 2015;).

The present review is aimed to explore the recent initiatives and the progress that has been occurred in the area of novel multi hurdle approaches with high pressure processing of processed meat products with respect to their quality and safety improvement aspects.

2. Role of high pressure processing in improving the shelf life and microbial safety of meat products

Ensuring the complete microbial safety of processed RTE meat products is been a great challenge to the meat industry (Bermúdez-Aguirre & Barbosa-Cánovas, 2011). The pathogenic microbiota that associates with meat and meat products, had a wide history in causing severe food borne infections to the humans in different countries throughout the world (Ahmadi, Anany, Walkling-Ribeiro, & Griffiths, 2015; Juck et al., 2012). The most common pathogens that associated with food borne illness for decades are *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus* *Clostridium botulinum* and emerging pathogens includes *Yersinia enterocolitica*, *Campylobacter* spp., *L. monocytogenes* and *Escherichia coli* O157 (Bover-Cid et al., 2012). The successive control of food borne outbreaks can be achieved through the complete inactivation of growth of pathogenic microflora in the respective food material (Jofré, Aymerich, Grèbol, & Garriga, 2009). Numerous studies have investigated the effects of HPP on the inactivation of various spoilage and pathogenic microorganisms in different cooked and cured meat products (Table 1). On the other hand, studies have also reported the efficacy of HPP in inactivation of microorganisms in low salt, low phosphate and low water activity meat products (Jofré et al., 2009; Stollewerk, Jofré, Comaposada, Arnau, & Garriga, 2012).

2.1. Ready to eat (RTE) convenient cooked and cured meat products

RTE cooked meat products are highly perishable in nature due to

high pH (around 6) and high water activity values (usually >0.97) that favors for the extensive and rapid growth of spoilage microorganisms especially lactic acid bacteria. Dry cured meat products are shelf stable due their low water activity (below 0.90) and high salt contents (up to 15% dry matter) (Hereu, Dalgaard, Garriga, Aymerich, & Bover-Cid, 2012b). As RTE products, these cooked and cured meat products usually sold as sliced and vacuum packed that involve handling, cutting, slicing and final packing operations. During these operations, vulnerable chances are posing for the recontamination of product with emerging pathogens and spoilage organisms from the external sources (Stollewerk et al., 2012). HPP has been permitted as post processing decontamination technology for RTE cooked and cured meat products in different countries in order to eliminate the risks; poses with the pathogens include *Salmonella* spp., *E. coli* O157:H7 and *L. monocytogenes*. In this regard, several studies have attempted to ensure the microbial safety of different RTE cooked and cured meat products (stored at 2 °C–8 °C) with intentionally spiked or naturally present pathogens. In most of the recent studies, the survival of *L. monocytogenes* has been widely tested followed by *Salmonella* and *E. coli* species apart from other pathogens (Simonin et al., 2012).

In this regard, Realini, Guàrdia, Garriga, Pérez-Juan, and Arnau (2011) intended to extend the shelf life of cured pork carpaccio using HPP (400 and 600 MPa at –35 and –15 °C) and reported that HPP significantly controlled the growth of lactic acid bacteria (LAB) (final count 2.99 log cfu/g-41 days) and psychrotrophs (4.70 log cfu/g) during storage of 41 days at 2 °C compared to control samples (final count 6.75 and 6.25 log cfu/g of LAB and psychotropic bacteria – 41 days). Rubio, Martinez, Garcia-Cachan, Rovira, and Jaime (2007) estimated the effects of HPP (500 MPa for 5 min at 18 °C) on the shelf life quality of vacuum packaged dry cured beef (Cecina de Leon) product for 210 days under refrigerated storage condition (6 °C). HPP showed its effectiveness in maintenance of better microbiological quality while no changes observed in sensory and other physical quality characteristics. In a detailed study by Garriga, Grèbol, Aymerich, Monfort, and Hugas (2004) different commercial meat products (sliced cooked ham-pH 6.25, a_w –0.978 and dry cured ham-pH 5.88, a_w –0.985) were subjected for HPP (600 MPa for 6 min at 31 °C) and the shelf life was evaluated for 120 days under refrigerated storage (4 °C) condition. The periodical microbiological reports for 90 days revealed that HPP exerted an important activity in delaying the growth of aerobic total count

Table 1
Effect of high pressure processing on the microbial quality of meat products.

