4

Novel Non Thermal Preservation Techniques in Meat Processing: High Hydrostatic Pressure as a Model Technology

4.1 Introduction

As a consequence of market globalization, the production and manufacture of meat products is at a stage of innovative dynamics. In order to keep or to reinforce their leading position, meat and food companies need to take into consideration the evolution of the purchasing and consumption habits of consumers, as well as the perception and definitively the trends of the consumers' demands. These consumers' demands are continuously changing, but some of the main parameters or axes are consolidating. Consumers demand high quality and convenient meat products, with natural flavour and taste, and very much appreciate the fresh appearance of minimally processed food. Besides, they require safe and natural products without additives such as preservatives and humectants.

To harmonize or to blend all these demands without compromising safety, it is necessary to implement new preservation technologies in the meat industry and in the food industry in general. High hydrostatic pressure (HHP) represents an attractive non-thermal process for meat products to avoid post-processing contamination. When combined with antimicrobials, like bacteriocins, the death rate may be increased because of sub lethal injuries to living cells. HPP is a powerful tool to control risks associated with *Salmonella spp*. and *Listeria monocytogenes* in raw or marinated meats. The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria.

Storage of chilled meats in air leads to rapid spoilage by psychotropic bacteria, predominantly *Pseudomonas* spp. and *Brochothrix thermosphacta*. Traditional packaging systems for meat products have been very successful in slowing the

rate of microbial spoilage and extending the shelf life of meats. These systems are designed to manipulate the gas environment surrounding the product. Such systems include oxygen - permeable overwrap for short – term retail display to maintain the bloom colour of red meats. For long-term storage, vacuum packaging (complete removal of headspace gases) or modified atmosphere packaging (MAP)/controlled atmosphere packaging (CAP) is employed. The success of these packaging systems is such that the majority of red meat produced in the United States is vacuum or MAP packaged (Siragusa et al. 1999).

The past decade has seen development of non - thermal technologies for the control of meat spoilage microorganisms and extension of shelf life. Information is now available on the types of microbes found on meats and conditions that lead to spoilage (Marshall and Bal'a 2001; Nychas et al. 2007). New information related to revolutionary packaging innovations such as gas scavenging and antimicrobial impregnation systems is now also available. So is information on recent developments in natural product biological interventions (phage, bacteriocins, chitosan, essential oils, and enzymes), chemical interventions (organic acid salts, acidified sodium chlorite, phosphates, ozone, and electrolyzed water), and physical interventions (ionizing irradiation, high pressure, hydrodynamic shockwave, pulsed electric fields, high intensity light, and cold plasma).

Many of the interventions remain at the theoretical stage and will require extensive validation and economic analysis before practical introduction to industry. Others, however, have found widespread use and will likely remain a mainstay in industry.

4.2 Description

4.2.1 Ionizing Irradiation

Irradiation is a safe and effective method to improve food safety and quality. Ionizing irradiation employs gamma rays (cobalt – 60 and caesium - 137 as radioactive sources), x - rays (machine - generated), and e beam (high - energy electrons, machine - generated) as treatments to successfully kill microbes in foods. Irradiation damages microbial DNA, resulting in cell death. According to Aymerichet al. (2008), viruses are most resistant to irradiation, followed by bacterial spores, yeasts, moulds, Gram - positive bacteria, and Gram - negative bacteria. This technology has excellent penetration power. For example, x - rays and gamma rays can penetrate 80 to100 cm while e beams have less penetrating power, ranging from 8 to 10 cm. None of these ionizing treatments make food radioactive, making questionable negative consumer fears about the technology. Irradiated foods should bear the internationally recognized radura symbol together with a "treated with irradiation" statement on the label to inform consumers.

4.2.2 Phage Technology

Bacteriophages (also known as phages), from "bacteria" and Greek *phagin*, "to eat" are viruses that infect bacteria. Phages consist of an outer protein shell with enclosed DNA or RNA. Phages infect, grow, and multiply only inside bacterial cells. Lytic phages cause bacterially is (cell death), which leads to the spread of more phage in the environment. Some phages lyse only a fraction of infected cells and keep other cells alive while continuously shedding new phages. Phages capable of lysogeny integrate phage DNA into the bacterial host DNA without causing cell death. Most reports on the use of phage technology focus on

applications to control meat -derived bacterial pathogens. For example, specific phages have been investigated against *Escherichia coli* O157: H7, *Listeriamonocytogenes*, *Campylobacter jejuni*, and *Salmonella enterica* Typhimurium (Bigwoodet al. 2008). In 2006, *L. monocytogenes* phage was approved by the FDA as a food antimicrobial (Stahl 2007). Several advantages of phage technology for meat spoilage control are described by others (Greer 2005; Hudson et al. 2005). For example, phages are self - reproducible and release more phage after bacterial lysis. Phage specificity may be an advantage if selective for spoilage micro flora only. On the other hand, specificity may diminish phage activity against broad - spectrum spoilage micro flora. Whitman and Marshall (1971a) noticed that phages from bacteriophage – host systems isolated from refrigerated food products usually attacked only those hosts upon which they were isolated. Phages are generally more stable than their hosts and can survive processing (Koo et al. 2000). Greer (1988) showed that phage concentration remained stable (5 to 6 log 10 PFU/cm 2) on the surface of refrigerated (4 ℃) beef rib – eye steaks during 14 days of storage in air. Whitman and Marshall (1971b) showed that some *Pseudomonas* phages isolated from beef may remain infectious after heating to 60 ℃, pH change to 4.0, and exposure to 4 MNaCl. Phages are naturally present entities and constitute part of the environment. Whitman and Marshall (1971a) isolated total of 38 host - phage pairings from ground beef, sausage, chicken, raw milk, and oysters. Phage concentration as high as 6.3 × 10 6 PFU/gas is found on chicken skin. Not surprisingly, most isolated were invaders of *Pseudomonas* spp., followed by Gram - positive cocci and members of the Enterobacteriacea family. Similarly, Atterbury et al. (2003) isolated 34 *Campylobacter* phages from retail chicken meat.

