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School of Food and Nutritional Sciences

HIGH PRESSURE PROCESSING AS A HURDLE TECHNOLOGY FOR DEVELOPMENT OF CONSUMER-ACCEPTED, LOW-SALT PROCESSED MEAT PRODUCTS WITH ENHANCED SAFETY AND SHELF-LIFE

Thesis presented by

Ciara O' Neill, BSc.

For the degree of
Doctor of Philosophy in Food Science and Technology

Under the supervision of
Professor Joseph P. Kerry
Dr. Malco Cruz-Romero

Head of School
Professor Paul Mc Sweeney

June 2018

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Declaration

I hereby declare that this thesis is my own work and contains no material that has been accepted for the award of any degree in University College Cork or elsewhere.

Certified by: _____

Ciara O' Neill

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Abstract

Processed meat manufacturers continuously seek new ways to reduce salt in meat products, without compromising consumer acceptability, but with enhanced safety and shelf-life. Response surface methodology (RSM) was used to develop optimised low-salt processed meat products (frankfurters and cooked ham). A box-Behnken experimental design was used to assess independent factor effects; salt replacer (Artisalt^{TM}) (0-100%), high pressure processing (HPP) (0.1-600 MPa) and a mix of organic acids (Inbac^{TM}) (0.2-0.4%) on measured responses for overall sensory acceptability (OSA). Optimum parameters to maximise salt reduction and produce cooked ham with similar OSA associated with these product types were Artisalt^{TM} (53%), HPP (535 MPa) and Inbac^{TM} (0.3%), while optimum parameters for frankfurters were Artisalt^{TM} (48%), HPP (580 MPa) and Inbac^{TM} (0.3%). Total salt contents for optimised low-salt cooked ham and frankfurters were 1.4% and 1.3%, respectively. Hurdles applied extended the shelf-life of low-salt frankfurters or cooked ham by 51% or 97%, respectively, compared to control samples. Consumers (n=100) assessed optimised low-salt and control frankfurters and cooked hams in comparison to 'gold standard' commercially-available products on the Irish market and results showed that optimised low-salt processed meat products were as acceptable, or better, than 'gold standard' equivalents, thereby confirming the potential for use of the salt replacer Artisalt^{TM} and hurdles HPP and Inbac^{TM} to produce consumer-acceptable low-salt processed meat products with enhanced safety and shelf-life.

A combination of HPP (300 MPa, 400 MPa or 500 MPa) and a mix of organic acids Inbac^{TM} (0.3%) were then used as hurdles to extend the shelf-life of marinated pork chops. Results showed that HPP ≥400 MPa increased (P<0.05) piri-piri marinade absorption which enhanced the flavour acceptability of the marinated pork chops; however, at 500 MPa, initial toughness was increased. The piri-piri marinade masked the whitening effect caused

by HPP and also increased (P<0.05) the tenderness of the marinated pork chops over storage time. Combined effects of HPP at 300, 400 or 500 MPa and Inbac™ (0.3%) extended (P<0.05) product shelf-life by 16, 22 and 29 days, respectively. Finally, the effects of griddle and steam cooking on the physicochemical and sensory characteristics of HPP piri-piri pork chops were investigated. Results indicated that the acceleration of marinade by HPP modified product fatty acid profile by increasing Oleic acid, as this was the main fatty acid present in the piri-piri marinade. Overall, steam cooking resulted in better quality marinated pork chops with improved physicochemical and sensory characteristics compared to griddled marinated pork chops.

Keywords

Processed meat, high pressure processing, frankfurters, cooked ham, low salt, salt replacers, piri-piri, pork chops, marination, organic acids, hurdle technology, shelf life, sensory, microbiology, physicochemical.

Publications List

1. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. The application of response surface methodology for the development of sensory accepted low-salt cooked ham using high pressure processing and a mix of organic acids. Accepted for publication in the Journal of Innovative Food Science and Emerging Technologies. 45: 401-411.

2. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. The application of response surface methodology for development of sensory-acceptable, low-salt, shelf-stable frankfurters using high pressure processing and a mix of organic acids. Submitted to the Journal of Meat Science.

3. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. Shelf life extension of vacuum-packed salt reduced frankfurters and cooked ham through the combined application of high pressure processing and organic acids. Accepted for publication in the Journal of Food Packaging and Shelf life. 17: 120-128

4. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. Comparative study on the acceptability and consumer appeal of commercial products and research optimised low-salt frankfurters and cooked ham manufactured using high pressure processing and organic acids. Submitted to the Journal of LWT Food Science and Technology.

5. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. Improving flavour absorption and shelf life of marinated pork chops through the application of high pressure processing as a hurdle. Submitted to the Journal of Food Packaging and Shelf life.

6. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry. 2018. Comparative effect of different cooking methods on the physicochemical and sensory characteristics of high pressure processed marinated pork chops. Submitted to the Journal of Innovative Food Science and Emerging Technologies.

Conference presentations

1. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry (2015). The Application of Response Surface Methodology in the Development of Low-Salt Content Frankfurters Using High Pressure Processing and Organic Acids as Hurdles. 29th EFFoST International Conference, Athens, Greece.

2. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry (2015). Response Surface Methodology Applied To Salt Reduction On Frankfurters Using High Pressure Treatment. 29th EFFoST International Conference, Athens, Greece.

3. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry (2016). Application of Response Surface Methodology for the Development of Low-Salt Hams Using High Pressure Processing and Organic Acids as Hurdles. 18th IuFoST World Congress of Food Science and Technology, Dublin, Ireland.

4. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry (2017). The Use of Hurdle Technology for the Development of Consumer Accepted Low-Salt Ham with Enhanced Shelf Life. 63rd ICoMST International Congress of Meat Science and Technology, Cork, Ireland.

5. Ciara O' Neill, Malco Cruz-Romero, Geraldine Duffy, Joe Kerry (2017). The Application Of Hurdle Technology (High Pressure Processing And Organic Acids) For The Development Of Salt Reduced Processed Meat Products With Enhanced Safety. Industry Workshop, UCC.

Abbreviations

a* - Redness

ALA - α-linolenic acid

A_w – Water activity

b* - Yellowness

CVD – Cardiovascular disease

DHA - Docosahexaenoic acid

E.Coli – *Escherichia Coli*

EM – Expressible moisture

EPA - Eicosapentaenoic acid

F-LS/1T - Optimised low-salt frankfurters containing 1.04%NaCl+ 0.96% Artisalt™, optimum levels of 1 treatment (a mix of organic acids (0.3 % Inbac™) without HPP).

F-LS/2T - Optimised low-salt frankfurters containing 1.04%NaCl+ 0.96% Artisalt™, optimum levels of 2 treatments (a mix of organic acids (0.3% Inbac™) and HPP at 580 MPa for 5 mins).

FAME - Fatty acid methyl esters

FSAI – Food safety authority of Ireland

GC – Gas chromatography

HDP – Hydrodynamic pressure

H-LS/1T - Optimised low-salt ham containing 1.06% Artisalt™ + 0.94% NaCl, optimum levels of 1 treatment (a mix of organic acids (0.3 % Inbac™) without HPP).

H-LS/2T - Optimised low-salt ham containing 1.06% Artisalt™ + 0.94% NaCl, optimum levels of 2 treatments (a mix of organic acids (0.3% Inbac™) and HPP at 535 MPa for 5 mins).

HP – High pressure

HPP – High pressure processing

IHF – Irish heart foundation

IS – Ionic strength

KCl – Potassium Chloride

L* - lightness

L. monocytogenes - *Listeria monocytogenes*

LAB – Lactic acid bacteria

LSCH – Low salt cooked ham

MDA – Malondialdehyde

MPC – Marinated pork chops

MRD - Maximum recovery diluent

MUFA – Monounsaturated fatty acids

NaCl – Sodium Chloride

OSA – Overall sensory acceptability

PCA – Principle component analysis

PUFA – Polyunsaturated fatty acids

ROLS- Research optimised low-salt

RSM – Response surface methodology

RTE – Ready to eat

S.aureus – *Staphylococcus aureus*

SC- Steam cooked

SFA – Saturated fatty acids

TBARS - Thiobarbituric acid reactive substances

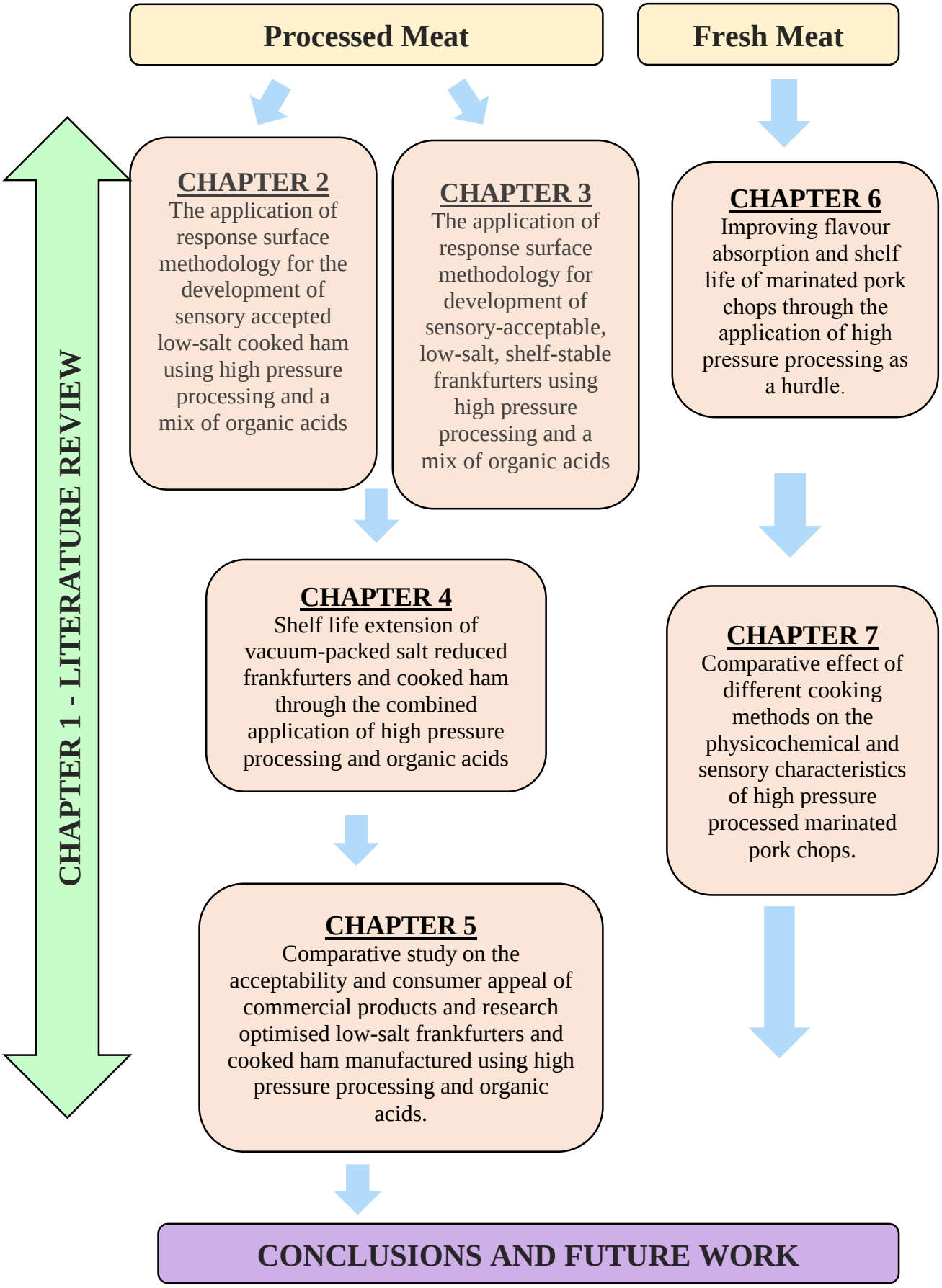
TVC – Total viable count

WBSF – Warner bratzler shear force

WHC – Water holding capacity

WHO – World health organisation

Schematic overview of thesis chapters



CHAPTER 1 - Literature Review

1.1 Introduction

The knowledge to preserve meat by making fermented sausages dates back to ancient times (Pederson, 1979). Such products were described in old Greek, Roman and even Babylonic scripts. In Northern and Central Europe "meat animals" were slaughtered before winter; however, not all this meat could be eaten at once, so the remaining part was processed to preserve the meat for later consumption (Vandendriessche *et al.* 2008).

It is said that the success of the Roman army in conquering nearly all the territories of the "Old World" was partly due to the knowledge of preserving meat (dry-cured ham and fermented sausages), which made the long distance supply of food to the troops feasible. In the 18th century, meat consumption increased significantly owing to agricultural innovations (Fiddes, 2004).

To this day, meat is still an integral part of the human diet in most cultures, often seen with a deep symbolic meaning and relative social function (Leroy and Praet, 2015). Due to richer and more diverse diets, the high-value protein that meat offers improves nutrition for the majority of the global population. Meat is also an important source of a wide range of essential micronutrients, including; zinc, iron and vitamins such as vitamin B-complex, α-tocopherol, retinol and vitamin K (Belitz *et al*. 2009). However, excessive consumption of meat-based products can lead to high intakes of saturated fat and salt (WHO, 2003a), which in turn can lead to an increase of chronic diseases, such as; obesity, diabetes, hypertension, stroke, cardiovascular disease, and some types of cancer. Consequently, the World health organisation (WHO) are currently driving measures to reduce salt and saturated fat content in foods by raising consumer awareness of such issues and setting guidelines for the food processing industry to follow.

1.2 Overview of the global meat industry

The world's livestock sector is growing at an unprecedented rate and the driving force behind this enormous surge is a combination of population growth, rising incomes and urbanization. Worldwide, meat production has tripled over the last four decades and has increased by 20% in the last 10 years due to a growing demand for high-value animal protein (WHO, 2003b; Worldwatch Institute, 2018). Annual meat production is projected to increase from 326 million tonnes in 2018 to 376 million tonnes by 2030 (WHO, 2003b; Statista, 2018).

Pork is currently the most widely consumed meat in the world, followed by poultry, beef, and mutton; however, poultry production is the fastest growing muscle-food producing sector, (Worldwatch Institute, 2018; Statista, 2018) and is predicted to rise to 181 million tonnes by 2050 (Figure 1.1).

The growing demand for livestock products is likely to have an undesirable impact on the environment as there will be more larger-scale industrial production, often located close to urban centres, which brings with it a range of environmental and public health risks (WHO, 2003b; Griffin, 2018). Animal waste also significantly contributes to global greenhouse gas emissions and is one of the main reasons why emissions are continuing to rise at the rate that they are as animal waste releases methane and nitrous oxide, greenhouse gases that are 25 and 300 times more potent than carbon dioxide, respectively (Worldwatch Institute, 2018; Griffin, 2018).

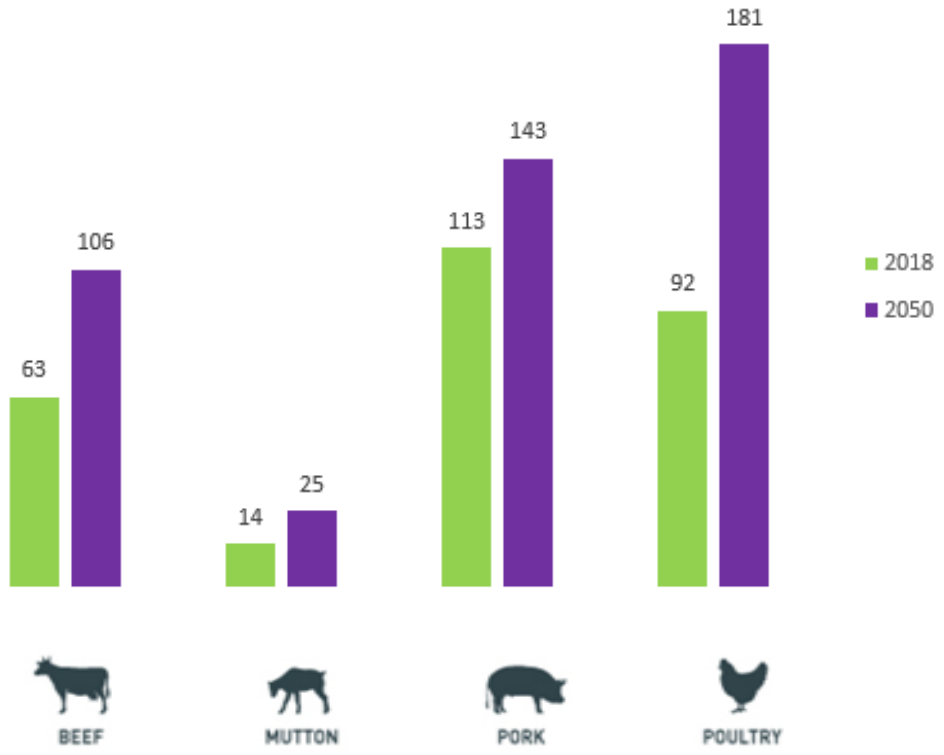


Figure 1.1 - Global demand for meat (in million tonnes) 2018 –V- 2050
Source: FAO, 2008; Statista, 2018.

1.3 The Irish Meat Industry

Despite the difficult market environment for some meats in 2015, most notably pigmeat, the value of meat and livestock exports grew by 2% to over €3.7 billion. This equates to over 32% of total Irish food and drink exports (Bord Bia, 2016a).

Ireland is the highest consumer of poultry meat in the EU. The Irish poultry industry is divided into two separate sections – poultry meat and egg production. Each year in Ireland, approximately 70 million chickens and four million turkeys are produced, while two million hens lay eggs (Enterprise Ireland, 2015).

Irish beef production is predominately a grass-based system, with around 588,000 tonnes produced in 2016. Beef self-sufficiency is estimated at over 650%. In 2016, Ireland exported an estimated 535,000 tonnes of beef worth approximately €2.38 billion (Bord Bia, 2018).

Sheep meat production in 2016 was over 61,000 tonnes and self-sufficiency is estimated at over 360%. During 2016, Ireland exported an estimated 50,000 tonnes of sheep meat which was valued at approximately €240 million (Bord Bia, 2018).

In regards to pork, Ireland exported an estimated 235,000 tonnes in 2016 and was worth an estimated €615 million, with the UK being the primary market for Irish pork, taking 56% of our total pig meat exports. Continental EU markets accounted for 16% of our pork exports, while the remaining 28% went to international markets (Bord Bia, 2018). In terms of self-sufficiency, Ireland is over 180% self-sufficient in pork production (Bord Bia, 2016a). In Ireland, bacon sales comprise 38% of total retail pork sales and are dominated by the sales of bacon joints and rashers. Pork retail sales make up around 23% and are comprised mainly of pork chops and joints, followed by pork casserole and mince. Sausages make up 19%, while sliced meats make up 20% of retail pork sales. (Bord Bia, 2016a).

1.4 Current trends on meat consumption and added value meat products

The global importance of the nutritional value of food has increased significantly in recent years. There is an ever increasing demand in the meat industry for minimally processed foods, which are lower in salt, preservatives, fat and calories, whilst maintaining good-quality products with respect to physicochemical, nutritional and sensory characteristics (Weiss *et al*, 2010).

The consumer demand for convenience is a driving force within the meat industry (Bord Bia, 2011). The work and leisure lifestyle patterns of the Western consuming culture, with high disposable incomes, have created a demand for pre-prepared foods (Purdy and Armstrong, 2007). Both retailers and foodservice businesses are responding to this demand with increasingly innovative solutions (Figure 1.2). Cook-in pouches offer a new solution to meal preparation and have emerged in the retail space over recent times (Bord Bia, 2011). Demands for convenience, snack foods, processed and ready-to-eat (RTE) meat products is expected to grow into the future (Brennen *et al*., 2013; Alberta agriculture and forestry, 2017).

Consumers are becoming increasingly motivated to protect the environment by enhancing sustainability in the meat industry. One way of achieving this is reducing food waste by extending shelf-life (Bord Bia, 2016b). This means more processing innovations and the necessity to link novel processing technologies more closely with packaging innovations.

Global trends in new and unique flavours and tastes are influencing cuts and varieties in foodservice and retail. Demand for new flavours is also impacting upon consumer expectations pertaining to pre-marinated and ready-to cook products. The growing popularity of Asian food in Western markets has driven consumer demands for more sophisticated and authentic Asian ingredients (Bord Bia, 2016b).

Clean label is now a consumer-driven movement, demanding a return to ‘real food’ and transparency through authenticity. Food products containing natural, familiar, simple ingredients that are easy to recognise, understand, and pronounce and contain no artificial ingredients or synthetic chemicals are now in greater demand (GoCleanLabel, 2018) and policed by the involvement of retailers.

Figure 1.2 - Examples of ready to eat snack foods and flavoured meat in a cook in pouch. (Bridgford foods, Dukes meats, Tyson fresh meats)

1.5 Morphology and chemical composition of meat

The unit of muscular tissue is a fibre, consisting of myofibrils between which resides a solution, the sarcoplasm, and a fine network of tubules (sarcoplasmic reticulum). The fibre is bound by a very thin membrane called the sarcolemma, which is attached by connective tissue on the outside (Lawrie and Ledward, 2006). Proteins in muscles can be divided into those which are either soluble in water or diluted salt solutions (sarcoplasmic proteins (11.5%); myoglobin and enzymes), those which are soluble in concentrated salt solutions

(myofibrillar proteins (5.5%); actin and myosin) or those which are completely insoluble (connective tissues (collagen and elastin) and membrane proteins (2%)).

Most of the sacroplamic proteins are enzymes involved in the glycolytic pathway. Myofibillar proteins, myosin and actin are responsible for the overall structure of the muscle (Greaser *et al*., 1981; Lawrie and Ledward, 2006).

Fat in meat can be either adipose tissue (triglyceride) or intramuscular fat (phospholipids and unsaponifiable constituents e.g cholesterol) (Lea, 1962). Despite meat containing only a small amount of carbohydrates, they are important in developing flavour during cooking through caramelization and Maillard-type reactions between reducing sugars and amino groups. Furthermore, carbohydrates are responsible for the brown colour of cooked meat (Belitz *et al*. 2009). Besides the excellent source of protein, meat contains all of the nine essential amino acids. Obtaining complete proteins in your diet is important for cell regrowth, hormone production, immune function and muscle gain (Sfgate, 2018). Meat is also a good source of all the B-complex vitamins (thiamin, riboflavin, niacin, biotin, vitamins B6 and B12, pantothenic acid and folacin), vitamins A, E and K (Belitz *et al*. 2009) and minerals including iron, zinc copper and manganese. Meat therefore plays an important role in the prevention of zinc deficiency, and particularly of iron deficiency which is widespread (Bender, 1992).

1.6 Health and safety concerns for processed meat products

The food industry is currently under pressure from the Food Standards Agency of Ireland (FSAI) to deliver reductions in the salt intake of the Irish population through the introduction of lower salt levels in processed foods (Gilbert and Heisler, 2004). A national guideline for the Irish meat industry was agreed by the FSAI (2017), with the target to decrease salt content. The following salt levels for processed meats were set; 0.68% salt

for uncured cooked meat products, 1.38% salt for sausages, 1.5% salt for frankfurters, 1.63% salt for ham and other cured meats, 2.88% salt for bacon and 0.75% salt for burgers (FSAI, 2017).

Salt is primarily consumed within processed foods, with the remaining salt intake from natural sources, discretionary salt and tap water (Kenten, 2013). Over 80% of salt intake in the UK, Ireland and the USA comes from processed food, meaning many consumers do not realise they are consuming such high quantities (Gray, 2013).

Government research indicates that meat and meat products as a food category are the second largest contributor to dietary salt after cereal products (Figure 1.3).

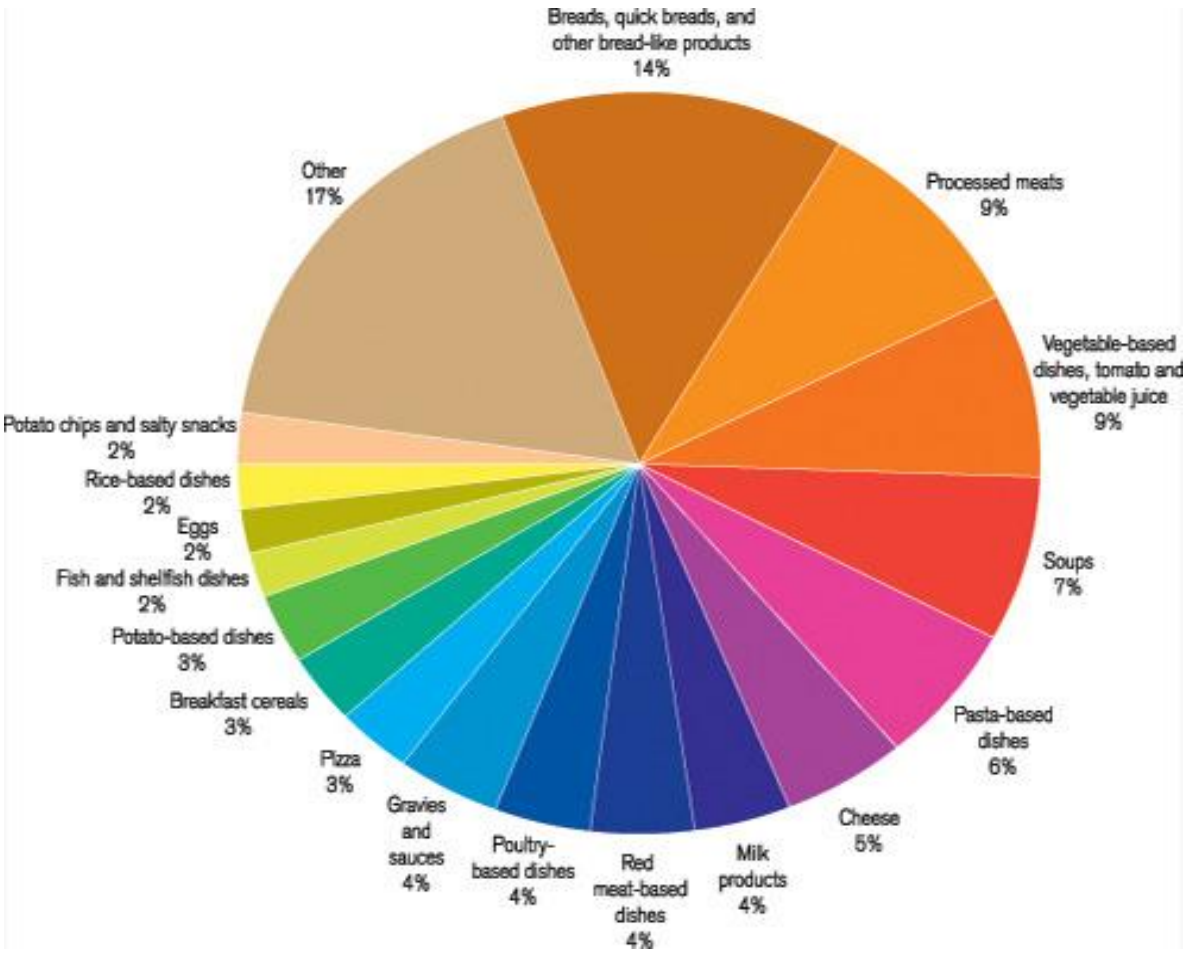


Figure 1.3 Major contributors of dietary sodium in our diet.
Source: Shrivastav, 2015.

1.6.1 Health Concerns of processed meat products

High salt consumption has been associated with hypertension (also known as high blood pressure) and which is the dominant cause of death and disability in adults worldwide (He and McGregor, 2008). The risk of developing cardiovascular disease (CVD) increases with increasing blood pressure (Cappuccio, 2013). CVD is the most common cause of death in Ireland, which accounts for 10,000 deaths per year (IHF, 2016).

Salt intake of less than 5g/day for adults has been recommended by the WHO to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart attack; however, in most European countries this recommended dietary intake is greatly exceeded, with an estimated salt consumption as high as 9-12g/day. It was reported that an estimated 2.5 million deaths could be prevented each year if global salt consumption was reduced to WHO recommended levels (WHO, 2016). The cost of CVD to the EU economy is estimated at €210 billion per annum (Luengo-Fernandez *et al.*, 2017), while in the US, the estimated total cost of CVD in 2015 was $565 billion and this amount is expected to increase by almost 100% by 2030 (Farmakis *et al*., 2016). The association between excessive sodium intake and the development of hypertension and CVD (MacGregor and Sever, 1992; De Wardener, and MacGregor, 2002) has prompted public health and regulatory authorities to recommend reducing dietary intake of salt (NaCl). Another health concern regarding processed meat products is obesity. Obesity is a global problem leading to chronic diseases such as diabetes and cardiovascular disease (Apovian, 2009). The Western dietary pattern includes high amounts of red and processed meat which are rich sources of saturated fatty acids and cholesterol and is therefore considered an ‘obesity inducing dietary pattern’. (Esmaillzadeh and Azadbakht, 2008). There is also increasing evidence that salt intake is related to diabetes, associated with renal stones and osteoporosis

and may play a role in the development of stomach cancer (He and MacGregor, 2009; Wang *et al*., 2009).

Due to these health concerns, there is an ever increasing demand in the meat industry for products which are lower in various salts, preservatives, fat and calories (Weiss *et al.*, 2010).

1.6.2 Microbiology of meat; pathogens and spoilage microorganisms

Many foodborne diseases are associated with consumption of meat and poultry (Mor-mor And Yuste, 2009). The pathogens of greatest concern in fresh and frozen meat and meat products are *Salmonella spp*., *Escherichia coli* O157:H7 and other enterohemorrhagic *E. coli* (EHEC*), L. monocytogenes, Staphylococcus aureus*, and the potential for *Clostridium botulinum* in cured hams and sausages. Emerging pathogens, include; *Campylobacter jejuni*, *Arcobacter butzleri*, *Mycobacterium avium* subsp. paratuberculosis, *Aeromonas hydrophila* and prions (Mor-mor And Yuste, 2009).

Many of these pathogens may cause severe gastroenteritis, and although they are typically short-lived, chronic complications and even fatalities can occur (Kroll, 2001). Examples of the complications, include; arthritis, Chrons disease, meningitis and septicaemia.

Fatal outbreaks of foodborne disease caused by *E. coli* O157:H7 and *L. monocytogen*es have increased consumer awareness and aroused interest by public health authorities and the food industry in improving sanitary conditions and controlling pathogens in meat and poultry production and processing (Mor-Mur and Yuste, 2009). The predominant bacteria associated with spoilage of beef and pork under refrigerated conditions are; *Brochothrix thermosphacta*, *Carnobacterium* spp., *Enterobacteriaceae*, *Lactobacillus spp*., *Leuconostoc spp*., *Pseudomonas spp*. and *Shewanella putrefaciens.* (Borsch *et al.*, 1996).

Many of these meat spoilage micro-organisms can also cause illnesses such as gastroenteritis (Mor-Mur and Yuste, 2009).

1.7 Meat Processing

Meat processing technologies can combine a variety of methods such as cutting, mincing, chopping, salting, injecting, tumbling, stuffing/filling into casings/moulds, heat treatments and ripening, drying or smoking. Based on the processing technologies used and taking into account the treatment of raw materials and the individual processing steps, it is possible to categorize processed meat products in six broad groups (Figure 1.4).

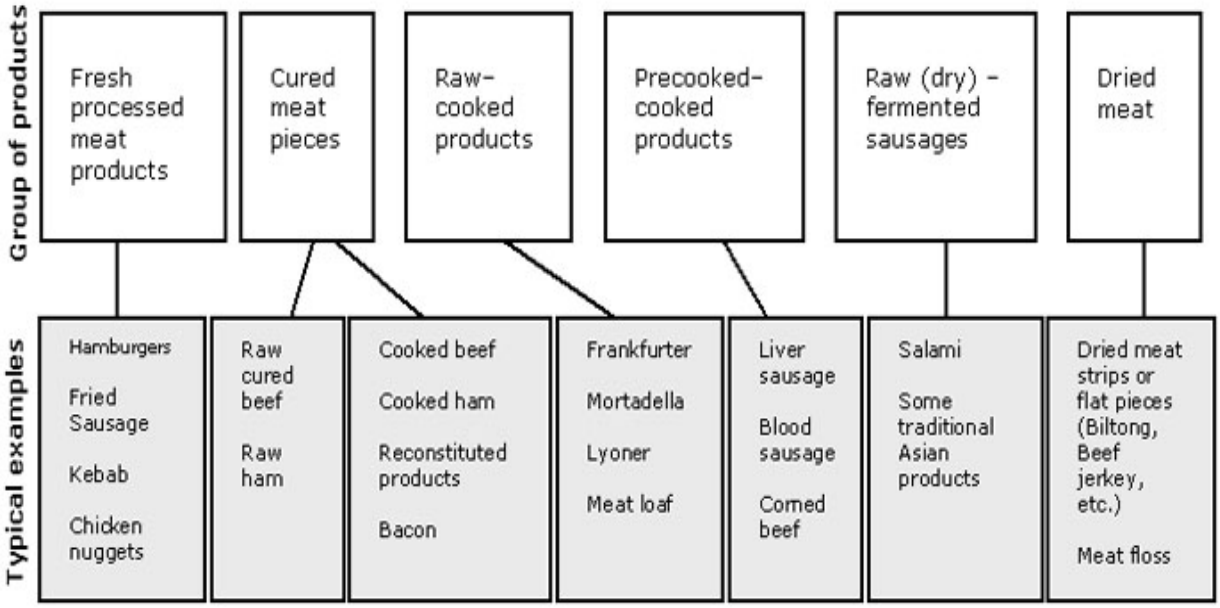


Figure 1.4 Meat products grouped according to the processing technology applied
Source: Heinz and Hautzinger, 2007.

Frankfurters (raw-cooked products) and cooked ham (cured meat pieces) are commercially important processed meat products which are very popular in Europe and were therefore chosen for investigation in the current thesis.

1.7.1 Frankfurters

Comminuted cooked meat products (gel/emulsion systems) are a commercially important group of processed meat products, of which frankfurters are among one of the more popular varieties (Delgado-Pando *et al*., 2010). Frankfurters are a type of highly seasoned sausage which can contain up to 30% fat with an industrial average of about 20% (Keeton, 1994) and a salt content of 2% or higher.

Emulsification ensures the physicochemical stability of the product, thereby, determining the characteristic structure of the batter. It also creates the typical sensory properties (appearance, texture, flavour) associated with such products. Low-value meat offcuts such as trimmings, parts with higher content of connective tissues or fat can be used (Sebranek, 2003). Through the addition of salt and other preservatives during the emulsification process, and following thermal treatment, the shelf-life of the final product is increased (Allais, 2010).

1.7.2 Cooked ham

Cooked ham is a reformed cured meat product made from pork leg meat. Brine curing is the most popular way to produce hams. It is a wet cure whereby fresh meat is injected with a curing solution before cooking. Brining ingredients, include; salt, sugar, sodium nitrite, sodium nitrate, sodium erythorbate, sodium ascorbate, sodium phosphate, potassium chloride, water and flavourings. Smoke flavouring (liquid smoke) may also be injected via the brine solution (USDA, 2016).

1.8 Functions of Sodium Chloride in meat processing

Sodium chloride (NaCl), commonly known as salt, plays a significant technological role in processed meat due to its preservation and antimicrobial properties provided by its ability to reduce water activity. Moreover, salt activates salt-soluble myofibrillar proteins to increase hydration and water-binding capacity; it increases the binding properties of these proteins to improve texture and it is essential for flavour (Terrell, 1983; Mariutti and Bragagnolo, 2017). Thus, salt reduction in processed meat products is challenging as quality of the final product can be compromised.

1.8.1 Physical Processing effects and texture development in processed meats

One of salt's main functions in processed meats is in the solubilisation of the functional myofibrillar proteins in meat (Desmond, 2006). A wide range of meat products depend on this property of muscle proteins to generate their characteristic texture (Matthews and Strong, 2005). Protein solubility in water depends on the distribution of polar and nonpolar groups in the amino acid lateral chain (Cheftel *et al*., 1989) and the ionic species present in solutions (Curtis and Lue, 2006).

The myofibrillar (contractile) proteins of muscle, actin and myosin, are insoluble at low-salt concentrations, but become soluble in concentrated salt solutions (Schmidt *et al.* 1987). This activates the proteins to increase hydration and water-binding capacity, ultimately increasing the binding properties of proteins to improve texture.

Increasing the water holding capacity of the meat reduces cook loss, thereby increasing tenderness and juiciness of the meat product (Desmond, 2006; Tobin *et al*., 2012). The resulting tenderising effect gives the characteristic texture of meat products such as cooked hams. Increased solubilisation results in extracted proteins which, on cooking, form stable cross links and bind pieces of meat together (Desmond, 2006).