Product	Microorganism inactivation outputs (log cfu/g)	Processing and storage conditions	Microbial inactivation outputs (log cfu/g)	Reference
Chicken breast fillet	<i>Salmonella typhimurium</i> (KCTC 1925) <i>Escherichia coli</i> (KCTC 1682) and <i>Listeria monocytogenes</i> (KCTC 3569)	450 MPa/5 min at 15 ± 3 °C 600 MPa/5 min at 15 ± 3 °C 7–14 days 3–14 days	450 MPa/5 min at 15 ± 3 °C- 6–8 log (cfu/g) 600 MPa/5 min at 15 ± 3 °C - 4–8 log (cfu/g)	Kruck et al. (2011)
Norwegian type dry-fermented sausages	VTEC O103:H25 Inoculated at 6.8 log ₁₀ CFU/g	600 MPa for 10 min Three cycles of 600 MPa for 200 s per cycle Immediate after pressure treatment	600 MPa for 10 min: 2.9 log ₁₀ CFU/g Three cycles of 600 MPa for 200 s per cycle: 0.3 log ₁₀ CFU/g	Omer et al. (2010)
Beef jerky	<i>L. monocytogenes</i> inoculated at 7.85 log ₁₀ CFU/strip <i>Salmonella</i> spp. inoculated at 7.43 log ₁₀ CFU/strip <i>E. coli</i> O157:H7 inoculated at 7.30 log ₁₀ CFU/strip <i>S. aureus</i> inoculated at 7.25 log ₁₀ CFU/strip	550 MPa for two consecutive, 60 s 14 days at room temperature	<i>L. monocytogenes</i> : 1.28 log ₁₀ CFU/strip <i>Salmonella</i> spp.: 6.83 log ₁₀ CFU/strip <i>E. coli</i> O157:H7: 4.45 log ₁₀ CFU/strip <i>S. aureus</i> : 1.32 log ₁₀ CFU/strip	Scheinberg et al. (2014)
Iberian and Serrano hams	<i>L. monocytogenes</i> spiked at 6 log CFU/g	450 MPa for 10 min 60 days at 4 °C or 8 °C	Iberian hams- final counts 3.24 log ₁₀ CFU/g Serrano hams- 2.73 log ₁₀ CFU/g	Morales, Calzada, & Nunez, 2006
Vacuum packaged frankfurters	Enterobacteriaceae	400 MPa for 10 min at 30 °C 2 °C for 141 days	Below the detection limit	Ruiz-Capillas, Carballo, and Jimenez-Colmenero (2007)
Sliced cooked ham	<i>L. monocytogenes</i> spiked at 5 log ₁₀ CFU/g	500 MPa for 10 min 2 °C for 70 days	Immediate after treatment: below detection limit 10 ² log CFU/g During storage period: 7–8 log ₁₀ CFU/g	Koseki, Mizuno, and Yamamoto (2007)

(2–3 log CFU/g), psychrotrophs count (2–5 log CFU/g), lactic acid bacteria (2–3 log CFU/g), yeasts and enterobacteriaceae (3 log CFU/g) with lesser counts compared to non treated samples.

Jofré et al. (2009) studied the effect of HPP in extending the shelf life of RTE convenient meat products. The products cooked ham, dry cured ham and marinated beef loin were subjected to HPP at 600 MPa for 6 min at 31 °C and observed the inactivation of pathogenic (*L. monocytogenes*, *Salmonella enterica*, *S. aureus*, *Y. enterocolitica* and *Campylobacter jejuni* spiked at 3.5 log cfu/g) and spoilage organisms ("*LAB*, *E. coli* and the yeast *Debaryomyces hansenii*" spiked at 3.5 log cfu/g) during 120 days storage period at 4 °C. The authors indicated that, the pressure treatment at 600 MPa level was effective in inactivation of both pathogenic and spoilage microorganisms and showed the counts below the detection limit during whole storage period compared to control. On the other hand, Gudbjornsdottir, Jonsson, Hafsteinsson, and Heinz (2010) investigated the efficiency of HPP (700–900 MPa for 10 s) on the inactivation of *Listeria* species (*Listeria innocua*, strains EU2173/E-34 and EU2172/E-33; *L. monocytogenes*, strains E1, E5, H-01-170-2, L-327, L-435 and L-462) in cold smoked salmon. Pressure treatment significantly reduced the *L. innocua* from 4500 cfu/g to non detectable level (<0.3 cfu/g). In another study inoculated growth of *L. monocytogenes* in RTE ham and turkey formulated with sodium nitrite (0 or 200 ppm) and sodium chloride (1.8 or 2.4%) processed at 0 and 600 MPa was investigated up to 182 days. The authors found that HPP (600 MPa for 3 min) resulted in a 3.85–4.35 log cfu/g reduction in pathogen compared to control. The authors did not observe any synergistic action of HPP and sodium nitrite/sodium chloride on the inactivation of the pathogen studied (Myers, Montoya, Cannon, Dickson, & Sebranek, 2013).

Subsequently, recent studies are focusing on the impact of HPP on microbial inactivation of low sodium meat products. These studies revealed the importance of salt in meat products for microbial inactivation while delivering the potential strategies for development of shelf stable low salt meat products through HP technology.