4.2.3 High Pressure Processing

High hydrostatic pressure (HHP) treatment involves placing packaged meat in a pressure vessel and applying isostatic water pressure of 100 to 900 MPa. HHP processing is considered non-thermal, since temperatures increase only 3 ℃ for every 100 MPa applied (Aymerich et al. 2008). Equipment for HHP is commercially available, including manufacturers Avure Technologies (United States) and Nicolas Correa Hyperbaric (Spain). HHP kills bacterial cells through a combination of actions, with the bacterial membrane the primary site of damage. Gram – negative bacteria are more susceptible, followed by Gram - positive bacteria and spores (Hugaset al. 2002). Linton et al. (2004) reported that the micro flora of chicken mince became less diverse and shifted to Gram - positive bacteria after HHP treatment. Regarding cell shape, rods (elongated) are more susceptible than cocci (round). It is generally believed that HHP does not significantly change the sensory quality of meats, although cooked colour (at 150 MPa), oxidation of ferrous myoglobin (at 400 MPa), and lipid oxidation has been reported in fresh and marinated meats (Hugas et al. 2002). Results of studies showing prevention of meat spoilage with HHP treatment are summarized in Table 4.1.

Table 4.1 *High hydrostatic pressure treatment of meat products.*

Product	Target bacteria	Results	Process	Reference
Minced beef muscle	Total micro flora	3 to 5 log 10 reduction	450 MPa, 20 min, 20 ℃	Carlez et al. 1994
Mechanically recovered poultry meat	Mesophilic bacteria	3.6 log 10 reduction	450 MPa, 15 min, 2 ℃	Yuste et al. 2001
Marinated beef loin Dry cured ham Cooked ham	Aerobic total count	> 4.5 log 10 reduction > 2.5 log 10 reduction > 6 log 10 reduction after 60 days at 4 ℃	600 MPa, 6 min, 31 ℃	Garriga et al. 2004
Minced chicken	Aerobic plate count	1 log 10 reduction	500 MPa, 15 min, 40 ℃	Linton et al. 2004

4.2.4 Hydrodynamic Shockwave Treatment

Hydrodynamic shockwaves (HDS) are generated either electrically (capacitor discharge system) or by using explosives in water. Besides tenderizing meat products by disrupting the myofibrillar structure (Schillinget al. 2003), HDS might influence bacterial counts as well, resulting in extended product shelf life (Raloff 1998). Explosively produced HDS are not commercially feasible because it is a batch - type process, has specific packaging requirements, and has potential worker safety concerns. In contrast, electrically generated HDS has been commercialized by Hydrodyne, Inc. (Claus et al. 2001). Mixed results are found in the literature on the effectiveness of HDS to inactivate microbes on meats. Williams - Campbell and Solomon (2002) showed that explosively generated shockwaves caused immediate reduction of aerobic plate counts by 1.5 to 2.0 log 10 CFU/g in fresh beef. After 14 days of storage, treated beef counts were 4.5 logs less than control samples. Schilling et al. (2003) showed that blade - tenderized beef treated with HDS had lower standard plate counts (0.5 log difference) compared to controls after 14 days of storage. On the other hand, Moeller et al. (1999) found no significant difference in aerobic plate counts and coliform counts between explosive HDS -treated pork muscle and control. Thus, aside from the obvious increase in tenderness, HDS treatment as a tool to decrease microbial loads and prolong the shelf life of meat products remains undetermined, and additional research is needed to support this concept.

4.2.5 Antimicrobials

(a) Bacteriocins

Bacteriocins are cationic and hydrophobic peptides produced by lactic acid bacteria, with antibacterial activity against related Gram-positive bacteria (Chen

and Hoover 2003). In addition to bacteriocins, lactic acid bacteria produce other antimicrobials, such as lactic acid, acetic acid, diacetyl, ethanol, and carbon dioxide among others (Davidson and Hoover 1993) Bacteriocins, usually named after the bacterium that produces it, can be classified into four major classes, with class I and class II being the most investigated (Hugas 1998). A brief summary of bacteriocins is presented in Table 4.2.

Table 4.2 *Summary of bacteriocins and their producing bacteria.*

Bacteriocin	**Producer**	**Bacteriocin**	**Producer**
Nisin, lacticin	*Lactococcus lactis*	Sakacin	*Lactobacillus sakei*
Lactocin	*Lactobacillus sakei*	Curvacin	*Lactobacillus curvatus*
Pediocin	*Pediococcus acidilactici*	Curvacitin	*Leuconostoc curvatus*
Enterocin	*Enterococcus faecium*	Bavaricin	*Lactobacillus bavaricus*
Brevicin	*Lactobacillus brevis*	Leucocin	*Leuconostoc gelidum*
Divergicin	*Carnobacterium divergens*	Carnobacteriocin/Piscicolin	*Carnobacterium piscicola*

Application of nisin in meat products is somewhat challenging due to its binding ability to meat components, low solubility (hydrophobic nature), and loss of efficacy at pH > 5 (Scannell et al. 1997; Murray and Richards 1998). For example, Rose et al. (1999) showed that glutathione, which is present in raw ground beef, can inactivate nisin. Scott and Taylor (1981) showed the need for greater nisin concentration to inactivate *Clostridium botulin* in cooked meat compared to microbiological medium. Finally, Chung et al. (1989) showed a 70% loss in nisin activity in raw meat during storage at 5 ℃ for 4 days. Hugas (1998) mentioned that pediocin might be more effective than nisin in meat applications, since it is derived from the meat-fermentation bacterium *Pediococcusacidilactici*. Another approach for bio preservation might be use of lactic acid – producing bacteria that also produce bacteriocins as direct protective cultures on meats (Hugas1998) due to the fact that lactic acid bacteria do not induce significant spoilage until large population numbers are reached (Nychas etal. 2007). Bloukas et al. (1997) extended shelf life of vacuum -

packaged frankfurters stored at 4 ℃ by one week using commercially available protective culture of *Lactobacillus alimentarius*.