In finely chopped or emulsified products such as frankfurters, bologna, etc., the salt-solubilised myofibrillar proteins, derived from the lean meat component, forms a sticky exudate on the surface of the highly comminuted meat pieces, which subsequently binds the meat pieces together through the formation of a gel-based continuous phase which forms a protein film around fat globules. This matrix of heat-coagulated protein entraps free water, thereby, retaining the fat during cooking which results in a product with acceptable quality characteristics (Desmond, 2006; The Salt institute, 2013).

Cooking yield also affects the cost of manufacture of processed meats (O' Flynn *et al*., 2014). The control of cook loss is also important because changes in the cooking yields may result in compositional changes in finished products, which may in turn affect palatability characteristics. As moisture is lost from meat, both during and after thermal processing, product yield and other quality attributes such as tenderness, texture, and flavour are negatively affected (Pietrasik, 1999).

1.8.2 Preservation effects

The use of salt for preservation originated long before the use of refrigeration (Matthews and Strong, 2005). The antimicrobial effects of salt is based on its ability to reduce water activity (aw), which is defined as the amount of free water available for the growth of microorganisms (Ingulgia *et al*., 2017). The effect of salt on microorganisms depends on the amount of salt present in the aqueous phase of the food (Ingulgia *et al*., 2017), therefore salt reduction increases water activity, which in turn increases water availability for microbial growth. The preservation effect of salt also suppresses the growth of pathogens, such as *Clostridium botulinum* and *Listeria monocytogenes* in vacuum packed and chilled products (Matthews and Strong, 2005).

1.8.3 Sensory properties

In addition to functional and microbial stability, salt can significantly affect sensory properties and subsequent flavour perception of meat (Liem *et al.*, 2011). Sensory properties of food products are the most important attributes as they are most apparent to consumers (Singham *et al*., 2015).

Salt has a flavour enhancing effect in meat products, with perceived saltiness primarily due to Na^+, with the Cl^- anion modifying the perception (Ruusunen and Puolanne, 2005; Miller and Barthoshuk, 1991). The perception of salt involves the activation of physiologic processes such as stimulation of salivation and secretion of gastric acid (Mattes, 1997).

A particular problem with low-salt meat products is that not only is perceived saltiness reduced, but so too is flavour intensity (Ruusunen and Poulanne 2004). While the loss of saltiness may be generally problematic for some products, additional challenges exist for those containing bitter-eliciting compounds, where lower sodium leads to a reduced capacity for bitterness suppression and may lead to an excessively bitter product (Gaudette and Pietrasik, 2017). In addition, saltiness enhances the perceived in-mouth aroma of products, and for meat, this equates to an enhancement of “meaty” flavour an important factor in the overall acceptability of meat products (Ruusunen and Puolanne, 2005). The effects of salt on the physical properties of meat products also results in an influence on sensory textural attributes (Matthews and Strong, 2005).

Considering the significant technological role of salt in meat processing, a global approach is necessary to reduce salt content in processed meat products (Albarracin *et al.*, 2011).

1.9 Approaches to salt reduction in processed meat products

Although the Irish food industry has had great achievements in lowering salt content in a wide variety of foods, further reductions are still required to meet the targets for healthy salt consumption levels. For frankfurters, the salt target set by the FSAI in 2017 is 1.5% salt and for cooked ham the salt target is 1.6% salt.

The main strategies used for salt reduction in processed meat products, include; product reformulation, compensation through use of substitutes, use of saltiness enhancers and use of salt replacers (Kilcast and Angus, 2007). Salt reduction in processed meat products has been thoroughly investigated by many authors using product reformulation and various salt replacers/substitutes and enhancers (Table 1.1). A number of commercial sodium replacers and flavour enhancers have entered the market to fully, or partially, replace NaCl in processed meats. However, it is difficult to assess their impact on sensory properties or to determine the best approach toward sodium reduction in specific meat products without direct comparison within a model system. In addition, due to their potential impact on sensory properties, the employment of replacers or enhancers may be appropriate and successful for some meat system applications, but not for others (Desmond, 2006; Fouladkhah *et al*., 2015). The main strategies for salt reduction in processed meat products are described as follows;

1.9.1 Product Reformulation

In product reformulation, a small gradual reduction of salt from the recipe might be unnoticed by assessors (Bertino *et al*., 1982; Puolanne, 2010). Larger reductions might be achieved with a gradual salt reduction over a period of years, thereby, allowing consumers to get used to lower salt levels in food. For example, to reduce the salt content in cooked sausages from 2.3 - 2.4% to 1.5 - 1.7% took approximately 20 years to achieve in Finland

(Ruusunen and Puolanne, 2005). However, international governments and regulatory agencies are now looking for significant salt reduction in foods in the short-term and cannot afford to wait for decades to achieve this overall goal. Therefore, the use of salt replacers and salt enhancers in food products may play an essential role in achieving this.

1.9.2 Salt substitutes or replacers

Among the chloride salts, potassium chloride (KCl) is the most commonly used salt alternative (Dötsch *et al*., 2009). However, at blends over 50:50 NaCl/KCl in solution, a significant increase in bitterness and loss of saltiness is observed (Desmond, 2006). The basis for using salt replacers is to reduce sodium cations by replacement with potassium, magnesium, calcium or to reduce the chloride anions with ingredients such as glutamates, phosphates, etc. as a means of providing salty tastes or flavours (Wheelock and Hobbiss, 1999). KCl contributes to some saltiness by itself, but sometimes imparts off-flavours such as bitterness and metallic flavour (Doyle and Glass, 2010). The bitterness perception of KCl can be suppressed by using it in combination with further salt replacers or flavour enhancers. Prime Favourites, a US company, has launched NeutralFres®, which the company claims naturally neutralises the characteristic taste or bitter alkaline off-flavour of KCl (Primefavourites, 2005).

The use of KCl in salt mixtures also gives an additional benefit, owing to the fact that potassium is a counter-ion to sodium and reduces the harmful effect of sodium on blood pressure (Ruusunen and Poulanne, 2005). Solubilisation of muscle proteins, which is critical in the manufacture of processed meats, can also be achieved using salt replacers such as KCl. According to the “Hofmeister Series” which ranks the relative influence of ions on the physical behaviour of a wide variety of aqueous processes (Zhang and Cremer,

2006), KCl has been found to be effective at solubilising meat protein (Puolanne and Halonen, 2010).

Research has also demonstrated that phosphates can be very useful in lowering the NaCl content in meat products (Ruusunen *et al*., 2002; Ruusunen *and* Poulanne. 2005). Phosphates are generally used in meat products to enhance water-holding capacity and improve cook yield. They increase water-holding capacity in fresh and cured meat products by increasing the ionic strength, which frees negatively charged sites on meat proteins, such that these proteins can bind more water.

Other salt replacers include Sodium or Potassium lactate which can maintain certain saltiness, while reducing the sodium content in products (Price *et al.*, 1997) and water binders such as starches or gums which are used to maintain the water binding function lost due to salt reduction (Desmond, 2006).

1.9.3 Flavour enhancers

There are a number of flavour enhancing and masking agents commercially available and the number of products coming to the market is increasing. These include; yeast extracts, lactates, monosodium glutamate (MSG) and nucleotides amongst others. Taste enhancers work by activating receptors in the mouth and throat, which helps compensate for salt reduction (Brandsma, 2006). A commonly used salt enhancer is MSG, which contains high levels of glutamic acid and imparts a ‘umami’-type taste to enhance the palatability and acceptability of savoury foods; however, it has been associated with the so-called ‘Chinese Restaurant Syndrome’ that may cause headaches, swelling and weakness (Durack *et al*., 2008). Yeast autolysates are also commonly used in low salt products, in particular, they mask the metallic flavour of KCl (Desmond, 2006).

1.9.4 Physical form of salt

The perception of salt in the solid form is affected by crystal size and shape. Research has been carried out using various forms (flaked versus granular) as a method of reducing salt content in meat products (Desmond, 2006). Leatherhead Food International have investigated optimising and altering the physical form of salt, thereby making it more taste bioavailable, with the consequence being able to add less of it to products. This involves increasing the efficiency of the salt, changing the structure and modifying the perception of the salt (Angus *et al*., 2005). Lutz (2005) has shown that flake salt can produce red meat batters with superior fat and water binding properties compared to regular vacuum evaporated salts.

1.9.5 High Pressure Processing

High pressure processing (HPP) may be of interest to improve protein functionality, where it is desired to reduce the sodium content of meat products (Cheftel and Culioli, 1997). Sensory analysis on reduced salt HPP frankfurter batters have shown that panellists preferred these products to controls. Results also indicated that the texture of these products was improved after HPP at 150 MPa (Crehan *et al*., 2000). These authors concluded that HPP is a viable technology that partially compensates for the reduction of salt levels in frankfurters.

Some studies have also shownthat HPP enhances the saltiness perception in meat products (Ken *et al*., 2006; Clariana *et al*., 2011) due to differential binding forces of NaCl within the product network and its release in the mouth (Tamm *et al*., 2016), which in turn would permit salt reduction, as the saltiness perception would be increased.

Table 1.1 – Previous salt reduction studies in processed meat products using various approaches.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Sofos (1983)	Product reformulation	Frankfurters	Salt content ranging from 1 - 2.5%	Reduction >20% resulted in softer and less firm texture
Sofos (1985)	Use of Salt replacer (Potassium Sorbate)	Frankfurters	Salt replacer compensated for the 20% NaCl reduction	Based on textural attributes
Dimitrakopoulou *et al*. (2005)	Product reformulation	Reformulated pork shoulder	Salt reduced from 2% to 1%	Based on acceptability of sensory attributes.
Aaslyng *et al*. (2014)	Product reformulation	Cooked ham	Salt reduced from 2.3% to 1.8%	Without altering the sensory properties, sliceability, production yield, shelf life and safety.
Fellendorf *et al*. (2016)	Product reformulation	White pudding	The critical acceptable limits were achieved at 0.6% sodium and 5% fat	Based on acceptable scores for sensory attributes
Fellendorf *et al*. (2016)	Product reformulation	Black pudding	The critical acceptable limits were achieved at 0.4% sodium and 5% fat	Based on acceptable scores for sensory attributes

Table 1.1 contd.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Tobin *et al*. (2013)	Product reformulation	Sausages	The sausages containing 1.4% and 1.0% salt were the most acceptable	Based on the highest scores for sensory attributes
Tobin *et al.* (2012)	Product reformulation	Frankfurters	Salt levels below 1.5% had a negative effect on consumer acceptability	Based on acceptable scores for sensory attributes
Tobin *et al*. (2012)	Product reformulation	Beef patties	Salt reduction of 50% achieved	Based on highest scores for overall sensory acceptability
Pietrasik *et al*. (2014)	The use of two commercial salt replacers: Oceans flavour sea salt™ OF45 or 0F60	Cooked ham	100% replacement of NaCl was achieved	Texture and cook loss were not affected; however, salt replaced ham was liked less in terms of flavour and aftertaste

Table 1.1 contd.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Tamm *et al*. (2016)	Partial replacement of NaCl with KCl and HPP 100MPa	Cooked ham	45% salt reduction was achieved	Acceptable in terms of texture, consistency and appearance but lower saltiness taste.
Aliño *et al*. (2009)	Partial replacement of NaCl with KCl	Dry cured loin	Up to 50% replacement was achieved	Similar physicochemical characteristics to control
Lorenzo *et al.* (2015)	Partial replacement of NaCl with KCl	Cooked ham	NaCl was 50% replaced with KCl.	Resulted in an increased bitterness taste.
Pietrasik *et al*. (2017)	Partial replacement of NaCl with modified KCl	Wieners	Substitution of 50% NaCl with modified KCl	Negative effects on textural and sensory characteristics
Frye *et al*. (1986)	Replacement of NaCl with KCl or $MgCl_2$	Ham	50% or 100% ionic strength replacement with KCl or $MgCl_2$	Control – best OSA NaCl/KCl – best bind and acceptable sensory scores NaCl/$MgCl_2$ – lowest bind and lowest sensory scores
Stanley *et al*. (2017)	The use of modified KCl based salts to replace NaCl	Sausage patties	NaCl reduced from 1.7% to 1.07% using modified KCl	Limited impact on physicochemical traits; however, scored lower in all sensory traits

Table 1.1 contd.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Skogsberg *et al*. (2017)	Partial replacement of NaCl with KCl	Frankfurters	Up to 40% of salt can be replaced	Without major quality or sensory changes
Gou *et al*. (1996)	Substitution of NaCl with glycine and potassium lactate	Fermented sausages	40% NaCl reduction achieved	Above this level a slight off taste or an unacceptable sweet taste was detected.
Guardia *et al*. (2008)	Substitution of NaCl by mixtures of KCl and potassium lactate	Fermented sausages	50% NaCl substitution was achieved	It was possible to achieve 50% reduction of NaCl to obtain sensory acceptable product
Gimeno *et al*. (2001)	Partial replacement of NaCl with calcium ascorbate	Fermented sausages	Replacement of 15%, 24%, 37% and 45% of NaCl	Replacement ≥ 24% resulted in higher lightness, redness and yellowness and lower hardness and gumminess.
Fulladosa *et al.* (2009)	Partial replacement of NaCl with potassium lactate and HPP	Dry-cured ham	50% replacement of NaCl with K-lactate	Did not have a negative effect on colour, flavour or texture

Table 1.1 contd.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Gelabert *et al*. (2003)	The substitution of NaCl with KCl, potassium lactate or glycine	Fermented Sausages	The critical level of salt substitution with KCL was 40%	Flavour and texture defects occurred when NaCl was replaced >40%
Morton Salt. (2004)	Partial replacement of NaCl with KCl	Ham, bacon, turkey	40% replacement of NaCl was achieved	Similar flavour scores to the control products and maintained protein hydration
Zanardi *et al*. (2010)	Partial replacement of NaCl by KCl, $MagCl_2$ and $CaCl_2$	Italian salami	40% replacement of NaCl	Limited detrimental effects on sensory attributes
O' Flynn *et al*. (2014)	Application of HPP to reduce NaCl	Sausages	HPP at 150 MPa has potential for reducing salt levels in sausages to 1.5%	Based on textural and sensory attributes
Verma *et al*. (2010)	Partial replacement of NaCl with a salt substitute blend (KCl, citric and tartaric acid, and sucrose) and apple pulp	Chicken nuggets	40% replacement of NaCl was achieved	Similar scores for saltiness and juiciness as control, although flavour, texture, and OSA were lower

Table 1.1 contd.

Reference	Salt reduction strategy	Processed meat product	Details	Findings/Based on
Paulsen *et al*. (2014)	The use of salt substitutes (KCl, Na-lactate, K-lactate/Na-diacetate and milk minerals)	Sausages	NaCl was reduced from 0.9% to 0.5% and replaced with substitutes	Control was preferred in terms of sensory attributes (saltiness, texture and after-taste)
Santos *et al*. (2014)	The use of salt replacers (KCl) and flavour enhancer (MSG) in combination with lysine, taurine, disodium inosinate and disodium guanylate	Sausages	NaCl was 50% or 75% replaced with KCl.	Flavour enhancers masked the undesirable sensory attributes associated with the replacement of 50% and 75% NaCl with KCl resulting in good sensory acceptance

1.10 Preservation of processed meat products

Meat provides favourable growth conditions for various microorganisms, but is also susceptible to spoilage due to chemical and enzymatic activities. The breakdown of fats, proteins and the limited content of carbohydrates present in meat due to microbiological, chemical and enzymatic activities results in the development of off-odours, off-flavour and slime formation, which makes the meat objectionable for human consumption. Therefore, it is necessary to control meat spoilage in order to increase its shelf-life and maintain its nutritional value, colour, texture and flavour (Dave and Ghally, 2011).

Traditionally, methods of meat preservation may be grouped into three broad categories based on temperature control, moisture control and, more directly, by inhibitory processes (bactericidal and bacteriostatic, such as ionising radiation, packaging, etc.) (Zhou *et al*. 2010). Methods of meat preservation can also be categorised based on physical and chemical treatments (Mor-Mur and Yuste, 2009) (Table 1.2).

Table 1.2 – Physical and Chemical preservation techniques. Adapted from: (Mor-Mur and Yuste, 2009).

Physical preservation techniques	Chemical preservation techniques
Chilling	Modified atmosphere packaging and
Freezing	Active packaging (e.g. edible coating films containing ascorbic acid or plant essential oils)
Heating	Spray washing with water, steam, or solutions (e.g., organic acids, such as lactic acid, trisodium phosphate)
Drying	Agents in solution, such as fatty acid esters, para-hydroxybenzoic acid esters, lysozyme, phenolic compounds, isothiocyanates, ascorbic acid
Packaging (e.g vacuum packaging)	
High pressure processing	Salts
Ultraviolet radiation	Nitrites
Ohmic heating	Sulphites
Ionizing radiation	Spices, condiments, and plant essential oils
Pulsed electric fields	Chitosan
Ultrasound	Bacteriocins
Oscillatory magnetic fields	Bacteriophages

1.10.1 Physical treatments

1.10.1.1 Heat treatments

Meat and meat products are considered cooked when the centre (coldest point) of the product is maintained at a temperature of 65-70°C for 10 minutes since the proteins will then be coagulated and the meat tenderised by partial hydrolysis of the collagen. (Bender, 1992). The vegetative form of bacteria, but not spores, will have been destroyed (thermoresistant spores can survive heating above 100°C) (Bender, 1992). Heat treatment at a minimum of 62.8 °C internal temperature is also sufficient for destruction of enteric pathogens such as *Salmonella spp*. and pathogenic *E. coli* (Van Schothorst, 1998; Mor-mor and Yuste, 2009).

1.10.1.2 Cooling and Freezing

The aim of cooling techniques is to slow or limit the spoilage rate of food, as temperature below the optimal range can inhibit microbial growth. Low temperature storage employ three approaches: (a) chilling (b) freezing and (c) superchilling. All these levels help to inhibit or completely prevent bacterial growth (Zhou *et al*., 2010) and the physicochemical and biochemical reactions that govern the deterioration of foods (George, 1993).

However, the growth of psychrophilic bacteria, yeasts and moulds is not prevented by all levels of refrigeration (Neumeyer *et al*., 1997) and both enzymatic and non-enzymatic changes will continue at a much slower rate (Berkel *et al*., 2004).

The loss of quality of frozen foods depends primarily on storage temperature, length of storage time, and thawing procedure. Microbial growth is completely prevented below -18°C, and both enzymatic and non-enzymatic changes continue at much slower rates during frozen storage (Rahman, 2004). Most pathogens (*Salmonella, Staphylococcus species* and

Clostridium perfringens) are inhibited by cooling, but *Listeria monocytogenes* can grow at +2°C, some *Salmonella* species at +5°C and *Campylobacter* at +7°C (Bender, 1992).

1.10.1.3 High pressure processing

High pressure processing is the technology by which a product is treated at or above 100 MPa of pressure. Pressure is transmitted uniformly and instantaneously throughout the food, but does not change the nutrient content, odour or taste of the product (Yuste *et al*., 2001). It is likely that modifications in cytoplasmic membrane (the primary site of pressure damage) are the main causes of sub-lethal injury generated by pressure treatment to some microorganisms. HPP effectively inactivates the spoilage microbiota of numerous food types, and important foodborne pathogens, such as; *Campylobacter jejuni, Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella spp.* (Yuste *et al*., 2001).

1.10.1.4 Irradiation

The potential application of ionising radiation in food processing is based on the fact that ionising radiation damages DNA very effectively, so much so that living cells become inactivated which consequently prevents microorganisms from reproducing, resulting in various preservative effects as a function of the absorbed radiation dose (Farkas, 2006).

High doses (50 kiloGrays (kGy)) are required for sterilisation of meat, while WHO recommendations and legislation in most countries limit the dose to 10 kGy (Bender, 1992). The 10 kGy dose does not sterilise the product, but substantially reduces bacterial load and is effective in destroying many pathogens, including *Salmonellae*. Although the preservation of food by irradiation has been intensively studied for many years, its commercial application is still in its infancy since the process calls for heavy investment in

factory plant and is regarded with some suspicion by consumers (Bender, 1992; Elhermann, 2006).

1.10.2 Chemical treatments

1.10.2.1 Salt

Salt addition is one of the oldest and most widely used meat preservation techniques. Common salt does not display antimicrobial action, but its capacity to reduce water activity values (aw) in foods slows down, or even interrupts, vital microbial processes (Albarracin *et al*., 2011). A high salt concentration generates changes in cellular metabolism because of its osmotic effect. Moreover, the salting process alone is inadequate as a sole preservation method in ready-to-eat products, necessitating its combination with other preservation processes (Lück and Pager, 2000).

1.10.2.2 Organic acids

Organic acids have been traditionally used as food preservatives but are becoming more popular in application as antimicrobial ingredients; particularly, for their ability to reduce *L. monocytogenes* in ready-to-eat meat products. Typical organic acids utilized for their antimicrobial properties include sodium lactate, potassium lactate, sodium citrate, sodium lactate combined with sodium diacetate, and combinations of sodium lactate with potassium lactate and diacetate (Alvardo and Mc Kee, 2007).

Organic acids have optimal inhibitory activity at low pH (Aslim *et al*., 2005; Nazer *et al*., 2005). It is believed that the inhibitory action of organic acids on a microorganism is as a result of the compound in the undissociated state being able to freely cross the plasma membrane to enter the bacterial or fungal cell (Brul *et al*., 1999) and the ability to inhibit essential metabolic reactions, such as the production of ATP and the production of essential

enzymes, however, they can also cause membrane disruption and stress on intracellular pH homeostasis (Lues, 2005). Organic acids can be applied to meat systems through inclusion in the formulation, packaging and also through marination via immersion or injection.

1.10.2.3 Nitrites

The nitrites used in meat preservation are always in the form of salts such as sodium nitrite or potassium nitrite (Dave and Ghally, 2011). They are long known for their antimicrobial properties, preventing the growth of the toxin producing *Clostridium botulinum*, *Staphylococcus aureus and Yersinia enterocolitica* which can grow under anaerobic environment in vacuum packages (Roberts, 1975; Cassen, 1994; Archer, 2002; Ray, 2004; Lövenklev *et al*., 2004; Sindelar and Houser, 2009). Nitrite salts are effective in controlling colour, lipid oxidation and odour, in addition to controlling anaerobic bacteria (Roberts, 1975; de Giusti and de Vito 1992; Archer, 2002; Lovenklev *et al*., 2004; Sindelar and Houser, 2009); however, nitrite is used in extremely small quantities in processed meat products due to the negative health effects of higher concentrations. (Alahakoon *et al*., 2015).

1.11 Hurdle Technology

The microbial safety and stability, as well as the sensory and nutritional quality of most food products is based on hurdle technology (Leistner, 2000). Hurdle technology intelligently combines different mild preservation techniques (hurdles) to control or eliminate pathogens (Rodriguez-Calleja *et al*., 2012) in order to achieve multi-target, mild, but reliable preservation effects (Leistner and Gorris, 1995). The hurdle technology concept fits well with the present consumer trend for minimally-processed foods and, as such, has gained much in popularity regarding practical application and research (Mukhopadhyay

and Gorris, 2014). Since the 1980's the intelligent application of hurdle technology became more prevalent, because the principles of major preservative factors for foods (e.g., temperature, pH, water activity, competitive flora) and their interactions became better known (Leistner, 2000).

Potential hurdles for food preservation, include; temperature (low or high), pH (low or high), water activity (low or high), redox potential (low or high), modified atmospheres (nitrogen, carbon dioxide, oxygen etc.), packaging systems (aseptic packaging, vacuum or modified atmosphere packaging, active packaging or coating systems), HPP, radiation (microwave, ultraviolet, irradiation), other physical processes (pulsed electric field, oscillating field pulses, radiofrequency energy), competitive flora (lactic acid bacteria), preservatives (organic acids (e.g lactate, acetate, sorbate, ascorbate), free fatty acids and their esters, ethanol, spices and their extracts, nitrite, nitrate, smoke, antioxidants, chitosan, nisin and other bacteriocins) (Leistner, 1999).

The application of hurdle technology has been thoroughly investigated by numerous authors applying various combinations of hurdles to many food products for shelf life extension/preservation and also in challenge testing for various food pathogens (Table 1.3).

Table 1.3 – Previous studies which use hurdle technology for shelf life extension of meat products.

Reference	Product	Combination of hurdles applied	Findings
Rodriguez calleja *et al*. (2012)	Raw chicken breast fillet	Applied HPP at 300 MPa for 5mins and an edible antimicrobial coating Articoat™ and MAP	Extended shelf life by 4 weeks in terms of total viable count (TVC) compared to control samples
Vercammen *et al*. (2011)	Cooked ham	Applied HPP at 600 MPa for 10 min and natural antimicrobials (Caprylic acid or Purasal)	Extended shelf life by at least 44 in terms of TVC days compared to control samples
Thomas *et al*. (2008)	Pork sausages	Lower pH, lower water activity, dipped in potassium sorbate solution and vacuum packaging	Extended shelf life by 6 days in terms of TVC compared to control samples
Chawla *et al*. (2006)	Natural lamb casing	Reduced water activity, packaging and gamma irradiation (10 KgY)	No viable count detected over 90 day storage; however, TVC of control samples were 3 log at this time
Karthikeyan *et al*. (2000)	Indian Keema	Lower water activity and pH	Extended shelf life by at least 2 days in terms of TVC compared to control samples
Rindhe *et al*. (2017)	Chicken sausage	Lower pH, addition of humectants and bio preservative Nisin	Extended shelf life by 4 days in terms of TVC compared to untreated control samples

Table 1.3 contd.

Reference	Product	Combination of hurdles applied	Findings
Gunasekaran *et al*. (2018)	BBQ chicken	Addition of glycerol, lactic acid, gluconodelta-lactone and irradiation	Reduced bacterial growth by 3 Log compared to control samples over 6 days storage
Liu *et al*. (2012)	Cooked ham inoculated with 10^4 CFU/g *L. monocytogenes* and *Salmonella enteritidis*	HPP (400 MPa for 10 min) and Enterocin (256/2560 AU/g)	Extended the shelf life above 90 days compared to control by inhibiting the growth of bacteria and pathogenic microorganisms
De Alba *et al.* (2013)	Dry cured ham inoculated with *E.Coli* 10^6 CFU/g	HPP (500 MPa for 10mins) combined with bio preservative (Nisin)	Reduced *E.Coli* by 4 Log over 60 day storage compared to control samples
Marcos *et al*. (2008)	Cooked ham inoculated with 10^4 CFU/g *L. monocytogenes*	HPP (400 MPa for 10 min) in combination with natural antimicrobials (enterocins and lactate-diacetate)	Reduced the levels of *L. monocytogenes* during storage by 2.7 log CFU/g.
Stratakos *et al*. (2015)	Chicken breast inoculated with *L. monocytogenes* 10^5 CFU/g	HPP (500 MPa for 1 min and active packaging (coriander oil active film)	Maintained *L. monocytogenes* count below the detection limit throughout the 60 day storage period
Rodrigues *et al.* (2016)	Marinated Beef inoculated with *L. innocua and E.faecium* 10^6 CFU/g	HPP (600 MPa for 5 min) and NaCl (1-2%) and Citric acid (1-2%)	The combination of HPP and 2% NaCl/ 2% Citric acid resulted in a six log cycle reduction of both microorganisms

1.12 High pressure processing of meat products

The application of HPP in the food industry traces back to the early 19th century (Medina-Meza *et al*., 2014) and HPP of meat has been a fascinating research subject ever since (Ledward, 1998). HPP is an alternative method for food preservation which subjects liquid semi-solid and solid foods, with or without packaging, to pressures between 100 and 800 MPa and (Bermúdez-Aguirre and Barbosa-Cánovas, 2011). For most food applications, the minimum and maximum limits of HPP are 200 MPa and 600 MPa, respectively. (Medina-Meza *et al*., 2014)

HPP treatment is currently being used in commercial meat products to eliminate pathogenic microorganisms, extend shelf-life, improve safety and increase sensory quality by improving texture and of these products (PFV, 2009). However, HPP treatment can also increase lipid oxidation, negatively affect texture and induce colour changes in red meat which can present a cooked appearance (Yagiz *et al*., 2009).

HPP is a technology of interest to the food industry and its advantages over thermal processing, include; reduced heat damage, shorter processing times, the retention of nutritive value, freshness, texture, flavour, colour, retention of vitamin C and fewer undesirable functional changes (Al-Khuseibi *et al*., 2005; Patterson *et al*., 2005; Vega-Gálvez *et al.*, 2011). HPP foods also have a distinct advantage over foods processed by other means, in that they have the potential to be marketed as value-added foods due to the retention of organoleptic and nutritional qualities similar to those of 'fresh' unprocessed products (Rastogi *et al*., 2007) and can also be marketed as novel foods as they fulfil two criteria: a new manufacturing process employed in their production, and their history of human consumption has been minimal (Hogan *et al*., 2005). Furthermore, HPP can fulfil consumer requirements for minimally processed additive-free products and can maintain nutritional properties (Watson, 2012).

Product yield is of immense economic importance to food manufacturers, and HPP treatment in general, provides a higher food product yield in uncooked products compared with heat treatment, with effects depending on product type and treatment intensity (Hugas *et al*., 2002).

Some studies have also pointed out that HPP applied to raw meat before processing can also maintain or improve protein functionality where it is desired to reduce the sodium content of processed meats (Cheftel and Culioli 1997; Mújica *et al*., 2011). An application of this concept to develop low salt meat products is that HPP could increase the solubility of myofibrillar proteins thereby improving the chances to lower the amount of salt for the development of healthier meat products (Sikes *et al*., 2009; Grossi *et al*., 2012; Tornberg, 2013). Of all foods and food constituents, muscle and muscle proteins are probably the most responsive to HPP. This is due to the relatively high sensitivities of muscle glycolytic processes and of the associations between myofibrillar proteins (actin and myosin) to pressure (Macfarlane, 1985).

1.12.1 The effects of high pressure on meat texture

HPP application up to 1000 MPa can influence meat protein conformation and induce protein denaturation, aggregation or gelation which can result in meat becoming either tenderised or toughened. These outcomes depend on the meat protein system, the rigor state, the temperature used, the pressure applied and its level of duration (Sun and Holley, 2010).

The effect of HPP on the texture of muscle foods has been known since 1973, when Macfarlane (1973) reported the potential use of HPP for pressure-induced tenderization of meat. Since then many authors have investigated muscle texture changes in meat, poultry and fish during HPP treatment and have also observed that HPP at lower levels (100-

400MPa) can tenderise meat when applied pre rigor (Bouton *et al*., 1980; Beilken *et al*., 1990; Angsupanich and Ledward 1998; Angsupanich *et al.,* 1999; Jung *et al*., 2000b; Chevalier *et al*., 2001; Iwasaki *et al*. 2006). Tenderisation postrigor with HPP can only be achieved at higher temperatures (up to 80 °C) (Sun and Holley, 2010). HPP had no beneficial effects on the tenderisation of post rigor meat at temperatures below 50 °C (Ma and Ledward, 2004).

Jung *et al*. (2000a) reported that in post rigor meat myofibrillar proteins appeared capable of increasing toughness and/or neutralizing the effect of HPP on meat tenderness in the absence of heat treatment. It is well known that HPP increases meat toughness proportionally with increasing pressure levels up to 600MPa (Macfarlane *et al*., 1980-81; Yuste *et al*., 1998; Jung *et al.,* 2000a; Ma and Ledward, 2004; Zamri *et al*., 2006; Del Olmo *et al.,* 2010; McArdle *et al*., 2011; Kruk *et al.,* 2011). The increased toughness with HPP has been attributed to an increasing incidence of sarcomeres, in which thick filaments have been compressed onto the Z-line, thus removing the I-band as a zone of weakness (Macfarlane *et al*., 1980). HPP primarily affects the physicochemical properties of raw/uncooked meat products and has minimum effects on cooked products (Considine *et al*., 2008; Neto *et al*., 2015; Bansal *et al*., 2015) as the myofibrillar proteins in cooked products have already been denatured due to the cooking process.

1.12.2 The effects of high pressure on meat colour

The colour of meat depends on the amount and type of myoglobin present in the muscle (Campus *et al*., 2010). HPP has minimum effects on the physicochemical properties of cooked products as the myofibrillar proteins have already been denatured due to the cooking process. (Considine *et al.*, 2008; Neto *et al.*, 2015; Bansal *et al*., 2015). In dry cured meat products the pigment responsible for the dry-cured meat colour is nitrosylmyoglobine, a compound which is hardly affected by HPP (Cheftel and Culioli, 1997). However, HPP causes drastic colour changes in fresh meat especially in redness, and thus is not considered suitable for commercial applications (Cheftel and Culioli, 1997). Souza *et al.* (2011) also stated that consumers' purchasing preferences are highly based on fresh meat colour and HPP treatment caused meat to appear lighter in colour meaning that more work was and is needed to investigate meat colour preservation.

Colour changes in fresh muscle food products after HPP have been reported to be related to the denaturation of myofibrillar and sarcoplasmic proteins (Zhou *et al.*, 2010; Ma and Ledward, 2013). Similar results have been reported by Carlez *et al*. (1995) who suggested that fresh meat discolouration after HPP at 200-350 MPa is due to a “whitening” effect (increase in L* values) caused by globin denaturation, haem release or displacement or by oxidation of ferrous myoglobin to ferric metmyoglobin when fresh meat is HPP at ≥400 MPa. Goutefongea *et al*., (1995) also suggested that discolouration occurs as a results of protein coagulation which would affect sample structure and surface properties. According to Ledward, (1971), myoglobin undergoes a pre-denaturational conformational change that makes the haem more exposed/available to other denatured or denaturing proteins in the system and that it co-precipitates with them.

The degree of discoloration in fresh meat is usually proportional to the level of protein denaturation which increases with increasing pressure level. Kruk *et al*. (2011) applied HPP to raw chicken breast fillets at 300, 450 or 600 MPa for 5 minutes and found that lightness and yellowness increased significantly and that this increase was proportional to the level of HPP applied. Another colour-related observation was that when HPP was applied to beef at extremely low levels (80 to 100 MPa 2 days post-slaughter), its colour stability increased during storage, however, treatment of beef 7 or 9 days post-slaughter led to no increase in colour stability at all (Cheah and Ledward, 1997). The authors suggested that this was because the treatment destroyed part of the catalytic system responsible for the oxidation of myoglobin to metmyoglobin.

1.12.3 The effects of high pressure on lipid oxidation

From a sensory point of view, lipid oxidation can impair quality and cause rancidity problems which are considered unpleasant by consumers (Jeremiah, 2001; Guyon *et al*., 2016). Lipid oxidation was reported to be linked to an increase in protein oxidation (Souza *et al*. 2013), a deterioration of meat texture (Estevez *et al*. 2005) and a potentially negative association with drip loss, discolouration, loss of nutrient value, decrease in shelf-life, and the accumulation of toxic compounds, which may be detrimental to the health of consumers (Richards *et al*. 2002; Chaijan, 2008; Mapiye *et al*., 2012)

The maximum acceptable limit for TBARS is 1 mg/kg meat (Warriss, 2000) which is regarded as the limit beyond which meat products will normally develop objectionable odours/tastes.

HPP can accelerate lipid oxidation in HPP-treated meat products (Cheah and Ledward, 1995; Andres *et al.*, 2004) by triggering intrinsic pro-oxidants such as myoglobin (Medina-Meza et al. 2014). Increased rates of lipid oxidation due to HPP has also been attributed to

pressure-induced protein denaturation, which leads to the release of free-radicals catalysing oxidation (Cheftel and Culioli, 1997), the release of metal ions from iron complexes promoting auto-oxidation of lipids in HPP meat and also due to membrane damage (Cheah and Ledward, 1996; Cheah and Ledward, 1997; Angsupanich and Ledward, 1998; Chevalier *et al*., 2001).

Cheah and Ledward (1996 and 1997) reported that the effect of HPP on oxidative stability of lipids in pork meat depends on the applied pressure, with a value between 300 and 400 MPa constituting the critical pressure to induce catalysis.

Previous studies have also shown that HPP decreases oxidative stability of meat. Ma *et al.* (2007) reported increased TBARS values in HPP beef at ≥ 400 MPa. Núñez *et al*. (2003) used response surface methodology (RSM) to model changes induced by HPP at 24 to 400 MPa, and holding times from 7 to 28 minutes, on lipid oxidation of vacuum-packed slices of dry-cured Iberian ham and pork loin and reported that significantly increased TBARS values were obtained as the pressure level and holding time increased. Cava *et al.* (2002) also reported that pressure level and holding time increased the extent of lipid oxidation in dry-cured Iberian ham and pork loin. Kruk *et al*. (2011) reported no significant increased rate of oxidation when raw chicken breast fillets were HPP at 300 MPa for 5 minutes; however, above this pressure the oxidation rate increased significantly with pressure intensity. Souza *et al.* (2011) found that lipid oxidation only increased slightly in HPP-treated pork samples; however, the pressure level applied (215 MPa) was lower than 300 MPa, which is the critical pressure required to accelerate lipid oxidation (Cheah and Ledward, 1997).