In this regard, Stollewerk et al. (2012) developed standard - S (NaCl 28 g/kg) and NaCl free - F (replaced NaCl with KCl (15.31) + potassium lactate (33.83)/kg) sliced smoked dry cured ham and studied the growth fate of *L. monocytogenes* and *Salmonella* (spiked at <100 cfu/g) with subsequent HPP treatment at 600 MPa for 5 min during refrigerated storage period (112 days). Pressurization successively led to elimination of pathogens in S type ham after 14 days whereas in F type ham the counts were identified even after 28 and 56 days respectively. These results indicated that the role of salt and efficiency of HPP in elimination of pathogenic microorganisms in cured meat products. Canto et al. (2014) attempted a study to develop low sodium restructured caiman steaks with different levels and combinations of salt (0.75%–1.5%), microbial transglutaminase (1%), KCl (0.375%) and MgCl₂ (0.375%). Fulladosa et al. (2012) developed reduced salt restructured dry cured ham with and without K- lactate and evaluated after resting period (16% weight loss) and after two drying levels (40 and 50% weight loss). The addition of K-lactate resulted in reduced a_w and microbiota in the inner parts of the processed hams. Authors claimed that the simultaneous application of HPP further reduced the microbial counts and increased the saltiness of the product.

Temperature abuse conditions are considered to be very frequent at house hold level refrigerators and some times during transports and at commercial displays. During temperature abuse conditions the sub lethally damaged cells can rejuvenate their growth in particular food. In respect to this few researchers have studied the potency of HPP in controlling the growth of microorganisms in RTE meat products stored under temperature abused conditions. The proportional increase in inactivation of food borne pathogen *Salmonella enteritidis* (1.06, 2.54 and 4.32 log units) was observed in sliced vacuum packed dry cured ham when subjected at different levels of HPP (400, 500 and 600 MPa for 5 min at 12 °C) stored at 8 °C with temperature abused conditions. Compared to control a reduction of 2.56 and 2.66 log units was observed in samples treated at 500 and 600 MPa after 60days of storage (de Alba

et al., 2012). With a well designed cold chain break (2 days at 4 °C, 4 h at 20 °C, and 8 days at 8 °C) study, Duranton, Guillou, Simonin, Chéret, and de Lamballerie (2012) investigated the effects of salt (0, 1.5 and 3%) and HPP (200, 350 and 500 at 20 °C for 6 min) alone and their interaction on the growth behavior of endogenous microflora (aerobic mesophiles, LAB, *Enterobacteriaceae*) in raw pork meat. The study concluded that the addition of salt during HPP favors in more inactivation of microbial growth.

3. Novel hurdle combinations to improve HPP efficiency in inactivation of microorganisms

The recent novel multi hurdle approaches attempted to increase the efficacy of HPP in extending the shelf life and microbial safety of meat products are shown in Table 2. The mode of action and the benefits of additional hurdles during HPP are also represented in Fig. 2.

3.1. Combination of natural antimicrobials and high pressure

The susceptibility of microorganism to the HPP depends on the physiological response of the particular organism present in the complex food matrix. Studies have reported that high pressure processing do have great potential on the inactivation of target microorganisms tested under controlled selective media conditions. The potential may not be same in the food matrix since it is a complex medium of moisture, fat, protein, minerals and sugars that can favor the microorganism to increase the resistance towards the applied pressure conditions (Huang, Lung, Yang, & Wang, 2014).

Plant extracts and essential oils of different plant parts includes seeds, leaves, flowers, buds and bark that are rich in phenolic compounds are proved to be a good antioxidant and antimicrobial agents in different fresh and processed meat products (Ahn, Grun,

& Mustapha, 2007; Brannan, 2008; Falowo, Fayemi, & Muchenje, 2014). Apart from the plant extracts, bacteriocins from selected microorganisms have also been tested for their effectiveness in delaying the growth of targeted spoilage and pathogenic microorganisms in different meat systems (Galvez, Abriouel, López, & Omar, 2007; Alves, Martinez, Lavrador, & De Martinis, 2006). Bacteriocins are the bioactive peptides generated from the microorganisms with the primary action of defense with other competitive microorganisms. The bacteriocins developed from LAB showed their wide spectrum in inhibition of pathogens especially towards the gram positive organisms (Cleveland, Montville, Nes, & Chikindas, 2001; Liu et al., 2012). The most tested bacteriocin in meat products is Nisin and only the bacteriocin approved as an additive in meat products in selected countries. The combined multi hurdle effect of high pressure and natural antimicrobials have observed in selective media conditions on the target pathogens (Khan, Flint, & Yu, 2010).

The shelf life extension of sliced cooked ham with HPP (200 and 400 MPa for 10 min at 17 °C) and enterocin combination during 3 months storage at refrigerated storage condition was studied by Liu et al. (2012). The authors identified that combined effects of enterocin (256/2560 AU/g) and HPP (400 MPa for 10 min at 17 °C) were effective in extending the shelf life of cooked ham above 90 days when compared to control by inhibiting the growth of bacteria and pathogenic micro organisms (*L. monocytogenes* and *S. enteritidis*) without affecting the sensory quality of the product. In contrast to this, de Alba, Bravo, and Medina (2013) also studied the effects of HPP combined with bio preservative (nisin) on the inactivation of *E. coli* O157:H7 in sliced dry cured ham (10⁶/g) stored under mild temperature abuse conditions (8 °C) for 60 days. Pressure treatment alone reduced 3 log units whereas nisin alone did not show any inhibitory effects on the pathogen. Interestingly synergistic effect of HPP (400 and 500 MPa for 10 min) and nisin

Table 2

Combined effects of novel hurdles and high pressure processing on the microbial population of meat and meat products.