(b) Lactic Acid, Sodium Lactate, Diacetate, and Acetate

Table 4.3 *Lactic acid - derived antimicrobials.*

Product	Antimicrobial	Result	Reference
Sliced poultry sausage	2% Na lactate	3 ×to 4 ×shelf – life extension, 5 to 7 ℃, air 7 ×shelf - life extension, 5 to 7 ℃, N2	Cegielska – Radziejewska and Pikul 2004
Pork chops	Na acetate Na lactate Na lactate/diacetate	Na lactate/diacetate treatment had lowest APC and least discoloration after 96 - h display	Jensen et al. 2003
Low - fat Chinese - style sausage	3% Na lactate	Lower microbial counts after 12 weeks storage at 4 ℃	Lin and Lin 2002
Retail beef cuts	1.2% acetic acid, 120 s 1.2% lactic acid, 120 s	Paler meat, but small sensory difference; 1 to 2 log 10 CFU/g reductions in Escherichia coli and APC count within 9 d storage	Kotula and Thelappurate 1994
Pork loin chop	2% Acetic acid 10% Na lactate dip	Pale soft exudates appearance, > 9 day shelf - life. Extended shelf - life by 3 days compared to control (9 vs. 6)	Lin and Chuang 2001
Vacuum packaged fresh pork sausage	1% Na lactate 2% Na lactate	1 to 2 weeks shelf – life Extension 2 week shelf - life extension	Brewer et al. 1993
Vacuum packaged cooked beef loins	4% Na lactate	Lower APC after 7 days at 10 ℃	Maca et al. 1999
Vacuum packaged beef bologna	3% Na lactate	Lower APC after 10 weeks storage at 4 ℃	Brewer et al. 1992
Vacuum packaged frankfurters	2% Na lactate	2 to 3 week shelf – life extension at 4 ℃	Bloukas et al. 1997

The U.S. government allows the use of lactic acid, sodium lactate (4.8%), sodium diacetate (0.25%), and sodium acetate (0.25%) on meat products as extensive research has shown their safety for human consumption (FDA 2000). Whether produced by lactic acid bacteria or chemically derived, the listed compounds are antagonists to food-borne pathogens and to general spoilage micro flora due to nonspecific mechanisms of action (Kim et al. 1995a, b; Marshall and Kim 1996; al' A and Marshall 1998; Kim and Marshall 2000). Numerous publications have documented the effectiveness of these compounds against *L. monocytogenes, E. coli O157:H7, Clostridium perfringens, and Salmonella spp*. (Glass et al. 2002; Porto et al. 2002; Juneja 2006; Michaelsen

et al. 2006; Paulsonet al. 2007). Lactate efficacy can be improved by combining with diacetate (Jensen et al. 2003; Serdengecti et al. 2006). The main drawback of using straight organic acids instead of their salts is lowered pH and the pale/watery appearance of fresh meats (Kotula and Thelappurate 1994; Lin and Chuang 2001). A summary of organic acid applications (with an emphasis on lactate) for meat product shelf life extension is presented in Table 4.3.

(c) Chitosan

Chitin is the second - most abundant natural biopolymer after cellulose and is a starting material for chitosan (deacetylated derivative of chitin) manufacturing. Since biodegradation of chitin is slow, its accumulation during crustacean processing (mainly shrimp and crab shell wastes) is a disposal challenge. The production of value - added chitin by - products, such as chitosan, could provide a solution to crustacean processing waste accumulation (Shahidi et al. 1999). Chitosan is reported to have antimicrobial properties. Factors that improve antimicrobial activity are a low degree of acetylation and a low pH, both of which increase solubility (Shahidi et al. 1999). Due to the highly reactive nature of polycationic chitosan, which readily interacts with proteins, fats, and other anionic compounds, chitosan antimicrobial activity is less in foods than in vitro (Rhoades and Roller 2000). Chitosan has achieved self - affirmed GRAS status (FDA - CFSAN 2004), removing regulatory restrictions on its use in some foods. Studies by Darmadji and Izumimoto (1994) showed that 1% chitosan addition to minced beef stored at 4 ℃ for 10 days inhibited growth of spoilage bacteria, reduced lipid oxidation and putrefaction, and resulted in better sensory quality. Specifically, an initial reduction of total bacterial count by 0.5 log 10 CFU/g was observed, with average count reductions after 10 days storage at 4 ℃ of 1.0, 2.6, 1.0, 1.4, > 2.0, and > 2.0 log 10 CFU/g for total bacterial, pseudomonad, staphylococci, coli form, Gram – negative bacteria, and micrococci counts,

respectively. Sagoo et al. (2002) showed that the addition of 0.3 and 0.6% chitosan to an unseasoned minced – pork mixture reduced total viable counts, yeasts and moulds, and lactic acid bacteria by up to3 log 10 CFU/g for 18 days at 4 ℃ compared with an untreated control. Juneja et al. (2006) found that addition of 3% chitosan to ground beef and ground turkey prevented growth of inoculated *C. perfringens* after cooking and inadequate cooling. Their results showed a 4 to 5 log 10 CFU/g reduction in *C. perfringens* spore germination and outgrowth over12 -, 15 -, and 18 - hour cooling cycles and a2 log 10 CFU/g reduction during a 21 – hour cooling cycle. Three treatments of fully cooked grilled pork (air packaged, vacuum packaged, or treated with chitosan and vacuum packaged) were investigated for the duration of shelf life (Yingyuad et al. 2006).