1.12.4 Effects of HPP on the sensory quality of meat products

Sensory properties of food products are the most important product attributes owing to the fact that they are the most apparent to consumers (Singham *et al*., 2015). Physicochemical changes are reflected in sensory characteristics; colour changes (Lightness, redness and yellowness) affect liking of appearance, WBSF changes affect liking of texture and tenderness and TBARS values >1mg/kg can decrease liking of flavour and increase off-flavour perception. Studies have reported that HPP of cooked meat products does not affect sensory acceptability as HPP has minimum effects on the physicochemical properties of cooked products as a consequence of the myofibrillar proteins already being denatured through the cooking process (Considine *et al*., 2008; Neto *et al*., 2015; Bansal *et al*., 2015). Furthermore, it was reported that HPP did not affect significantly the sensory quality of various cooked meat products (low-fat pastrami, strassburg beef, export sausage, cooked ham, wieners and cajun beef) (Hayman *et al.*, 2004; Karlowski *et al.*, 2002; Pietrasik *et al*., 2017), even if the products were HPP at 600 MPa.

However, in fresh meat products, HPP can significantly alter the sensory attributes such as appearance, texture, juiciness and overall sensory acceptability. HPP can decrease liking of appearance due to the whitening effect described previously and which can decrease consumer acceptability of HPP processed fresh meats (Cheftel and Culioli, 1997; Souza *et al.*, 2011). The appearance and colour of food has been shown to significantly influence consumer sales (Considine *et al*, 2008). The ability of HPP to either toughen or tenderise meat can also result in positive or negative textural effects (Chevalier *et al*., 2001; Iwasaki *et al.*, 2006; Zamri *et al*., 2006; Kruk *et al*., 2011). HPP has been shown to improve juiciness by Crehan *et al*. (2000), who demonstrated that the application of 300 MPa significantly increased juiciness of frankfurters. This may be due to the fact that HPP can lower the cook loss of meat products (Rodriguez- Calleja *et al*., 2012; Souza *et al*., 2011),

which in turn would increase product juiciness. With respect to flavour, Crehan *et al*. (2000) stated that HPP does not markedly alter taste, flavour or the nutrient content of foods. Interestingly, studies have also suggested that HPP enhances saltiness perception in meat products, which can positively affect flavour and OSA (Ken *et al*., 2006; Clariana *et al*., 2011). This effect may be due to differential binding forces of NaCl within the product network and its release in the mouth (Tamm *et al.*, 2016). This offers a unique opportunity for HPP to assist in salt reduction within processed meats.

1.12.5 Effects of HPP on microorganisms – spoilage and pathogens

One of the principal advantages of HPP is through its ability to extended shelf-life and improve food safety due to its inactivation of microbial populations (Rendueles *et al*. 2011). The loss of viability of microorganisms through HPP is the result of a combination of factors, so cell death is due to multiple or accumulated damage inside the cell (Simpson and Gilmour, 1997).

For most forms of vegetative bacteria and spoilage microorganisms, significant reductions (usually higher than 4 log units) in the microbial population are achieved when 400-600 MPa is applied at room temperature (Campus, 2010). Pressure level, temperature and time are the critical factors that determine the lethality of microorganisms in a particular food matrix (Bajovic *et al*., 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 microorganisms and reduce the extent of bacterial inactivation, thereby leading to the recovery of sub-lethally damaged cells during product storage (Rendueles *et al*., 2011; Szerman *et al*., 2011). In general, Gram-negative

bacteria and cells in the growth phase, are more sensitive than Gram-positive bacteria and cells in the stationary phase (Campus *et al*., 2010).

The resistance of microorganisms to pressure is highly variable, depending mainly on the type of organism and the food matrix being considered. Prokaryotes are usually more resistant when compared to eukaryotes (Yuste *et al*., 2001). The destruction of protozoa and parasites is achieved with relatively low pressures (100-400MPa) (Collins *et al*., 2005; Lindsay *et al*., 2006; Rosypal *et al*., 2007; Brutti *et al*., 2010). Moulds and yeasts have intermediate resistance (Palou *et al*., 1997; Shimoda *et al*., 2002). Viruses possess a wide range of pressure resistance which appears to be dependent upon their structural diversity. Enveloped viruses are usually more sensitive to pressure than naked viruses (Hygreeva and.Pandey, 2016).

However, bacterial spores show great resistance to inactivation by HPP. The genera *Bacillus* and *Clostridium* comprise significant species as foodborne spore-forming pathogens, such as *Clostridium botulinum*, *Clostridium perfringens* and *Bacillus cereus* (Rendueles *et al*., 2011).

One strategy has been successfully employed to induce germination of spores and subsequently inactivate the bacteria by HPP. The strategy was developed and applied to poultry meat (Atkar *et al.*, 2009) and consisted of the following: (1) a primary heat treatment (80°C, 10 min) to pasteurize and denature meat proteins and to activate *Clostridium Perfringens* spores for germination, (2) cooling of the product to 55°C for 20 min and further incubation at 55°C for 15 min for spore germination (3) inactivation of vegetative from germinated spores by pressure-assisted thermal processing (586 MPa at 73°C for 10 min). This approach is not new as the principal is based upon the process of Tyndallisation, but using pressure rather than a temperature application.

The pathogenic microbiota that associates with meat and meat products has a wide history in causing severe food borne infections for humans in different countries throughout the world (Juck *et al*., 2012; Ahmadi *et al*., 2015). The most common pathogens that are associated with food borne illness are *Salmonella* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium botulinum*, *Yersinia enterocolitica*, *Campylobacter* spp., *Listeria 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 food product (Jofré *et al*., 2009).

Listeria monocytogenes is a bacterial pathogen of major concern to the processed meat industry, because this pathogen is ubiquitous and will grow under refrigerated conditions in the presence of both salt and nitrite (Myers *et al*. 2013). HPP has been permitted as a post-processing decontamination technology for RTE cooked and cured meat products in different countries in order to eliminate the riskspose by pathogens, including; Salmonella spp., *E. coli* O157:H7 and *Listeria monocytogenes* (Hygreeva and Pandey, 2016). The following recommended microbiological limits are applied for cook-chill products examined at the point of consumption before reheating or cooking is applied: Aerobic plate counts < $5x10^5$ CFU/g of product; E. coli< 10 CFU/g of product; LAB < 10^9 CFU/g of product, Salmonella: absent in 25 g of product, (FSAI, 2014). For fresh meat products the recommended microbiological limits are: Aerobic plate counts < $5x10^6$ CFU/g of product; E. coli< 10 CFU/g of product, Salmonella: absent in 25 g of product (FSAI, 2015).

Numerous studies have investigated the effects of HPP on the inactivation of various spoilage and pathogenic microorganisms in different meat products (Table 1.4). The majority of these studies apply HPP at 600 MPa, which is the standard pressure level used in commercial meat applications (Myers *et al*., 2013).

Table 1.4 - Previous studies investigating the effects of HPP on the inactivation of various spoilage and pathogenic microorganisms in meat products.

Reference	Meat product	HPP	Findings
Carlez *et al.* (1994)	Minced beef	450 MPa for 20 mins	Resulted in a delay of 13–15 days for TVC growth compared to untreated control samples
Carpi *et al*. (1999)	Cooked ham	600 MPa for 5 mins	Extended shelf life up to 75 days compared to untreated control samples which spoiled at 15 days
Lopez – Caballero *et al.* (1999)	Cooked ham	400 MPa for 20 mins	Extended shelf life by at least 14 days compared to untreated control samples
Diez *et al*. (2008)	Blood sausage	600 MPa for 10 mins	Resulted in 15 days shelf life extension compared to untreated control samples based on TVC
Pietrzak *et al.* (2007)	Cooked ham	600 MPa for 10 mins	After 8-week storage, spoilage microorganisms in the HPP samples were 4–5 log cycles lower than in untreated control samples

Table 1.4 contd.

Reference	Meat product	HPP	Findings
Han *et al*. (2011)	Cooked ham	600MPa for 10 mins	Reduced initial TVC by 2 log compared to untreated control samples
Pietrasik *et al*. (2017)	Wieners	600 MPa for 3 mins	Control samples spoiled at 8 weeks. TVC and LAB of wieners were below the limit of detection for 12 weeks.
Yanqing *et al*. (2009)	Smoked ham	400MPa and 600 MPa for 10 mins	Based on TVC and LAB, Control spoiled at 2 weeks, 400MPa spoiled at 8 weeks, 600MPa spoiled at 10 weeks
Garriga *et al*. (2004)	Marinated beef loin	600 MPa for 6 mins	Reduced TVC and LAB at least 4 log cycles compared to untreated control samples
Wang *et al*. (2015)	Honey garlic pork chops	450 MPa for 3 mins	Shelf life extension by 21 days based on TVC compared to untreated control samples
Karlowski *et al*. (2010)	Cooked ham and Smoked pork loin	600 MPa for 10 mins	Reduced TVC by 5-6 logs and extended shelf life to 6-8 weeks.

Table 1.4 contd.

Reference	Meat product	HPP	Findings
Hugas *et al*. (2002)	Cooked ham and Marinated beef	600MPa for 6 mins	Reduced initial *S.aureus* counts by 2 log. Reduced LAB by 1 log
Rubio *et al*. (2007)	Cured beef	500 MPa for 5 mins	TVC was 2 log lower over 210 day storage compared to untreated control samples
Kruk *et al*. (2011)	Chicken fillets inoculated with *Salmonella typhimurium*, *Escherichia coli*, and *Listeria monocytogenes* 10^{8-9} CFU/g	600 MPa for 5 mins	Reduced the total bacterial count by 6–8 logs improving shelf-life for 7–14 days.
Myers *et al*. (2013)	Sliced ham inoculated with *L. monocytogenes*	600 MPa	Reduced *L. monocytogenes* by 3 log cycles
Hayman *et al*. (2004)	Pastrami, Strassburg beef, export sausage, Cajun beef inoculated with *L. monocytogenes* 10^4 CFU/g.	600 MPa for 3 mins	Below detection limit for TVC, LAB and *L. monocytogenes* for 90 day storage

Table 1.4 contd.

Reference	Meat product	HPP	Findings
Tanzi *et al*. (2004)	Dry-cured ham inoculated with *L. monocytogenes* $10^{4.65}$ CFU/g	600 MPa for 9 min	Total inactivation (<1 cfu/g)
Scheinberg *et al*. (2014)	Beef jerky inoculated with 10^7 CFU/g *L. monocytogenes*, *Salmonella spp, E.Coli 0157:H7, S.aureus*	HPP 550 MPa for 1 min (x2)	6.83 and 4.45 log reduction in *Salmonella spp.* and *Escherichia coli O157: H,* respectively. 1.28 and 1.32 log reduction in *L. monocytogenes* and *Staphylococcus aureus*, respectively
Porto-Fett *et al*. (2010)	Salami inoculated with 10^7 CFU/g *E.Coli*	HPP 600 MPa for 5 mins	>5 log reduction

1.13 Commercial applications of HPP

Research into HPP in the 1980's led to it being commercially exploited with the development of rigs capable of processing large volumes of food at pressures of several hundred MPa. Initial success was with fruit- and vegetable-based products such as orange juice and guacamole. For such products HPP applied at 500 MPa inactivated both bacteria and enzymes, fully or partially, so that products could be stored for several weeks at refrigeration temperatures with no loss of quality (Patterson *et al*., 2005).

Currently, the meat industry has the greatest number of industrial scale HPP rigs in the food industry, followed by vegetable-based processors (Figure 1.5). Commercially-available HPP meat products, include; dry cured ham, tapas, parma ham, chorizo, salami, and turkey, chicken and beef (Campus, 2010). These HPP meat products are currently commercialised mainly for export purposes. Consequently, HPP offers the possibility of increasing commercial commodities and expanding product portfolios for meat companies (Campus, 2010). Fresh meat is not typically commercially HPP, primarily owing to negative colour changes in the meat. (Cheftel and Culioli, 1997).

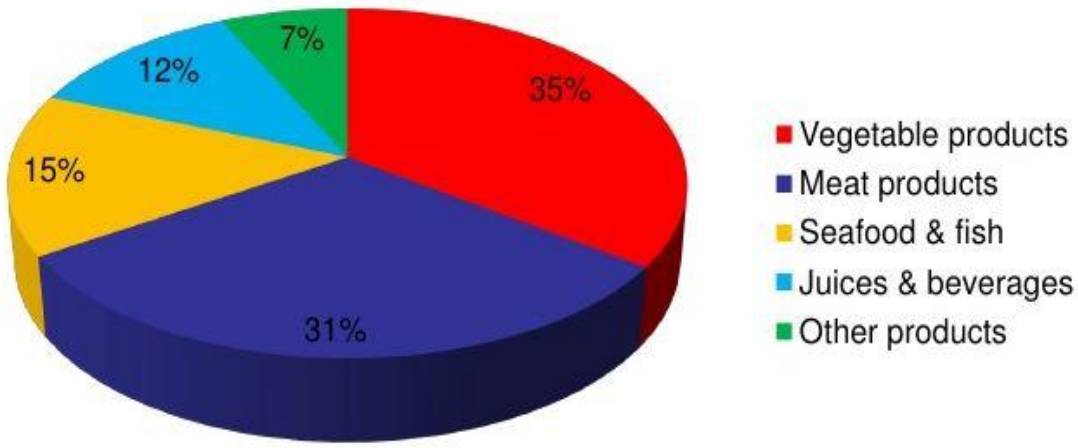


Figure 1.5 Industrial HPP machines in different food industries.
Source: Campus, 2010

Custom HPP systems range between $500,000 and $2.5 million; however numerous facilities now offer HPP tolling services (Balasubramaniam *et al*., 2008; Patterson *et al*., 2005). Industrial HPP units can range in size up to 525 litre capacity (Figure 1.6).

HPP service costs will vary due to different operating parameters, product specifications, packaging, volume, and labour costs; however, available sources have estimated treatment costs ranging between $0.04 and $0.11 per pound of product (Patterson *et al.*, 2005; Balasubramaniam *et al*., 2008). In more recent times, the development of high-efficiency HPP machines has reduced the processing costs to more acceptable levels and HPP as a low-temperature treatment is also viewed as an environmentally friendly and waste-free technology (Campus, 2010).

Figure 1.6 Industrial HPP unit (HPP Tolling, Dublin)

1.14 Marination of meat products

Marinade technology has been used in the meat industry for several decades. The role and perception of marinades has evolved from flavouring and tenderising to enhancing yield, colour, shelf-life and meat quality (Yusop *et al*., 2011).

1.14.1 Types of marinades

Based on their functionality marinade ingredients are classified into two categories; 1) Ingredients that affect the water-binding or textural properties, and condition the meat to bind water via ionic strength and pH such as water, salt, phosphates, organic acids, hydrocolloids, protein isolates, curing aids and enzymes and 2) ingredients which affect consumer appeal and eating quality of marinated meat products such as herbs and spices, flavour extracts and sweeteners. (Toledo, 2007).

1.14.2 Marinating techniques

Marinades diffuse from the meat surface into the interior of the meat due to the gradient formed from the higher concentration of marinade to the lower concentration of fluid in the interior of the meat (Yusop *et al*,. 2011).

Consumers generally incorporate marinades into meat via immersion. This consists of immersing the meat in a liquid marinade and allowing penetration of the meat through diffusion over time. Dry/paste marinating is also a common method of marinate delivery for consumers; however, injection processing and tumbling/massaging are operating marinade processes more commonly employed by the meat industry (Yusop *et al.*, 2011).

1.14.3 Effects of marination on physicochemical characteristics of fresh meat

1.14.3.1 Flavour enhancement

There has been an increase in the range of commercially available marinade products (Yusop *et al.*, 2011) and flavour components such as barbeque and piri piri marinade are in high consumer demand (Nachay, 2011). Ethnic marinades and ethnic flavour-marinated meat products are very popular due to the increased demand for such products by consumers who are more adventurous, demand products possessing more authenticity and desire a more flavourful experience when eating meat (Yusop *et al*., 2009a; Yusop *et al.*, 2009b; Yusop *et al*., 2010). Marinades can increase the sensory acceptability of meat products by enhancing flavour (Yusop *et al*., 2011). Kim *et al.* (2010) found that pork marinated with garlic and onion juice had significantly higher flavour attributes than control samples which were not marinated. Similarly, Kingsley *et al*. (2015) found that a combination of Sriracha® hot sauce flavouring and HPP at 600MPa for 5mins yielded a raw oyster with improved sensory quality in regards to flavour.

1.14.3.2 Texture

With respect to texture, marinades containing organic acids are primarily applied for tenderisation. Many authors have demonstrated the ability of marinades to tenderise meat products such as beef, chicken and pork (Lewis and Purslow, 1991; Oreskovich *et al*., 1992; Berge *et al*., 2001; Aktas *et al*., 2003; Burke and Monahan 2003; Sheard and Tali, 2004; Bowkler *et al*., 2010; Birk *et al*., 2010; Wang *et al.*, 2015; Rodrigues *et al.*, 2016). Marinades increase tenderness due to marinade uptake by muscle proteins and through solubilisation of collagen (Burke and Monahan, 2003).

1.14.3.3 Yield

Marinades containing Phosphates have been applied to muscle foods to increase product yield. Phosphates increase water-holding capacity in fresh and cured meat products by increasing the ionic strength, which frees negatively charged sites on meat proteins, allowing them to bind more water (Desmond, 2006).

Many products are marinated by vacuum tumbling meat with a mixture of water, salt, and phosphates to increase cook yield (Smith and Young, 2007). Sheard and Tali, (2004) reported that injection of a marinade consisting of salt, tripolyphosphate and bicarbonate increased the yield of pork loin. Alvarado and Sams (2004) found that vacuum tumbling with phosphate increased the yield of broiler chicken breast fillets. Other authors have also reported an increase in product yield in muscle foods after vacuum tumbling with phosphates (Landes, 1972; Smith *et al*., 1991; Young and Lyon, 1997; Young *et al*., 2004; Smith and Young, 2007).

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1.14.3.4 Colour

Marinating can also be employed to incorporate colours into meat products (Yusop *et al*. 2011). Colour enhancement through marination is particularly beneficial in the case of HPP treated processed meats, but might also be a potential approach to dealing with fresh meat discolouration associated with the application of HPP.

Wang *et al.* (2015) demonstrated the potential of marinades to mask the whitening effect/discolouration of HPP on raw meat as the authors concluded that honey garlic marinade partially masked HPP-associated meat discolouration up to 600MPa.

1.14.4 Preservation

Marinades containing organic acids are becoming more popular as antimicrobial ingredients; particularly, for their ability to reduce *Listeria monocytogenes* in ready-to-eat meat products. Typical marinades utilized for their antimicrobial properties include sodium lactate, potassium lactate, sodium citrate, sodium lactate combined with sodium diacetate, and combinations of sodium lactate with potassium lactate and diacetate (Alvardo and Mc Kee, 2007). The inhibitory action of organic acids on a microorganism is believed to be a result of the compound in the undissociated state being able to freely cross the plasma membrane to enter the bacterial or fungal cell (Brul *et al*., 1999) and the ability to inhibit essential metabolic reactions, such as the production of ATP and the production of essential enzymes, they can also cause membrane disruption and stress on intracellular pH homeostasis (Lues, 2005).

Organic acids are typically employed in hurdle technology in combination with another method of preservation (Marcos *et al*., 2008; Jofre *et al*., 2009; Vercammen *et al.*, 2011; Rodriguez calleja *et al*., 2012; De Alba *et al.*, 2013; Gunasekaran *et al*., 2018).

Rodrigues *et al.* (2016) inoculated marinated beef with 10^6 CFU/g of *Listeria innocua* and *Escherichia faecium*, and marinated it with solutions in different concentrations of NaCl (1 or 2%) and citric acid (1 or 2%) for 18 hrs followed by HPP (300, 450 or 600 MPa) and found that the different marinating solutions were not sufficient to reduce initial microbial loads in the non-pressurized samples, but the combination with HPP caused six log cycle reductions of both microorganisms.

1.15 Cooking methods of meat products

Cooking of meat is essential to achieve a palatable and safe product (Tornberg, 2005) as it enhances flavour and tenderness and inactivates pathogenic microorganisms (Rodríguez-Estrada *et al*., 1997; Broncano *et al*., 2009). Cooking denatures proteins and increases the digestibility and bioavailability of nutrients (Davey and Gilbert 1974; Meade *et al*., 2005). Temperature and time play an important role in the characteristics of cooked meat and fish (Sobral *et al*., 2018). The major components of myofibrillar protein (myosin and actin) start to denature at ∼40–60 °C and ∼80 °C, respectively (Ishiwatari *et al*., 2013). Myofibrillar and connective tissue proteins (collagen and elastin) control the toughness of muscle tissues and during heating, these proteins are denatured, causing destruction of cell membranes, shrinkage of fibres, aggregation, and gelling of myofibrillar and sarcoplasmic proteins, and shrinkage and solubilisation of connective tissue (Tornberg 2005; Yu *et al*., 2017).

The most common methods of cooking meat include roasting, boiling, grilling, broiling, frying, braising, steaming, griddling, poaching, microwaving, baking, poaching, barbequing, *sous*vide and *confit* (AMSA, 2018; Sobral *et al.*, 2018) and the three main factors that differ among various cooking techniques are the temperature on the surface of the meat, the temperature profile through the meat and the method of heat transfer (convection or conduction by contact, air or steam) (Bejerholm and Aaslyng, 2004).

Steam cooking is a widely used, convenient and healthy cooking method as the typical characteristics of colour, flavour, texture, palatability and nutrients are retained (Kahlon *et al.*, 2008). Steaming relies on cooking with steam heat resulting from boiling water. The meat has direct contact only with steam which contributes to the moist texture of steam cooked meat (Sobral *et al*., 2018). Air convection is often coupled with steam injection in the oven chamber to improve meat tenderness and to reduce cooking losses (Murphy *et al*., 2001). Griddle cooking is gaining popularity in meat research, especially in industry

settings. The griddle cooks meat through conduction heating as the heat is transferred directly from the hot griddle surface to the meat (Yancey *et al*., 2011).

1.15.1 Effects of cooking methods on physicochemical quality of meat products

The physical properties and quality of cooked meat are strongly affected by the degree of protein denaturation resulting from different heat treatment conditions, such as temperature, time and contact method (Ishiwatari *et al*., 2013). Many studies have shown that protein denaturation due to cooking causes structural changes in meat and affects its physical properties such as water-holding capacity, texture, and colour (Bendall and Restall, 1983; Palka and Daun, 1999; Tornberg, 2005; Garcia-Segovia *et al.*, 2007) and as a result all sensory attributes can be influenced by changes in the cooking technique (Bejerholm and Aaslyng, 2004). Cooking method can also alter the fatty acid composition in meat products (Badiani *et al*., 2004; Maranesi *et al*., 2005; Sarriés *et al*., 2009) due to increased cook loss or oxidation (Weber *et al*., 2008).

It is well known that different cooking techniques result in different eating qualities (Fjelkner-Modig, 1986; Heymann *et al.*, 1990; Wood *et al*., 1995). Dreeling *et al*. (2000) examined the effect of various cooking methods (grilling, frying, griddling, roasting or deep fat frying) on the quality of low-fat beef burgers and found that the cooking method significantly affected the cook loss with deep fat frying and grilling resulted in the highest cooking losses and deep fat frying also resulted in beef burgers with the lowest moisture content. The sensory characteristics of overall sensory acceptability, tenderness, flavour, appearance, texture and juiciness were significantly affected by the cooking method and griddling was the most acceptable cooking method in terms of overall sensory acceptability. Latif (2010) concluded that the most suitable cooking methods for marinated chicken breast meats were roasting and boiling as they reduced the cook loss compared to

microwaving and frying. Barbanti and Pasquini, (2005) reported that marination, followed by air-steam cooking is the best combination to obtain the most tender chicken breast slices.

1.16 Consumer attitudes towards HPP meat products

When introducing new technologies in food processing, consumer opinion plays a significant and deciding role (Lyndhurst, 2009). In the past few years, research organizations and social media have been actively working to promote consumer awareness about newer food processing technologies and associated benefits relating to their health and convenience aspects. Recent reports have indicated positive responses from consumers who are ready to accept the foods that are being processed by novel processing techniques (Sorenson *et al*., 2011).

Butz *et al.* (2003) surveyed 3000 consumers in France, Germany and UK in relation to their perceptions of HPP and found that HPP was acceptable to the majority of consumers in France and Germany; however, it was important that the product price does not exceed that of conventional products and that there is a health and convenience benefit.

Overall, the evidence presented above highlights the great potential of salt replacers to significantly reduce salt content in processed meat products and also the potential of HPP for enhancement of safety and shelf life with minimum effects on the quality characteristics of cooked meat products. However, HPP and organic acids have not been previously applied through reponse surface methodology as a hurdle technology to compensate in terms of safety and shelf life for significant salt reduction in processed pork meat products such as frankfurters or cooked ham.

HPP can also improve the safety and shelf life of fresh meat products; however, the drawbacks of applying HPP to fresh meat products include the whitening effect which can decrease consumer acceptability and also the ability of HPP to increase fresh meat toughness. A combination of HPP, organic acids and marinades have the potential not only to enhance the safety and shelf life of fresh meat products but also to mask the discolouration and improve the tenderness. Furthermore, the ability of HPP to accelerate marinade absorption in order to enhance the yield and flavour acceptability of marinated piri-piri pork chops has not been examined previously; therefore two novel approaches are investigated in the current thesis.

1.17 Thesis objectives 1-6

- Use RSM to develop a sensory-acceptable, low-salt cooked ham using salt replacers (Artisalt™) and hurdles including HPP and a mix of antimicrobial organic acids (Inbac™).

- Use RSM to develop sensory-acceptable, low-salt frankfurters with enhanced safety and shelf-life using salt replacers (Artisalt™) and hurdles including HPP and a mix of antimicrobial organic acids (Inbac™).

- Establish the efficacy of a combination of HPP and a mix of organic acids Inbac™ as hurdles to extend the shelf life of previously sensory optimised low-salt frankfurters and low-salt cooked ham from a microbiological and physicochemical point of view.

- Assess the acceptability and consumer appeal of previously sensory optimised low-salt frankfurters and cooked ham with enhanced safety and shelf life compared to research control and commercial gold standard frankfurters and cooked ham products available in the Irish market.

- Determine the efficacy of HPP to accelerate the marinade absorption of piri piri pork chops and to study these effects on the physicochemical, sensory and microbiological characteristics over storage time.

- Compare the effects of griddle and steam cooking on the physicochemical and sensory characteristics of HPP marinated pork chops.

CHAPTER 2

The application of response surface methodology for the development of sensory accepted low-salt cooked ham using high pressure processing and a mix of organic acids.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

The objective of this study was to develop sensory accepted low-salt cooked ham by the application of response surface methodology (RSM). A Box-behnken experimental design was used to assess the effects of the independent factors salt replacer (Artisalt^{TM}) (0-100%), high pressure treatment (0.1-600 MPa) and a mix of organic acids (Inbac^{TM}) (0.2-0.4%) on hardness, flavour, saltiness and overall sensory acceptability (OSA) of the cooked ham. The main factor that affected all response variables was salt replacement. The optimum parameters to maximise salt reduction and produce hams with similar OSA associated with this type of products were Artisalt^{TM} (53%), HPP (535 MPa) and Inbac^{TM} (0.3%) and the cooked ham manufactured using the optimum parameters contained 1.4% total salt which is a 46% reduction compared to control samples which contained 2.6% total salt. Overall, a combination of salt replacer, HPP and organic acids showed great potential for the development of cooked ham with significantly reduced salt content.

Industrial Relevance

Consumer studies have shown that meat consumption is being more and more influenced by health, nutritional and environmental considerations; therefore, companies are constantly searching for new and emerging technologies to reduce salt in meat products and enhance shelf life to reduce food waste. In this study we used a novel approach which showed great potential in salt reduction of ham as the quality and sensory acceptability of the ham were similar and/or better after salt was replaced by 53%. The hurdle approach used in this study is expected to improve the safety and shelf life of the low-salt optimised ham and this confirmatory study is underway.

2.1 Introduction

Sodium chloride (NaCl), commonly known as salt plays a significant technological role in processed meat due to its preservation and antimicrobial properties provided by its ability to reduce water activity. Moreover, salt activates proteins to increase hydration and water-binding capacity; it increases the binding properties of proteins to improve texture and it is essential for flavour (Terrell, 1983; Mariutti and Bragagnolo, 2017). Thus salt reduction in processed meat products is challenging as quality of the final product can be compromised. High salt consumption has been associated with cardiovascular disease (CVD) which is the most common cause of death in Ireland which accounts for 10,000 deaths per year (IHF, 2016). Salt intake of less than 5 g/day for adults has been recommended by the World Health Organisation (WHO) to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart attack; however, in most European countries this recommended dietary intake is greatly exceeded with an estimated salt consumption as high as 9-12 g/day. It was reported that an estimated 2.5million deaths can be prevented each year if global salt consumption is reduced to the WHO recommended levels (WHO, 2016).

Due to the preservation properties of salt, when salt is reduced in meat products, the safety and shelf-life can be compromised. Hurdle technology combines intelligently different mild preservation techniques (hurdles) such as high pressure processing (HPP) to control or eliminate pathogens (Rodriguez-Calleja *et al*., 2012). HPP can fulfil consumer requirements for minimally processed additive-free products, maintain sensory and nutritional properties and can contribute to the development of meat products with lower salt content (Watson, 2012). Furthermore, Karłowski *et al.*, (2002) reported that HPP did not have an effect on the sensory quality of cooked ham; indicating that HPP affect minimally the physicochemical characteristics of cooked meat products as the protein had been denatured by cooking. Some studies have also pointed out that HPP enhances the

saltiness perception in meat products (Ken *et al*., 2006; Clariana *et al*., 2011) due to differential binding forces of NaCl within the product network and its release in the mouth (Tamm *et al.*, 2016).

The main strategies used for salt reduction in processed meat products include product reformulation, compensation by the use of substitutes, use of saltiness enhancers and the use of salt replacers (Kilcast and Angus, 2007). Dimitrakopoulou *et al*. (2005) successfully reduced salt in reformulated pork shoulder from 2% to 1% based on acceptability of sensory attributes. Aaslyng *et al.* (2014) found that through product reformulation the salt in cooked ham can be reduced from 2.3% to 1.8% without altering the sensory properties, sliceabilty, production yield, shelf life and safety; however, further reductions affected significantly product quality and would therefore require other measures such as the substitution of salt with other functional ingredients such as salt replacers.

Potassium Chloride (KCl) is probably the most common salt substitute/replacer used in reduced-salt meat products and has been extensively examined for salt reduction in cooked ham (Tamm *et al.*, 2016; Lorenzo *et al.*, 2015; Aliño *et al*., 2009; Ruusunen and Puolanne., 2005; Hand *et al*., 1982); however, one of the major problems when replacing NaCl with KCl is the bitterness and the use of higher concentrations of KCl can leave a metallic aftertaste (Albarracín *et al.*, 2011). Lorenzo *et al.* (2015) found that partial replacement (50%) of NaCl with KCl in the manufacture of hams resulted in an increased bitterness.

Pietrasik *et al.* (2014) examined the effects on the physicochemical characteristics and sensory acceptability of cooked ham when NaCl was fully replaced (100%) with two commercial salt replacers: Oceans flavour sea salt^{TM} OF45 or 0F60, which are natural sea salts that contain 45% and 60% less sodium than table salt, respectively. The authors reported that the texture and cook loss of the cooked ham were not significantly affected;

however, the hams containing the sea salt replacers were liked significantly less compared to control for flavour and aftertaste. The authors concluded that further flavour optimisation through the application of bitter masking agents or flavour enhancers was required to suppress undesirable levels of bitterness elicited by the ingredients used. Tamm *et al.* (2016) achieved a 45% salt reduction in ham through the use of KCl combined with a pressurisation step at 100 MPa after tumbling, these salt-reduced hams were acceptable in terms of texture, consistency and appearance but a lower saltiness taste was detectable by the sensory panel, which can potentially reduce product acceptability.

To the best of our knowledge no previous studies have been carried out on the application of product optimisation using a combination of salt replacers, HPP and antimicrobials in the development of low-salt cooked ham; therefore, the objective of this study was to use response surface methodology (RSM) to develop a sensory accepted low-salt cooked ham using salt replacers (Artisalt™) and hurdles including high pressure processing (HPP) and a mix of antimicrobial organic acids (Inbac™).

2.2 Materials and Methods

2.2.1 Materials

Pork Silverside was obtained from Ballyburden meats, Ballincollig, Cork. NaCl, Sodium Nitrite, Sodium Nitrate, Sodium Ascorbate and Sodium tripolyphosphate hydrated food grade, Carfosel 990 (Prayon, Belgium) were obtained from All in All ingredients Ltd, Dublin. Artisalt™ (a mix of Potassium chloride 41%, Ammonium chloride 40% and flavour enhancers - yeast extract, onion and celery 19%) is a commercially available salt replacer used in processed meat products. which was obtained from Chemital Ltd, (Chemital Ltd, Barcelona, Spain). According to the manufacturer specification sheet Artisalt™ can replace all (100%) or part (50%) of common salt in meat products without giving any off-taste and allowing meat proteins solubilisation which is an essential factor in producing products with good texture and palatability. A commercial antimicrobial Inbac™ (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%,) was obtained from Chemital Ltd and used as recommended by the manufacturer (2-4 g/kg of product).

2.2.2 Methods

2.2.2.1 Experimental Design

A three-factor experimental design (Box-Behnken) was used to optimise salt reduction and consisted of the manufacture of 15 different formulations (Table 2.1). The centre point of the experimental design was repeated 3 times. The independent factors were Salt replacer Artisalt™ (0-100%), HPP (0.1-600MPa) and organic acid Inbac™ (0.2-0.4%). The full polynomial model involving the main effects (linear terms), interaction terms (cross

products) and quadratic or squared terms were defined to fit the responses as is shown in Equation 1.

Equation 1:

$$Y=b_0 + b_1A + b_2B + b_3C + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

Where: Y represents the dependent or response variable (overall sensory acceptability (OSA), flavour, saltiness or hardness), b_0 is a constant coefficient of the models and A, B and C represents the independent coded variables; A = *S*alt replacement (%), B = HPP (MPa) and C = Inbac (%)) ranging from -1 to +1and b_0–b_9 are the regression coefficients to be determined.; b_1–b_3 are the linear coefficient terms; b_4–b_6 are the interaction coefficient effects; and b_7–b_9 are the quadratic coefficient effects of the model estimated by multiple regression analysis, respectively. The effect of variables at the linear, quadratic, and interactive levels on individual responses was described using a significance level of confidence set at 5%.

Table 2.1 Experimental design of uncoded and coded parameters.

Independent variables	Symbols		Levels	
	Uncoded	Coded	Uncoded	Coded
Salt replacement (%)	A	X1	0	-1
			50	0
			100	+1
High Pressure treatment (MPa)	B	X2	0.1	-1
			300	0
			600	+1
Concentration of Inbac (%)	C	X3	0.2	-1
			0.3	0
			0.4	+1

2.2.2.2 Characterisation of salt replacer and antimicrobial

Ammonium Chloride was determined using the methods outlined in 23rd Joint FAO/WHO Expert Committee on Food Additives (1979). KCl, Nitrites and Nitrates concentration of the salt replacer Artisalt™ and concentration of sodium acetate and malic acid of the antimicrobial Inbac™ was determined by a commercial external analytical testing facility (ALS laboratories, Little Island, Cork). For the determination of KCl, Artisalt™ was homogenized and mineralized by acids and hydrogen peroxide prior to analysis by atomic emission spectrometry with inductively coupled plasma and stoichiometric calculations of compounds concentration carried out from measured values. Nitrites and Nitrates were determined by flow-injection analysis and gas-phase molecular absorption spectrometry. Results were expressed as a percentage of the salt replacer Artisalt™. Organic acids (Sodium acetate and Malic acid) were determined by high pressure liquid chromatography with UV detection and results were expressed as percentage of the antimicrobial Inbac™.

2.2.2.3 Brine preparation and injection

The brine composition for the 15 ham formulations contained 13% NaCl, 2% Sodium tripolyphosphate hydrated food grade (Carfosel 990), 0.3% Sodium ascorbate, 0.15% Nitrate and 0.15% Nitrite with varying levels of NaCl replacer Artisalt™ (0% replacement, 50% replacement or 100% replacement) and Inbac™ (0.2 – 0.4%) (Table 2.1). The brine containing 13% salt was injected using a multineedle injector (Machine factory Hollstein and Fuhrmann, Vienna, Austria) to obtain a 10% weight gain. After injection pork meat pieces were placed in a vacuum tumbler (Inject Star, Austria) and tumbled at 4°C for 2 hours at a speed of 6 rpm and vacuum of -0.9 bar. The pork pieces (~1kg) were packed into stainless steel moulds which were then sealed, clamped and cooked at full steam (100°C)

in a Zanussi oven (Zanussi Professional, Italy) and temperature monitored using a thermocouple data logger (Omega Engineering Ltd, Manchester, UK) inserted into the coldest point of the ham until an internal temperature of 74°C was reached. The cooked hams were then removed from the oven and final end-point temperature of the moulded hams were re-checked using a Testo hand-held food thermometer. The cooked hams were cooled down at room temperature and stored overnight in a chill room at 4°C before high pressure processing was carried out.