Product	Microorganism inactivation outputs (log cfu/g)	Combination processing and storage conditions	Research outputs	Reference
Fuet- low acid fermented sausage	<i>Listeria monocytogenes</i> , <i>Salmonella enterica</i> , and <i>Staphylococcus aureus</i>	Enterocin AS-48 (148 AU ⁻⁸) and HPP at 400 for 10 min at 17 °C Stored at 7 °C for 30days	Synergistic effect of natural antimicrobial and HPP was observed in inactivation of the growth of pathogens	Ananou et al. (2010)
Sliced cooked ham	<i>Listeria monocytogenes</i> , <i>Salmonella enteric</i> subsp. <i>enterica</i> serovar <i>Enteritidis</i> and <i>Escherichia coli</i> O157:H7	Reuterin, lactoperoxidase system (LPS) and lactoferrin (LF) and HPP- 450 MPa for 5min Storage conditions: 4 °C and 10 °C for 35 days	Combination of HPP and Reuterin, LPS inhibited the growth of organisms at 4 °C and 10 °C for 35 days In individual treatments recovery of the pathogens during storage was observed	Montiel, Martín-Cabrejas, and Medina (2015)
Chicken breast fillets	<i>Escherichia coli</i> O157:H7 and <i>Pseudomonas fluorescens</i>	Bovine lactoferrin 0.5 mg/g and HPP- 200,300,400 and 500 MPa for 10 min AT 10 °C Storage conditions: 5 °C for 9 days	The combined effects of HPP and Lactoferrin was observed in additional reduction of 2.3 log CFU/g compared to HPP only treated samples at 300 MPa The counts of <i>Escherichia coli</i> O157:H7 was maintained below 0.5 log CFU/g in combined treated samples.	Del Olmo, Calzada and Nuñez (2012)
Cured beef carpaccio	<i>Escherichia coli</i> O157:H7	Freezing (−40 °C) HPP- 400 or 600 MPa for 5 min Storage conditions:1 °C for 12 days	Immediate after treatment a 2 log reduction of <i>Escherichia coli</i> O157:H7 was observed in frozen beef carpaccio whereas in fresh product it was 1.19 log reduction	Masana, Barrio, Palladino, Sancho, and Vaudagna (2015)

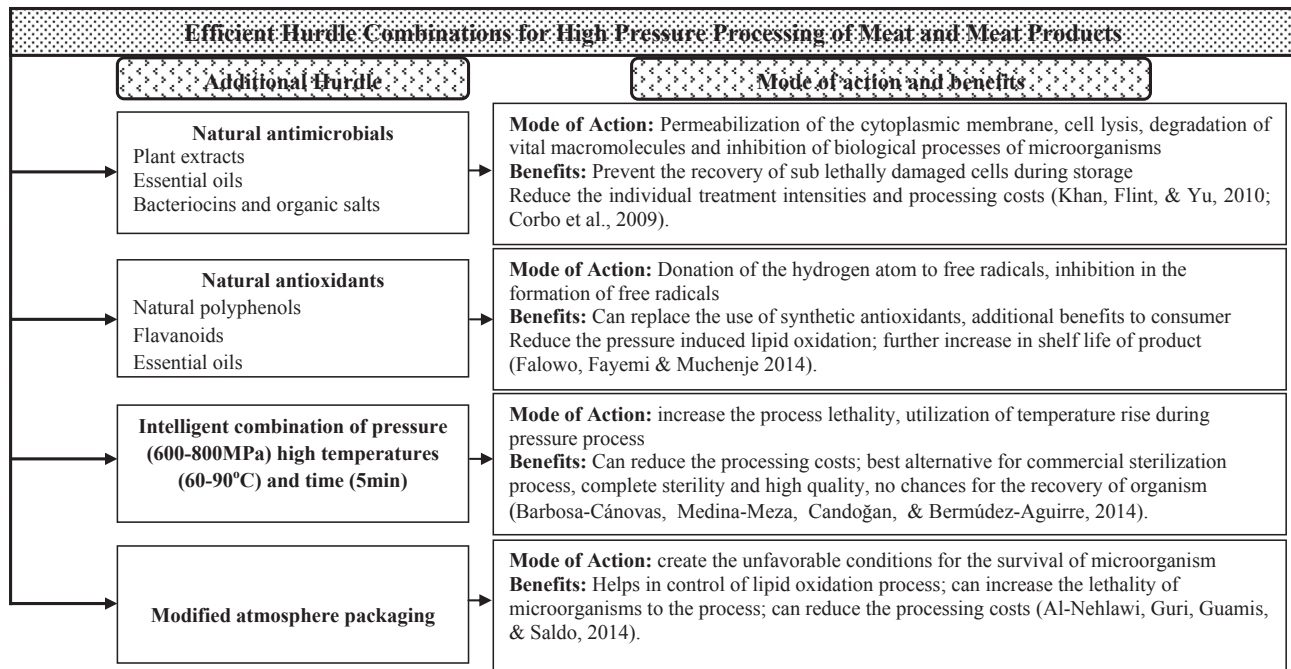


Fig. 2. Novel hurdle approaches to increase the efficacy of high pressure processing in inactivation of microorganisms (Al-Nehlawi, Guri, Guamis, & Saldo, 2014; Corbo et al., 2009).