(d) Essential Oils

Plant - derived essential oil components may be active against bacteria but are difficult to apply in foods due to significant changes in sensory quality (Davidson 2001). Seydim and Sarikus (2006) compared the antimicrobial activity of oregano, rosemary, and garlic essential oils in whey protein isolate films (1.0 to 4.0% wt/vol) against *E. coli O157:H7, Staphylococcus aureus, almonella* Enteritidis, *L. monocytogenes*, and *Lactobacillus plantarum* on agar plates. Film with 2% oregano essential oil was the most effective compared to films with garlic effective at 3% and 4%) or rosemary extracts (no effect). Oussalah et al. (2004, 2006) also showed that alginate - based or protein – based edible films containing oregano essential oil were more effective than cinnamon orpimento in the extension of shelf life of whole beef muscle. They found that application of oregano oil edible film caused 0.9 and1.1 log 10 CFU/g reductions in *Pseudomonas* and *E. coli O157* counts, respectively after 7days of storage at 4 ℃ (Oussalah et al. 2004) ikewise, Skandamis and Nychas (2002) found that oregano essential oil extract extended shelf life of refrigerated MAP -stored fresh meat.

Allyl isothiocyanate is one of many volatile natural antimicrobials found in cruciferous plants, such as horseradish, black mustard, cabbage, and turnip. Nadarajah etal. (2005a) prepared paper disks containing1 ml of 65% allyl isothiocyanate mixed with corn oil. They then applied the paper disks to ground beef patties that were then vacuum packaged and stored for 15 days at 4 ℃. Results showed a delay in natural micro flora growth and significant population reduction in inoculated *E. coli O157: H7*. They argued that the antimicrobial might have use as vapour. When 5% to 20% mustard flour was used as a natural source of allyl isothiocyanatein ground beef, inoculated *E. coli O157: H7* population declined but no effect on spoilage micro flora was noted (Nadarajahet al. 2005b). Sensory evaluation results showed that panellists could detect mustard treatment, but considered mustard – treated meat to be acceptable.

(e) Enzymes

Lysozyme is a naturally occurring (human saliva, egg white), 14.6 kDa, single – peptide protein that has antimicrobial activity due to its enzymatic ability to hydrolyse β (1 – 4) glycosidic linkages in bacterial cell walls Proctor and Cunningham 1988). It is more active against Gram - positive bacteria, and activity against Gram - negatives can be increased by use of membrane disrupting agents (detergents and chelators), such as EDTA (Padgett et al. 1998). Because of this narrow activity range, most studies use lysozyme in combination with other antimicrobials. Gill and Holley (2000) showed that combined lysozyme, nisin, and EDTA treatment of ham and bologna sausages reduced populations of *B. thermosphacta* to no detectable levels for up to 4 weeks, while during storage at 8 ℃, growth of *Lactobacillus curvatus*, *Leuconostoc mesenteroides*, and *Listeriamonocytogenes* was slowed for up to 3, 2, and 2 weeks, respectively. Cannarsi et al. (2008) showed that the combination of 0.5% lysozyme and 2% EDTA extended the shelf life of chilled buffalo meat, with an antimicrobial effect

on all micro flora present, including *B. thermosphacta*. Nattressand Baker (2003) combined nisin and lysozyme as an antimicrobial treatment on pork loins, with successful inhibition of lactic acid bacteria and preferential growth of Enterobacteriacea. However, the authors noticed that aerobically displayed nisin - lysozyme treated meat spoiled sooner than untreated meat. They attributed this to inhibition of lactic acid bacteria and a resultant shift to putrefactive bacterial spoilers. In summary, a combined lysozyme/nisin/EDTA mixture may be a promising tool for extension of the shelf life of anaerobically packaged meats by inhibiting lactic acid bacteria, which is the predominant bacterial spoilage group capable of growth in such conditions.

4.3 General Analysis

4.3.1 Shelf Life Extension in Meat Products Treated with HPP

(a) Fresh Products

The application of HPP to fresh meat products results in a cooked-like aspect, and sometimes the products may develop a rubbery consistency. Murano, Murano, Brennan, Shenoy, and Moreira (1999) tested the usefulness of applying a mild heat treatment at 50 ℃ simultaneously with HPP in ground pork patties to lower the D values of Listeria monocytogenes obtained with only HPP. With a treatment of 414 MPa and 50 ℃ for 6min they obtained a 10-log^{-1} reduction in the most resistant strain of Listeria monocytogenes. Shelf life studies were also conducted, spoilage levels for control samples were reached after 5 days of storage at 4 ℃ and after 28 days for treated samples. Sensory evaluation of uninoculated grilled patties showed that panellists could not distinguish between those treated by heat and HPP and untreated controls. Thus, treatment by HPP in combination with mild heating can be used successfully to produce safer, long-