2.2.2.4 High Pressure Processing

Chilled whole cooked hams were packed individually in combivac vacuum pouches (20 polyamide/70 polyethylene bags; Alcom, Campogalliano, Italy) and vacuum-sealed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, Germany). Samples were placed in a second vacuum pouch and this was again vacuum-sealed. Packaged samples were HP treated in a Stansted Fluid Power Iso-Lab 900 Power High Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK), using an ethanol-castor oil (90:10) as the pressure transmitting medium. The speed of pressurisation was 300 MPa per minute, the speed of depressurisation was 600 MPa per minute and holding time at the required pressure level was 5 minutes. Pressurisation was carried out at room temperature 20°C which was monitored by the temperature sensor contained in the HP units pressure transmitting medium. Adiabatic heating resulted in a ~3°C increase per 100 MPa.

2.2.2.5 Compositional Analysis and pH

Fat and moisture were determined using the SMART Trac and CEM Analysis System (CEM Corporation, Matthews, NC 28105, USA) (Bostian *et., al*, 1984). Protein content was determined according to AOAC Procedures (1997) (method 981.10). The ash content of the cooked hams were determined by overnight incineration in a furnace (Nabertherm, Model L9/C6, Nabertherm, Germany) at 550 °C. Each value represents the average of 4 measurements (two independent trials x two samples).

The pH of the cooked hams were measured using a digital pH-metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the cooked ham. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

2.2.2.6 Expressible moisture

The expressible moisture was determined as previously described by Grau and Hamm (1953) with some modifications. Briefly, 300 mg of grounded ham sample was placed on a filter paper (Whatman No. 1) and compressed for 2 min with a force of 1kg using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK). The compressed sample was then removed and the filter paper was weighed. Expressible moisture was calculated as the quantity of water released by the sample and expressed in percentage using the following equation.

% EM = (weight of filter paper and sample – weight of filter paper after compression) / (weight of filter paper and sample before) * 100.

Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

2.2.2.7 Salt content and Ionic strength

Salt content was determined using the DiCromat II Salt Analyser (The Noramar Co, US). Before use, the instrument was calibrated using a 2% NaCl (Sigma, Ireland) solution. For the determination of salt content in the cooked ham samples, 25g of blended cooked ham was weighed to which 225 mls of distilled water was added and then blended for 1 min using an Ultraturrax homogeniser (IKA-Werke GmbH and Co, Germany). The homogenate was then filtered through a Watmann no.1 filter paper and the filtrate received in a 250 ml beaker. The dip-in probe of the DiCromat II Salt Analyser was immersed in the filtrate and the percentage of salt in the sample was read in the instrument display. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

The ionic strength_was calculated using the Debye and Huckel formula as described by Stanley (2017) using the following equation;

$$IS = \frac{1}{2} n \sum I\,(C_i\,Z_i)^2$$

Where IS = ionic strength, n = number of ions in solution, I = the specific ion in solution, C_i= concentration to the species (M), Z_i = the valence or oxidation number of the species. Results were expressed as (M).

2.2.2.8 Cook Loss

The cooking loss of the hams were determined prior to HP treatment and expressed as a percentage of the differential weight between hams before and after cooking. Briefly, the initial weight of the raw ham was recorded, after cooking the ham was patted dried with a paper towel to remove excess moisture and re-weighed. Cook loss was calculated as follows:

% Cook loss = (cooked weight – initial raw weight) / (initial raw weight) * 100

Each value represents the average of 4 measurements (two independent trials x two samples).

2.2.2.9 Sliceability

Sliceability of the cooked hams was determined as previously described by O' Neill *et al.* (2003). Briefly, 10 slices of 2 mm thickness were obtained from the cooked ham and breakage of the slices observed. The results were expressed as a percentage of slice breakage out of 10 during the slicing procedure. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

2.2.2.10 Colour

The colour of the surface of the cooked ham was measured using a Minolta Chromameter CR-300 (CR-300, Minolta Camera Co., Osaka, Japan). Before measurement, the Chromameter was calibrated using a white tile (Y = 86, X = 0.3166, y = 0.3237). CIE L*, a* and b* values (Lightness, redness and yellowness, respectively) are reported. Each value

represents the average of 8 measurements (two independent trials x two samples x two measurements)

2.2.2.11 Texture profile analysis (TPA)

Cylindrical sections of the cooked ham (2.5 cm x 4 cm) were extracted using a corer and texture measurements were performed at room temperature (20°C) using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK). The cooked ham samples were subjected to a two-cycle compression to 40% of their original height with a cylindrical probe (SMSP/35 Compression plate) 35 mm diameter using a 25 kg load cell at a cross-head speed of 1.5 mm/s. Texture profile parameters measured were hardness (N), adhesiveness (N x mm), springiness (mm), gumminess, chewiness (N) and cohesiveness (dimensionless). Each value represents the average of 8 measurements (two independent trials x four samples)

2.2.2.12 Sensory evaluation

A 25 member semi-trained taste panel was used to evaluate the cooked hams over three sessions using a 9-point hedonic scale. The panellists were recruited from staff and postgraduate students at the School of Food and Nutritional Sciences, University College Cork and chosen based on their experience in the sensory analysis of processed meat products and on their availability. The panellists have partaken in sensory analysis of processed meat products on numerous occasions and are familiar with the sensory terminology. The tested attributes included; Appearance (1= extremely dislike, 9= extremely like), Texture (1= extremely dislike, 9= extremely like), Flavour (1= extremely dislike, 9= extremely like), Juiciness (1= extremely dry, 9= extremely juicy) Tenderness

(1= extremely tender, 9= extremely tough,), Saltiness (1= not salty, 9= extremely salty), Off-flavour intensity (1= imperceptible, 9= extremely pronounced), metallic taste (1= imperceptible, 9= extremely pronounced), Overall acceptability (1= extremely dislike, 9= extremely like). Cooked ham samples were labelled with a three digit random numbers and 10 random samples per session were served to each of the 25 panellists.

2.2.2.13 Statistical analysis

The experiment was performed twice and the software STATGRAPHICS® centurion XV (Statpoint, Inc., USA) was used for the design of the experiment (DOE) which consisted of 15 ham formulations (Table 2.1), for prediction of the full model (Equation 1), ANOVA of the 4 response variables for optimisation and their interactions (Table 2.5) and to carry out product optimisation (Figure 2.2). The DOE was randomised by the software; however; the formulations have been arranged in order in the tables to allow for better understanding.

One-way ANOVA of all physicochemical (colour, texture, cook loss, pH, expressible moisture, sliceability, salt content) and sensory data was carried out using the SPSS 21 for Windows (SPSS Statistical software, IBM Corp., Armonk, NY, USA) software package. Differences between pairs of means was resolved by means of confidence intervals using Tukey's test; the level of significance was set at $P < 0.05$. Two independent trials were carried out consisting of the manufacture of 15 formulations of ham and all analysis was carried out in duplicate.

2.3 Results and Discussion

2.3.1 Composition of salt replacer and antimicrobial

The results of the quantitative chemical analysis of Artisalt™ indicated it contains 41% Potassium chloride, 40% Ammonium chloride, and 19% flavour enhancers (yeast extract, onion and celery (calculated by difference)). The contents of nitrates and nitrites in Artisalt™ were 5.8 and 2.3 ppm, respectively. This may be due to the fact that Artisalt™ contains celery and celery is a natural source of nitrates and nitrites (Sebranek *et al*., 2012).

The results of the quantitative chemical analysis of Inbac™ indicated it contains 43% Sodium acetate, 7% Malic acid, ~50% (emulsifier-mono and diglycerides of fatty acids, and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide (calculated by difference)). Small quantities of Inbac™ (0.2-0.4%) were used in the formulation therefore its inclusion in such small amounts would not affect the sodium content significantly nor contribute to the ionic strength.

Ionic strength of 2% NaCl, 2% Artisalt™ and 1% NaCl / 1% Artisalt™, concentrations used in the ham formulation, was calculated using the Debye and Huckel formula and the results indicated that the control (2% NaCl) formulation had an ionic strength of 0.34M, the 50% replacement formulation (1% NaCl and 1% Artisalt™) had an ionic strength of 0.31M and the 100% replacement formulation (2% Artisalt™) had an ionic strength of 0.26M. The results indicates that control samples that contained 2% NaCl and samples that were 50% NaCl replaced with Artisalt™ were quite similar which would result in similar protein solubility and binding of the ham which in turn would increase the water holding capacity (WHC) and reduce cook loss. These results are in agreement with O' Flynn *et al*., (2014) who reported that lower cook losses were observed in sausage samples with higher salt concentration due to increased ionic strength. Frye *et al*. (1986) also found that a

combination of KCl/NaCl gave the best physical bind in ham and concluded that partial replacement of NaCl ionic strength with 50% or less of KCl in tumbled ham can be accomplished while maintaining acceptable sensory and physical attributes.

Solubilisation of muscle proteins can also be achieved using salt replacers such as KCl. According to the "Hofmeister Series" which ranks the relative influence of ions on the physical behaviour of a wide variety of aqueous processes (Zhang and Cremer, 2006), KCl has been found to be effective at solubilising meat protein (Puolanne and Halonen, 2010).

2.3.2 Compositional analysis

Compositional analysis of cooked hams as affected by HP treatment, salt content and Inbac^{TM} showed no significant differences in protein, ash or fat content between samples (Table 2.2).The moisture content of the cooked ham decreased significantly ($P<0.05$) when the NaCl was 100% replaced with Artisalt^{TM}, this may be due to a significantly higher ($P<0.05$) cook loss as a result of reduced protein solubility and binding due to a lower ionic strength. These results are in agreement with the findings of O' Flynn *et al.* (2014) who reported that the fat content in sausages was not affected by salt content or high pressure treatment and that higher moisture loss due to cooking were observed in sausage samples with lower salt concentration due to decreased ionic strength.

The results in this study also indicated that there was no significant differences in the proximate composition when 50% of NaCl was replaced with Artisalt^{TM} compared to control samples, suggesting that the 50/50 combination of NaCl and Artisalt^{TM} produces hams of a similar quality to that of the control but with significantly less ($P<0.05$) salt content.

Similar results were reported by Bansal *et al.* (2015) who found that HP treatment produces minimal changes in cooked meat products; indicating that the physicochemical differences between treatments was mainly due to the level of salt replacement and subsequent total salt content. Several authors have reported that HPP primarily affects the physicochemical properties of raw/uncooked meat products and has minimum effects on cooked products (Considine *et al*., 2008; Neto *et al*., 2015).

In this study the main purpose of using HPP as a factor in the product optimisation is to enhance the safety and shelf life of the low-salt ham and this confirmatory study is underway.

Table 2.2 Effects of different ham formulations on the proximate composition of the cooked ham*

Formulation	Salt replacement	HPP	Inbac	Fat	Moisture	Protein	Ash
	(%)	(MPa)	(%)	%	%	%	%
1	0	0.1	0.3	2.51 ± 0.48 [a]	68.56 ± 0.15 [a]	24.46 ± 0.18 [a]	3.18 ± 0.43 [a]
2	0	300	0.2	2.68 ± 0.56 [a]	69.11 ± 0.15 [a]	24.42 ± 0.74 [a]	3.26 ± 0.85 [a]
3	0	300	0.4	2.60 ± 0.51 [a]	68.61 ± 0.23 [a]	24.70 ± 0.65 [a]	3.32 ± 0.82 [a]
4	0	600	0.3	2.84 ± 0.67 [a]	68.73 ± 0.08 [a]	24.34 ± 0.68 [a]	3.82 ± 0.4 [a]
5	50	0.1	0.2	2.53 ± 0.51 [a]	68.65 ± 0.34 [a]	24.76 ± 0.27 [a]	3.53 ± 0.96 [a]
6	50	0.1	0.4	2.46 ± 0.46 [a]	69.60 ± 0.24 [a]	24.46 ± 0.48 [a]	3.48 ± 0.09 [a]
7	50	300	0.3	2.57 ± 0.53 [a]	68.91 ± 0.18 [a]	24.87 ± 0.57 [a]	3.32 ± 0.53 [a]
8	50	300	0.3	2.45 ± 0.43 [a]	69.49 ± 0.26 [a]	24.79 ± 0.34 [a]	2.95 ± 0.57 [a]
9	50	300	0.3	2.59 ± 0.27 [a]	69.45 ± 0.09 [a]	24.8 ± 0.26 [a]	3.35 ± 0.54 [a]
10	50	600	0.2	2.51 ± 0.15 [a]	69.69 ± 0.15 [a]	24.26 ± 0.19 [a]	3.75 ± 0.33 [a]
11	50	600	0.4	2.53 ± 0.54 [a]	69.45 ± 0.35 [a]	24.27 ± 0.66 [a]	3.72 ± 0.37 [a]
12	100	0.1	0.3	2.68 ± 0.65 [a]	66.61 ± 0.11 [b]	24.76 ± 0.24 [a]	3.21 ± 0.24 [a]
13	100	300	0.2	2.50 ± 0.46 [a]	66.27 ± 0.23 [b]	24.95 ± 0.41 [a]	3.37 ± 0.35 [a]
14	100	300	0.4	2.76 ± 0.91 [a]	65.91 ± 0.07 [b]	24.30 ± 0.69 [a]	3.38 ± 0.53 [a]
15	100	600	0.3	2.75 ± 0.73 [a]	66.58 ± 0.09 [b]	25.03 ± 0.6 [a]	2.69 ± 0.06 [a]

[*] Values are Mean ± standard deviation, [a,b] Different superscripts in the same column indicate significant difference ($P < 0.05$) between treatments.

2.3.3 Salt content and pH

The total salt content significantly ($P<0.05$) increased as the level of replacement decreased hence cooked ham that were 0% replaced with Artisalt™ (control samples) contained the highest salt content (2.6% total salt), compared to samples that NaCl was 50% or 100% replaced with Artisalt™ which contained 1.4% and 0.5% total salt, respectively (Table 2.3).

The pH of the raw pork meat was 5.6. After injection, there were no significant differences in pH values between the control ham and ham which NaCl was 50% replaced with Artisalt™ however, significantly higher pH values ($P<0.05$) were noticed in samples that 100% NaCl was replaced with Artisalt™ (Table 2.3). The measured pH of Artisalt™ is 7.4 in 2% which is higher than the measured pH of NaCl which is 6.6 in 2% and this may explain why the ham in which NaCl was 100% replaced with Artisalt™ had a significantly higher ($P<0.05$) pH value than any other treatment.

The results found in this study are in agreement with the findings of Aaslyng *et al.* (2014) who reported that the pH of ham was not affected when salt was reduced from 2.3% to 1.3%. Conversely an earlier study by Lee and Chin (2011) showed that a reduction in salt in ham from 1.5% to 0.5% did not change the pH; however, in this study significant higher pH values were noticed in samples containing 0.5% salt (100% salt replaced with Artisalt™). The higher pH values in the latter samples may have been due to the composition of the Artisalt™ which contains celery extract. Similar results were found by Pietrasik *et al.,* (2016) who reported that hams manufactured containing celery powder had significantly ($P<0.05$) higher pH due to high pH (9.8) of celery powder solution; furthermore, Sebranek *et al*. (2012) and Horsch *et al.* (2014) also reported that the pH was as much as 0.4 units higher in products manufactured using celery concentrate.

2.3.4 Cook loss and sliceability

The cook loss of the ham was determined prior to HP treatment and is shown in Table 2.3. There were no significant differences in cook loss between the control ham and ham that NaCl was 50% replaced with Artisalt^{TM}; however, the ham in that NaCl was 100% replaced with Artisalt^{TM} produced significantly ($P<0.05$) higher cook loss values which was due to a significantly ($P<0.05$) lower salt content and ionic strength which may have affected the protein solubility and binding of the ham which in turn increased the cook loss.

The sliceability of the ham was significantly affected ($P<0.05$) when NaCl was 100% replaced with Artisalt^{TM} as this product contained the lowest total salt content (Table 2.3). The negative effects on sliceability may be due to the fact that salt increases protein solubility providing binding strength between adjacent pieces of meat (The Salt Institute, 2013) and that full replacement of NaCl with KCL had a detrimental effect on water binding properties of restructured ham possibly due to lower protein solubilisation of KCl salt (Pietrasik *et al*, 2016). The significant effect of NaCl reduction on the water binding characteristics in this study supports the findings of a number of authors that binding properties are strongly influenced by NaCl content in processed muscle foods (Tamm *et al.*, 2016; Desmond, 2006; Pietrzak *et al*., 2007; Pietrasik *et al*., 2014; Jimenez-Colmenero *et al*., 2010; Lee and Chin, 2011; Ruusunen and Puolanne, 2005).

Conversely, Frye *et al.* (1986) reported that replacement of 50% of NaCl (2%) with KCl had the best binding when compared to the control samples which contained 2% NaCl indicating that the combination of NaCl and KCl can improve the binding ability of salt reduced hams which is in agreement with our findings as no significant differences in the cook loss and sliceability were found between the ham in 50% NaCl was replaced with Artisalt^{TM} and control ham samples. Overall, apparently similar values for ionic strength

resulted in no significant differences in the cook loss or sliceability between control and samples that 50% of NaCl was replaced with Artisalt™ suggesting that the combination of NaCl and Artisalt™ produces hams of a similar quality to that of the control but with significantly ($P<0.05$) less salt.

2.3.5 Expressible moisture

The results showed that expressible moisture (%EM) was significantly ($P<0.05$) affected when the NaCl was 100% replaced with Artisalt™ (Table 2.3). The WHC of meat is the inherent ability of the cellular and subcellular structures of meat to retain excess water compared with the amount of the other muscular constituents (Honikel and Hamm, 1994). The EM is directly related to WHC as a higher EM indicates a lower WHC. Salt performs an important function in the production of tumbled meat products by the extraction of salt soluble proteins to the surface of ham pieces and their subsequent coagulation during cooking (Pietrasik *et al.*, 2007). The significant ($P<0.05$) effect of salt content on water binding characteristics in this study supports the findings of a number of authors who reported that binding properties are strongly influenced by salt content in processed muscle foods (Pietrasik *et al*., 2016; Pietrasik and Gaudette, 2014; Desmond, 2006; Pietrzak *et al*., 2007; Ruusunen and Puolanne, 2005) as reducing salt content limits protein extractability and alters thermal protein denaturation and aggregation patterns of the major muscle proteins (Trout and Schmidt, 1986), which affects the binding characteristics of meat products and subsequently the WHC.

2.3.6 Colour

Colour changes of cooked hams are shown in Table 2.3. The results showed that lightness and redness were significantly ($P<0.05$) affected when NaCl was 100% replaced by Artisalt™; however, results indicated that there were no significant differences between the control samples and hams that were 50% replaced with Artisalt™ which indicates that when the total salt was reduced from 2.6% to 1.4% the lightness and redness were not affected (Table 2.2).

These results are in agreement with Dimitrakopoulou *et al.* (2005) who reported that the lightness of the cooked restructured pork shoulder increased ($P<0.05$) while the redness significantly ($P<0.05$) decreased when the salt level was reduced from 2% to 1%. Wettasinghe and Shahidi (1997) also reported that darker pork meat was obtained when salt was increased from 1% to 2% due to oxidised products of meat pigments which have a brown and darker colour. In cooked ham samples that 100% of NaCl was replaced with Artisalt™ the increase in lightness and decrease in redness may be due to this effect.

Table 2.3 Effects of different ham formulations on the physicochemical characteristics of the cooked ham*

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Lightness (L*)	Redness (a*)	Yellowness (b*)	Salt content (%)	pH	Sliceablity (%)	EM (%)	**Cook loss (%)
1	0	0.1	0.3	63.76 ± 1.26 [a]	12.45 ± 0.76 [ab]	8.03 ± 0.84 [a]	2.5 ± 0.07 [a]	6.27 ± 0.03 [a]	98 ± 4.47 [a]	29.8 ± 0.56 [a]	16.0 ± 0.7 [a]
2	0	300	0.2	63.20 ± 1.28 [a]	12.23 ± 1.09 [ab]	8.91 ± 0.95 [a]	2.6 ± 0.08 [a]	6.25 ± 0.04 [a]	96 ± 5.31 [a]	29.6 ± 1.76 [a]	17.4 ± 0.89 [a]
3	0	300	0.4	63.71 ± 1.55 [a]	12.85 ± 1.23 [ab]	8.14 ± 1.5 [a]	2.5 ± 0.08 [a]	6.26 ± 0.07 [a]	94 ± 5.47 [a]	29.4 ± 0.6 [a]	17.0 ± 1.58 [a]
4	0	600	0.3	62.95 ± 2.12 [a]	12.83 ± 0.16 [ab]	7.51 ± 0.54 [a]	2.6 ± 0.08 [a]	6.23 ± 0.03 [a]	90 ± 5.2 [abc]	29.1 ± 0.04 [a]	17.4 ± 1.14 [a]
5	50	0.1	0.2	64.58 ± 2.15 [a]	13.01 ± 0.08 [ab]	8.32 ± 0.77 [a]	1.4 ± 0.07 [b]	6.28 ± 0.03 [a]	90 ±7.07 [abc]	29.7 ± 0.5 [a]	17.2 ± 0.94 [a]
6	50	0.1	0.4	62.89 ± 0.82 [a]	12.60 ± 0.61 [ab]	8.39 ± 1 [a]	1.3 ± 0.04 [b]	6.29 ± 0.02 [a]	90 ± 7.09 [abc]	29.1 ± 0.83 [a]	17.8 ± 0.84 [a]
7	50	300	0.3	65.24 ± 2.14 [a]	12.11 ± 2.05 [b]	7.22 ± 1.04 [a]	1.4 ± 0.07 [b]	6.28 ± 0.04 [a]	94 ± 4.17 [ab]	29.2 ± 1.88 [a]	17.1 ± 1.43 [a]
8	50	300	0.3	64.18 ± 2.65[a]	11.92 ± 2.14 [b]	8.12 ± 0.81 [a]	1.3 ± 0.05 [b]	6.26 ± 0.06 [a]	93 ± 5.44 [ab]	30.1 ± 0.42 [a]	17.1 ± 1.43 [a]
9	50	300	0.3	64.27 ± 2.74 [a]	11.81 ± 2.45 [b]	7.92 ± 0.94 [a]	1.4 ± 0.1 [b]	6.22 ± 0.05 [a]	93 ± 6.47 [ab]	29.9 ± 1.87 [a]	17.1 ± 1.43 [a]
10	50	600	0.2	64.80 ± 1.47 [a]	13.12 ± 1.21 [a]	8.92 ± 1.24 [a]	1.3 ± 0.05 [b]	6.29 ± 0.07 [a]	94 ± 5.61 [a]	29.7 ± 0.5 [a]	16.8 ± 1.48 [a]
11	50	600	0.4	64.67 ± 1.81 [a]	12.13 ± 1.01 [ab]	8.52 ± 0.87 [a]	1.4 ± 0.04 [b]	6.29 ± 0.05 [a]	94 ± 5.47 [a]	29.4 ± 0.94 [a]	16.6 ± 1.24 [a]
12	100	0.1	0.3	74.16 ± 1.16 [b]	10.18 ± 0.27 [c]	8.63 ± 0.59 [a]	0.5 ± 0.1 [c]	6.62 ± 0.03 [b]	78 ± 8.36 [cd]	33.3 ± 1.2 [b]	23.6 ± 1.14 [b]
13	100	300	0.2	74.39 ± 1.17 [b]	9.95 ± 0.35 [c]	8.91 ± 0.24 [a]	0.6 ± 0.08 [c]	6.42 ± 0.46 [b]	76 ± 5.47 [d]	33.8 ± 0.98 [b]	24.6 ± 1.11 [b]
14	100	300	0.4	74.51 ± 0.91 [b]	10.12 ± 0.21 [c]	8.85 ± 0.48 [a]	0.5 ± 0.08 [c]	6.60 ± 0.02 [b]	80 ± 7.07 [bcd]	33.5 ± 0.63 [b]	24.6 ± 1.13 [b]
15	100	600	0.3	73.68 ± 1.11 [b]	10.32 ± 0.24 [c]	8.43 ± 0.59 [a]	0.5 ± 0.07 [c]	6.61 ± 0.04 [b]	78 ± 4.47 [cd]	32.9 ± 0.21 [b]	25.0 ± 0.7 [b]

*Values are Mean ± standard deviation, **this analysis was carried out before HPP treatment. [a,b,c,d] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments

2.3.7 Texture Profile Analysis

The texture profile analysis of the cooked hams is shown in Table 2.4. The results showed that hardness, chewiness, springiness, cohesiveness values were significantly lower ($P<0.05$) when NaCl was 100% replaced with Artisalt^{TM}. A similar salt effect on hardness was reported on dry cured ham muscles (Gou *et al.*, 2008; Morales *et al.*, 2007). Bonbrum *et al* (2014) reported that in cooked ham, molecular bonds are able to form inside the exudate matrix due to gel cohesion and between the exudate and the muscle; therefore when the NaCl concentration is reduced the protein solubility is limited due to the solubility of the myosin (Offer, 1988) which subsequently effects the binding ability resulting in ham in which the hardness/firmness is reduced. Patana-Anake *et al.* (1985) suggested that springiness is affected by the type and amount of protein solubilised and this may explain the lower springiness observed in cooked ham samples in which 100% NaCl was replaced with Artisalt^{TM}. Texture represents another meat quality parameter which was not compromised when NaCl was replaced with 50% Artisalt^{TM} compared to control samples

Table 2.4 Effects of different ham formulations on the texture parameters of the cooked ham*.

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Hardness (N)	Adhesiveness (N)	Springiness (mm)	Cohesiveness	Gumminess (N)	Chewiness (N-mm)
1	0	0.1	0.3	16.97 ± 1.23 [a]	-.089 ± 0.16 [a]	.846 ± 0.04 [a]	.548 ± 0.02 [ab]	9.70 ± 2.68 [a]	9.05 ± 0.9 [a]
2	0	300	0.2	17.23 ± 1.19 [a]	-.022 ± 0.03 [a]	.842 ± 0.04 [a]	.568 ± 0.14 [a]	9.76 ± 2.03 [a]	8.49 ± 1.21 [ab]
3	0	300	0.4	17.72 ± 0.80 [a]	-.231 ± 0.40 [a]	.880 ± 0.02 [a]	.527 ± 0.03 [b]	8.07 ± 1.28 [a]	8.02 ± 1.16 [ab]
4	0	600	0.3	16.99 ± 1.08 [a]	-.027 ± 0.02 [a]	.869 ± 0.47 [a]	.566 ± 0.02 [a]	8.14 ± 0.74 [a]	7.56 ± 1.41 [ab]
5	50	0.1	0.2	17.50 ± 0.79 [a]	-.052 ± 0.06 [a]	.837 ± 0.03 [ab]	.536 ± 0.01 [ab]	6.99 ± 0.83 [a]	8.62 ± 0.97 [ab]
6	50	0.1	0.4	17.46 ± 1.13 [a]	-.003 ± 0.02 [a]	.845 ± 0.02 [a]	.565 ± 0.08 [a]	7.18 ± 0.4 [a]	8.79 ± 1.0 [ab]
7	50	300	0.3	17.74 ± 0.6 [a]	-.174 ± 0.07 [a]	.849 ± 0.06 [a]	.541 ± 0.01 [ab]	8.51 ± 1.15 [a]	8.16 ± 0.77 [ab]
8	50	300	0.3	17.30 ± 0.79 [a]	-.168 ± 0.14 [a]	.848 ± 0.04 [a]	.561 ± 0.02 [ab]	8.42 ± 2.16 [a]	7.96 ± 0.18 [ab]
9	50	300	0.3	17.70 ± 0.51 [a]	-.171 ± 0.16 [a]	.851 ± 0.07 [a]	.558 ± 0.01 [ab]	8.66 ± 2.19 [a]	7.99 ± 0.28 [ab]
10	50	600	0.2	17.39 ± 0.46 [a]	-.024 ± 0.04 [a]	.835 ± 0.03 [ab]	.539 ± 0.04 [ab]	7.81 ± 0.6 [a]	8.03 ± 0.32 [ab]
11	50	600	0.4	17.68 ± 0.60 [a]	-.191 ± 0.26 [a]	.842 ± 0.04 [a]	.551 ± 0.02 [ab]	7.85 ± 1.48 [a]	8.05 ± 1.01 [ab]
12	100	0.1	0.3	13.04 ± 0.69 [b]	-.034 ± 0.03 [a]	.788 ± 0.03 [c]	.497 ± 0.02 [c]	8.62 ± 2.03 [a]	5.96 ± 0.73 [c]
13	100	300	0.2	13.28 ± 1.2 [b]	-.024 ± 0.4 [a]	.787 ± 0.04 [c]	.500 ± 0.17 [c]	6.90 ± 0.62 [a]	5.88 ± 0.58 [c]
14	100	300	0.4	12.72 ± 0.3 [b]	-.051 ± 0.07 [a]	.794 ± 0.01 [bc]	.471 ± 0.04 [c]	8.71 ± 1.55 [a]	6.36 ± 0.49 [c]
15	100	600	0.3	12.15 ± 1.14 [b]	-.052 ± 0.06 [a]	.786 ± 0.02 [c]	.492 ± 0.01 [c]	9.22 ± 1.4 [a]	5.69 ± 0.57 [c]

*Values are Mean ± standard deviation. [a,b,c,d] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

2.3.8 Sensory Analysis

The results of the sensory analysis of the cooked ham showed that sensory attributes were not affected when NaCl was 50% replaced with Artisalt^{TM}; however, when NaCl was 100% replaced with Artisalt^{TM}, significantly (P<0.05) lower values for the sensory attributes including flavour, texture, saltiness and tenderness and higher values for off-flavour and metallic taste were observed (Table 2.5); however, due to the fact that the OSA of these samples scored above 4.5 on the 9 point scale indicated that they could not be considered as unacceptable. The control hams were formulated to contain similar ingredients and salt content as hams available in the Irish market therefore no significant differences between the control ham and ham which NaCl was 50% replaced with Artisalt^{TM} indicated that the 50/50 combination of NaCl/Artitsalt^{TM} produces ham of similar quality to commercial products.

Many studies (Keeton, 1984; Aliño *et al.*, 2009; Costa-Corredor *et al.*, 2009; Fulladosa *et al.*, 2009; Lorenzo e*t al.*, 2015) reported that partial replacement (up to 50%) with salt replacers such as KCl can adversely affect the flavour and overall acceptability of meat products by producing a bitter off-flavour and according to Crehan *et al.* (2000) overall flavour intensity and perceived saltiness are decreased by salt reduction; however, in this study when NaCl was 50% was replaced with Artisalt™ none of the sensory attributes were negatively affected nor did the panel perceive a reduction in saltiness. This may be due to the composition of the salt replacer Artisalt^{TM} which contains flavour enhancers (yeast extract, celery and onion) which can mask the bitterness associated with KCl and enhance the flavour and saltiness perception of the ham.

Table 2.5 Effects of different ham formulations on the sensory characteristics of the cooked ham*

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Appearance	Texture	Flavour	Saltiness	Tenderness	Juiciness	Off-flavour	Metallic Taste	OSA
1	0	0.1	0.3	6.12 ^{a}	6.57 ^{a}	6.36 ^{a}	5.79 ^{a}	6.57 ^{a}	6.44 ^{a}	1.55 ^{a}	1.35 ^{a}	7.01 ^{abc}
2	0	300	0.2	6.24 ^{a}	6.78 ^{a}	6.15 ^{a}	5.68 ^{a}	6.37 ^{a}	6.47 ^{a}	1.53 ^{a}	1.24 ^{a}	7.11 ^{abc}
3	0	300	0.4	6.32 ^{a}	6.50 ^{a}	6.34 ^{a}	5.85 ^{a}	6.44 ^{a}	6.34 ^{a}	1.42 ^{a}	1.55 ^{a}	7.26 ^{abc}
4	0	600	0.3	6.18 ^{a}	6.82 ^{a}	6.31 ^{a}	5.74 ^{a}	6.66 ^{a}	6.38 ^{a}	1.56 ^{a}	1.12 ^{a}	7.34 ^{ab}
5	50	0.1	0.2	6.14 ^{a}	6.50 ^{a}	6.30 ^{a}	5.75 ^{a}	6.28 ^{a}	6.30 ^{a}	1.52 ^{a}	1.38 ^{a}	6.88 ^{c}
6	50	0.1	0.4	6.20 ^{a}	6.80 ^{a}	6.15 ^{a}	5.71 ^{a}	6.44 ^{a}	6.38 ^{a}	1.58 ^{a}	1.47 ^{a}	6.92 ^{bc}
7	50	300	0.3	6.29 ^{a}	6.70 ^{a}	6.21 ^{a}	5.76 ^{a}	6.48^{a}	6.49 ^{a}	1.60 ^{a}	1.25 ^{a}	7.17 ^{abc}
8	50	300	0.3	6.19 ^{a}	6.67 ^{a}	6.24 ^{a}	5.74 ^{a}	6.50 ^{a}	6.29 ^{a}	1.50 ^{a}	1.11 ^{a}	7.07 ^{abc}
9	50	300	0.3	6.24 ^{a}	6.66 ^{a}	6.33 ^{a}	5.8 ^{a}	6.59 ^{a}	6.37 ^{a}	1.42 ^{a}	1.26 ^{a}	7.09 ^{abc}
10	50	600	0.2	6.26 ^{a}	6.78 ^{a}	6.16 ^{a}	5.72 ^{a}	6.52 ^{a}	6.30 ^{a}	1.44 ^{a}	1.89 ^{a}	7.27 ^{abc}
11	50	600	0.4	6.24 ^{a}	6.52 ^{a}	6.14 ^{a}	5.78 ^{a}	6.60 ^{a}	6.40 ^{a}	1.38 ^{a}	1.25 ^{a}	7.39 ^{a}
12	100	0.1	0.3	6.18 ^{a}	4.84 ^{bc}	5.23 ^{b}	3.30 ^{b}	4.36 ^{b}	6.42 ^{a}	3.02 ^{b}	4.12 ^{b}	4.66 ^{d}
13	100	300	0.2	6.28 ^{a}	4.78 ^{c}	5.22 ^{b}	3.31 ^{b}	4.34 ^{b}	6.36 ^{a}	3.08 ^{b}	3.05 ^{b}	4.93 ^{d}
14	100	300	0.4	6.24 ^{a}	5.40 ^{b}	5.24 ^{b}	3.31 ^{b}	4.40 ^{b}	6.38 ^{a}	3.00 ^{b}	3.15 ^{b}	4.90 ^{d}
15	100	600	0.3	6.22 ^{a}	5.41 ^{b}	5.25 ^{b}	3.42 ^{b}	4.35 ^{b}	6.28 ^{a}	3.19 ^{b}	3.54 ^{b}	4.98 ^{d}

Values are Mean ± standard deviation ^{a,b,c,d} Different superscripts in the same column indicate significant difference (P<0.05) between treatments

2.3.9 Modelling and Optimisation

The fitness of the models were evaluated using the coefficients of determination (R^2) and lack of fit test. It has been suggested that for the good fit of a model R^2 should be ≥ 80% (Joglekar *et al.*, 1987). However, the adjusted R-squared statistic is more suitable for comparing models with different numbers of independent variables. The lack of fit test is designed to determine whether the selected model is adequate to describe the observed data, of the P value is greater than or equal to 0.05 then the model appears to be adequate for the observed data at the 95% confidence level. (STATGRAPHICS® Centurion XV User Manual, Statpoint, Inc., USA)

ANOVA was carried out on ham hardness, OSA, flavour and saltiness (Table 2.6). A, B and C indicates Salt replacement, HPP and Inbac, respectively. In the Pareto charts (Figures 2.1a-1d) the length of the horizontal bars are proportional to the significance of the effect of each factor. Each figure shows that Salt replacement had the most significant effect on all response variables. The vertical line is the threshold for significant effects at the level $P < 0.05$ thus the effects are statistically significant when the respective bars exceed this vertical line.

For hardness, the Pareto chart (Figure 2.1a) shows that the linear effects of the independent factors A and B, the interactive effects of AB and AC and the quadratic effects of AA, BB and CC affected significantly ($P<0.05$) the hardness of the cooked ham. For overall acceptability, the Pareto chart (Figure 2.1b) shows that the linear effects of A and B and quadratic effects of AA affected significantly ($P<0.05$) the OSA of the cooked ham. For flavour, the Pareto chart (Figure 2.1c) shows that the linear effects of A and B, the interactive effects of AC and the quadratic effects of AA affected significantly ($P<0.05$) the flavour of the cooked ham. For saltiness, the Pareto chart (Figure 2.1d) shows that the

linear effects of A and the quadratic effects of AA affected significantly ($P<0.05$) the saltiness of the cooked ham. The following regression equations predict the value of each response variable when the independent factors are varied;

Equation 2: Hardness = 17.41 + 0.0797205*A + 0.00176317*B - 5.61525*C - 0.00103705*A^2 - 0.00001514*A*B - 0.05257*A*C - 0.00000358083*B^2 + 0.00275333*B*C + 12.7575*C^2.