were observed in reduce the counts of pathogens. Finally, the authors concluded that HPP at 500 MPa for 10 min combined with nisin can be effective in inactivation of *E. coli* O157:H7 in dry cured ham and maintained better microbial safety during 60 days storage period.

In another study the combined effects of natural antimicrobials (800AU/g nisin (N), 1.8% potassium lactate (L) and 800AU/g nisin +1.8% potassium lactate (NL)) and HPP (600 MPa for 5 min at 10 °C) on the inactivation of *Salmonella* sp., *L. monocytogenes* and *S. aureus* spiked with 4 log cfu/g was studied in vacuum packed sliced cooked ham. The effectiveness of the treatments was evaluated after 3 months of storage period at 1 and 6 °C. The study concluded that HPP and low temperature preservation can be effective in control the growth of *L. monocytogenes* and *Salmonella* sp, whereas the combination of pressure treatment and nisin could be better for the inactivation of pressure resistant *S. aureus* in cooked ham at low temperature preservation (Jofré, Aymerich, & Garriga, 2008).

Marcos, Aymerich, Monfort, and Garriga (2008) investigated the effect of HPP (400 MPa for 10 min) in combination with natural antimicrobials (enterocins and lactate-diacetate) to prevent the *L. monocytogenes* growth in cooked ham during storage (84 days) at 6 and 1 °C with temperature abuse conditions. The temperature abuse was created by keeping the samples for 24 h at room temperature on 40 and 60 day of 6 and 1 °C stored samples. The results showed that the storage temperature, HPP and antimicrobial combination resulted in reduce of 2.7 log cfu/g of *L. monocytogenes* during storage period of samples. Interestingly even after storage period least counts of pathogen with 4 MPN/g was observed in samples treated with HPP and enterocin stored at 1 °C.

3.2. Combination of active packaging and high pressure processing

The process involves in the controlled release and diffusion of the active compounds present in sustainable packaging material to the intact food material in order to maintain the desired quality and safety aspects is called active packaging of food (Raouche et al.,

2011). The consumers demand for the meat products with added natural preservatives has thrive the utilization of natural antioxidants and antimicrobials especially from plant resources and selected microorganisms. To increase the efficacy of HPP in extending the shelf life of meat products, as a novel multi hurdle approach, incorporation of natural antioxidants and antimicrobials into polymer matrix is gaining importance in recent years. Research studies have mentioned that the antibacterial and antioxidant activity of the active compounds will be better through packaging systems rather than direct addition to the meat products (Marcos et al., 2013). The most recent studies have demonstrated that development of active packaging in combination with high pressure processing presents a promising multi hurdle approach in complete elimination of food borne pathogens in RTE meat products during refrigerated storage conditions. The other important active packaging concepts include moisture absorbers, carbon dioxide emitters and oxygen scavengers (Stratakos, Delgado-Pando, Linton, Patterson, & Koidis, 2015; Véronique, 2008). The present paper aimed to explore the antimicrobial active packaging in combination with HPP on the safety of RTE meat products.

3.2.1. Antimicrobial active packaging and high pressure processing

Marcos et al. (2013) manufactured sodium salt free fermented sausages and inoculated *L. monocytogenes* (10^7 cells/g) on the surface of sausages. The combined effects of HPP and antimicrobial packaging with polyvinyl alcohol (PVOH) films containing nisin was assessed for inactivation of *Listeria* during storage period of 90 days at 4 °C. The authors mentioned that either HPP alone/nisin alone did not control the growth of organism during storage period where the combination of both (HPP and Nisin) resulted in reduce the levels of *L. monocytogenes* in the sausages.

In another study two different ready to eat dry cured hams varied with water activity and fat content (a_w of 0.92 and 14.25% fat and a_w of 0.88 and 33.26% fat) were subjected to HPP at 600 MPa for 6 min with or without application of nisin and analyzed for (*L. monocytogenes* CTC1034 inoculated at 10^7 cells/g) during 60 days

storage at 8 °C. The nisin was applied directly (200 AU/cm²) or through active packaging films (PVOH films 200 AU/cm².) during HPP. HPP resulted in immediate reduction of *L. monocytogenes* ranged from 1.85 to 3.58 log units depend on the type of ham. The results clearly showed that the effect was better in directly applied nisin samples moreover a_w had significant role in inactivation of the pathogen indicated lower the a_w less was the inactivation rates (Hereu et al., 2012).