lasting fresh pork without affecting quality. Marinated beef loin, which is a raw uncooked meat product with high water activity, a low level of salt and without nitrite, harbours a mixed flora of spoilage and pathogenic microorganisms from the slaughterhouse cutting and trimming operations. Sliced, skin vacuum-packaged marinated beef loin was treated by HPP at 600 MPa for 6 min at 31 ℃. Aerobic, psychrophilic and lactic acid bacteria counts showed at least a 4 log 10 cycle reduction after treatment and remained below the detection limit ($<10^2$ cfu g^{-1}) during the chilling storage of 120 days, helping to prevent the sour taste and off-flavours while untreated samples reached 10^8 cfu g^{-1} after 30 days in the same conditions. Enterobacteriaceae were kept below 10 cfu g^{-1} during the whole storage period in HPP treated samples, while untreated samples reached 10^5 cfu g^{-1} after 30 days. HPP is a powerful tool to control risks associated with Salmonella spp. and Listeria monocytogenes in raw or marinated meats. Most of the untreated samples showed presence in 25 g from one or both of the pathogens, whereas all pressurized samples showed absence in 25 g (Garriga, Aymerich, & Hugas, 2002). The HPP treatment could extend the shelf life of the marinated beef loin by controlling the growth of both spoilage and pathogenic bacteria.

Main Technological Effects of HHP in Meat

About colour:

- In fresh or marinated meat, the iron in the myoglobin changes rom ferrous to ferric and globin is denatured: the red colour is lost.

About texture:

- Inhibition or stimulation of the proteolytic activity in muscles activity muscles (depending on processing conditions).

- Proteins are partially denaturized in products where proteins have not been previously modified by other process: heating, drying and fermentation.

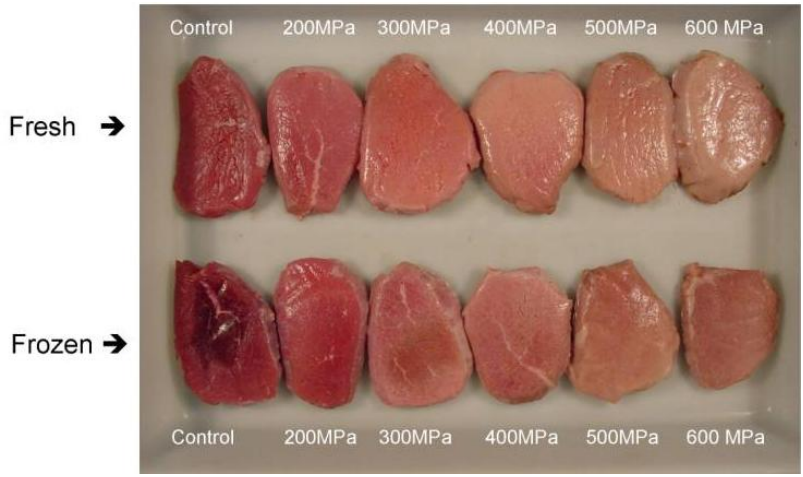

Figure 4.1 *Top view of the fresh and frozen beef samples treated by HHP Source: IRTA.*

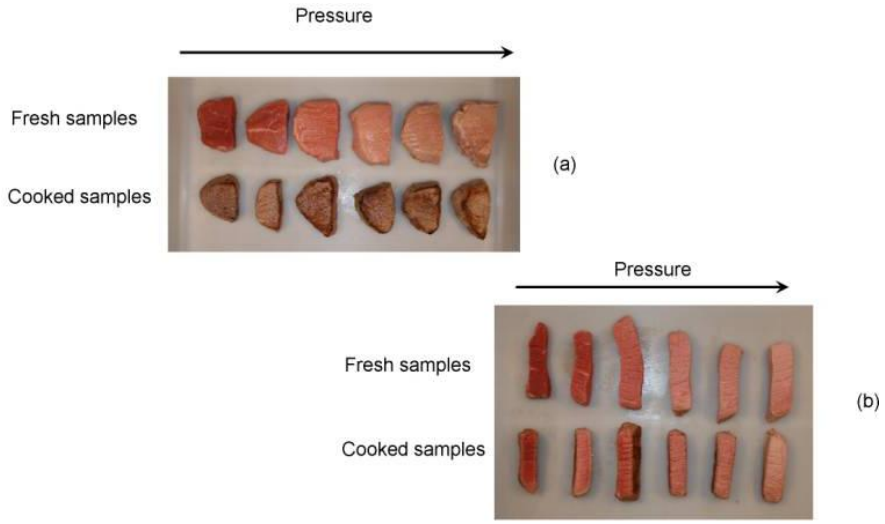

Figure 4.2 *Cooked samples: view from the top (a); view of the inside (b) Source: IRTA.*

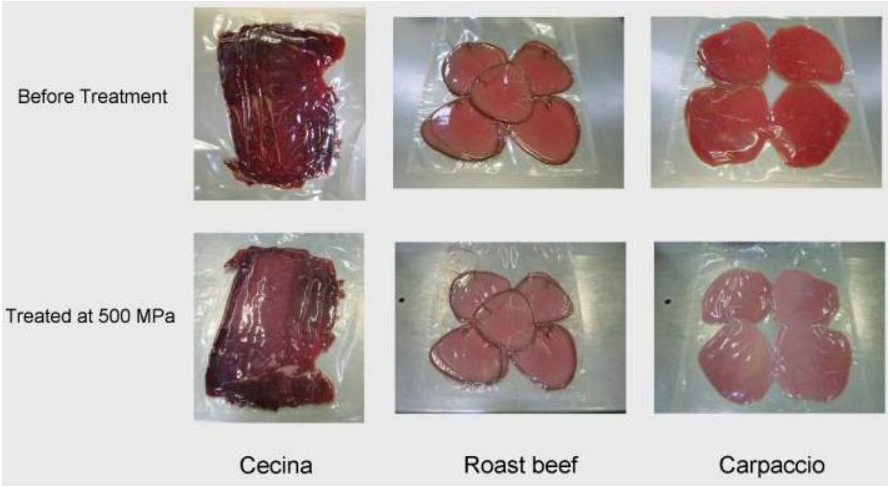

Figure 4.3 *Commercial beef products before and after HHP treatment, Source IRTA.*