Equation 3: OSA = 7.2125 + 0.02225*A + 0.0004375*B - 2.3125*C - 0.000415*A^2 - 8.33333E-7*A*B - 0.0125*A*C + 0.0*B^2 + 0.000833333*B*C + 5.0*C^2.

Equation 4: Flavour = 6.78525 - 0.00134*A + 0.00114917*B - 0.3225*C - 0.0002949*A^2 + 0.00000546667*A*B + 0.0452*A*C + 4.91667E-7*B^2 - 0.00458333*B*C - 0.175*C^2

Equation 5: Saltiness = 5.597 + 0.02482*A - 0.000448333*B + 1.045*C - 0.0004764*A^2 + 0.000003*A*B - 0.008*A*C + 1.44444E-7*B^2 + 0.000866667*B*C - 1.1*C^2

After generating the model polynomial equation to relate the dependant and independent variable, the combination was optimised for all 4 responses. The final optimal experimental parameter was calculated using the optimisation technique in the Statgraphics® software.

For hardness, OSA and flavour, the absolute values of partial regression coefficient were A > B > C within the range of the experimental design, demonstrating the greatest effects of Salt replacement on hardness followed by HPP and Inbac^{TM}, respectively. For saltiness, the absolute values of partial regression coefficient were A > B and C within the range of the experimental design, demonstrating the greatest effects of salt replacement on the overall acceptability followed by HPP and Inbac^{TM} equally.

For hardness, OSA, flavour and saltiness the adjusted R^2 of the predicted models was 99.68%, 98.85%, 95.33% and 99.64%, respectively indicating that the predicted model can reasonably represent the observed values shown in the regression equations 2-5. For OSA, the lack of fit value was 0.33 which was insignificant and therefore indicates that the selected model is adequate to describe the observed data.

The 3-Dimentional Response Surface Plots were formed based on the polynomial function and show how varying the salt replacement and HPP level s can affect the measured responses. The relationship between the dependent and independent variables can be clearly understood by these plots (Figure 2.2). For each response one 3-D response plot was produced. The yellow area of Figure 2.2a represents the optimum hardness and the corresponding factors required in order to achieve this level of hardness. The results showed that the best combination of the variables in order to maximise hardness were; Salt replacement 26%, HPP 345MPa and Inbac^{TM} 0.3%. The red area of Figure 2.2b represents the optimum OSA and the corresponding factors required in order to achieve this level of OSA. The results showed that the best combination of the variables in order to maximise OSA were; Salt replacement 53%, HPP 535MPa and Inbac^{TM} 0.3%. The yellow area of Figure 2.2c represents the optimum flavour and the corresponding factors required in order to achieve this level of flavour. The results showed that the best combination of the variables in order to maximise flavour were; Salt replacement 44%, HPP 455MPa and Inbac^{TM} 0.3%. The red area of Figure 2.2d represents the optimum saltiness and the corresponding factors required in order to achieve this level of saltiness. The results showed that the best combination of the variables in order to maximise saltiness were; Salt replacement 24.6%, HPP 600MPa and Inbac^{TM} 0.3%.

Sensory properties of food products are the most important attributes as they are most apparent to consumers (Singham *et al.*, 2015). While attributes such as hardness, flavour

or saltiness can be predicted by the models; a higher level of salt replacement and HPP was achieved when product optimisation was carried out based on OSA which subsequently produced a lower salt product with increased safety and shelf life; therefore, manufacture of the optimised cooked ham was carried out based on maximising the OSA.

Table 2.6 ANOVA of the independent factors and their interactive effects on each response variable.

	Sum of Squares	*Df*	*Mean Square*	*F-Ratio*	*P-Value*	*SL*
Hardness						
A:Salt replacement	78.4907	1	78.4907	5314.51	0.0000	*
B:HPP	0.144096	1	0.144096	9.76	0.0056	*
C:Inbac	0.00896809	1	0.00896809	0.61	0.4454	NS
AA	49.6372	1	49.6372	3360.8	0.0000	*
AB	0.412595	1	0.412595	27.94	0.0000	*
AC	0.552721	1	0.552721	37.42	0.0000	*
BB	0.766975	1	0.766975	51.93	0.0000	*
BC	0.0545821	1	0.0545821	3.70	0.0697	NS
CC	0.120187	1	0.120187	8.14	0.0102	*
Blocks	0.0	1	0.0	0.00	1.0000	NS
Total error	0.280614	19	0.0147691			
Total (correlation)	130.535	29				
R-squared = 99.78%						
R-squared (adjusted) = 99.68%						
OSA						
A:salt replacement	21.6225	1	21.6225	2594.7	0.0000	*
B:HPP	0.600625	1	0.600625	72.07	0.0011	*
C:Inbac	0.015625	1	0.015625	1.87	0.2427	NS
AA	7.94885	1	7.94885	953.86	0.0000	*
AB	0.00125	1	0.00125	0.15	0.7183	NS
AC	0.03125	1	0.03125	3.75	0.1249	NS
BB	0.0	1	0.0	0.00	1.0000	NS
BC	0.005	1	0.005	0.60	0.4818	NS
CC	0.0184615	1	0.0184615	2.22	0.2109	NS
Blocks	0.003	1	0.003	0.36	0.5808	NS
Total error	0.0333333	4	0.00833333			
Total (correlation)	30.6337	29				
R-squared = 99.2%						
R-squared (adjusted) = 98.84%						
Flavour						
A:salt replacement	9.77188	1	9.77188	257.68	0.0000	*
B:HPP	0.168921	1	0.168921	4.45	0.0483	*
C:Inbac	0.033489	1	0.033489	0.88	0.3591	NS
AA	4.01382	1	4.01382	105.84	0.0000	*
AB	0.053792	1	0.053792	1.42	0.2483	NS
AC	0.408608	1	0.408608	10.78	0.0039	*
BB	0.0144595	1	0.0144595	0.38	0.5442	NS
BC	0.15125	1	0.15125	3.99	0.0603	NS
CC	0.0000226154	1	0.0000226154	0.00	0.9808	NS
Blocks	0.0175692	1	0.0175692	0.46	0.5043	NS
Total error	0.720515	19	0.0379218			
Total (correlation)	15.4326	29				
R-squared = 95.33%						
R-squared (adjusted) = 93.23%						

Saltiness						
A:salt replacement	23.6585	1	23.6585	5280.1	0.0000	*
B:HPP	0.003364	1	0.003364	0.75	0.3970	NS
C:Inbac	0.009604	1	0.009604	2.14	0.1595	NS
AA	10.4749	1	10.4749	2337.8	0.0000	*
AB	0.0162	1	0.0162	3.62	0.0725	NS
AC	0.0128	1	0.0128	2.86	0.1073	NS
BB	0.001248	1	0.001248	0.28	0.6038	NS
BC	0.005408	1	0.005408	1.21	0.2857	NS
CC	0.000893538	1	0.000893538	0.20	0.6602	NS
Blocks	0.00188813	1	0.00188813	0.42	0.5240	NS
Total error	0.0851339	19	0.00448073			
Total (correlation)	34.3891	29				
R-squared = 99.75%						
R-squared (adjusted) = 99.64%						

SL = Significance level, NS = Not Significant, * = $P<0.05$

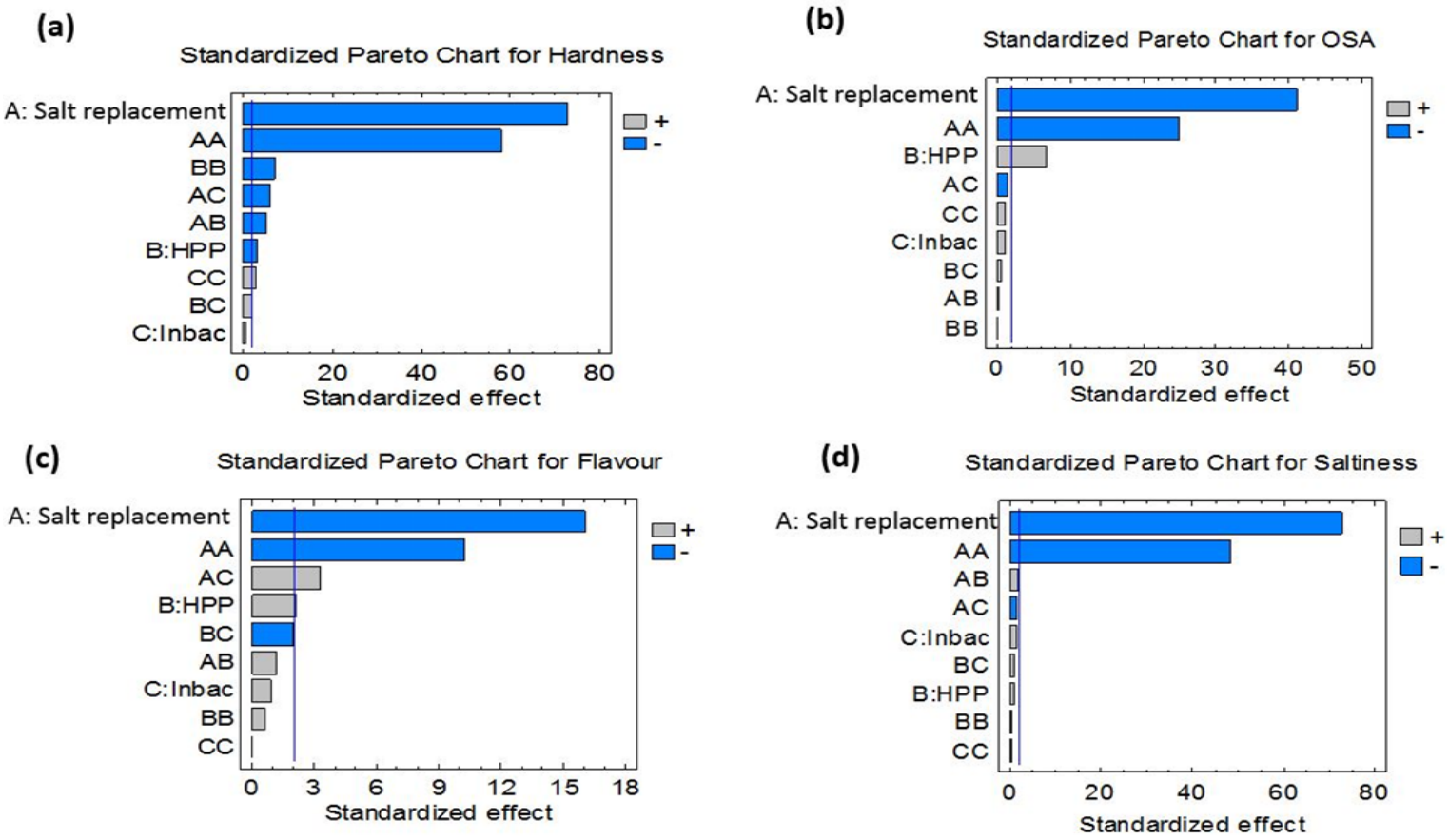

Figure 2.1 – Pareto charts of the significance of the effects of the independent factors and their interactions on the (a) hardness, (b) OSA, (c) flavour and (d) saltiness of cooked ham.

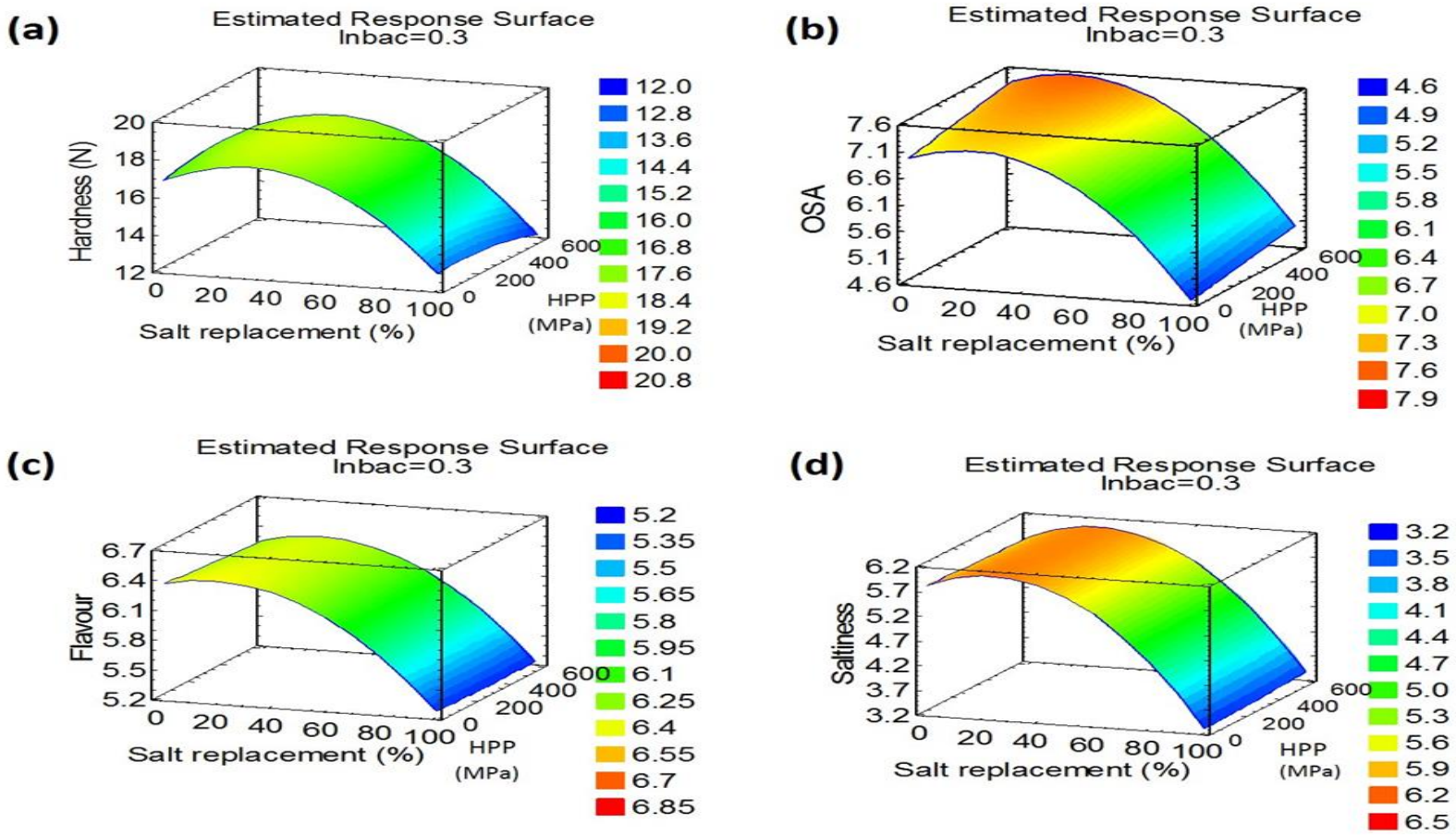

Figure 2.2 – Effect of salt replacement and high pressure processing on the (a) hardness, (b) OSA, (c) flavour and (d) saltiness of cooked ham.

2.3.10 Validation experiments

The robustness of the model used for the product optimisation was validated carrying out three independent confirmatory trials to ascertain difference between predicted and experimental values. Optimised hams (53% salt replacement, 535MPa and 0.3% Inbac^{TM}) were produced and analysed on three occasions by a 25 member semi-trained panel. The predicted and average values for all sensory attributes (flavour, saltiness and OSA) and hardness were similar (Table 2.7). The results of the validation indicated that the RSM approach was effective for modelling and optimizing the operational conditions for the manufacture of low salt cooked ham.

Table 2.7 Predicted and observed responses of the ham manufactured using the optimised OSA parameters (53% salt replacement. 535 MPa and 0.3% Inbac^{TM}).*

Response	Y (predicted)	Y (observed)
OSA	7.1	7.21 ± 0.55
Hardness (N)	17.5	17.07 ± 0.87
Saltiness	5.6	5.29 ± 1.02
Flavour	6.7	6.38 ± 0.77

*Values are Mean ± standard deviation

2.4 Conclusion

In general, quality parameters such as pH, cook loss, sliceability, expressible moisture, colour, texture or sensory attributes were not significantly affected when NaCl was partially replaced (50%) with Artisalt^{TM} compared to control samples; however, when added NaCl was fully replaced (100%) with Artisalt^{TM} all quality parameters were significantly affected and the obtained cooked ham were significantly less acceptable to the sensory panel.

Response Surface Methodology can be successfully used to develop low-salt cooked ham products and the results indicated that the main independent factor that significantly influenced the assessed response variables of the cooked ham was salt replacement. The optimisation process carried out maximising the OSA indicated that the best formulation to obtain low-salt cooked ham was: Salt replacer Artisalt^{TM} (53%), HPP (535MPa) and concentration of Inbac™ (0.3%).

The validation process indicated that the observed responses were found to be quite similar to the predicted values for the OSA optimised low salt cooked meat products indicating that the model obtained can accurately predict changes on the OSA of the cooked ham. Therefore, a 53% added salt reduction was achieved reducing the total salt content in the cooked hams from 2.6% to 1.4% and the developed product can be classified as 'salt reduced' cooked ham. This significant salt reduction in cooked ham was achieved through the use of salt replacer Artisalt^{TM} which contains flavour enhancers along with HPP and Inbac^{TM} without compromising the physicochemical or sensory quality of the cooked hams. The addition of the hurdles HPP and antimicrobial Inbac^{TM} are expected to compensate the reduction in safety and shelf life as a result of the extensive salt reduction and this study is underway.

CHAPTER 3

The application of response surface methodology for development of sensory-acceptable, low-salt, shelf-stable frankfurters using high pressure processing and a mix of organic acids.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

Response surface methodology (RSM) was used to develop sensory-acceptable, low-salt, shelf-stable frankfurters. A Box-behnken experimental design assessed the effects of three independent factors; salt replacer (Artisalt^{TM}) (0-100%), high pressure processing (HPP) (0.1-600 MPa) and a mix of organic acids (Inbac^{TM}) (0.2-0.4%). Measured responses included: hardness, flavour, saltiness and overall sensory acceptability (OSA) of the frankfurters. The primary factor affecting (P<0.05) all responses was the salt replacer. The optimum parameters to maximise salt reduction and produce frankfurters with OSA similar to commercial-type products were; Artisalt^{TM} (48%), HPP (580 MPa), Inbac^{TM} (0.3%) which contained a total salt content of 1.3%, compared to control samples which contained 2.5% total salt. The hurdle approach used in this study extended product shelf-life by 51% compared to control samples. Overall, a combination of salt replacer, HPP and organic acids showed great potential for the development of low-salt frankfurters with enhanced shelf-life, without compromising on sensory attributes.

Industrial Relevance

Processed meat manufacturers are constantly looking for new ways to reduce salt levels without compromising food safety, shelf-life or consumer acceptability. In this study we used a novel approach which showed great potential for reducing salt in frankfurters. Frankfurters manufactured in this study were similar or better than frankfurter controls after 48% of Sodium Chloride (NaCl) was replaced with Artisalt™ and the hurdles of HPP and an organic acid mix were applied. The optimised frankfurter formulation, in addition to possessing lower salt levels, was accepted sensorially and had a shelf-life that was extended

by 51%. This finding is not just of commercial and processing interest, but of public health significance also.

3.1 Introduction

The global importance of the nutritional value of food has increased significantly in past years. There is an ever increasing demand in the meat industry for products which are lower in salt, preservatives, fat and calories, whilst maintaining good-quality products in regards to physicochemical, nutritional and sensory characteristics (Weiss *et al.*, 2010). Cardiovascular disease (CVD) which is largely associated with high salt consumption accounts for 33% of deaths in Ireland (IHF, 2016) and 31% of deaths worldwide (World Health Organisation (WHO), 2016). It has also been suggested that there is increasing evidence that salt intake is related to obesity, associated with renal stones and osteoporosis and may play a role in the development of stomach cancer (He and MacGregor, 2009; Wang *et al*., 2009). The WHO recommends a salt intake of less than 5g/day for adults; however, the recommended dietary intake is greatly exceeded and estimated to be as high as 9-12g NaCl/day (WHO, 2016). The work and leisure lifestyle patterns of the Western consuming culture, with high disposable incomes, have created demand for pre-prepared foods (Purdy and Armstrong, 2007). Over 80% of salt intake in the UK, Ireland and the USA comes from processed food, meaning many consumers do not realise they are consuming such high quantities (Gray, 2013). Therefore, processed meat manufacturers require new solutions in the reformulation of low-sodium processed meat products as a means of reducing dietary sodium in consumer foods.

Comminuted cooked meat products (gel/emulsion system) are a commercially important group of processed meat products, of which frankfurters are among one of the more popular

varieties (Delgado-Pando *et al*., 2010). Frankfurters are a type of highly seasoned sausage which can contain up to 30% fat with an industrial average of about 20% (Keeton, 1994) and a salt content of 2% or higher. In processed meat products, salt provides essential functions as it affects flavour, preservation, safety, texture characteristics and consumer acceptability (Inguglia, 2017; Xiong, 1997). The salt-solubilised myofibrillar proteins derived from the lean meat component used in frankfurter processing forms a sticky exudate on the surface of the highly comminuted meat pieces which binds the meat pieces together through the formation of a gel-based matrix after cooking. This matrix of heat-coagulated protein entraps free water. In finely chopped or emulsified products such as frankfurters, bologna, etc. the solubilised protein in the continuous phase forms a protein film around fat globules, thereby retaining the fat during cooking (Desmond, 2006) which results in a product with acceptable quality characteristics. Therefore, salt reduction in these products can have negative effects in terms of quality and safety if the reduction of salt is not compensated for in another way. Considering the significant technological role of salt in meat processing, it was suggested that a global approach is necessary to reduce salt content in meat products (Albarracin *et al.*, 2011).

Several approaches to reduce the sodium content in processed meat products have been reported: (i) dietary salt reduction based on sensory evaluation and acceptance of products, either by a complete or partial replacement of NaCl (Fellendorf *et al*., 2016; Liem *et al.*, 2011; Tobin *et al.*, 2012 and 2013), (ii) replacement with a low-sodium mixture (Paulsen *et al.*, 2014), (iii) use of flavour enhancers such as monosodium glutamate or yeast extract (Santos *et al*., 2014), (iv) changes in the physical form of salt (Rama *et al*., 2013), (v) improvement of salt diffusion via HPP or ultrasound technology (McDonnell *et al*., 2014; Ojha *et al*., 2016).

HPP is an alternative method for food preservation which subjects liquid and solid foods, with or without packaging, to pressures between 100 and 800 MPa (Bermúdez-Aguirre and Barbosa-Cánovas, 2011) which can also be used for the development of meat products with lower salt contents (Watson, 2012). It was reported that HPP can maintain or improve protein functionality where it is desired to reduce the sodium content of processed meats (Mújica *et al*., 2011; Cheftel and Culioli 1997) and improve safety through the application of hurdle technology (Rodriguez-Calleja *et al*., 2012) which advocates the intelligent use of combinations of different preservation factors or techniques ('hurdles') in order to achieve multi-target, mild, but reliable preservation effects (Leistner and Gorris, 1995) and this concept fits well with the present consumer trend for minimally processed foods and, as such, has gained much in popularity regarding practical application and research (Mukhopadhyay, 2014).

Crehan *et al.* (2000) found that HPP (150MPa) can be used to improve the functionality of frankfurters formulated with lower salt levels (1.5%). This conclusion was based on the fact that no significant effect on colour was observed and sensory attributes such as juiciness and also textural attributes were found to have improved. A recent study carried out by Pietrasik *et al.* (2017) found that substitution of 50% NaCl with modified KCl (modified to create a single crystal that significantly reduced the bitter/metallic note associated with normal KCl) had a negative effect on textural and sensory characteristics of conventionally cured wieners; however; consumer acceptability results indicated that HPP did not impact sensory acceptability.

A study carried out by Diez *et al*. (2008) examined independently the application of organic acids (L-potassium lactate, L-potassium lactate/sodium lactate or L-potassium lactate/sodium acetate) and HPP (300, 500 or 600 MPa for 10 mins) to improve the shelf-life of blood sausage. An increased shelf-life of blood sausages by 15 days was achieved

using L-potassium/sodium lactate or HPP at 600 MPa for 10 mins. The authors suggested that the synergistic effects of HPP and Inbac™ could improve the effectiveness of the treatments; however, they did not investigate this further. In our previous study (Chapter 2) a low-salt reformed meat product (cooked ham) was developed using RSM to optimise salt replacer Artisalt™, HPP and Inbac™ based on maximizing overall sensory acceptability (OSA).

To the best of our knowledge, no previous studies have been carried out on the use of RSM to optimise salt replacers, HPP and organic acids for the development of sensory accepted low-salt comminuted meat products (frankfurters). Furthermore, a combination of HPP and organic acids as hurdles has not previously been used as a methodology to enhance the safety and shelf life of low-salt processed meat products. Therefore, the study objective was to use RSM to develop sensory-acceptable, low-salt frankfurters with enhanced safety and shelf-life using salt replacers (Artisalt™) and hurdles including HPP and a mix of antimicrobial organic acids (Inbac™).

3.2 Materials and Methods

3.2.1 Materials

Pork oyster meat (90-95% VL) and pork fat were obtained from Ballyburden meats, Ballincollig, Cork. NaCl, starch, farina (milled wheat), paprika, Sodium caseinate, tomato powder, Sodium tripolyphosphate hydrated food grade (Carfosel 990, Prayon, Belgium), carmine, Sodium nitrite and Sodium ascorbate were sourced from All in All ingredients (All in All ingredients, Ltd, Ireland). Frankfurter spice and artificial cellulose casings

(26mm) were obtained from Fispak (Fispak Ltd, Ireland) and Viscofan (Viscofan, Spain), respectively.

A commercially available salt replacer used in processed meat products Artisalt™ (a mix of Potassium chloride 41%, Ammonium chloride 40% and flavour enhancers - yeast extract, onion and celery 19%) was obtained from Chemital (Chemital Ltd, Barcelona, Spain). According to the manufacturer specification sheet Artisalt™ can replace all (100%) or part (50%) of common salt in meat products without giving any off-taste and allowing meat proteins solubilisation which is an essential factor in producing products with good texture and palatability. A commercial antimicrobial Inbac™ (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%,) was obtained from Chemital Ltd and used as recommended by the manufacturer (2-4g/kg of product).

3.2.2 Methods

3.2.2.1 Experimental Design

A three-factor experimental design (Box-Behnken) was used to optimise salt reduction and consisted of the manufacture of 15 different formulations (Table 3.1). The centre point of the experimental design was repeated 3 times. The independent factors were Salt replacer Artisalt™ (0-100%), HPP (0.1-600MPa) and organic acid Inbac™ (0.2-0.4%) and the measured response variables included Hardness (N) and sensory characteristics flavour, saltiness and OSA. The full polynomial model involving the main effects (linear terms), interaction terms (cross products) and quadratic or squared terms is shown in Equation 1.

Equation 1:

$$Y=b_0 + b_1A_1 + b_2B + b_3C_3 + b_4AB + b_5AC + b_6BC + b_7A^2 + b_8B^2 + b_9C^2$$

Where: Y represents the dependent or response variable (overall sensory acceptability (OSA), Hardness, flavour and saltiness), and A, B and C represents the independent variables (A) = *S*alt replacement (%), B= HPP (MPa) and C = Inbac (%) and b_0–b_9 are the regression coefficients to be determined. The regression coefficient b_0 is the constant or intercept term; b_1–b_3 are the linear coefficient terms; b_4–b_6 are the interaction coefficient terms; and b_7–b_9 are the quadratic coefficient terms, respectively. The effect of variables at the linear, quadratic, and interactive levels on individual responses was described using a significance level of confidence set at 5%.

3.2.2.2 Characterisation of salt replacer and antimicrobial

Ammonium Chloride was determined using the methods outlined in 23rd Joint FAO/WHO Expert Committee on Food Additives (1979). KCl, Nitrites and Nitrates concentration of the salt replacer Artisalt™ and concentration of sodium acetate and malic acid of the antimicrobial Inbac™ was determined by a commercial external analytical testing facility (ALS laboratories, Little Island, Cork). For the determination of KCl, Artisalt™ was homogenized and mineralized by acids and hydrogen peroxide prior to analysis by atomic emission spectrometry with inductively coupled plasma and stoichiometric calculations of compounds concentration carried out from measured values. Nitrites and Nitrates were determined by flow-injection analysis and gas-phase molecular absorption spectrometry. Results were expressed as a percentage of the salt replacer Artisalt™. Organic acids (Sodium acetate and Malic acid) were determined by the high pressure liquid

chromatography with UV detection and results were expressed as percentage of the antimicrobial Inbac™.

3.2.2.3 Frankfurters manufacture

Frankfurters were manufactured according to the formulation shown in Table 3.2. Each batch included varying levels of NaCl replacer Artisalt™ (0% replacement, 50% replacement or 100% replacement) and Inbac™ (0.2 – 0.4%) (Table 3.1). For each treatment, four kilograms of batter was prepared on two separate occasions.

Pork meat and pork fat were minced separately through a 3-mm plate using a Talsa mincer (Talsabell, Valencia, Spain).The minced pork meat was placed in a bowl chopper (Seydelmann, Germany) and chopped at low speed for 3 minutes and then the curing ingredients, seasonings and half of the ice were added. The mixture was then chopped for 2 minutes at high speed and the minced pork fat and remaining ice was added and then chopped for a further 2 minutes. The batter was then stuffed into a 26 mm diameter cellulose casings using a Mainca vacuum filler (Mainca, Barcelona, Spain). The frankfurters were hand-linked (~12cm in length) and heat-treated at 100ºC in an electric steam-convection oven (Zanussi Professional, Italy) until an internal temperature of 74°C was achieved. Final internal end-point temperatures were re-checked using a hand-held food thermometer (Testo, Germany). The frankfurters were cooled down by immersion in icy cold water (1-2ºC) for 5 minutes and then stored at 4°C overnight. Before packaging, the casing of the frankfurters were aseptically removed and 7 frankfurters were placed into a combivac vacuum pouch (20 polyamide/70 polyethylene bags (Alcom, Campogalliano, Italy), vacuum packed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, German) and then stored at 4°C.

Table 3.1 Experimental design of uncoded and coded parameters.

Formulation	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*	*12*	*13*	*14*	*15*
Independent Factors															
Salt replacement (%)	50	50	0	100	0	50	0	50	50	50	50	100	0	100	100
	(0)	(0)	(-1)	(+1)	(-1)	(0)	(-1)	(0)	(0)	(0)	(0)	(+1)	(-1)	(+1)	(+1)
High Pressure (MPa)	300	600	300	600	600	0.1	300	0.1	300	300	600	300	0.1	300	0.1
	(0)	(+1)	(0)	(+1)	(+1)	(-1)	(0)	(-1)	(0)	(0)	(+1)	(0)	(-1)	(0)	(-1)
Organic Acids (%)	0.3	0.4	0.2	0.3	0.3	0.2	0.4	0.4	0.3	0.3	0.2	0.2	0.3	0.4	0.3
	(0)	(+1)	(-1)	(0)	(0)	(-1)	(+1)	(+1)	(0)	(0)	(-1)	(-1)	(0)	(+1)	(0)

Table 3.2 Standard frankfurter formulation

Ingredient	%
Pork oyster meat	65
Pork fat	19
Water/Ice	10.2
NaCl	2
Starch	0.92
Farina	0.92
Frankfurter Spice	0.5
Paprika	0.5
Sodium Caesinate	0.35
Tomato powder	0.25
Phosphate	0.25
Sodium Ascorbate	0.05
Sodium nitrite	0.0075
Carmine	0.05

3.2.2.4 High Pressure Processing

For samples that were HP treated, vacuum-packed frankfurters were placed in a second vacuum pouch (20 polyamide/70 polyethylene bags; Alcom, Campogalliano, Italy) and vacuum-sealed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, Germany). Packaged samples were HP treated in a Stansted Fluid Power Iso-Lab 900 Power High Pressure Food Processor (Stansted Fluid Power Ltd., Stansted, UK), using an ethanol-castor oil (90:10) as the pressure transmitting medium. The speed of pressurisation was 300 MPa per minute, the speed of depressurisation was 600 MPa per minute and holding time at the required pressure level was 5 minutes. Pressurisation was carried out at room temperature 20°C which was monitored by the temperature sensor contained in the HP units pressure transmitting medium. Adiabatic heating resulted in a ~3°C increase per 100 MPa.

3.2.2.5 Compositional Analysis and pH

Proximate composition was carried out on the raw batter and the cooked frankfurter. Fat and moisture were determined using the SMART Trac and CEM Analysis System (CEM Corporation, Matthews, NC 28105, USA) (Bostian *et al.*, 1985). Protein content was determined according to AOAC Procedures (1997) (method 981.10). The ash content of the frankfurters was determined by overnight incineration in a furnace (Nabertherm, Model L9/C6, Nabertherm, Germany) at 550 °C. Each value represents the average of 8 measurements (two independent batches x two frankfurters per batch x two readings per sample).

The pH was measured using a digital pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the frankfurter. Each value represents

the average of 8 measurements (two independent batches x two frankfurters per batch x two readings per sample).

3.2.2.6 Salt content and Ionic strength

Salt content was determined using the DiCromat II Salt Analyser (The Noramar Co, US). Before use, the instrument was calibrated using a 2% NaCl (Sigma, Ireland) solution. For the determination of salt content in the frankfurter samples, 25g of sample was weighed to which 225 mls of distilled water was added and then blended using an Ultraturrax homogeniser (IKA-Werke GmbH and Co, Germany) for 1 min. The homogenate was then filtered through a Watmann no.1 filter paper and the filtrate received in a 250 ml beaker. The dip-in probe of the DiCromat II Salt Analyser was immersed in the filtrate and the percentage of salt in the sample was read in the instrument display. Each value represents the average of 8 measurements (two independent batches x two samples per batch x two readings per sample).

The ionic strength was calculated using the Debye and Huckel formula as described by Stanley (2017) using the following equation;

$$IS = \frac{1}{2} n \sum I\,(C_i\,Z_i)^2$$

Where IS = ionic strength, n = number of ions in solution, I = the specific ion in solution, C_i= concentration to the species (M), Z_i = the valence or oxidation number of the species. Results were expressed as (M).

3.2.2.7 Emulsion stability

The emulsion stability was determined on the raw frankfurter batter prior to HPP and was measured by the method described by Hughes *et al*. (1997). Briefly, 25g of raw batter was placed in a centrifuge tube and centrifuged for 1 min at 704 x g in a Beckman centrifuge (model J2-21) (Beckman Coulter, USA) to remove unbound water prior to cooking. The samples contained in the centrifuge tube were then heated in a water bath for 30min at 70°C and centrifuged for 3 min at 704 x g. The pelleted samples were removed and weighed and the supernatants poured into pre-weighed crucibles and dried overnight at 100°C and re-weighed. Each value represents the average of 8 measurements (two independent batches x two samples per batch x two readings per sample).

The volumes of total expressible fluid (TEF) and the percentage fat were calculated as follows:

TEF = Weight of centrifuge tube and sample –Weight of centrifuge tube and pellet

%TEF = TEF / Sample weight $*$ 100

% Fat = (Weight of crucible + dried supernatant – Weight of empty crucible / TEF) $*$ 100.

3.2.2.8 Cook Loss

The cooking loss of the frankfurters were determined prior to HP treatment. The initial weight of a string of five raw frankfurters was recorded, after cooking the frankfurters were patted dry with a paper towel to remove excess water and re-weighed. Cook loss was then expressed as a percentage of the original raw weight. Calculation for cook loss was as follows:

% cook loss = (cooked weight – initial raw weight) / (initial raw weight) * 100

Each value represents the average of 8 measurements (two independent batches x two strings of five frankfurters per batch x two readings per sample).

3.2.2.9 Colour

The Colour of the cross section of the frankfurter was measured using a Minolta Chromameter CR-300 (CR-300, Minolta Camera Co., Osaka, Japan). Before use, the Chromameter was calibrated using a white tile (Y = 86, X = 0.3166, y = 0.3237). CIE L*, a* and b* values (Lightness, redness and yellowness, respectively) are reported. Each value represents the average of 8 measurements (two independent batches x two samples x two measurements).

3.2.2.10 Texture profile analysis (TPA)

Cylindrical sections of the frankfurter (2.6 cm diameter x 5 cm length) were analysed at room temperature (20°C) using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK). The samples were subjected to a two-cycle compression using a 25 kg load cell. The samples were compressed to 40% of their original height twice with a cylindrical probe (SMSP/35 Compression plate) 35 mm diameter at a cross-head speed of 1.5 mm/s. Texture profile parameters measured were hardness (N), adhesiveness (N), springiness (mm), chewiness (N-mm) and cohesiveness (dimensionless). Each value represents the average of 8 measurements (two independent batches x four samples)

3.2.2.11 Sensory evaluation

A 25 internally semi-trained taste panel of the School of Food and Nutritional Sciences, University College Cork was used to evaluate the frankfurters over two sessions using a 9-point hedonic scale. The panellists were recruited from staff and postgraduate students at the School of Food and Nutritional Sciences, University College Cork and chosen based on their experience in the sensory analysis of processed meat products and on their availability. The panellists have partaken in sensory analysis of processed meat products on numerous occasions and are familiar with the sensory terminology. Each frankfurter sample was labelled with a three digit random number and re-heated in a bain marie at 65ºC before being served on labelled polystyrene plates. The tested attributes included; Appearance (1= extremely dislike, 9= extremely like), Texture (1= extremely dislike, 9= extremely like), Flavour (1= extremely dislike, 9= extremely like), Juiciness (1= extremely dry, 9= extremely juicy) Tenderness (1= extremely tender, 9= extremely tough,), Saltiness (1= not salty, 9= extremely salty), Off-flavour intensity (1= imperceptible, 9= extremely pronounced), Overall acceptability (1= extremely dislike, 9= extremely like).