In a study Marcos et al. (2008) examined the efficacy of HPP (400 MPa for 10 min at 17 °C) and active packaging (alginate films containing 200 AU/cm² enterocin (AE)) combination on the intentionally spiked 3 strain cocktail of *L. monocytogenes* at 10⁴ CFU/g in cooked ham during 90 days storage at 6 °C with temperature abuse conditions (24 h at room temperature after 60 days). The HPP treated cooked ham containing AE successively prevented the growth of *L. monocytogenes* and maintained at undetectable levels (5 CFU/g) compared to control groups (8.2–8.8 log CFU/g) during 90 days storage period at 6 °C.

In a very recent study, Stratakos et al. (2015) evaluated the synergistic and individual effects of active packaging (10% coriander oil active film - AP) and HPP (500 MPa for 1 min) on the growth of *L. monocytogenes* in a ready to eat chicken breast product. It was observed that HPP sample reached >8 log CFU/g at day 30 whereas AP resulted in low reduction (0.8 log CFU/g) of pathogen after one day of storage period. However the authors observed that combination of both the, active packing and HPP exhibited a synergistic activity in maintaining the *L. monocytogenes* count below the detection limit (1.69 log CFU/g) throughout the storage period (60days) at 4 °C.

In all the above mentioned studies, the authors demonstrated that combination of natural antimicrobials (either direct addition or through active packaging) with high pressure processing will be an important multi hurdle approach for the complete inactivation of spoilage and pathogenic microorganisms in RTE meat products stored at refrigerated conditions. Moreover the above studies evaluated the efficacy of this hurdle approach on the RTE meat products stored under temperature abuse conditions and in low salt meat products with important results. With this the authors strongly recommended the multi hurdle approach with combination of natural antimicrobials with HPP to increase the safety and quality of processed meat products.

3.3. High pressure processing: combination of antioxidants and antioxidant packaging

After microbial spoilage mechanism, oxidation process is primarily concerned for deteriorative changes in meat products (Gram et al., 2002). High pressure processing has been notified in increase the lipid oxidation rates of meat products at higher pressure levels (300–600 MPa). The increase in oxidation process leads to deteriorative changes that finally affect the shelf life and eating quality (Medina-Meza, Barnaba, & Barbosa-Cánovas, 2014) of the product. In general addition of synthetic antioxidants such as BHA, BHT, TBHQ to meat products are proved to be suitable in decrease the oxidation process. But in recent years the continuous alarming from the health organizations about the safety of synthetic antioxidants on the human health has led to the extensive research for the exploration and usage of natural antioxidants into meat products from plant sources (Stratakos et al., 2015). Several studies have proved the efficacy of natural antioxidants (essential oils and plant extracts rich in natural polyphenols) in controlling the lipid oxidation process in different meat products (Gallo, Ferracane, & Naviglio, 2012; McCarthy, Kerry, Kerry, Lynch, & Buckley, 2001). In regard to this very few studies have attempted to study the combined multi hurdle effects of natural antioxidants (either

through direct addition/active packaging) and HPP on the quality characteristics of meat products (Alves, Bragagnolo, da Silva, Skibsted, & Orlien, 2012).

In a very recent study, de Oliveira et al. (2015) tested the efficacy of phenolic carvacol (200 ppm) as natural additive in HP treated low sodium vacuum packaged sliced turkey breast ham stored at 4 °C for 60 days. The samples are prepared as follows; control with 20 g/kg- F_C, reduced sodium chloride 14 g/kg- F_{SR} and reduced sodium chloride with carvacol (200 ppm-F_{SRE}) and subjected for HPP (MPa for 180 s at 25 °C). The lipid oxidation was estimated through TBARS for every 10 days interval of storage period. The results indicated that HP induced lipid oxidation in F_C and F_{SR} samples which reached 0.173 and 0.148 MDA/kg during 60th day of storage period whereas F_{SRE} showed 0.071 MDA/kg. The authors recommended phenolic carvacol as multi hurdle approach to control the lipid oxidation process in vacuum packaged sliced turkey breast ham.

In another study the effect of antioxidant active packaging (AAP) system on the lipid oxidation profile of high pressure processed chicken patties prepared with breast and thigh portions. The extent of lipid oxidation process was determined from the surface and inner parts of the meat during 25 days storage at 5 °C. It was found that rate of lipid oxidation was dependent on the AAP that could control the extent of lipid oxidation in both surface and inner parts of meat patties compared to control (Bolumar, Andersen, & Orlien, 2011).

The research studies related to the combination of natural antioxidants during HPP of meat products still is in beginning stages. More research studies need to be conducted in order to explore the possible interventions with this multi hurdle approach. This approach seems to be potent in controlling the lipid oxidation process in RTE meat products.