(b) Cooked Ham

Sliced vacuum-packaged cooked ham is a highly perishable product due to its composition, pH and water activity and the lack of a background flora competing with spoilage or pathogenic microorganisms. The physico-chemical and microbiological characteristics of cooked ham do not represent any hurdles to bacterial growth. Its shelf life depends on the hygienic characteristics of the final product after post-processing as well as to the packaging methods where cross-contamination is more likely to occur. The techniques used to reduce cross-contamination include good manufacturing practices, post-pasteurization after packaging or even the use of ‘‘white rooms’’ at the slicing and packing stage. Sliced, skin vacuum-packed cooked ham treated by HPP at 600 MPa for 6 min showed a significant delay in the growth of spoilage associated microorganisms compared with untreated samples, thus contributing to the maintenance of organoleptic freshness for at least 60 days after treatment (Garriga, Aymerich, & Hugas, in press). The HPP process helped to prevent any sour taste, off-flavours, ropiness and colour changes. Thus, HPP processing on cooked ham in the

conditions mentioned earlier was an effective process to avoid the growth of yeasts and Enterobacteriaceae, with the potential to produce off-flavours and gas. Accordingly, it contributed to the shelf life extension in this highly perishable meat product. Dry cured ham is a dry, bone-in, salted and dried, non-fermented meat product. Because of the low water activity and high salt content of this type of product, spoilage microorganisms are mainly gram-positive cocci and yeasts. They may be present on the surface of whole hams and reach the sliced product during final boning, slicing and packaging operations. Sliced, skin vacuum-packed dry cured ham samples, treated by HPP at 600 MPa for 6min, showed a significant reduction of at least two log10 cycles for spoilage associated microorganisms after treatment. The surviving microbiota was kept at low levels during the storage period; contributing to the preservation of the organoleptic freshness during shelf life (120 days) and helping to prevent off-flavours, sour taste and gas formation. Enterobacteriaceae and Escherichia coli were below the detection limit, both in HPP and untreated samples. Listeria monocytogenes was present (in 25 g) in one untreated sample, but absent in all HPP treated samples during the whole storage period. (Garriga, Aymerich, & Hugas, 2002). Demonstration of the substantial equivalence of HPP meat products after evaluating the proximate composition of marinated beef loin, cooked ham and dry cured ham pressurized at 600 MPa for 10 min at 30 ℃ compared with control non-pressurized samples (Table 4), small differences have been observed which could be more related with the variability of samples and raw materials than with the technological procedures. A slight decrease in phosphate content was detected in samples of HPP-treated dry cured ham, indicating a possible enhancement of phosphatase activity. The differences in chloride and phosphate contents ($P<0.001$) fell within the typical variability between samples in whole muscle meat products. As a general conclusion HPP did not show any influence in the proximate composition of cooked ham, dry cured ham and marinated beef loin.

Non-significant differences were found in the non-protein nitrogen fraction in the three meat products studied when HPP treated and compared with controls. In the same sense, no differences were observed in their amino acid content (Garcı´a-Regueiro, Sa´rraga, Horto´s, Dı´az, Valero, & Rius, 2002). These results agree with a lack of protein breakdown due to HPP. For the fatty acid composition and the cholesterol content in the three products studied no significant differences between samples were found, with the exception of acid. With this fatty acid, an increased stability was observed in pressurized marinated beef loin ($P<0.05$). According to the levels obtained in cholesterol oxides, less cholesterol oxidation was obtained in pressurized products. 7 Ketocholesterol which was high in beef control samples was strongly reduced in beef subjected to HPP. However, it is necessary to study if HPP processing could have some influence on the recovery of cholesterol oxides by analytical methods. The vitamin content did not present any significant differences between HPP-treated and untreated samples, at least on the B group vitamins. In general, no significant differences were found in the mineral composition of pressurized samples compared with control. The decrease of calcium content in HPP cooked ham is difficult to explain and more experiments should be carried out to verify if the solubility of some ions is modified by HPP. An increase in the iron content of HPP beef loin can be explained according to the results of Ledward (2001), who reported a release of iron from non-heme complexes at pressures higher than 400 MPa as well as from the heme proteins denaturation above 300 MPa. Such changes do not apparently occur in cured meats. As a general conclusion it can be stated that from a physico-chemical point of view, cooked pork ham, dry cured pork ham and marinated beef loin, vacuum packed and high pressure treated at 600 MPa for 10 min at 30 ℃, are substantially equivalent to the same untreated products. The effect on the bioavailability of nutrients was also assessed. The solubility of proteins in cold 1% SDS was higher in marinated meat HPP than in untreated samples, whereas

no differences were found in dried cured ham or cooked ham. The proteins solubilised in this medium are representative of the cytoplasmic fraction, excluding most of the myofibrillar proteins. The solubility of the myofibrillar protein fraction in a selective solvent (1 M KCl) was markedly reduced by pressure treatment, but it is even more dramatically decreased by traditional cooking. Analysis by SDS– PAGE of different conditions of protein extraction, showed only minor differences confirming that pressure did not affect the primary structure of proteins. Nevertheless, precipitation by TCA after KCl extraction as well as solubilisation by 6M urea and SDS–PAGE confirmed the lower major proteins' solubility in the pressurized materials except in dry cured ham.