3.2.2.12 Microbiological analysis

Microbiological analyses were carried out after process optimisation. In order to obtain a representative sample, 10 g of frankfurters were weighted aseptically into a stomacher bag in a vertical laminar-flow cabinet and a primary 10-fold dilution was performed by addition (90 ml) of sterile maximum recovery diluent (Oxoid, Basingstoke, U.K.), stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min and homogenates were 10-fold serially diluted using maximum recovery diluent solution. For the enumeration of total viable counts (TVC) 1 ml of each appropriate dilution was inoculated on duplicated plates

in the centre of compact dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan) following incubation at 37°C for 48 hours. 24 hr. Results were expressed as log_{10} colony-forming units (CFU) g^{-1} frankfurters. Each value represents the average of 8 measurements (two independent batches x two samples x two readings).

3.2.2.13 Statistical analysis

The software STATGRAPHICS® centurion XV (Statpoint, Inc., USA) was used for the experimental design which consisted of 15 treatment combinations (Table 3.1). The DOE was randomised by the software and the experimental work carried out on random order as presented by the software However, for the presentation of the results the formulations have been arranged in order to allow for better understanding.

For the process optimisation, RSM was used. Both the independent variables and responses were fitted to the quadratic model by performing the analysis of variance (ANOVA). The experimental results were analysed to determine the lack of fit and the significance of the quadratic model and the effect of interaction between the independent variables and responses. The statistical significance of the terms in the regression equations was examined by ANOVA for each response and the significance test level was set at 5% ($P < 0.05$).

All physicochemical results (colour, texture, cook loss, pH, emulsion stability, salt content) and sensory data were tested using one way ANOVA and significance assessed using Tukey's test at 5% significance level using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA). Two independent batches of frankfurters per treatment were manufactured and all analysis carried out at least in duplicate.

3.3 Results and Discussion

3.3.1 Composition of salt replacer and antimicrobial

The results of the quantitative chemical analysis of Artisalt™ indicated it contains 41% Potassium chloride, 40% Ammonium chloride, and 19% flavour enhancers (yeast extract, onion and celery (calculated by difference)). The contents of nitrates and nitrites in Artisalt™ were 5.8 and 2.3 ppm, respectively. This may be due to the fact that Artisalt™ contains celery and celery is a natural source of nitrates and nitrites (Sebranek *et al*., 2012).

The results of the quantitative chemical analysis of Inbac™ indicated it contains 43% Sodium acetate, 7% Malic acid, ~50% (emulsifier-mono and diglycerides of fatty acids, and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide (calculated by difference)). Small quantities of Inbac™ (0.2-0.4%) were used in the formulation therefore its inclusion in such small amounts would not affect the sodium content significantly nor contribute to the ionic strength.

Ionic strength of 2% NaCl, 2% Artisalt™ and 1% NaCl / 1% Artisalt™, concentrations used in the ham formulation, was calculated using the Debye and Huckel formula and the results indicated that the control (2% NaCl) formulation had an ionic strength of 0.34M, the 50% replacement formulation (1% NaCl and 1% Artisalt™) had an ionic strength of 0.31M and the 100% replacement formulation (2% Artisalt™) had an ionic strength of 0.26M. The results indicates that control samples that contained 2% NaCl and samples that were 50% NaCl replaced with Artisalt™ were quite similar which would result in similar protein solubility and stability of the emulsion which in turn would increase the water holding capacity (WHC) and reduce cook loss. These results are in agreement with O' Flynn *et al*. (2014) who reported that lower cook losses were observed in sausage samples with higher salt concentration due to increased ionic strength.

3.3.2 Compositional analysis

Compositional analysis was carried out on the raw batter and also on the cooked frankfurters (Table 3.3 and 3.4). There were no significant compositional differences between treatments in the raw batter. After cooking, in general the fat and moisture were significantly lower ($P<0.05$) while protein was significantly ($P<0.05$) higher which was due to the cook loss. It has been reported that aggregation of the meat protein with increased temperature on cooking causes the meat protein matrix to shrink (Trout and Schmidt, 1986) which reduces the amount of water that the batter can bind, thereby decreasing the moisture content of cooked samples. Sheard *et al*. (1999) and Tornberg (2005) reported that during cooking water is lost not only by evaporation from the surface or exudation; and also as a result of heat protein denaturation which reduces the WHC of meat proteins.

In cooked frankfurters, fat, ash or protein content was not significantly affected by the level of salt replacement, however; when 100% of NaCl was replaced with Artisalt™ the moisture content frankfurters was significantly lower ($P<0.05$) compared to control or frankfurters that 50% of NaCl was replaced with Artisalt™ and this correlates to a significantly ($P<0.05$) higher cook loss for these products due to reduced protein solubility, emulsion stability and WHC due to a lower ionic strength.

Our results are in agreement with the findings of O' Flynn *et al.* (2014) who reported that the fat content in sausages was not affected by salt content or HPP and also found that lower moisture losses due to cooking were observed in samples with higher salt concentration, indicating improved batter stability due to increased ionic strength. Tobin *et al.* (2012) also reported that fat and moisture levels in frankfurters decreased after cooking which in turn increased the percentage of protein in the samples. The proximate composition results indicated that there were no significant differences when 50% of NaCl was replaced with

Artisalt™ in the manufacture of frankfurters compared to control samples suggesting that the combination of NaCl and Artisalt™ produces frankfurters of a similar quality to that of control samples but with significantly ($P<0.05$) less salt content.

Several studies have reported that HPP primarily affects the physicochemical properties of raw/uncooked meat products; however, HPP produces minimal changes in cooked meat products (Considine *et al*., 2008; Neto *et al*., 2015; Bansal *et al*., 2015). Therefore, the physicochemical differences observed in this this study may have been mainly due to the level of NaCl replacement and subsequent total salt content in the frankfurters.

Table 3.3 Effects of different frankfurter formulations on the proximate composition of the raw frankfurter batter*

Formulation	Salt replacement	HPP	Inbac	Fat	Moisture	Protein	Ash
	(%)	(MPa)	(%)	%	%	%	%
1	0	0.1	0.3	18.29 ± 0.24 [a]	60.18 ± 0.60 [a]	16.33 ± 0.28 [a]	2.59 ± 0.34 [a]
2	0	300	0.2	17.97 ± 0.35 [a]	61.12 ± 0.53 [a]	16.11 ± 0.25 [a]	2.55 ± 0.26 [a]
3	0	300	0.4	18.31 ± 0.45 [a]	60.35 ± 0.99 [a]	16.16 ± 0.30 [a]	2.48 ± 0.12 [a]
4	0	600	0.3	18.08 ± 0.29 [a]	61.14 ± 0.65 [a]	16.33 ± 0.37 [a]	2.51 ± 0.18 [a]
5	50	0.1	0.2	18.18 ± 0.35 [a]	60.49 ± 0.57 [a]	16.52 ± 0.40 [a]	2.54 ± 0.07 [a]
6	50	0.1	0.4	18.45 ± 0.36 [a]	60.10 ± 0.41 [a]	16.46 ± 0.32 [a]	2.39 ± 0.15 [a]
7	50	300	0.3	18.25 ± 0.31 [a]	60.72 ± 0.76 [a]	16.27 ± 0.41 [a]	2.34 ± 0.15 [a]
8	50	300	0.3	18.33 ± 0.40 [a]	60.62 ± 0.50 [a]	16.22 ± 0.26 [a]	2.41 ± 0.14 [a]
9	50	300	0.3	18.29 ± 0.30 [a]	60.62 ± 0.49 [a]	16.31 ± 0.31 [a]	2.50 ± 0.25 [a]
10	50	600	0.2	18.37 ± 0.38 [a]	60.95 ± 0.28 [a]	16.41 ± 0.32 [a]	2.52 ± 0.23 [a]
11	50	600	0.4	18.41 ± 0.45 [a]	60.46 ± 0.69 [a]	16.38 ± 0.35 [a]	2.48 ± 0.19 [a]
12	100	0.1	0.3	18.54 ± 0.86 [a]	61.42 ± 0.62 [a]	16.37 ± 0.31 [a]	2.68 ± 0.21 [a]
13	100	300	0.2	18.55 ± 0.32 [a]	60.85 ± 0.86 [a]	16.52 ± 0.32 [a]	2.53 ± 0.27 [a]
14	100	300	0.4	18.36 ± 0.41 [a]	60.75 ± 0.32 [a]	16.76 ± 0.28 [a]	2.66 ± 0.19 [a]
15	100	600	0.3	18.51 ± 0.42 [a]	60.91 ± 0.70 [a]	16.57 ± 0.24 [a]	2.68 ± 0.37 [a]

*Values are Mean ± standard deviation, [a] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments. Analysis was carried out before HPP

Table 3.4 Effects of different frankfurter formulations on the proximate composition of the cooked frankfurters*

Formulation	Salt replacement	HPP	Inbac	Fat	Moisture	Protein	Ash
	(%)	(MPa)	(%)	%	%	%	%
1	0	0.1	0.3	17.68 ± 0.41 [a]	58.99 ± 0.52 [a]	17.68 ± 0.41 [a]	2.62 ± 0.15 [a]
2	0	300	0.2	17.57 ± 0.27 [a]	58.86 ± 0.18 [a]	17.55 ± 0.23 [a]	2.58 ± 0.25 [a]
3	0	300	0.4	17.49 ± 0.32 [a]	58.79 ± 0.40 [a]	17.65 ± 0.31 [a]	2.48 ± 0.19 [a]
4	0	600	0.3	17.83 ± 0.36 [a]	58.78 ± 0.38 [a]	17.58 ± 0.29 [a]	2.55 ± 0.24 [a]
5	50	0.1	0.2	17.51 ± 0.31 [a]	58.94 ± 0.44 [a]	17.77 ± 0.27 [a]	2.56 ± 0.20 [a]
6	50	0.1	0.4	17.56 ± 0.30 [a]	58.45 ± 0.23 [a]	17.67 ± 0.35 [a]	2.54 ± 0.18 [a]
7	50	300	0.3	17.7 ± 0.14 [a]	58.79 ± 0.27 [a]	17.28 ± 0.31 [a]	2.7 ± 0.22 [a]
8	50	300	0.3	17.84 ± 0.31 [a]	58.99 ± 0.43 [a]	17.49 ± 0.31 [a]	2.56 ± 0.30 [a]
9	50	300	0.3	17.80 ± 0.51 [a]	58.94 ± 0.88 [a]	17.55 ± 0.30 [a]	2.58 ± 0.32 [a]
10	50	600	0.2	17.47 ± 0.36 [a]	58.45 ± 0.47 [a]	17.53 ± 0.25 [a]	2.61 ± 0.21 [a]
11	50	600	0.4	17.57 ± 0.49 [a]	59.18 ± 0.60 [a]	17.66 ± 0.27 [a]	2.58 ± 0.14 [a]
12	100	0.1	0.3	17.68 ± 0.46 [a]	56.16 ± 0.41 [b]	17.49 ± 0.18 [a]	2.63 ± 0.24 [a]
13	100	300	0.2	17.60 ± 0.46 [a]	56.22 ± 0.63 [b]	17.65 ± 0.23 [a]	2.68 ± 0.17 [a]
14	100	300	0.4	17.44 ± 0.43 [a]	56.17 ± 0.42 [b]	17.70 ± 0.19 [a]	2.55 ± 0.16 [a]
15	100	600	0.3	17.45 ± 0.28 [a]	56.45 ± 0.60 [b]	17.64 ± 0.28 [a]	2.64 ± 0.23 [a]

*Values are Mean ± standard deviation [a,b] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

3.3.3 Salt content and pH

As the level of salt replacement increased, the total NaCl content significantly ($P<0.05$) decreased; therefore, frankfurters that were manufactured without NaCl replacement (control samples) contained the highest NaCl content (2.5% total salt), compared to frankfurters manufactured with 50% or 100% NaCl replacement with Artisalt™, which contained 1.3% and 0.5% total salt, respectively (Table 3.5). According to the FSAI the recommended NaCl concentration in pork sausages was set to 1.37% (FSAI, 2010) and in this study the set target was achieved when 50% of NaCl was replaced with Artisalt™.

The pH values did not change significantly when NaCl was 50% replaced with Artisalt™; however, significantly higher pH values ($P<0.05$) were noticed in frankfurters that 100% of NaCl was replaced with Artisalt™. When measured the pH or Artisalt™ and NaCl at the same concentration, the pH of a 2% Artisalt™ solution was 7.4 while the pH of a 2% NaCl was 6.6 and this high pH of Artisalt™ may explain why the when 100% NaCl was replaced by Artisalt™ in the frankfurter had a significantly higher ($P<0.05$) pH value than any other treatment.

Previous studies have reported that increasing salt content did not affect significantly the pH or neither increased the pH of sausages (Aaslyng *et al.*, 2014; O' Flynn *et al.*, 2014; Clarke *et al*., 1987; Poulanne and Terrell, 1983; Sofos, 1983); however, in this study, the higher pH values in the 100% NaCl replaced samples may have been due to the composition of the Artisalt™ which has a pH value of 7.4 in 2% and also as it contains celery extract and the concentration of celery extract present in each formulation increased as the concentration of Artisalt™ increased. The significant effect of celery extract on increasing pH in this study supports the findings of a number of previous studies (Pietrasik *et al.*,

2016; Sebranek *et al*., 2012; Horsch *et al.*, 2014) which reported that celery concentrate has a pH value of 9.2.

3.3.4 Cook loss

The cook loss of the frankfurters was determined prior to HP treatment and is shown in Table 3.5. There were no significant differences in cook loss between the control and frankfurters that 50% of NaCl were replaced with Artisalt™, however; the frankfurters that 100% of NaCl was replaced with Artisalt™ produced significantly ($P<0.05$) higher cook loss values which was due to a significantly ($P<0.05$) lower salt content and subsequently a lower ionic strength and WHC.

Cooking yield affects the cost of manufacture of processed meats (O' Flynn *et al*., 2014). The control of cook loss is also important because changes in the cooking yields may result in compositional changes in the finished products that may affect the palatability characteristics. As moisture is lost from meat during and after thermal processing, product yield and other quality attributes such as tenderness, texture, and flavour are negatively affected (Pietrasik, 1999). The significant effect of salt reduction on the cooking loss in this study supports the findings of a number of authors that WHC and therefore the cook loss is significantly influenced by NaCl content in processed meats (O' Flynn *et al*., 2014; Tobin *et al.*, 2012; Desmond, 2006).

The results also showed that the cook loss and therefore the WHC were not significantly affected although, a significantly ($P<0.05$) lower total salt content was obtained when 50% of NaCl was replaced with Artisalt™ compared to control samples. The results indicated that the combination of NaCl and Artisalt™ can produce frankfurters of a similar quality to that of the control samples but with significantly ($P<0.05$) less salt. Crehan *et al*. (2000)

and Hongsprabhas and Barbut (1999) found that salt levels in meat batters could be reduced to 1.5% without affecting cooking losses; however, in our study no significant effect on cook loss was found between the frankfurters that 50% of NaCl replaced with Artisalt™ which contained 1.3% total salt content and to control samples which contained 2.5% of total salt and this may be due to the fact they had similar ionic strengths.

3.3.5 Emulsion stability

The emulsion stability of the frankfurter batter was determined prior to cooking and HPP and is shown in Table 3.5. The results of TEF showed that emulsion stability was significantly ($P<0.05$) affected when NaCl was 100% replaced by Artisalt™, however, the emulsion stability was not significantly affected when 50% of NaCl was replaced with Artisalt™ compared to control samples.

It was reported that the level of fluid loss is directly related to the degree of emulsion stability as less water is lost during cooking if the emulsion is stable. (The Salt Institute, 2013). In sausage manufacture, stable emulsions are formed when the salt-soluble protein coat the finely-formed globules of fat, providing a binding gel consisting of meat, fat and moisture (The Salt Institute, 2013). Terrell (1983) also reported that salt increases the viscosity of meat batters, facilitating the incorporation of fat to form stable meat batters; therefore, if salt content is reduction in the manufacture of comminuted products will reduce the stability of meat batters. Our results are in agreement with the results reported by O' Flynn *et al*. (2014) who found that the stability of sausage batter was unaffected when salt was reduced up to 1.0%. In general, when salt content is reduced, the WHC and therefore the emulsion stability is affected negatively; however; in this study the combination of NaCl and Artisalt™ produced frankfurters of a similar quality to control

samples. This may be due to similar ionic strengths which increases protein solubility and subsequent emulsion stability.

3.3.6 Colour

The results showed that lightness, redness and yellowness were significantly ($P<0.05$) affected when 100% NaCl was replaced with Artisalt™ resulting in a lighter, yellower and less red frankfurters compared to control samples. However, when 50% of NaCl was replaced with Artisalt™, generally there was no significant differences in lightness, redness and yellowness compared to control frankfurters even though the total salt was reduced from 2.5% to 1.3% (Table 3.5). Similar results were found by Wettasinghe and Shahidi (1997) who reported a decrease in the lightness of pork meat values due to increased salt levels (1.0 to 2.0%). The darker colour was attributed to oxidised products of meat pigments (due to increased salt levels) which have a brown and darker colour. Skogsberg (2017) reported that colour was not significantly affected in sausages when salt was replaced with up to 30% KCl; however; in our study a higher level of salt replacement was achieved (50%) without significantly affecting the colour of frankfurters.

Our findings are also in agreement with the results reported by Grossi *et al*. (2012) who found that the salt content of pork sausages can be reduced from 1.8 to 1.2% salt by addition of hydrocolloids (either carrot fibres or potato starch) and subsequent HPP at 600 MPa without negative effects on colour. Conversely, Tobin *et al.* (2013) reported a paler sausage was observed when salt content was decreased while O' Flynn *et al*. (2014) found that colour in sausages were significantly affected ($P<0.05$) when salt levels were reduced below 1.5%. Similarly, Crehan *et al*. (2000) found that salt reduction from 2.5 to 1.5% significantly ($P<0.05$) reduced the redness and yellowness of frankfurters.

Table 3.5 Effects of different frankfurter formulations on the physicochemical characteristics*

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Lightness (L*)	Redness (a*)	Yellowness (b*)	Salt content (%)	pH	TEF** (%)	Cook loss** (%)
1	0	0.1	0.3	70.36 ± 1.05 ^{a}	9.27 ± 0.55 ^{a}	12.35 ± 0.62 ^{a}	2.48 ± 0.08 ^{a}	5.97 ± 0.07 ^{a}	2.22 ± 0.13 ^{a}	2.4 ± 0.46 ^{a}
2	0	300	0.2	71.13 ± 1 ^{ab}	8.86 ± 0.22 ^{ab}	12.52 ± 0.37 ^{ab}	2.52 ± 0.13 ^{a}	5.99 ± 0.04 ^{a}	2.42 ± 0.14 ^{a}	2.7± 0.4 ^{a}
3	0	300	0.4	70.52 ± 0.45 ^{ab}	8.78 ± 0.29 ^{ab}	12.36 ± 0.73 a	2.52 ± 0.08 ^{a}	6.00 ± 0.09 ^{a}	2.38 ± 0.19 ^{a}	2.5 ± 0.49 ^{a}
4	0	600	0.3	70.88 ± 0.54 ^{ab}	8.71 ± 0.5 ^{ab}	12.84 ± 0.08 ^{ab}	2.54 ± 0.05 ^{a}	5.95 ± 0.05 ^{a}	2.37 ± 0.17 ^{a}	2.5 ± 0.4 ^{a}
5	50	0.1	0.2	71.05 ± 1.04 ^{ab}	8.49 ± 0.25 ^{b}	12.18 ± 0.68 ^{a}	1.28 ± 0.08 ^{b}	6.03 ± 0.02 ^{a}	2.46 ± 0.11 ^{a}	2.5 ± 0.23 ^{a}
6	50	0.1	0.4	71.04 ± 1.03 ^{ab}	8.44 ± 0.27 ^{b}	12.55 ± 0.23 ^{ab}	1.31 ± 0.1 ^{b}	5.97 ± 0.06 ^{a}	2.42 ± 0.19 ^{a}	2.5 ± 0.58 ^{a}
7	50	300	0.3	71.18 ± 1.02 ^{ab}	8.74 ± 0.66 ^{ab}	12.22 ± 0.63 ^{a}	1.34 ± 0.16 ^{b}	6.01 ± 0.03 ^{a}	2.31 ± 0.16 ^{a}	2.6 ± 0.61^{a}
8	50	300	0.3	71.28 ± 0.83 ^{ab}	8.67 ± 0.21 ^{ab}	12.32 ± 0.56 ^{a}	1.29 ± 0.08 ^{b}	5.99 ± 0.02 ^{a}	2.64 ± 0.16 ^{a}	2.5 ± 0.37 ^{a}
9	50	300	0.3	70.59 ± 0.49 ^{ab}	8.56 ± 0.26 ^{ab}	12.19 ± 0.06 ^{a}	1.24 ± 0.05 ^{b}	6.02 ± 0.02 ^{a}	2.50 ± 0.11 ^{a}	2.3 ± 0.46 ^{a}
10	50	600	0.2	71.54 ± 0.34 ^{b}	8.82 ± 0.47 ^{ab}	12.37 ± 0.56 ^{a}	1.28 ± 0.08 ^{b}	6.03 ± 0.04 ^{a}	2.46 ± 0.31 ^{a}	2.4 ± 0.23 ^{a}
11	50	600	0.4	71.10 ± 0.61 ^{ab}	8.60 ± 0.19 ^{ab}	12.57 ± 0.32 ^{ab}	1.29 ± 0.08 ^{b}	6.04 ± 0.05 ^{a}	2.42 ± 0.29 ^{a}	2.4 ± 0.51 ^{a}
12	100	0.1	0.3	72.64 ± 0.72 ^{c}	6.74 ± 0.4 ^{c}	14.02 ± 0.64 ^{d}	0.52 ± 0.08 ^{c}	6.35 ± 0.05 ^{b}	6.66 ± 0.36 ^{b}	5.0 ± 0.27 ^{b}
13	100	300	0.2	73.01 ± 0.81 ^{c}	6.87 ± 0.65 ^{c}	13.71 ± 0.51 ^{cd}	0.48 ± 0.09 ^{c}	6.33 ± 0.04 ^{b}	6.90 ± 0.82 ^{b}	5.3 ± 0.28 ^{b}
14	100	300	0.4	72.64 ± 0.47 ^{c}	7.26 ± 0.41 ^{c}	13.57 ± 0.52 ^{cd}	0.56 ± 0.08 ^{c}	6.38 ± 0.05 ^{b}	6.44 ± 0.83 ^{b}	5.3 ± 0.46 ^{b}
15	100	600	0.3	73.31 ± 0.55 ^{c}	7.26 ± 0.21 ^{c}	13.23 ± 0.55 ^{bc}	0.50 ± 0.1 ^{c}	6.36 ± 0.06 ^{b}	6.58 ± 0.31 ^{b}	5.1 ± 0.9 ^{b}

*Values are Mean ± standard deviation, ** Analysis were carried out before HPP.

^{a,b,c,d} Different superscripts in the same column indicate significant difference (P<0.05) between treatments.

3.3.7 Texture Profile Analysis

The texture profile of the frankfurters is shown in Table 3.6. The results showed that hardness, chewiness, springiness, cohesiveness values were significantly lower ($P<0.05$) when the NaCl was 100% replaced with Artisalt™; however, when 50% of NaCl was replaced with Artisalt™ no significant differences on the texture profile was noticed compared to control samples. Negative effects of salt reduction on the texture of processed meats has been well documented (Hand *et al*., 1987; Matulis *et al*., 1995; Corral *et al*., 2013 ; Desmond 2006 ; Gimeno *et al*., 2001). In regards to salt replacement, Gelabert (2003) reported that in sausages the critical level of salt substitution with KCL was 40%; however, in our study a higher level of salt replacement was successfully achieved (50%). Grossi *et al.* (2012) found that the salt content of pork sausages could be reduced from 1.8 to 1.2% salt by addition of hydrocolloids (either carrot fibres or potato starch) and subsequent HPP at 600 MPa without negative effects on texture.

The results obtained in this study suggest that the combination of NaCl and Artisalt™ produced frankfurters of a similar texture to that of the control samples containing full NaCl content; which may be due to similar ionic strengths which resulted in the cook loss or emulsion stability not being significantly affected when 50% of NaCl was replaced with Artisalt™ as these parameters are known to affect the texture of the final product after cooking.

Table 3.6 Effects of different frankfurter formulations on the texture parameters*.

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Hardness (N)	Adhesiveness (N)	Springiness (mm)	Cohesiveness	Chewiness (N-mm)
1	0	0.1	0.3	15.62 ± 1.22 ^{abc}	-.077 ± 0.07 ^{a}	.845 ± 0.04 ^{a}	.535 ± 0.02 ^{a}	13.15 ± 0.81 ^{abc}
2	0	300	0.2	16.42 ± 0.98 ^{a}	-.112 ± 0.1 ^{a}	.843 ± 0.03 ^{a}	.535 ± 0.03 ^{a}	13.83 ± 0.94 ^{a}
3	0	300	0.4	14.92 ± 0.89 ^{bcd}	-.111 ± 0.06 ^{a}	.854 ± 0.03 ^{a}	.538 ± 0.02 ^{a}	12.73 ± 0.77 ^{abc}
4	0	600	0.3	15.94 ± 0.82 ^{ab}	-.136 ± 0.1 ^{a}	.859 ± 0.03 ^{a}	.542 ± 0.01 ^{a}	13.70 ± 1.1 ^{ab}
5	50	0.1	0.2	14.24 ± 0.93 ^{d}	-.119 ± 0.05 ^{a}	.861 ± 0.02 ^{a}	.524 ± 0.02 ^{a}	12.26 ± 0.9 ^{c}
6	50	0.1	0.4	14.64 ± 0.90 ^{cd}	-.071 ± 0.09 ^{a}	.849 ± 0.02 ^{a}	.544 ± 0.03 ^{a}	12.44 ± 1.12 ^{bc}
7	50	300	0.3	14.84 ± 1.11 ^{bcd}	-.115 ± 0.03 ^{a}	.859 ± 0.02 ^{a}	.541 ± 0.02 ^{a}	13.04 ± 1.31 ^{abc}
8	50	300	0.3	15.24 ± 1.02 ^{bcd}	-.116 ± 0.56 ^{a}	.848 ± 0.04 ^{a}	.529 ± 0.03 ^{a}	12.78 ± 1.07 ^{abc}
9	50	300	0.3	15.01 ± 0.82 ^{bcd}	-.115 ± 0.05 ^{a}	.867 ± 0.02 ^{a}	.531 ± 0.04 ^{a}	12.74 ± 1.23 ^{abc}
10	50	600	0.2	15.26 ± 1.01 ^{abcd}	-.520 ± 0.65 ^{a}	.856 ± 0.04 ^{a}	.515 ± 0.01 ^{ab}	13.06 ± 0.86 ^{abc}
11	50	600	0.4	14.64 ± 1.28 ^{cd}	-.307 ± 0.4 ^{a}	.867 ± 0.01 ^{a}	.544 ± 0.02 ^{a}	12.69 ± 1.11 ^{abc}
12	100	0.1	0.3	12.34 ± 0.46 ^{e}	-.257 ± 0.26 ^{a}	.799 ± 0.02 ^{b}	.483 ± 0.04 ^{bc}	9.86 ± 0.57 ^{d}
13	100	300	0.2	11.78 ± 0.49 ^{e}	-.559 ± 0.25 ^{a}	.795 ± 0.02 ^{b}	.474 ± 0.03^{c}	9.35 ± 0.32 ^{d}
14	100	300	0.4	12.02 ± 0.85 ^{e}	-.338 ± 0.31 ^{a}	.769 ± 0.03 ^{b}	.480 ± 0.04 ^{bc}	9.24 ± 0.79 ^{d}
15	100	600	0.3	11.82 ± 0.57 ^{e}	-.055 ± 0.04 ^{a}	.796 ± 0.02 ^{b}	.471 ± 0.04 ^{c}	9.40 ± 0.65 ^{d}

*Values are Mean ± standard deviation ^{a,b,c,d,e} Different superscripts in the same column indicate significant difference (P<0.05) between treatments.

3.3.8 Sensory analysis

The results of the sensory analysis of the frankfurters showed that all sensory attributes were not significantly affected when 50% of NaCl was replaced with Artisalt^{TM}; however, when 100% of NaCl was replaced with Artisalt^{TM} significantly ($P<0.05$) lower scores for sensory attributes including flavour, texture, saltiness, tenderness and juiciness and higher ($P<0.05$) scores for off-flavour were obtained (Table 3.7); however, due to the fact that the OSA of these samples scored above the limit of acceptability set at 4.5 on the 9 point scale indicated that these frankfurters could not be considered as unacceptable. The control frankfurters were formulated to contain similar ingredients and salt content as frankfurters available in the Irish market therefore no significant differences between the control frankfurters and frankfurters which NaCl was 50% replaced with Artisalt^{TM} indicated that the 50/50 combination of NaCl/Artitsalt^{TM} produces frankfurters of similar quality to commercial products.

Jaenke *et al.* (2017) examined salt reduction, salt replacement or compensation in processed meats. Random effects meta-analyses conducted on salt-reduced products showed that salt in processed meats can be reduced by approximately 70% without significantly impacting consumer acceptability; however; physicochemical quality was not assessed. Mc Gough *et al.*, (2012); Skogsberg (2017) and Gelabert (2003) reported that in frankfurters up to 40% of salt can be replaced without major quality or sensory changes. However, in this study a higher level of salt replacement (50%) with Artisalt^{TM} was achieved without negatively affecting the sensory attributes of the frankfurters nor did the panel perceive a reduction in saltiness. This may be due to the composition of the salt replacer Artisalt^{TM} which contains flavour enhancers (yeast extract, celery and onion) which can mask the bitterness associated with KCl and enhance the flavour and saltiness perception of the frankfurters.

Table 3.7 Effects of different frankfurter formulations on the sensory characteristics*

Formulation	Salt replacement (%)	HPP (MPa)	Inbac (%)	Sensory attributes							
				Appearance	Texture	Flavour	Saltiness	Tenderness	Juiciness	Off-flavour	OSA
1	0	0.1	0.3	6.22 [a]	6.40 [ab]	7.22 [a]	6.26 [a]	6.80 [a]	6.73 [a]	1.36 [a]	7.33 [a]
2	0	300	0.2	6.37 [a]	6.22 [ab]	6.94 [a]	5.90 [a]	6.86 [a]	7.00 [a]	1.59 [a]	7.44 [a]
3	0	300	0.4	6.50 [a]	6.00 [b]	7.09 [a]	6.22 [a]	6.80 [a]	6.86 [a]	1.37 [a]	7.36 [a]
4	0	600	0.3	6.40 [a]	6.87 [a]	7.32 [a]	6.56 [a]	6.86 [a]	7.06 [a]	1.57 [a]	7.23 [a]
5	50	0.1	0.2	6.26 [a]	6.40 [ab]	7.22 [a]	6.53 [a]	6.80 [a]	7.13 [a]	1.52 [a]	7.82 [a]
6	50	0.1	0.4	6.33 [a]	6.05 [b]	7.18 [a]	6.05 [a]	6.73 [a]	7.00 [a]	1.46 [a]	7.24 [a]
7	50	300	0.3	6.38 [a]	6.34 [ab]	7.11 [a]	6.29 [a]	6.98 [a]	6.87 [a]	1.24 [a]	7.38 [a]
8	50	300	0.3	6.55 [a]	6.45 [ab]	7.41 [a]	6.11 [a]	6.86 [a]	6.95 [a]	1.39 [a]	7.24 [a]
9	50	300	0.3	6.71 [a]	6.29 [ab]	7.24 [a]	6.36 [a]	6.59 [a]	7.14 [a]	1.55 [a]	7.68 [a]
10	50	600	0.2	6.86 [a]	6.36 [ab]	7.38 [a]	5.91 [a]	6.80 [a]	7.06 [a]	1.53 [a]	7.51 [a]
11	50	600	0.4	6.23 [a]	6.47 [ab]	6.90 [a]	6.04 [a]	6.53 [a]	6.93 [a]	1.57 [a]	7.58 [a]
12	100	0.1	0.3	6.65 [a]	4.33 [d]	4.95 [b]	3.60 [b]	7.00 [a]	3.80 [b]	2.07 [ab]	4.06 [c]
13	100	300	0.2	6.35 [a]	4.20 [d]	5.28 [b]	3.73 [b]	6.86 [a]	3.46 [b]	2.93 [b]	4.96 [b]
14	100	300	0.4	6.60 [a]	4.73 [cd]	5.10 [b]	3.66 [b]	6.45 [a]	2.86 [b]	2.46 [b]	5.02 [b]
15	100	600	0.3	6.44 [a]	5.06 [c]	4.75 [b]	3.23 [b]	6.66 [a]	3.06 [b]	2.70 [b]	5.28 [b]

*Values are Mean ± standard deviation

[a,b,c,d] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

3.3.9 Modelling and Optimisation

The fitness of the models were evaluated through the coefficients of determination (R^2). It has been suggested that for the good fit of a model R^2 should be ≥ 80% (Joglekar *et al.* 1987). The adjusted R-squared statistic is more suitable for comparing models with different numbers of independent variables. The lack of fit test is designed to determine whether the selected model is adequate to describe the observed data, of the P value is greater than or equal to 0.05 then the model appears to be adequate for the observed data at the 95% confidence level. (STATGRAPHICS® Centurion XV User Manual, Statpoint, Inc., USA. 2005).

ANOVA was carried out on frankfurters hardness, OSA, flavour and saltiness (Table 3.8). Salt replacement, HPP and Inbac were represented by A, B and C, respectively. In the Pareto charts (Figures 3.1a-1d) the length of the horizontal bars are proportional to the significance of the factor. The vertical line is the threshold for significant effects at the level $P < 0.05$ thus the effects are statistically significant when the respective bars exceed this vertical line. The Pareto charts indicated that Salt replacement had the most significant effect on all response variables.

For hardness, the Pareto chart (Figure 3.1a) shows that the linear effects of the independent variable salt replacement (A), the interactive effects of salt replacement and Inbac (AC) and the quadratic effects of salt replacement (AA) affected significantly ($P<0.05$) the hardness of the frankfurters. For OSA, the Pareto chart (Figure 3.1b) shows that the linear effects of A and B, the interactive effects of AB and the quadratic effects of AA and CC affected significantly ($P<0.05$) the OSA of the frankfurters. For flavour and saltiness, Figures 3.1c and 3.1d showed that the linear effects of A and the quadratic effects of AA affected significantly ($P<0.05$) the flavor and saltiness of the frankfurters. The regression

equations which predicted the value of each response variable when the independent factors are varied were as follows:

Equation 2: Hardness = 14.42 - 0.01905*A + 0.004225*B + 10.75*C - 0.000402*A^2 - 0.000014*A*B + 0.087*A*C - 0.00000105556*B^2 - 0.0085*B*C - 24.0*C^2

Equation 3: OSA = 9.35 + 0.0149167*A - 0.000180556*B - 13.0*C - 0.000486667*A^2 + 0.0000233333*A*B + 0.005*A*C - 7.40741E-7*B^2 + 0.000833333*B*C + 20.8333*C^2

Equation 4: Flavour = 7.0 + 0.0275*A + 0.00104167*B - 0.625*C - 0.00042*A^2 - 0.000005*A*B - 0.015*A*C + 2.77778E-7*B^2 - 0.00333333*B*C + 2.5*C^2

Equation 5: Saltiness = 5.775 + 0.031*A- 0.00104167*B + 3.625*C - 0.0005*A^2 - 0.0000116667*A*B - 0.015*A*C - 2.77778E-7*B^2 + 0.005*B*C - 7.5*C^2

For hardness and flavour, the absolute values of partial regression coefficient were A > C > B within the range of the experimental design, demonstrating the greatest effects of Salt replacement followed by Inbac^{TM} and HPP, respectively. For OSA and saltiness, the absolute values of partial regression coefficient were A > B > C within the range of the experimental design, demonstrating the greatest effects of salt replacement followed by HPP and Inbac^{TM} respectively.

For hardness, OSA, flavour and saltiness the adjusted R^2 of the predicted models was 96.63%, 99.22%, 95.39% and 95.5%, respectively indicating that the predicted model can reasonably predict the observed values shown in the regression equations 2-5. For OSA, the lack of fit value was 0.19 which was insignificant and therefore indicates that the selected model is adequate to describe the observed data.