3.4. Combination of high temperature and high pressure

The intelligent combination of pressure and temperature for shorter periods of time has been evolved as an emerging hurdle for the complete inactivation of microorganisms in food products. The bacterial spores that are pressure resistant at low or moderate temperatures can be completely inactivated through high temperature assisted pressure processing of foods (Ramasmamy, Zaman, & Smith, 2008; Zimmermann, Schaffner, & Aragão, 2013). This process is being called as pressure assisted thermal sterilization (PATS) that absolutely acts as an alternative for traditional sterilization process and approved by FDA in 2009 Bermúdez-Aguirre and Barbosa-Cánovas (2011). The research studies in this area are still in nascent stage with respect to meat products, however very few authors tested the potentiality of PATS in complete sterilization of meat products with positive reports. The process has been proposed as high temperature low time process with the advantage of utilization of self generated heat while compression process and applied pressure that contributes to the process lethality (Barbosa-Cánovas, Medina-Meza, Candoğan, & Bermúdez-Aguirre, 2014).

The effects of temperature assisted pressure processing on the inactivation of five strain cocktail of *L. monocytogenes* in RTE turkey breast meat was investigated by Chen (2007). In this study the vacuum packed RTE turkey breast meat was pressure treated at different processing conditions (pressure time and temperature as variables 300 MPa/2 min, 400 MPa/1 min and 500 MPa/1 min at 1, 10, 20, 30, 40, 50, 55 °C). It was found that processing temperature (low temperature and above 30 °C) and time had significant effects on the inactivation of *L. monocytogenes* (eg: 500 MPa/1 min at 40 °C and 20 °C reduced 3.8 log₁₀ and 0.9 log₁₀ CFU/g respectively).

4. Role of HPP in development of low salt meat products with improved technological quality characteristics

Salt in meat products plays important multifunctional roles in providing desired flavor, texture and taste (Desmond, 2006). The ultimate technological quality characteristics such as water holding capacity, cook loss and firmness were majorly influenced by the soluble protein content, which has its action in forming the better matrix for storage of water and fat (Garcia-Garcia & Totosaus 2008). The amount of NaCl in meat product directly influences the solubility extent of myofibrillar proteins (actin and myosin) (Weiss et al., 2010). On the other hand the gelation behavior of meat proteins under pressure called as 'pressure assisted gelation' may improve binding of meat particles, water and fat holding capacity in comminuted meat products (Chattong & Apichartsrangkoon, 2009). In respect to this previous studies have been demonstrated the possibilities with high pressure to improve the muscle protein functionalities in different meat products. Muscle proteins are highly responsive to high pressure with aggregation, denaturation and solubility properties (Bajovic et al, 2012). An application of this concept to develop low salt meat products is that HP could increase the solubility of myofibrillar proteins thereby chances to lower the amount of salt for the development of healthy meat products (Grossi et al., 2012; Sikes et al., 2009; Tornberg, 2013). So application of HPP seems to be a promising approach for the development low salt meat products with desired quality characteristics.

4.1. Water holding capacity

The retention of the moisture in meat and meat product matrix when subjected for external forces such as heating, cutting and slicing is called water holding capacity that directly influences the fate of cook loss of the final product. It is one of the important physical quality indicators for fresh and processed meat products that primarily influence the consumer acceptability and market value of the product. It also imparts the nutritional and physical composition of the finished products and palatability attributes such as texture, appearance, juiciness and overall eating quality (Hughes, Oiseth, Purslow, & Warner, 2014).

The real effects of high pressure processing on the water binding properties of meat products are still unclear and moreover contradictory in their nature. Some studies revealed the positive effects of HPP on water binding properties (WBP) and some recorded no effects and others indicated there is negative approach with HPP. In all these studies there are different factors such as type and nature of product, processing pressure-holding time-temperature, ingredients such as phosphates, salts nitrates and nitrites are found to be highly influential factors in determining the end result of HPP effect on the quality characteristics of meat and meat products. The present review aimed to explore the positive approaches of HPP in improving the water holding capacity (WHC) of different low salt meat products. The improvement in WHC of meat products attained through intelligent combination of pressure, temperature and other non meat ingredients that were briefly discussed in the coming literature.

Yang, Han, Bai, et al. (2015) and Yang, Han, Wang, et al. (2015) developed sausages with reduced fat (0, 5, 10, 5, 20, 25, and 30%) and salt (1%) levels and subjected for HPP at 0.1 and 200 MPa for 10 min. The changes in water holding capacity was observed using LF-NMR (low field nuclear magnetic resonance) relaxation measurements. The results indicated that HPP at 200 MPa resulted in greater water retention in the fast relaxation compartment. This was further confirmed with enhanced tenderness and scanning and transmission electron microscopy results (HPP resulted in more

protein denaturation) of the sausage.