Table 4.4 *Proximate composition of pressurized meat products: marinated beef loin (A), cooked ham (B) and dry cured ham (C) pressurized at 600 MPa, 10 min 30 ℃; Garcı´a-Regueiro et al., 2002.*

		Control	SD[a]	HPP[b]	SD[a]
(A)	Marinated beef loin				
	Moisture (%)	74.11	0.60	73.78	0.65
	Fat (%)	4.54	0.76	3.68	0.46
	Protein (%)	20.64	0.83	21.43	0.50
	Hydroxyproline (ppm)	677.0	316.7	558.6	130.3
	NO_2 (ppm)	5.00	0.00	5.00	0.00
	NO_3 (ppm)	9.67	2.31	15.67	4.04
	Chloride (%)	0.74	0.03	0.83	0.09
	Ash (%)	1.68	0.13	1.96	0.08
	Carbohydrate (%)	0.71	0.04	0.65	0.06
	Phosphate (ppm)	4786	411	3795	320
	Ascorbate (ppm)	<10	0.00	<10	0.00
	pH	5.44	0.01	5.80	0.03
(B)	Cooked Ham				
	Moisture (%)	75.20	0.24	74.02	0.40
	Fat (%)	2.63	0.38	2.97	0.89
	Protein (%)	22.67	0.58	20.64	1.44
	Hydroxyproline (ppm)	993.7	136.3	1043.3	56.52
	NO_2 (ppm)	103.3	6.66	91.0	3.00

	Control	SD[a]	HPP[b]	SD[a]
NO_3 (ppm)	38.33	3.06	38.0	3.61
Chloride (%)	2.06	0.04	1.80	0.01
Ash (%)	3.16	0.05	3.18	0.09
Carbohydrate (%)	0.52	0.03	0.52	0.02
Phosphate (ppm)	4592	74	3051	269
Ascorbate (ppm)	234	16	219	14
pH	6.42	0.02	6.52	0.04
(C) Dry Cured Ham				
Moisture (%)	50.64	0.28	50.17	1.03
Fat (%)	12.9	1.46	14.6	1.36
Protein (%)	30.56	0.70	28.88	0.50
Hydroxyproline (ppm)	2035.3	144.3	1873.0	18.08
NO_2 (ppm)	5.00	0.00	7.67	0.58
NO_3 (ppm)	98.67	3.51	81.67	12.7
Chloride (%)	3.76	0.10	4.63	0.14
Ash (%)	6.24	0.09	6.41	0.11
Carbohydrate (%)	0.19	0.02	0.22	0.04
Phosphate (ppm)	4590	360	3663	980
Ascorbate (ppm)	58	1	74	6
pH	5.48	0.44	6.11	0.03

Key:

a. SD, standard deviation

b. HHP, high pressure processing

*. P<0.5

**. P<0.01

***. P<0.001

4.4 Actualisation

Study by J. Yuste, M. Mor-Mur, I M. Capellas, B. Guamis, and R. Pla- Mechanically Recovered Poultry Meat Sausages Manufactured with High Hydrostatic Pressure; 1999 Poultry Science 78:914–921.

The effect of high pressure processing at high temperature on texture and colour of frankfurter type sausages made with different contents of mechanically recovered poultry meat (MRPM) was evaluated and compared with that of a standard cooking process. Five types of sausages containing 100, 75, 50, 25, and 0% MRPM and 0, 25, 50, 75, and 100% of minced pork meat (MPM), respectively, were manufactured. They were pressurized at 500 MPa for 30 min at 50, 60, 70, and 75 C or cooked at 75 C for 30 min. Pressure treated sausages were less springy and firm, but more cohesive. Moreover, colour of pressurized sausages was lighter and more yellow than that of conventionally cooked sausages. Addition of MPM increased cohesiveness, hardness, and force at 80% compression. Minced pork meat also caused the appearance of sausages to be lighter, less red, and less yellow. Cooked sausages made with MRPM can have an attractive appearance and texture via high pressure processing.

Compared to a standard cooking process, high pressure processing at high temperature yielded less springy and firm but more cohesive sausages, which were also lighter and more yellow. The addition of MPM increased cohesiveness, hardness, and force at 80% compression. It also caused lighter, less red and less yellow sausages. In this study, formulation influenced textural parameters more than type of treatment; this effect was very clear, particularly in the case of absence of MRPM. Significant differences were caused by the three variables (formulation, temperature of treatment, and type of treatment) and also by the interactions among them. Thus, pressurization could be a good choice to achieve desirable characteristics in the case of meat products containing MRPM, because two of the main drawbacks of this meat as an ingredient are its appearance (too dark) and texture (too pasty and soft). Tartarisation of MRPM would possibly increase the range of products prepared from this raw material (Froning, 1976; Jones, 1988). Moreover, a certain

amount of MPM can help to solve the disadvantages and to improve the properties of these products, but this raw material should not be added excessively because it could lead to very firm products.

Dhillon and Maurer (1975), Froning (1976), Newman (1981), and Radomyski and Niewiarowicz (1987) stated that combinations of MRPM and hand deboned poultry meat gave desirable sensory and functional properties and economic advantages. From the results obtained, it can be stated, as reported by Cheftel and Culioli (1997), that pressure treatment with previous, simultaneous, or subsequent cooking is the most suitable way of processing fresh whole or minced meat, taking into account the modifications induced by pressurization.

Final cooked meat products would be obtained directly from this process. Cooked sausages containing MRPM with better appearance and texture than the traditional ones can be obtained by means of high pressure processing. Moreover, the ability of pressurization to inactivate microorganisms and, therefore, to enhance the safety and to extend the shelf-life of some food products must be emphasized (Hoover *et al*., 1989; Hayashi, 1991; Ludwig *etal*., 1992; Yuste *et al*., 1998). Thus, high pressure processing is a technique with a promising future in the processing of meat and meat products and, in general, in food technology.