The 3-Dimentional Response Surface Plots were formed based on the polynomial function depicting the variation in the parameter modelled (measured response) as the two factors (salt replacement and HPP) levels changed along the plots, while holding the third factor constant at the optimum point predicted for that factor. In particular, in Figure 3.2, the response variable correlates salt replacement (%) and pressure as a function of Inbac™ set at 0.3%. The relationship between the dependent and independent variables can be clearly understood by these plots (Figure 3.2). For each response one 3-D response plot was produced.

The yellow area of Figure 3.2a represents the highest hardness of the frankfurters and the corresponding combination of the independent factors required in order to achieve this level of hardness. The results showed that the best combination of the variables in order to maximise hardness were: Salt replacement 19%, HPP 403 MPa and Inbac™ 0.3%. The yellow area of Figure 3.2b represents the highest OSA and the corresponding combination of the independent factors required in order to achieve this level of OSA. The results showed that the best combination of the variables in order to maximise OSA were; Salt replacement 48%, HPP 580MPa and Inbac™ 0.3%. The orange area of Figure 3.2c represents the highest liking of flavour and the corresponding combination of the independent factors required in order to achieve this level of flavour. The results showed that the best combination of the variables in order to maximise flavour were; Salt replacement 26%, HPP 600MPa and Inbac™ 0.3%. The orange area of Figure 3.2d represents the highest saltiness and the corresponding combination of independent factors required in order to achieve this level of saltiness. The results showed that the best combination of the variables in order to maximise overall acceptability were; Salt replacement 36%, HPP 305MPa and Inbac™ 0.3%.

It is well known that sensory properties of food products are the most important attributes as they are the most apparent to consumers (Singham *et al.* 2015). While attributes such as hardness, flavour or saltiness were predicted by the models; a higher level of salt replacement and HPP was achieved when product optimisation was carried out based on OSA which subsequently produced a product with lower salt content an increased safety due to the use of a combination of HPP and antimicrobial organic acids; therefore, production of the optimised frankfurters was carried out based on maximising the OSA.

Table 3.8 ANOVA of the independent factors and their interactive effects on each response variable.

	Sum of Squares	*Df*	*Mean Square*	*F-Ratio*	*P-Value*	*SL*
Hardness						
A:Salt replacement	27.9005	1	27.9005	345.05	0.0000	*
B:HPP	0.08405	1	0.08405	1.04	0.3547	NS
C:Inbac	0.2738	1	0.2738	3.39	0.1251	NS
AA	3.72932	1	3.72932	46.12	0.0011	*
AB	0.1764	1	0.1764	2.18	0.1997	NS
AC	0.7569	1	0.7569	9.36	0.0281	*
BB	0.0333231	1	0.0333231	0.41	0.5492	NS
BC	0.2601	1	0.2601	3.22	0.1329	NS
CC	0.212677	1	0.212677	2.63	0.1658	NS
Total error	0.4043	5	0.08086			
Total (correlation)	33.6838	14				
R-squared = 98.78%						
R-squared (adjusted) = 96.63%						
OSA						
A:salt replacement	12.7513	1	12.7513	1177.04	0.0000	*
B:HPP	0.45125	1	0.45125	41.65	0.0013	*
C:Inbac	0.0	1	0.0	0.00	1.0000	NS
AA	5.46564	1	5.46564	504.52	0.0000	*
AB	0.49	1	0.49	45.23	0.0011	*
AC	0.0025	1	0.0025	0.23	0.6512	NS
BB	0.0164103	1	0.0164103	1.51	0.2731	NS
BC	0.0025	1	0.0025	0.23	0.6512	NS
CC	0.160256	1	0.160256	14.79	0.0120	*
Total error	0.0541667	5	0.0108333			
Total (correlation)	19.556	14				
R-squared = 99.72%						
R-squared (adjusted) = 99.22%						
Flavour						
A:salt replacement	8.405	1	8.405	197.76	0.0000	*
B:HPP	0.00125	1	0.00125	0.03	0.8706	NS
C:Inbac	0.06125	1	0.06125	1.44	0.2837	NS
AA	4.07077	1	4.07077	95.78	0.0002	*
AB	0.0225	1	0.0225	0.53	0.4995	NS
AC	0.0225	1	0.0225	0.53	0.4995	NS
BB	0.00230769	1	0.00230769	0.05	0.8250	NS
BC	0.04	1	0.04	0.94	0.3765	NS
CC	0.00230769	1	0.00230769	0.05	0.8250	NS
Total error	0.2125	5	0.0425			
Total (correlation)	12.9133	14				
R-squared = 98.35%						
R-squared (adjusted) = 95.39%						

Saltiness						
A:salt replacement	14.58	1	14.58	216.00	0.0000	*
B:HPP	0.06125	1	0.06125	0.91	0.3845	NS
C:Inbac	0.00125	1	0.00125	0.02	0.8971	NS
AA	5.76923	1	5.76923	85.47	0.0002	*
AB	0.1225	1	0.1225	1.81	0.2358	NS
AC	0.0225	1	0.0225	0.33	0.5887	NS
BB	0.00230769	1	0.00230769	0.03	0.8606	NS
BC	0.09	1	0.09	1.33	0.3004	NS
CC	0.0207692	1	0.0207692	0.31	0.6030	NS
Total error	0.3375	5	0.0675			
Total (correlation)	21.004	14				
R-squared = 98.39%						
R-squared (adjusted) = 95.5%						

SL = Significance level, NS = Not Significant, * = $P<0.05$

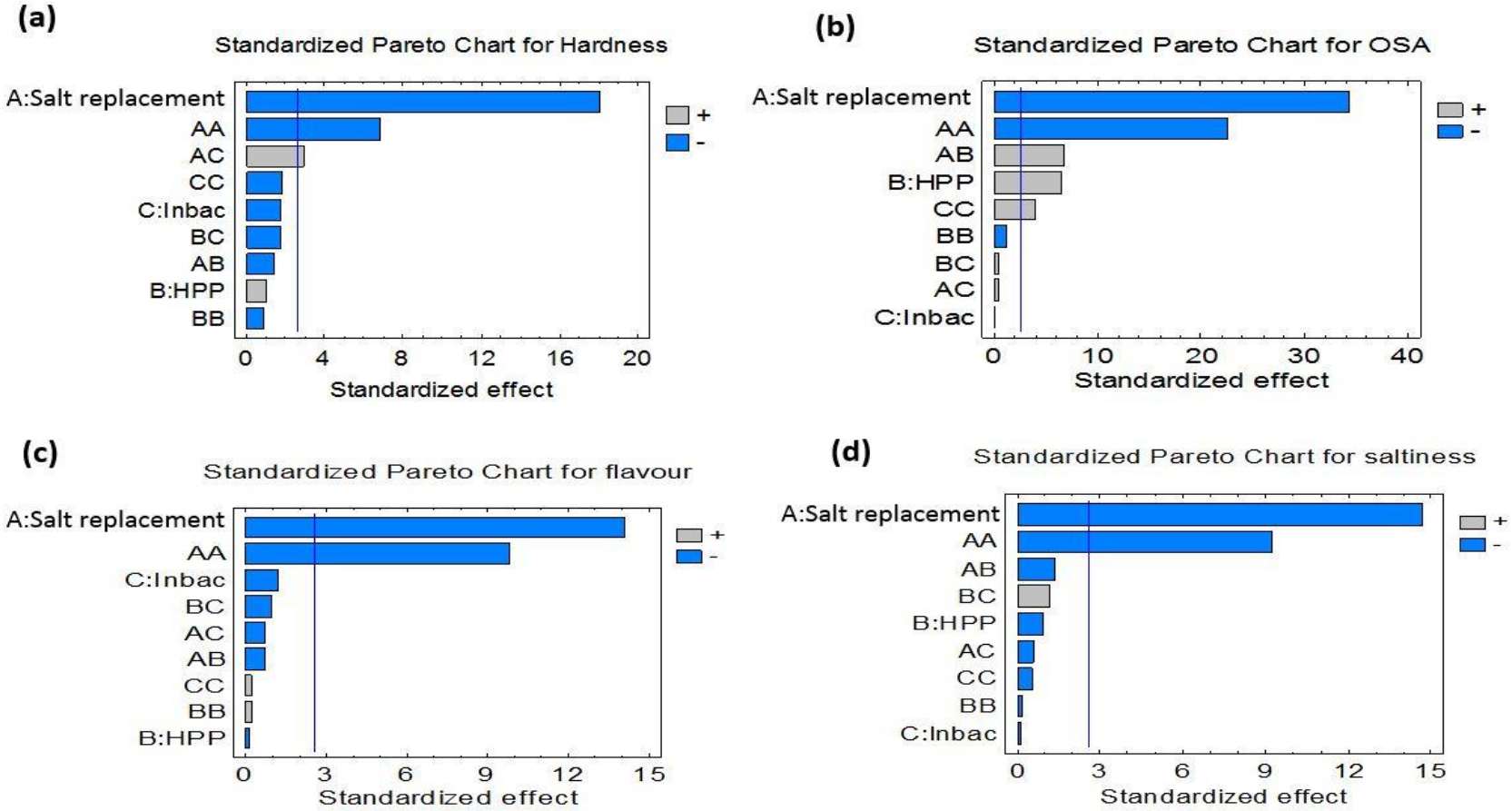

Figure 3.1 – Pareto charts of the significance of the effects of the independent factors and their interactions on the (a) hardness, (b) OSA, (c) flavour and (d) saltiness.

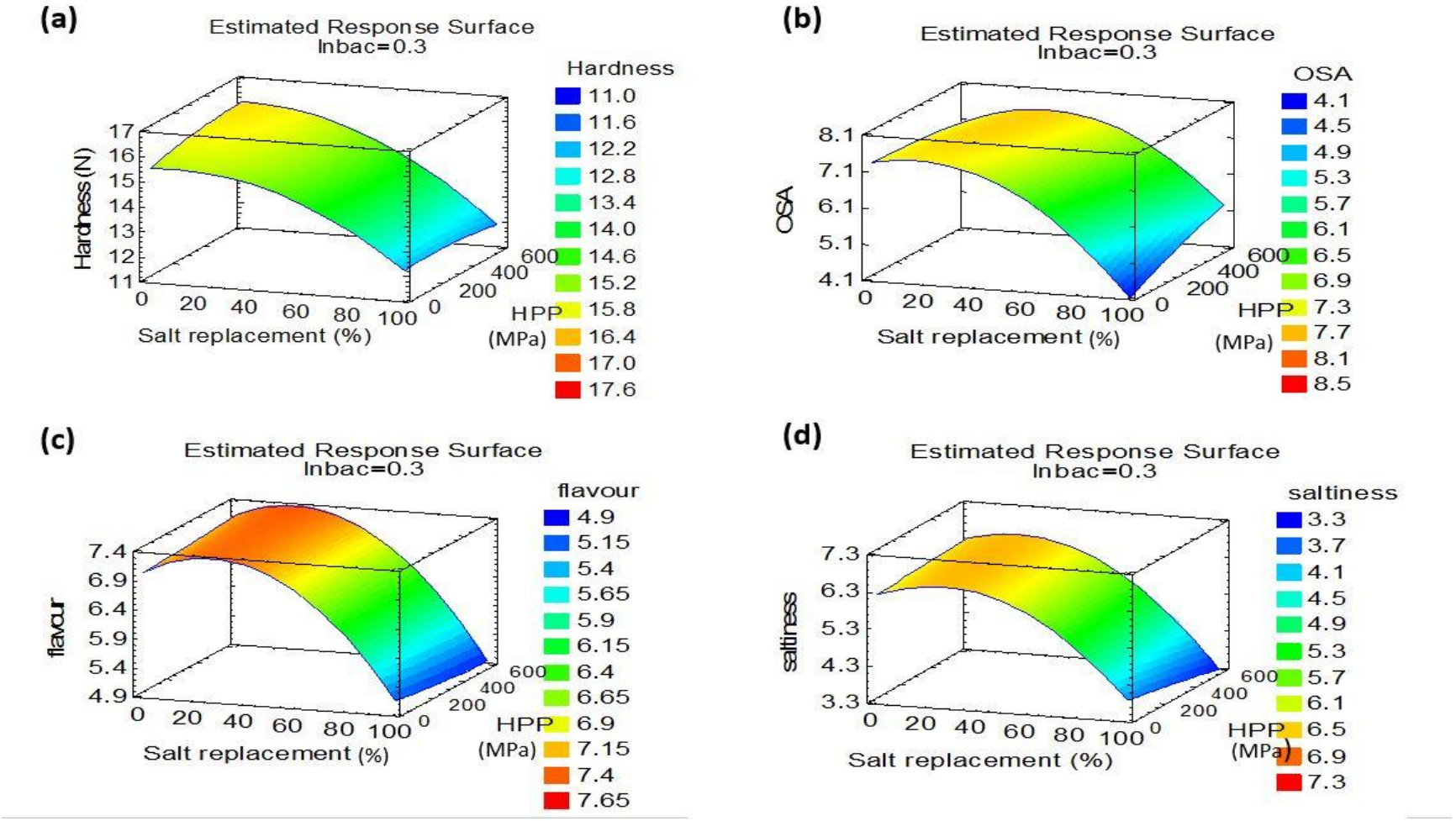

Figure 3.2 – Effect of salt replacement and high pressure processing on the (a) hardness, (b) OSA, (c) flavour and (d) saltiness of frankfurters.

3.3.10 Validation experiments

The robustness of the model used for the process optimisation was validated by carrying out three independent confirmatory trials to ascertain differences between predicted and experimental values. Optimised frankfurters (48% Salt replacement, 580MPa and 0.3% Inbac^{TM}) were produced and analysed on three different occasions by a 25 member semi-trained sensory panel. The predicted and average response values for all sensory attributes (flavour, saltiness and OSA) obtained by the sensory panel and instrumental measurement of hardness were similar to the observed values obtained using regression equations 1-4 (Table 3.9). The results of the validation experiments indicated that the RSM approach used was effective for modelling and optimizing the operational conditions for the manufacture of low salt frankfurters.

Table 3.9 Predicted and observed responses of the frankfurters manufactured using the optimised OSA parameters (48% salt replacement. 580 MPa and 0.3% Inbac^{TM})*

Response	Y (predicted)	Y (observed)
Hardness (N)	15.3	15.4 ± 0.96
Saltiness	6.0	5.62 ± 1.02
Flavour	7.2	7.01 ± 0.67
OSA	7.4	7.14 ± 0.68

*Values are Mean ± standard deviation

3.3.11 Microbiological analysis

The microbiological changes for TVC during chilled storage (4°C) of vacuum packed frankfurters (Low-salt optimised frankfurter and Control) are shown in Figure 3.3. The recommended microbiological limit for Aerobic plate counts is < $5x10^5$ CFU/g of product and is applied for cook-chill products examined at the point of consumption before reheating or cooking is applied (FSAI, 2014)

The initial microbiological quality of the both control and optimised frankfurters were of good quality with a TVC below the limit of detection <10 CFU/g. The limit of acceptability for control frankfurters was reached after 53 day storage; however, the limit of acceptability for the low-salt optimised frankfurter manufactured using HPP and Inbac™ as hurdles was reached after 80 days of storage which had a 51% longer shelf life than control samples (Figure 3.3).

The application of HPP to increase the shelf life of processed meat products has been well documented (Diez *et al.*, 2008; Garriga *et al.*, 2004; Hayman *et al.*, 2004); however, in this study the combined hurdle effects of HPP and a mix of organic acids extended significantly (P<0.05) the shelf life of low-salt frankfurters in which the safety and shelf life had been reduced due to significant salt reduction and the reduction on the preservative effects of salt. Our findings are in agreement with the results reported by Rodriguez-Calleja et al. (2012) who demonstrated the strongly potential synergetic interaction of HPP and a mix of organic acids as hurdles extending the shelf-life of skinless chicken breast fillets up to four weeks. This confirms the potential utility of the hurdle strategy for improving the shelf-life and safety of low-salt processed meat products.

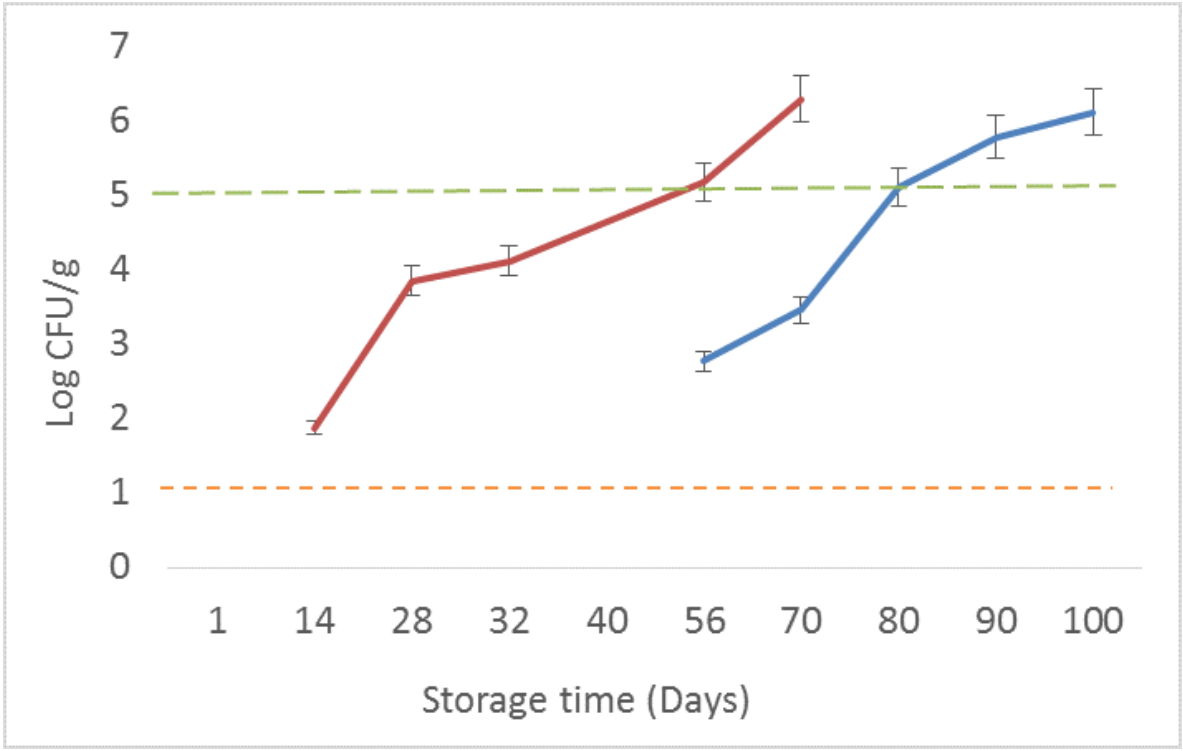

Figure 3.3 - Microbiological changes of TVC during chilled storage at 4°C of vacuum packed optimum formulation (—) or control (—) frankfurters. Each point shown is the average value from two different trials (n=8). The dotted lines show the limits of detection and acceptability, respectively.

3.4 Conclusion

When NaCl was partially replaced (50%) with Artisalt™ quality parameters such as cook loss, pH, emulsion stability, colour, texture and sensory attributes were not significantly affected compared to control samples; however, when NaCl was 100% replaced with Artisalt™ all frankfurter quality parameters were negatively ($P<0.05$) affected resulting in a less ($P < 0.05$) acceptable product by the sensory panel.

Response Surface Methodology was successfully used to develop sensory accepted low-salt frankfurters and the results indicated that the main independent factor that significantly ($P<0.05$) influenced the assessed response variables of the frankfurters was salt replacement. The product optimisation carried out maximising the OSA indicated that the best formulation to obtain low-salt cooked frankfurters was: Salt replacer Artisalt™ (48%), HPP (580MPa) and concentration of Inbac™ (0.3%).

The validation process indicated that the observed responses were found to be quite similar to the predicted values for the OSA optimised low salt cooked meat products indicating that the model obtained can accurately predict changes on the OSA of the frankfurters. Therefore, a 48% added salt reduction was achieved reducing the total salt content in the cooked frankfurters from 2.5% to 1.3% and the developed product can be classified as 'salt reduced' frankfurters. This significant salt reduction in cooked frankfurters without compromising the physicochemical, OSA or safety and shelf life of the product was achieved through the use of salt replacer Artisalt™ which contain flavour enhancers and the application of HPP and Inbac™ as hurdles. The hurdle approach used in this study extended significantly the shelf life of low salt frankfurters by 51% compared to control samples, indicating the potential utility of the hurdle strategy for improving the shelf-life and safety of low-salt processed meat products.

CHAPTER 4

Shelf life extension of vacuum-packed salt reduced frankfurters and cooked ham through the combined application of high pressure processing and organic acids.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

The objective of this study was to assess the efficacy of a combination of high pressure processing (HPP) and a mix of organic acids Inbac™ as hurdles to extend the shelf life of previously optimised sensory accepted frankfurters and cooked ham with significantly ($P<0.05$) lower salt content. The optimum parameters for the manufacture of low-salt frankfurters were; Salt replacer Artisalt™ (48%), HPP (580 MPa) and Inbac™ (0.3%) and for manufacture of low-salt cooked ham the optimum parameters were; Salt replacer Artisalt™, HPP (535 MPa) and Inbac™ (0.3%). Physicochemical changes ($P<0.05$) occurred over storage time; however, the sensory acceptability did not change significantly. From the microbiological point of view, the results indicated that the hurdles (HPP and Inbac™) applied in the manufacture of low-salt processed meat products extended ($P<0.05$) the shelf-life of low-salt frankfurters by 51% and low-salt cooked ham by 97%, compared to control samples which contained full salt content. These results highlight the potential use of the hurdle strategy for extending the shelf-life and safety of low-salt processed meat products.

4.1 Introduction

The functions of salt in meat processing fall into three broad categories; enhancing sensory properties, providing specific physical processing effects and affecting preservation (Matthews and Strong, 2005), therefore salt reduction in processed meats can be problematic (Pietrasik *et al.*, 2017) as the sensory acceptability and the safety and shelf life can be compromised. The antimicrobial effects of salt is based on its ability to reduce water activity (a_w) (Sofos, 1984; Ingulgia *et al*., 2017). The effect of salt on microorganisms depends on the concentration of salt present in the aqueous phase of the food (Ingulgia *et al*., 2017). The concentration of salt in the water phase has to be high enough to inhibit the growth of pathogenic micro-organisms such as *Clostridium botulinum* and *Listeria monocytogenes* in vacuum packed and chilled food products (Matthews and Strong, 2005). However, salt reduction increases a_w reducing the preservative effects of salt which in turn increases water availability for microbial growth.

There is strong evidence that our current salt consumption is the major factor increasing blood pressure and thereby cardiovascular disease (He and McGregor, 2009). Regardless of this, in most European countries the recommended dietary salt intake of <5g/day is greatly exceeded with an estimated salt consumption as high as 9-12g/day (WHO, 2016) with 75% of dietary salt coming from processed foods (Appel and Anderson, 2010). As a result, the food industry is currently under pressure from food standards agencies to deliver reductions in the salt intake of the population through the introduction of lower salt levels in processed foods (Phillips, 2003) without compromising consumer acceptability or food safety and shelf life. Salt replacers such as Potassium Chloride (KCl) are commonly used to reduce salt in meat products; however, health concerns regarding the replacement of Sodium chloride (NaCl) with KCl have been highlighted by Steffensen *et al.* (2018) and include renal malfunctioning, hypoaldosteronism and Addison disease.

Shelf life is the period of time during which a food retains acceptable characteristics of flavour, colour, aroma, texture, nutritional value, and safety under defined environmental conditions (Lee *et al*., 2009). During storage, the main factors of deterioration leading to unacceptable food quality or safety issues of cooked food products are physical, chemical and microbiological, such as; discoloration, oxidative rancidity, increase in the numbers of spoilage microorganisms or the presence of food pathogens (Robertson, 2009; Lee *et al.*, 2009).

Hurdle technology combines intelligently different mild preservation techniques (hurdles) to control or eliminate pathogens (Rodríguez-Calleja *et al*., 2012). One of the potential hurdles to assure the safety of reduced sodium ready-to-eat (RTE) meat products is HPP (Han *et al*., 2011; Rendueles *et al*., 2011; Myers *et al*., 2013; Oliveira *et al*., 2015). Application of HPP at 600 MPa has demonstrated the inactivation of most pathogens and spoilage bacteria resulting in substantial extension of shelf-life of RTE meat products such as low-fat pastrami, strassburg beef, export sausage, cajun beef, cooked ham, dry cured ham and marinated beef loin (Jofré *et al*., 2009; Hayman *et al*., 2004). Marcos *et al*. (2007) improved the microbial quality of fermented sausages without affecting the quality applying HPP at 400 MPa for 10 mins at 17 °C. Pietrasik *et al*. (2017) reported that HPP does not impact the sensory acceptability of reduced sodium naturally cured wieners and can also successfully extend the shelf-life up to 12 weeks without compromising eating quality. Garriga *et al*. (2004) examined microbial inactivation on cooked ham after HPP at 600 MPa and found that after 60 days storage lactic acid bacteria (LAB) count was 6 log (CFU/g) lower in HPP cooked ham than in untreated samples. A study carried out by Diez *et al*. (2008) examined independently the application of organic acids (L-potassium lactate, L-potassium lactate/sodium lactate or L-potassium lactate/sodium acetate) and high-pressure treatments (300, 500 or 600 MPa for 10 mins) to improve the shelf life of blood

sausage. The longest shelf life of 15 days was achieved using L-potassium/sodium lactate or HPP at 600 MPa for 10 mins. The authors suggested that the synergetic effects of the organic acids and HPP might further improve the effectiveness of these treatments.

In Chapters 2 and 3, sensory accepted low-salt frankfurters and cooked ham were developed through the application of response surface methodology (RSM). The optimum parameters to maximize the overall sensory acceptability (OSA) of frankfurters were salt replacer Artisalt™ (48%), HPP (580 MPa) and Inbac™ (0.3%) and for cooked ham the optimised parameters were Artisalt™ (53%), HPP (535 MPa) and Inbac™ (0.3%). As processed meat manufacturers are constantly looking for new ways to reduce salt levels without compromising food safety, shelf-life or consumer acceptability; in our previous work a novel approach which showed great potential for reducing salt in frankfurters and ham was used; however, the shelf life of these low-salt products was not investigated. The use of HPP as additional post packaging processing and a mix of organic acids Inbac™ as hurdles was expected to not only increase the shelf life of the significantly reduced salt processed meat products but also increase the safety of these products which is necessary to compensate for the loss of safety and shelf life due to significant salt reduction. Extending the shelf life of these low-salt processed meat products can also reduce food waste of these products which will enhance sustainable food production

Moreover, most of the studies reported in the literature were carried out using lab scale HPP to treat processed samples (Vercammen *et al*., 2011; Rodriguez-calleja *et al*., 2012; O'Flynn *et al*., 2014; Crehan *et al*., 2000; Andres *et al*., 2004; Han *et al*., 2011; Cava *et al*., 2009) with a few studies using industrial HPP units for treating processed meat products. (Garriga *et al*., 2004; Jofre *et al*., 2009; Marcos *et al*., 2007). In the present study an industrial scale HPP unit and commercially available mix of organic acids Inbac™ were

used in the manufacture of frankfurters and cooked ham which have the advantage of scaling the manufacture of these products up easily.

While there are studies that use a combination of HPP and organic acids to extend the shelf life of meat products such as chicken and sausages (Rodrigues-Calleja *et al*., 2012; Diez *et al*., 2008; Vercammen *et al.,* 2011); to the best of our knowledge, a combination of HPP and organic acids as hurdles has not been used as a methodology to enhance the safety and shelf life of low salt processed meat products. Therefore, the objective of this study was to assess the efficacy of a combination of HPP and a mix organic acids Inbac^{TM} as hurdles to extend the shelf life of previously sensory optimised low-salt frankfurters and cooked ham from a microbiological and physicochemical point of view".

4.2 Materials and Methods

4.2.1 Materials

Pork oyster meat (90-95% VL), pork silverside and pork fat were obtained from Ballyburden meats, Ballincollig, Cork, Ireland. NaCl, starch, Farina (milled wheat), paprika, Sodium caseinate, tomato powder, Sodium tripolyphosphate hydrated food grade (Carfosel 990, Prayon, Belgium), carmine, Sodium nitrite, Sodium nitrate and Sodium ascorbate were sourced from All in All ingredients (All in All ingredients, Ltd, Ireland). Frankfurter spice and artificial cellulose casings (26 mm) were obtained from Fispak (Fispak Ltd, Ireland) and Viscofan (Viscofan, Spain), respectively. Combivac vacuum pouches (20 polyamide/70 polyethylene bags were obtained from Alcom, Campogalliano, Italy. The barrier characteristics of the vacuum pouches were: oxygen permeability 50 cm^3/m^3/ 24 hr at STP) and water vapour transmission rate 2.2 g/m^2/ 24 hr at STP.

A commercially available salt replacer Artisalt™ (a mix of Potassium chloride 41%, Ammonium chloride 40% and flavour enhancers - yeast extract, onion and celery 19%) and a commercial antimicrobial mix of organic acids Inbac™ (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%,) used in processed meat products were obtained from Chemital (Chemital Ltd, Barcelona, Spain).

4.2.2 Methods

4.2.2.1 Frankfurters manufacture

The formulation of control frankfurters were as follows: pork oyster (65%), pork fat (19%), ice/water (10.15%). Additional ingredients were as follows: NaCl (2%), starch (0.92%), Farina (milled wheat) (0.92%), frankfurter spice (0.5%), paprika (0.5%), Sodium caesinate (0.35%), tomato powder (0.25%), Phosphate (0.25%), Sodium ascorbate (0.05%), Sodium nitrite (0.0075%) and carmine (0.005%). For the manufacture of optimised frankfurters 48% of the NaCl was replaced with Artisalt™ and included 0.3% Inbac™.

Pork meat and pork fat were minced separately through a 3 mm plate using a Talsa mincer (Talsabell, Valencia, Spain).The minced pork meat was placed in a bowl chopper (Seydelmann, Germany) and chopped at low speed for 3 minutes and then the curing ingredients, seasonings and half of the ice were added. The mixture was then chopped for 2 minutes at high speed and the minced pork fat and remaining ice was added and then chopped for a further 2 minutes. The batter was then stuffed into a 26 mm diameter cellulose casings using a Mainca vacuum filler (Mainca, Barcelona, Spain). The frankfurters were hand-linked (~12cm in length) and heat-treated at full steam (100 ºC) in

an electric steam-convection oven (Zanussi Professional, Italy) until an internal temperature of 74 °C was achieved. Final internal end-point temperatures were re-checked using a hand-held food thermometer (Testo, Germany). The frankfurters were rapidly cooled down by immersion in icy cold water (1-2 ºC) for 5 minutes and then stored at 4 °C overnight. Before packaging, the casing of the frankfurters were aseptically removed and 7 frankfurters were placed into a combivac vacuum pouch, vacuum packed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, German) and then stored at 4 °C in a chill room. The treatments used for the shelf life analysis are presented in Table 4.1.

4.2.2.2 Cooked ham manufacture

The treatments used for the shelf life analysis are presented in Table 4.1. The cooked ham was manufactured as previously described in Chapter 2. Briefly, the brine was injected into pork to obtain a 10% weight gain, tumbled at 6rpm for 2 hours, packed into stainless steel moulds and then cooked at full steam (100 °C) until an internal temperature of 74 °C was reached. The cooked hams were cooled down at room temperature, then placed into vacuum pouches, vacuum packed and stored at 4 °C in a chill room.

4.2.2.3 High Pressure Processing

Vacuum-packed frankfurters or cooked ham requiring HPP were removed from the chill room and were HPP at the HPP Tolling facilities (HPP tolling, St. Margaret's, Dublin) using an industrial Hiperbaric 420 litre unit (Burgos, Spain) which uses water as the pressure transmitting medium. The speed of pressurisation was 130 MPa per minute, the speed of depressurisation was instantaneous (~ 1 second) and the holding time was 5

minutes. Initial temperature of the pressure transmitting medium (water) was 10°C and an increase of ~2-3 °C per 100 MPa during HPP due to adiabatic heating was recorded. Optimised low salt samples that required HPP was carried out according to Table 4.1.

Table 4.1 Frankfurter and cooked ham treatments.*

Product	Treatment	Salt replacer (Artisalt™) (%)	HPP (MPa)	Inbac™ (%)
Frankfurters	Control	0	0.1	0
	F-LS/2T	48	580	0.3
	F-LS/1T	48	0.1	0.3
Cooked Ham	Control	0	0.1	0
	H-LS/2T	53	535	0.3
	H-LS/1T	53	0.1	0.3

*Control frankfurter = Untreated frankfurters with 0% Artisalt™ (2% NaCl)

F-LS/2T = Optimised low-salt frankfurters containing 1.04%NaCl+ 0.96% Artisalt™, optimum levels of 2 treatments (a mix of organic acids (0.3% Inbac™) and HPP at 580 MPa for 5 mins).

F-LS/1T = Optimised low-salt frankfurters containing 1.04%NaCl+ 0.96% Artisalt™, optimum levels of 1 treatment (a mix of organic acids (0.3 % Inbac™) without HPP).

Control ham = Untreated ham with 0% Artisalt (2% NaCl)

H-LS/2T = Optimised low-salt ham containing 1.06% Artisalt™ + 0.94% NaCl, optimum levels of 2 treatments (a mix of organic acids (0.3% Inbac™) and HPP at 535 MPa for 5 mins).

H-LS/1T= Optimised low-salt ham containing 1.06% Artisalt™ + 0.94% NaCl, optimum levels of 1 treatment (a mix of organic acids (0.3 % Inbac™) without HPP).

4.2.2.4 Salt content

Salt content was determined as described in Chapter 2. Briefly, a 1/10 dilution of samples was made and filtered before the dip-in probe of the DiCromat II Salt Analyser (The Noramar Co, US) was immersed in the filtrate and the percentage of salt in the sample was read in the instrument display. Each value represents the average of 8 measurements (two independent trials x two samples x two readings per sample).

4.2.2.5 Microbiological analysis

Microbiological analysis was carried out throughout the shelf life. In order to obtain a representative sample, 10 g of sample (frankfurters or cooked ham) was weighed aseptically into a stomacher bag in a vertical laminar-flow cabinet and a primary 10-fold dilution was performed by addition (90 ml) of sterile maximum recovery diluent (Oxoid, Basingstoke, U.K.), stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min and homogenates were 10-fold serially diluted using maximum recovery diluent solution (MRD). For the enumeration of TVC 1 ml of each appropriate dilution was inoculated on duplicated plates in the centre of compact dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan) following incubation at 37 °C for 48 hours. LAB was determined on overlaid de Man Rogosa Sharpe medium (Oxoid), after incubation at 30 °C for 72 hours. *Escherichia coli* (*E. Coli*) and total coliforms were determined using Compact Dry EC plates (Nissui Pharmaceutical, Japan) after incubation at 37ºC for 24 hours. At the start and the end of the shelf life, frankfurters or cooked ham were tested also for the presence or absence of *Salmonella* in Compact dry SL plates (Nissui Pharmaceutical, Co. Ltd., Japan). Compact dry SL is a dry medium for *Salmonella* detection, which contains chromogenic substrate and Novobiocin. The presence of

Salmonella is detected by the combination of different test principles: 1) Alkalinisation of the medium by *Salmonella's* lysine decarboxylase ability (medium colour will change blue purple to yellow) 2) Greening colony caused by decomposition of chromogenic substrate with specific enzyme of *Salmonella* (black colonies are generated by hydrogen sulphide producing *Salmonella*) and 3) motility of *Salmonella* Pre-enrichment process was carried out by weighting 25 g of sample into a sterile filter stomacher bag and then 225 ml of Buffered Peptone water (Oxoid) was added and homogenised with a stomacher for 1 min and incubated at 37 °C for 24 hr. The bag was taken from the incubator and 0.1 ml of enriched specimen was then dropped on the sheet gently 1 cm from the edge of the plate. After inoculation of the enriched culture, 1 ml of sterilized water was dropped at the opposite point were the specimen was dropped. The sterilised water diffused automatically and the sheet was wetted uniformly. The innoculated compact dry SL plates were incubated at 42 °C for 24 hrs. All results (except *Salmonella*) were expressed as $\log_{10}$ colony-forming units (CFU/g). Each value represents the average of 8 measurements (two independent trials x two samples x two readings per sample).

4.2.2.6 pH

The pH of frankfurters or cooked ham was measured using a digital pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the sample. The pH was measured throughout the shelf life and each value represents the average of 8 measurements (two independent trials x two samples x two readings per sample).

4.2.2.7 Texture analysis

Hardness (N) and Springiness (mm) of the cooked hams or frankfurters were determined as previously described in Chapters 2 and 3. Briefly, cylindrical sections of the frankfurter (2.6 cm diameter x 5 cm length) or cooked ham (2.5 cm diameter x 4 cm length) were analysed using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK). The texture was analysed throughout the shelf life and each value represents the average of 8 measurements (two independent trials x two samples x two readings per sample).