Apart from the ingredient effects the pressure levels and pressure gradient (PG) had a significant impact on the WHC of meat products. In this sense, to study the effects of pressure gradient levels (low- 2.5 MPa/s and high 40 MPa/s) of high pressure on the water binding properties of reduced salt (NaCl-0.5–2% and phosphate 0.3%) and phosphate (NaCl 1% and phosphate 0.15%) frankfurters, Tintchev et al. (2013) formulated sausage batters and subjected to high pressure processing. The WHC was evaluated by using drip loss parameter for the batters treated at 300 and 600 MPa with the initial temperatures up to 40 °C. Pressure gradient levels significantly influenced the WHC of all the batters, processed with low PG and minimum loss was observed at 600 MPa with 40 °C as initial temperature. Interestingly the authors observed highly significant improvement in WHC of reduced salt batter (0.5% NaCl) processed with low PG levels might be due to structural changes induced through low PG level.

The unfolding nature meat proteins (myosin) under pressurized conditions lead to the formation of more flexible structure that can able to hold the maximum of moisture content. Chattong and Apichartsrangkoon (2009) subjected the ostrich meat yor (thai sausage) at different pressure levels (400 and 600 MPa), time (40 and 60 min) and temperature (40 and 50) and evaluated the WHC in terms of released plus total expressible water. The sausage processed at 200 MPa at 40 °C for 40 min showed 27.44% total plus released water; whereas the product processed at 400 MPa at 50 °C for 60 min exhibited a great decrease in total expressible water with 12.69%. Authors determined that the more the severity levels of the processing conditions had more influence in improving the water holding capacity of the product.

To study the combined effects of pressure and heat treatment on the cook yield and tenderness of beef steaks Sikes and Tume (2014) applied heat treatment (60, 64, 68, 72 or 76 °C for 20 min) with and without simultaneous pressure treatment at 200 MPa for 20 min and reported that heat treatment alone resulted in more than 30% water losses compared with pressure assisted heat treatment (<10%). Improved tenderness (peak force) was obtained for the pressure treated samples at 64 °C and was most favorable at 76 °C treated samples.

4.2. Effect of HPP on cook loss/cook yield of the meat and meat products

Cook loss is an essential physical quality parameter of meat products which determines the composition and palatability characteristics of the finished product and further influences the consumer acceptability level in the market. The results of Crehan, Troy, and Buckley (2000) showed that HPP (150 MPa) of frankfurters formulated with reduced salt (1.5%) significantly decreased the cook loss compared with non treated ones, and ultimately increased the cook yield of the product. This could be due to the increase in the solubilization of myofibrillar proteins as a result of depolymerisation induced by HPP (Cheftel & Culioli, 1997). The disaggregation and unfolding behavior of meat proteins under pressure treatment results in increased binding of actin and myosin fragments which subsequently results in decrease in cook loss of the product (Macfarlane, 1985). Proper binding of comminuted meat particles during heating will determine ultimate product yield. The study of Macfarlane, McKenzie, Turner, and Jones (1984) reported that HPP of patties up to 150 MPa improved the binding capacity of meat particles and reduced the cook loss in finished product. The interaction of salt levels and HPP on the cook loss of meat products were studied by different authors and indicated that, use of HPP has definitely reduced the use of higher salt levels while maintaining better quality attributes.

Reduced phosphate breakfast sausages developed with different levels of salt (0.5, 1, 1.5, 2 and 2.5%) and subsequent application of HPP at 0 and 150 MPa delivered useful results for cook loss. The reduction of salt levels from 2.5% to 0.5% without HPP resulted in increase cook loss (15.78–21.51%), whereas the same treated along with HPP at 150 MPa 5 min decreased the cook loss of the product (12.15–23.18%). The authors recommended that HPP can be successively adapted to develop low salt meat products to 1.5% with better organoleptic properties compared to control (2.5% salt) (O'Flynn et al., 2014).

Sikes et al. (2009) formulated meat batters with different levels of NaCl (0, 0.5, 1 and 2%) and subjected to different levels of pressures at 10 °C for 2 min. Both the high pressure treated (HPT) and non pressure treated (NPT) samples were subsequently cooked and analyzed for cook loss. The non high pressure treated batters containing 2% salt (non treated) showed 9% weight loss during cooking, and the loss was linearly increased up to 36% in 0% NaCl added batters. Whereas the same batters treated at 200 MPa for 2 min resulted in reduced cook loss in the same manner (0.5, 1 and 2%) except in batters with 0% NaCl. The study concluded that meat batters with 1% salt treated at 200 MPa for 2 min resulted in equal cook loss of control samples containing double the NaCl content.

The above studies have clearly demonstrated the importance of salt and high pressure conditions on the quality characteristics of meat products. HPP can be adopted as efficient strategy to reduce the salt levels in meat products while maintaining better quality characteristics compared to high salt meat products.

5. Conclusion

Multi hurdle approach with HPP in order to improve the quality and safety of meat products seems to be an efficient strategy for development of healthier meat products. The use of multi hurdle approaches can definitely bring down the operation and energy costs and can yield better quality meat products. Use of natural antimicrobials and antioxidants can be important hurdles in HPP of meat products to ensure the safety of meat products during whole storage period. It can be concluded that HPP technology can be a good platform to apply and study the multi hurdle effects for the development of high quality and safe RTE meat products for the modern society.

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