4.5 Discussion

In the near future, the new non-thermal technologies will very likely replace current technologies. However this may cause confusion to the consumer. Does this mean that current technologies are not guaranteeing the safety of foods we are consuming every day? New technologies can tackle the problem of new emergent pathogens which concern the consumers but they could also be very

useful for the development of new products. A representative survey (Baron et al., 1999) of consumer attitudes concerning HPP of foods was carried out among 300 adults aged 14 years and over in France, Germany and the United Kingdom in face-to-face computer assisted personal interviews. The variable to be predicted using the model was the willingness to buy products preserved by HPP. The acceptability values were 71% for France, 74% for Germany and 55% for the UK. The average acceptability rate of 67% was clearly above the threshold value of 60% (a pragmatic market research threshold) which is extremely positive for such an emerging technology. The best predictor which optimizes the classification result of potential buyers and non-buyers in the three countries is mainly the hope for more personal advantage from this new technology. Before the total implementation of the new preservation technologies, several issues need to be addressed such as: the mechanisms of microbial resistance and adaptation to these new technologies, the mechanisms of microbial and enzyme inactivation, the identification of the most resistant and relevant microorganisms in every food habitat, the role of bacterial stress, the robustness of the technologies, the increased safety versus current technologies and last but not least, the legislation needed to implement them. In some years, there will be new technologies to be used: gamma irradiation, electron beams, microwave heating, ohmic heating, high pressure, pulsed electric field, submerged arcing, pulse lights on surfaces, etc. Some of them have a high likelihood of being used in combination with other technologies. The applications in the real world of the new technologies are new challenges to the food technologists and food researchers. The need to convince consumers and stakeholders about the improvement these new technologies represent is a must. To do so, it is very important to present convincing data, to identify stakeholders and to provide clear, objective and unbiased information including the potentially negative aspects and their limitations. It is very important to

demonstrate that the technology is available or that there is existing potential to develop a given technology. Pressure treatment is maybe, the most available emergent technology. However, it is still costly, mainly because of the initial capital expenditure, and this may limit its application. It is expected than these costs will go down as a consequence of further progress in technology, the acceptance of and resultant investment in the requisite equipment for HPP by an increasing number of manufacturers. As an example, the treatment cost of cooked ham is 0.1E per kg which is a cost quite affordable for the consumer.

4.6 General Recommendations

1. The use of HP as a possible alternative processing method to thermal treatment has brought about the need to study the pressure–temperature behaviour of macromolecular food ingredients since, for example, the mechanisms of protein denaturation under pressure are far from fully understood.

2. It is well known that HP can modify the activity of some enzymes and the structure of some proteins. Although covalent bonds are not affected, hydrogen bonds as well as hydrophobic and intermolecular interactions may be modified or destroyed. From this perspective, some concern about the potential risks of HP may arise. It is necessary to compile data in order to clarify the role of HP towards toxicity, allergenicity, loss of digestibility and the eating and nutritional quality of foods (Hugas et al., 2002).

3. There have been many studies of the use of HP as a pre-treatment method to improve the textural properties of food products. As a pre-treatment tool, HP processing appears effective in improving gelation properties of meat, egg or soy proteins, as well as improving the coagulating properties of

milk (Galazka et al., 2000). Further studies are also required to understand the potential of the technology for rheological control in food protein systems, as well as to optimize the operating conditions that should be used during actual processing.

4. Before any food product can be produced commercially using HP, optimization of processing conditions is essential to ensure product safety (McClements et al., 2001).

5. Food companies must be able to make a realistic cost-benefit analysis of the potential rewards in investment in HP processing. The value of HP in terms of increasing food safety assurance, in some cases, may alone be sufficient to justify such investment.

4.7 Conclusion

The application of any new technology presents significant challenges to food technologists and food researchers. HP processing offers the food industry a technology that can achieve the food safety of heat pasteurization while meeting consumer demand for fresher-tasting minimally-processed foods. In addition, other favourable organoleptic, nutritional and rheological properties of foods have been demonstrated following HP, in comparison to heat processing. The retention of colour and aroma and the preservation of nutritive components are enormous benefits to both the food processing industry and consumers. Also, from a food processing/engineering perspective, key advantages of high-pressure applications to food systems are the independence of size and geometry of the sample during processing, possibilities for low temperature treatment and the availability of a waste-free, environmentally-friendly technology. Application of HP can inactivate microorganisms and enzymes and

modify structures, while having little or no effects on nutritional and sensory quality aspects of foods. HP food processing is today being used on an ever-increasing commercial basis. Opportunities clearly exist for innovative applications and new food product development. HP can affect the functionality of protein and carbohydrate molecules in often unique ways, which may allow the optimization of food manufacturing processes and the production of novel foods. The range of commercially-available HP-processed products is relatively small at present but there are opportunities for further development and the production of a wide range of HP-treated products. The main drawbacks of pressure treatment of solid foods are the use of batch or semi continuous (the latter for liquids only) processing and the high cost of pressure vessels. HP is an environmentally-friendly, industrially-tested technology that offers a natural alternative for the processing of a wide range of different food products. This method prolongs product shelf-life while at the same time preserving organoleptic qualities, by inactivating microorganisms and enzymes while leaving small molecules such as flavours and vitamins intact. It is a technology with many obvious advantages, especially for food products with a high added value, targeted at a growing group of consumers that demand maximum safety and quality in the products they purchase.

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