4.2.2.8 Colour

Colour of ham was determined as previously described in Chapter 2 while the colour of the cross section of the frankfurter was measured as described in Chapter 3. CIE L*, a* and b* values (Lightness, redness and yellowness, respectively) are reported. Each value represents the average of 12 measurements (two independent trials x two samples x three readings per samples).

4.2.2.9 Sensory evaluation

Sensory analysis was carried out as described in Chapters 2 and 3. To ensure that all samples were safe for consumption, microbiological analysis was carried out before each sensory test. Sensory analysis was carried out at day 1 and at the time when samples reached Log 4 CFU/g of sample which indicated end of shelf life based on the microbiological limit for aerobic plate count (< $5x10^5$ CFU/g of product) (FSAI, 2014). For control samples, sensory analysis for frankfurters was carried out on day 31 while that for control cooked ham was carried out on day 22. For F-LS/1T samples, sensory analysis was carried out on

day 16 while for H-LS/1T samples the sensory analysis was carried out on day 18. For F-LS/2T samples, sensory analysis was carried out on day 72 while for H-LS/2T samples the sensory analysis was carried out on day 55.

Briefly, samples were labelled with a three digit random number, frankfurters were re-heated in a bain marie at 65 ºC and sliced cooked ham was served cold on labelled polystyrene plates. The tested attributes were: Liking of Appearance, Liking of Texture, Liking of Flavour, Juiciness, Tenderness, Saltiness, Off-flavour intensity and Overall acceptability.

4.2.2.10 Lipid oxidation

Throughout storage, lipid oxidation of frankfurters or cooked ham was measured using the 2-thiobarbituric acid (TBARS) assay (Siu and Draper, 1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56 × 105 L mol−1 cm−1 and results were expressed in mg MDA/kg sample. Each value represents the average of 8 readings (two independent trials x two samples x two readings per sample).

4.2.2.11 Statistical analysis

All physicochemical results (colour, texture, TBARS, pH and sensory) were tested using one way ANOVA, sensory data was also analysed using t-test analysis and significance assessed using Tukey's test at 5% significance level using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA). Two independent trials were carried out and all analysis was carried out in duplicate.

4.3 Results and Discussion

4.3.1 Compositional analysis and salt content

The results for proximate composition in our previous chapters (Chapters 2 and 3) in which the same ingredients and formulations were used in the manufacture of frankfurter and cooked hams indicated there were no significant differences in fat, moisture, protein or ash between control and low-salt frankfurters or ham. The total salt content of the low salt frankfurter and cooked ham was 1.3% and 1.4%, respectively, the control frankfurter and cooked ham had significantly higher ($P<0.05$) total salt contents of 2.5% and 2.6%, respectively.

4.3.2 Colour

At day 1, in both low salt frankfurters and cooked ham that were not HPP (F-LS/1T and H-LS/1T) had the lowest L* values; however, the results showed that these differences were not significantly different in the CIE L*, a* and b* values between any of the treatments (Tables 4.2 and 4.3). These results are in agreement with our previous findings (Chapters 2 and 3) where no significant differences on the CIE L*, a* and b* values on the low-salt products compared to control untreated frankfurters or cooked ham. Conversely, Crehan *et al*. (2000) found that salt reduction from 2.5 to 1.5% significantly ($P<0.05$) reduced the redness and yellowness of frankfurters manufactured using HPP raw pork meat. Tobin *et al*. (2013) also reported a paler sausage when salt content was decreased while O' Flynn *et al.* (2014) found that colour in sausages were significantly affected ($P<0.05$) when salt levels were reduced below 1.5% on breakfast sausages manufactured using HPP pork meat. The differences on the colour changes between our study and the studies mentioned above may be due to the fact that in those studies salt content was reduced without the use of any

salt replacer and manufactured using HPP raw meat while in the present study salt replacer Artisalt^{TM} was used and the HPP on both products was carried out after cooking.

During storage time, the colour parameters CIE L*, a* and b* values of the frankfurters did not change significantly in control, F-LS/2T or F-LS/1T. During storage of cooked ham, significant (P<0.05) changes in the CIE L* and a* values were noticed, as control, H-LS/2T and H-LS/1T became lighter (P<0.05) and less red (P<0.05) towards the end of storage time. These results are in agreement with the results reported by Lopez-Lopez *et al*. (2009) who found that storage time had little effect on the lightness of low-fat frankfurters and Garcia-estaban *et al*. (2004) who reported that lightness of vacuum packed cooked ham increased significantly over chilled storage while Parra *et al*. (2010) and Cava *et al*. (2009) found that during chilled storage vacuum packed cooked ham became less red. The changes in the redness during storage of the untreated control, H-LS/2T or H-LS/1T samples may be due to the oxidation of nitrosylmyoglobin as Lindahl *et al*., (2001) reported that colour fading in ham was attributed to the oxidation of nitrosylmyoglobin (MbFe(II)NO) resulting in the formation of metmyoglobin which is primarily responsible for meat browning.

4.3.3 Texture

While in both frankfurters and cooked ham, initially at day 1, the low salt samples that were not HPP (F-LS/1T and H-LS/1T) had the lowest hardness values; however, the results showed that at day 1, these differences in hardness and springiness were not significantly different between any of the treatments assessed (Tables 4.2 and 4.3). No significant differences in hardness or springiness between the low-salt samples with or without HPP may be due to the fact that HPP primarily affects raw meat and causes minimal changes in cooked products (Bansal *et al*., 2015). The results found in this study are in agreement with

our previous findings (Chapters 2 and 3) where hardness and springiness were not significantly different between low-salt and control frankfurters or cooked ham and this may be due to the calculated IS of a 50/50 combination of Artisalt^{TM}/NaCl was similar to that the IS of 2% NaCl. This similar ionic strength resulted in the development of optimised sensory accepted low-salt products without compromising the physiochemical characteristics and sensory acceptability associated with these type of products. Conversely, Corral *et al*. (2013) and Gimeno *et al*. (2001) reported the negative effects of salt reduction on the texture of processed meats; however, these studies did not use HPP or salt replacers such as Artisalt^{TM} which has a similar ionic strength to NaCl (Chapters 2 and 3) which apparently maintained the desired texture of the processed meat products even when the salt content of these products was significantly reduced.

However, during storage, significant changes ($P<0.05$) in the hardness and springiness were noticed resulting in the frankfurters and cooked ham becoming harder and less springy. In cooked ham, while the increase in hardness occurred after 18 days in H-LS/1T, in the case of untreated control ham significantly higher hardness ($P<0.05$) was noticed after 28 days and in H-LS/2T samples the significant increase ($P<0.05$) in hardness was noticed after 42 days. In frankfurters, for untreated control, F-LS/2T and F-LS/1T the increased ($P<0.05$) hardness was noticed after 32 days chilled storage. The increase in hardness during storage may be attributed to the formation of protein cross-links as Herrera (2006) reported that during storage ham can be hardened due to formation of protein cross-links and/or between collagen fibres. The results found in this study are in agreement with the findings of Garcia-estaban *et al*. (2004), Martinez *et al*. (2004), Lopez-Lopez *et al*. (2009) and Silva *et al*. (2014) who reported that the hardness of processed meat products (vacuum packed cooked ham, low-fat frankfurters, salted pork loin, bacon and goat blood sausage) increased significantly ($P<0.05$) over storage time.

4.3.4 pH

In regards to pH, the results showed that in both frankfurters and cooked ham, there were no significant differences between the three treatments on day 1 (Tables 4.2 and 4.3). These results are also in agreement with our previous findings (Chapters 2 and 3) where no significant differences in pH between low-salt and control frankfurters or cooked ham were observed. Similarly, previous studies have reported that increasing salt content did not significantly affect the pH of sausages (Aaslyng *et al.*, 2014; O' Flynn *et al.*, 2014).

Over the storage time, the pH of frankfurters or cooked ham decreased significantly ($P<0.05$) in all treatments. In general, when the main spoilage micro-organism LAB reached ~Log4 in all treatments of cooked ham and frankfurters, the pH began to decrease ($P<0.05$). For frankfurters, this significant ($P<0.05$) decrease in pH began on day 32 for control frankfurters, at day 28 for F-LS/1T and day 70 for F-LS/2T. In ham, significant ($P<0.05$) decrease in pH occurred on day 28 for control samples, day 14 for H-LS/1T and day 56 for H-LS/2T samples.

It was reported that LAB, produce acids such as lactic acid, acetic acid and formic acid; the levels of which depending on genus, species and growth conditions which cause decrease in pH (Borch *et al.*, 1991). The decrease in pH in meat products depends on the presence of fermentable carbohydrate. Pexara *et al*. (2002) noted a drop in the pH of turkey fillets during storage time from the initial 6.2 to 5.5; however; in piroski sausages which contain a lower amount of carbohydrate, the pH decreased at a slower rate than the turkey fillets. In the present work the pH decrease in cooked ham was less than in frankfurters and this is possibly due to a lower carbohydrate content in cooked ham than frankfurters. Han *et al*. (2011) also reported that the pH of vacuum packed untreated and HPP at 400 or 500 MPa cooked ham decreased significantly over storage time.

Table 4.2 Physicochemical changes of frankfurters during storage at 4°C*

	Day 1	Day 14	Day 28	Day 32	Day 40	Day 56	Day 70	Day 80
Lightness (L*)								
Control	71.00 ± 1.04 ^{aA}	71.11 ± 0.74 ^{A}	70.60 ± 0.60 ^{A}	71.42 ± 1.74 ^{A}	70.75 ± 1.34 ^{A}	70.53 ± 0.64 ^{A}	/	/
F-LS/2T	71.07 ± 0.61 ^{aA}	70.99 ± 1.24 ^{A}	70.34 ± 0.90 ^{A}	71.20 ± 1.19 ^{A}	71.16 ± 1.08 ^{A}	71.16 ± 0.97 ^{A}	71.55 ± 0.85 ^{A}	71.36 ± 0.71^{A}
F-LS/1T	70.75 ± 0.70 ^{aA}	70.63 ± 0.72 ^{A}	70.45 ± 1.39 ^{A}	69.18 ± 1.13 ^{A}	/	/	/	/
Redness (a*)								
Control	8.90 ± 0.23 ^{aA}	8.81 ± 0.26 ^{A}	9.10 ± 0.32 ^{A}	8.81 ± 0.30 ^{A}	9.06 ± 0.36 ^{A}	8.91 ± 0.18 ^{A}	/	/
F-LS/2T	8.83 ± 0.29 ^{aA}	8.89 ± 0.48 ^{A}	8.75 ± 0.50 ^{A}	8.92 ± 0.64 ^{A}	9.06 ± 0.46 ^{A}	8.68 ± 0.21 ^{A}	9.02 ± 0.31 ^{A}	8.93 ± 0.29 ^{A}
F-LS/1T	8.63 ± 0.34 ^{aA}	8.71 ± 0.48 ^{A}	9.03 ± 0.29 ^{A}	8.67 ± 0.26 ^{A}	/	/	/	/
Yellowness (b*)								
Control	12.29 ± 0.60 ^{aA}	12.18 ± 0.62 ^{A}	12.51 ± 0.20 ^{A}	12.43 ± 0.24 ^{A}	12.44 ± 0.33^{A}	12.46 ± 0.42 ^{A}	/	/
F-LS/2T	12.86 ± 0.38 ^{aA}	12.48 ± 0.45 ^{A}	12.39 ± 0.21 ^{A}	12.46 ± 0.34 ^{A}	12.50 ± 0.30 ^{A}	12.66 ± 0.49 ^{A}	12.39 ± 0.21^{A}	12.50 ± 0.32 ^{A}
F-LS/1T	12.57±0.69 ^{aA}	13.02 ±0.60 ^{A}	12.72 ± 0.58 ^{A}	13.02 ± 0.60 ^{A}	/	/	/	/
Hardness (N)								
Control	14.10 ± 0.10 ^{aA}	14.15 ± 0.13 ^{A}	14.12 ± 0.11 ^{A}	14.52 ± 0.16 ^{B}	14.50 ± 0.27 ^{B}	14.65 ± 0.29 ^{B}	/	/
F-LS/2T	14.12 ± 0.15 ^{aA}	14.11± 0.18 ^{A}	14.46 ± 0.15 ^{AB}	14.68 ± 0.21 ^{B}	14.74 ± 0.25 ^{B}	14.59 ± 0.34 ^{B}	14.84 ± 0.47 ^{B}	14.81 ± 0.27 ^{B}
F-LS/1T	14.01 ± 0.11 ^{aA}	14.17 ± 0.16 ^{AB}	14.18 ± 0.29 ^{AB}	14.62 ± 0.54 ^{B}	/	/	/	/
Springiness (mm)								
Control	0.854 ± 0.01 ^{aA}	0.861 ± 0.02 ^{A}	0.852 ± 0.01 ^{A}	0.820 ± 0.01 ^{B}	0.821 ± 0.01 ^{B}	0.817 ± 0.01 ^{B}	/	/
F-LS/2T	0.854 ± 0.01 ^{aA}	0.853 ± 0.02 ^{A}	0.853 ± 0.01 ^{A}	0.851 ± 0.02 ^{A}	0.859 ± 0.01 ^{A}	0.825 ± 0.01 ^{B}	0.820 ± 0.01 ^{B}	0.793 ± 0.01 ^{C}
F-LS/1T	0.859 ± 0.02 ^{aA}	0.859 ± 0.01 ^{A}	0.824 ± 0.01 ^{B}	0.821 ± 0.01 ^{B}	/	/	/	/
pH								
Control	5.80 ± 0.05 ^{aA}	5.71 ± 0.06 ^{AB}	5.72 ± 0.04 ^{AB}	5.65 ± 0.07 ^{BC}	5.58 ± 0.05 ^{C}	5.60 ± 0.02 ^{C}	/	//
F-LS/2T	5.83 ± 0.04 ^{aA}	5.81 ± 0.02 ^{A}	5.79 ± 0.04 ^{A}	5.80 ± 0.03 ^{A}	5.78 ± 0.04 ^{A}	5.76 ± 0.02 ^{A}	5.69 ± 0.05 ^{B}	5.61 ± 0.02 ^{C}
F-LS/1T	5.82 ± 0.02 ^{aA}	5.80 ± 0.03 ^{A}	5.71 ± 0.03 ^{B}	5.68 ± 0.03 ^{B}	/	/	/	/

*Values are Mean ± standard deviation.[a] Different lower case superscripts in the same column indicate significant difference ($P < 0.05$) between different treatments.

[A, B, C,] Different capital superscripts in the same row indicate significant differences ($P<0.05$) in the same treatment over time.

(/) indicates analysis was not determined on this day as end of shelf life was reached.

Table 4.3 Physicochemical changes of cooked ham during storage at 4°C*.

	Day 1	Day 14	Day 18	Day 20	Day 28	Day 42	Day 56	Day 70
Lightness (L*)								
Control	61.40 ± 1.11 ^{aAB}	61.10 ± 0.92 ^{A}	61.70 ± 1.07 ^{AB}	62.24 ± 1.02 ^{AB}	62.66 ± 1.56 ^{AB}	63.52 ± 1.40 ^{B}	/	/
H-LS/2T	61.33 ± 0.54 ^{aA}	61.49 ± 0.87^{AB}	61.45 ± 1.23 ^{AB}	61.66 ± 1.01 ^{AB}	61.83 ± 0.79 ^{AB}	62.34 ± 1.19 ^{AB}	62.61± 1.01 ^{AB}	63.39 ± 1.59 ^{B}
H-LS/1T	61.13 ± 0.86 ^{aA}	61.19 ± 0.84 ^{A}	64.60 ± 0.86 ^{B}	63.7 1 ± 1.69 ^{B}	/	/	/	/
Redness (a*)								
Control	13.51 ± 0.96 ^{A}	13.57 ± 1.18 ^{A}	12.18 ± 0.63 ^{AB}	12.27 ± 0.77 ^{AB}	11.58 ± 0.81 ^{B}	11.74 ± 0.58 ^{B}	/	/
H-LS/2T	13.39 ± 0.51 ^{A}	13.56 ± 0.62 ^{A}	12.65 ± 1.16 ^{AB}	12.77 ± 0.82 ^{AB}	12.70 ± 0.38 ^{AB}	12.06 ± 0.77 ^{B}	11.84 ± 0.58 ^{B}	12.14 ± 0.48 ^{B}
H-LS/1T	13.48 ± 1.01 ^{aA}	13.80 ± 0.56 ^{A}	11.89 ± 0.50 ^{B}	12.20 ± 0.62 ^{B}	/	/	/	/
Yellowness (b*)								
Control	7.99 ± 0.87 ^{aA}	8.11 ± 1.24 ^{A}	8.37 ± 1.27 ^{A}	8.33 ± 0.64 ^{A}	7.80 ± 0.75 ^{A}	8.10 ± 0.96 ^{A}	/	-
H-LS/2T	8.23 ± 1.11 ^{aA}	8.06 ± 0.49 ^{A}	8.30 ± 0.35 ^{A}	8.08 ± 0.87 ^{A}	8.29 ± 0.71 ^{A}	7.88 ± 1.14 ^{A}	7.77 ± 0.84^{A}	8.07 ± 0.87 ^{A}
H-LS/1T	8.09 ± 1.07 ^{aA}	8.70 ± 0.59 ^{A}	8.29 ± 0.77 ^{A}	8.18 ± 0.93 ^{A}	/	-	-	-
Hardness (N)								
Control	16.12 ± 0.70 ^{aA}	16.29 ±0.69 ^{A}	16.06 ± 0.53 ^{A}	16.62 ± 0.54 ^{AB}	17.27 ± 0.44 ^{BC}	17.47 ± 0.26 ^{C}	/	/
H-LS/2T	16.24 ± 0.47 ^{aA}	16.26 ± 0.48 ^{A}	16.42 ± 0.52 ^{A}	16.56 ± 0.34 ^{AB}	16.78 ± 0.29 ^{AB}	17.16 ± 0.94 ^{BC}	17.57 ± 0.29 ^{C}	17.54 ± 0.56 ^{C}
H-LS/1T	15.93 ± 0.39 ^{aA}	16.49 ± 0.60 ^{AB}	17.51 ± 0.28 ^{B}	17.45 ± 0.41 ^{B}	/	/	/	/
Springiness (mm)								
Control	0.861 ± 0.01 ^{aA}	0.86 ± 0.01^{A}	0.852 ± 0.01 ^{A}	0.793 ± 0.04 ^{B}	0.765 ± 0.05 ^{B}	0.781 ± 0.03 ^{B}	/	/
H-LS/2T	0.855 ± 0.02 ^{aAB}	0.861 ± 0.01 ^{A}	0.865 ± 0.01 ^{A}	0.83 ± 0.02 ^{B}	0.816 ± 0.05 ^{B}	0.768 ± 0.02 ^{C}	0.758 ± 0.02 ^{C}	0.744 ± 0.02^{C}
H-LS/1T	0.864 ± 0.01 ^{aA}	0.848 ± 0.01 ^{A}	0.788 ± 0.03 ^{B}	0.757 ± 0.02 ^{B}	/	/	/	/
pH								
Control	6.28 ± 0.02 ^{aA}	6.27 ± 0.04 ^{A}	6.28 ± 0.07 ^{A}	6.25 ± 0.03 ^{AB}	6.21 ± 0.02 ^{BC}	6.19 ± 0.02 ^{C}	/	/
H-LS/2T	6.27 ± 0.03 ^{aA}	6.25 ± 0.01	6.25 ± 0.01^{A}	6.27 ± 0.02 ^{A}	6.26 ± 0.02 ^{A}	6.27 ± 0.01 ^{A}	6.17 ± 0.03 ^{B}	6.16 ± 0.02 ^{B}
H-LS/1T	6.29 ± 0.02 ^{aA}	6.19 ± 0.01 ^{B}	6.18 ± 0.02 ^{B}	6.18 ± 0.01 ^{B}	/	/	/	/

*Values are Mean ± standard deviation.[a] Different lower case superscripts in the same column indicate significant difference (P<0.05) between different treatments.

[A, B, C,] Different capital superscripts in the same row indicate significant differences (P<0.05) in the same treatment over time.

(/) indicates analysis was not determined on this day as end of shelf life was reached.

4.3.5 Lipid oxidation

From the sensory point of view, lipid oxidation cause rancidity problems which are considered unpleasant for consumers (Jeremiah, 2001). Lipid oxidation was also reported to be linked to the increase in protein oxidation (Souza *et al*., 2013), the deterioration of texture (Estevez *et al*., 2005) and the discolouration of meat (Faustman and Cassens, 1990; Skibsted *et al*., 1998). The results for lipid oxidation showed that in both frankfurters and cooked ham; at day 1 the low-salt samples which had been HPP (F-LS/2T and H-LS/2T) had the highest TBARS values (Figure 4.1) compared to control and low-salt samples which were not HPP (F-LS/1T and H-LS/1T). This may be due to the use of HPP in the F-LS/2T and H-LS/2T formulations which has been reported that HPP can accelerate lipid oxidation on HPP meat products (Andres *et al*., 2004; Cheah and Ledward, 1995) by triggering intrinsic pro-oxidants such as myoglobin (Medina-Meza *et al*., 2014). The findings on this study are in agreement with the results reported by Núñez *et al.* (2003) who used response surface methodology (RSM) to create models of the changes induced by HPP at 24 to 400 MPa and holding time from 7 to 28 min on lipid oxidation of vacuum-packed slices of dry-cured Iberian ham and pork loin and reported that significantly increased TBARS values were obtained as the pressure level and holding time increased. Cava *et al*. (2002) also reported that pressure level and holding time increased the extent of lipid oxidation in dry-cured Iberian ham and pork loin.

Throughout storage, TBARS increased significantly ($P<0.05$) in untreated control and low-salt frankfurter and cooked ham which were HPP or not HPP (F-LS/2T and H-LS/2T, F-LS/1T and H-LS/1T) samples. While in all frankfurter and cooked ham samples the TBARS values increased significantly during storage, the frankfurters and cooked ham that were HPP (F-LS/2T and H/LS/2T) had higher initial TBARS and also the highest TBARS values throughout storage (Figure 4.1). Independent of the formulation used to manufacture

frankfurters or cooked ham, throughout storage the TBARS values remained below the maximum acceptable limit of 1 mg/kg (Warriss, 2000) which is regarded as the limit beyond which processed meat products will normally develop objectionable odours/tastes. Similar results were reported by Parra *et al*. (2010) and Ospina *et al*. (2015) where TBARS values of dry-cured Iberian ham and frankfurters increased during chilled storage, respectively.

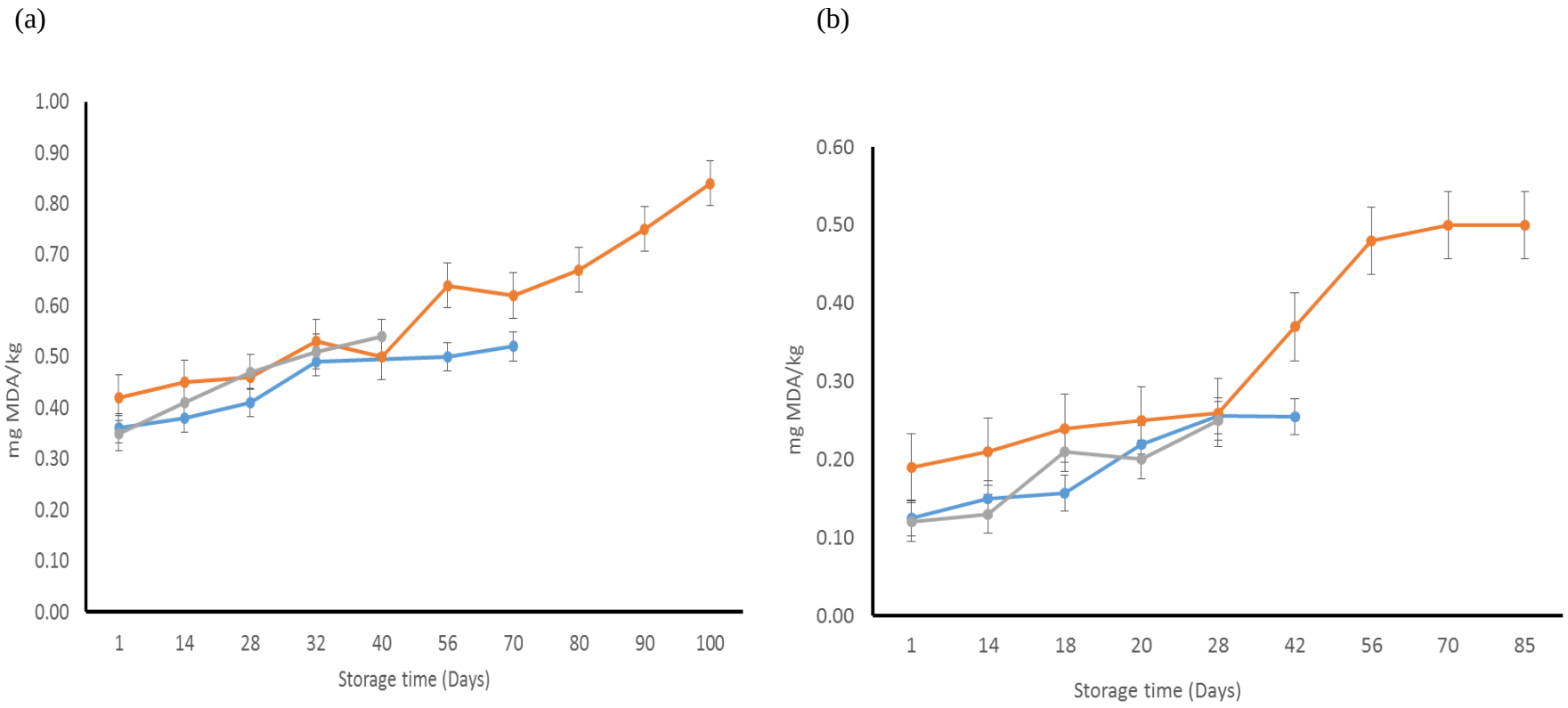

Figure 4.1 – Lipid oxidation (TBARS) of (a) vacuum packed frankfurters; control (—),F-LS/2T (—) and F-LS/1T (—) and (b) vacuum packed ham; control (—), H-LS/2T (—) and H-LS/1T (—) during chilled storage at 4°C. Each point shown is the mean value from two different trials.

4.3.6 Sensory Analysis

Sensory properties of food products are the most important attributes as they are most apparent to consumers (Singham *et al.*, 2015). The results for sensory analysis at day 1 showed that there were no significant differences between any of the treatments of frankfurters or cooked ham (Table 4.4). These results are in agreement with our previous studies (Chapters 2 and 3) where no significant differences in regards to sensory attributes between low-salt and control frankfurters or cooked ham were obtained when optimising the manufacture of these products using RSM. Conversely, authors have reported a decreased sensory acceptability in sausages, frankfurters and cooked ham due to reduced salt content (Crehan *et al.*, 2000; Aaslyng *et al.*, 2014); however, these studies did not use RSM to sensory optimise the manufacture of these products and also did not use salt replacers such as Artisalt^{TM} which contains flavour enhancers.

At the end of storage, the results showed that sensory acceptability was not significantly affected as all sensory attributes (Liking of appearance, Liking of texture, Liking of flavour, Juiciness, Tenderness, Saltiness, Off-flavour intensity and OSA) did not change. These results are in agreement with Sink and Hsu (1979) who reported that storage time generally had little effect on the sensory attributes of frankfurters. Parra *et al.* (2010) and Yanqing *et al.* (2009) also found that sensory attributes of dry cured Iberian ham and smoked cooked ham did not vary significantly throughout storage under chilling conditions.

During storage, TBARS values were below the acceptability limits and sensory acceptability did not change significantly; therefore, the end of shelf life for all frankfurter and cooked ham formulations was determined based on the recommended microbiological limits for cook-chill products.

Table 4.4 – Sensory evaluation of frankfurters and cooked ham during chilled storage*

Sensory Attribute	Frankfurters	Day 1	End of storage	Ham	Day 1	End of storage
Appearance	Control	6.81 ^{aA}	6.85 ^{aA}	Control	6.70 ^{aA}	6.38 ^{aA}
	F- LS/2T	6.78 ^{aA}	6.40 ^{aA}	H-LS/2T	6.43 ^{aA}	6.72 ^{aA}
	F- LS/1T	6.79 ^{aA}	6.62 ^{aA}	H-LS/1T	6.80 ^{aA}	6.70 ^{aA}
Flavour	Control	6.45 ^{aA}	6.70 ^{aA}	Control	6.42 ^{aA}	6.63 ^{aA}
	F- LS/2T	6.40 ^{aA}	6.56 ^{aA}	H-LS/2T	6.47 ^{aA}	6.46 ^{aA}
	F- LS/1T	6.67 ^{aA}	6.47 ^{aA}	H-LS/1T	6.51 ^{aA}	6.70 ^{aA}
Texture	Control	6.71 ^{aA}	6.49 ^{aA}	Control	6.42 ^{aA}	6.34 ^{aA}
	F- LS/2T	6.82 ^{aA}	6.58 ^{aA}	H-LS/2T	6.61 ^{aA}	6.66 ^{aA}
	F- LS/1T	6.55 ^{aA}	6.20 ^{aA}	H-LS/1T	6.54 ^{aA}	6.55 ^{aA}
Saltiness	Control	4.96 ^{aA}	4.96 ^{aA}	Control	5.72 ^{aA}	5.58 ^{aA}
	F- LS/2T	4.66 ^{aA}	4.51 ^{aA}	H-LS/2T	5.43 ^{aA}	5.55 ^{aA}
	F- LS/1T	4.85 ^{aA}	4.79 ^{aA}	H-LS/1T	5.67 ^{aA}	5.59 ^{aA}
Juiciness	Control	6.07 ^{aA}	6.40 ^{aA}	Control	6.15 ^{aA}	6.27 ^{aA}
	F- LS/2T	6.37 ^{aA}	6.12 ^{aA}	H-LS/2T	6.32 ^{aA}	6.15 ^{aA}
	F- LS/1T	6.25 ^{aA}	6.30 ^{aA}	H-LS/1T	6.41 ^{aA}	6.04 ^{aA}
Tenderness	Control	6.27 ^{aA}	6.60 ^{aA}	Control	6.18 ^{aA}	6.42 ^{aA}
	F- LS/2T	6.00 ^{aA}	6.25 ^{aA}	H-LS/2T	6.04 ^{aA}	6.51 ^{aA}
	F- LS/1T	6.30 ^{aA}	6.64 ^{aA}	H-LS/1T	6.28 ^{aA}	6.46 ^{aA}
Off-flavour	Control	1.30 ^{aA}	1.56 ^{aA}	Control	1.48 ^{aA}	1.37 ^{aA}
	F- LS/2T	1.53 ^{aA}	1.62 ^{aA}	H-LS/2T	1.50 ^{aA}	1.44 ^{aA}
	F- LS/1T	1.53 ^{aA}	1.41 ^{aA}	H-LS/1T	1.45 ^{aA}	1.41 ^{aA}
OSA	Control	7.00 ^{aA}	6.85 ^{aA}	Control	7.10 ^{aA}	6.97 ^{aA}
	F- LS/2T	7.13 ^{aA}	7.04 ^{aA}	H-LS/2T	7.06 ^{aA}	6.91 ^{aA}
	F- LS/1T	6.96 ^{aA}	6.79 ^{aA}	H-LS/1T	7.03 ^{aA}	6.69 ^{aA}

*Values are Mean ^{a} Different lower case superscripts in the same column indicate significant difference (P<0.05) between different treatments.
^{A} Different capital superscripts in the same row indicate significant differences (P<0.05) in the same treatment over time.

4.3.7 Microbiological Analysis

The microbiological changes for TVC and LAB during chilled storage (4 °C) in all treatments of vacuum packed frankfurters or cooked ham is shown in Figures 4.2 and 4.3. The following recommended microbiological limits are applied for cook-chill products examined at the point of consumption before reheating or cooking is applied: Aerobic plate counts < $5x10^5$ CFU/g of product; *E. coli*< 10 CFU/g of product; LAB < 10^9 CFU/g of product, *Salmonella*: absent in 25 g of product (FSAI, 2014). For this study, the recommended microbiological limits of acceptability for the frankfurters and cooked ham were set as above with reference to TVC, *E. coli* and *Salmonella*. The initial microbiological quality of all treatments of frankfurters or cooked ham were of good quality with a TVC below the limit of detection <10 CFU/g, *E. coli* < 10 CFU/g and absence of *Salmonella* in 25 g of sample. Throughout storage *Salmonella* and *E.Coli* remained absent.

For frankfurters, the limit of acceptability in terms of TVC for the reformulated low-salt frankfurters which contained the antimicrobial Inbac™ but was not HPP (F-LS/1T) was reached after 31 days of storage. The limit of acceptability in terms of TVC for control frankfurters was reached after 53 days of storage. However, the limit of acceptability in terms of TVC for the low-salt optimised frankfurter manufactured using a combination of HPP and Inbac™ as hurdles (F-LS/2T) was reached after 80 days of storage. These results indicated that F-LS/2T had 51% longer shelf life compared to control samples and 158% longer shelf life than F-LS/1T samples which contained antimicrobial Inbac™ but were not HPP (Figure 4.2a).

For cooked ham, the limit of acceptability in terms of TVC for low-salt cooked ham samples which contained antimicrobial Inbac™ but were not HPP (H-LS/1T) was reached after 18 days of storage. For control cooked ham samples the limit of acceptability was

reached after 32 days of storage and the limit of acceptability in terms of TVC for the low-salt sensory optimised cooked ham manufactured using a combination of antimicrobial Inbac™ and HPP as hurdles (H-LS/2T) was reached after 63 days of storage. These results indicated that H-LS/2T samples had 97% longer shelf life than control samples and 250% longer shelf life than H-LS/1T cooked ham samples which contained antimicrobial Inbac™ but were not HPP (Figure 4.3a). Overall, these results indicated the effectiveness of the combined effect of HPP and a mix of organic acids in enhancing the safety and shelf life of processed meat products which contained significantly low salt content and that the combined effect of the hurdles used can compensate the preservation effect lost due to salt reduction.

Previous studies conducted on cooked ready to eat products indicated that HPP can significantly extend shelf-life of vacuum-packed meat products such as wieners, turkey breast ham, cooked pork ham, dry-cured ham and marinated beef loin. (Pietrzak *et al*., 2007; Jofré *et al.*, 2009; Han *et al*., 2011; Vercammen *et al*., 2011; Myers *et al*., 2013; Oliveira *et al*., 2015).

Apparently, the main spoilage micro-organism in all frankfurter and cooked ham treatments was LAB (Figures 4.2b and 4.3b) which increased significantly ($P<0.05$) over storage time at a rate similar to TVC. It is well known that LAB is the major group associated with spoilage of refrigerated vacuum or modified atmosphere packed cooked meat products (Korkeala and Björkroth, 1997) and vacuum packed HPP meat products (Pietrasik *et al*., 2017; Yanqing *et al*., 2009) Pietrasik *et al*. (2017) reported that HPP at 600MPa resulted in the TVC and LAB of wieners remaining below the limit of detection for 12 weeks; however, for control samples LAB reached 7 Log (CFU/g) after 8 weeks of storage. Yanqing *et al*. (2009) examined the shelf life of HPP smoked ham and found that untreated samples were spoiled by LAB after 2 weeks of refrigerated storage; however, the shelf-life

of smoked ham HPP at 400 or 600 MPa was extended to 8 or 10 weeks, respectively. Vercammen *et al.* (2011) used a combination of HPP at 600 MPa at 10 °C for 10 min and natural antimicrobials (Caprylic acid (0.15%) or Purasal® (2.5%)) as hurdles to enhance the shelf life of sliced cooked ham. The results showed that untreated sliced ham with or without antimicrobials reached 6 log (CFU/g) after 40 days and HPP further delayed this initiation of spoilage to 59 days in absence of antimicrobials; however the sliced ham that were HPP and also contained either Caprylic acid or Purasal® remained < 1 log (CFU/g) up to 84 days. The authors indicated that this was due to the synergetic effect of these two hurdles.

While the shelf life in the study reported by Vercammen *et al.* (2011) which applied the hurdles HPP and organic acids was longer than the shelf life obtained in the present study; the differences may be due the higher pressure level and holding time applied as it is known that the effect on the microbiological load is affected significantly by these parameters. Our group also have demonstrated the synergetic interaction of HPP and a mix of organic acids as hurdles extending the shelf-life of skinless chicken breast fillets up to four weeks (Rodriguez-Calleja *et al*., 2012). This results confirms the potential utility of the hurdle strategy for improving the shelf-life and safety of low-salt processed meat products.

The results of this study indicated that a combined effect of HPP at 580 MPa or 535 MPa for 5 min and Inbac™ (0.3%) for frankfurters and cooked ham, respectively, were a feasible alternative for the preservation of low-salt frankfurters and cooked ham compared to control samples which contained full salt content and the preservative effects of salt.

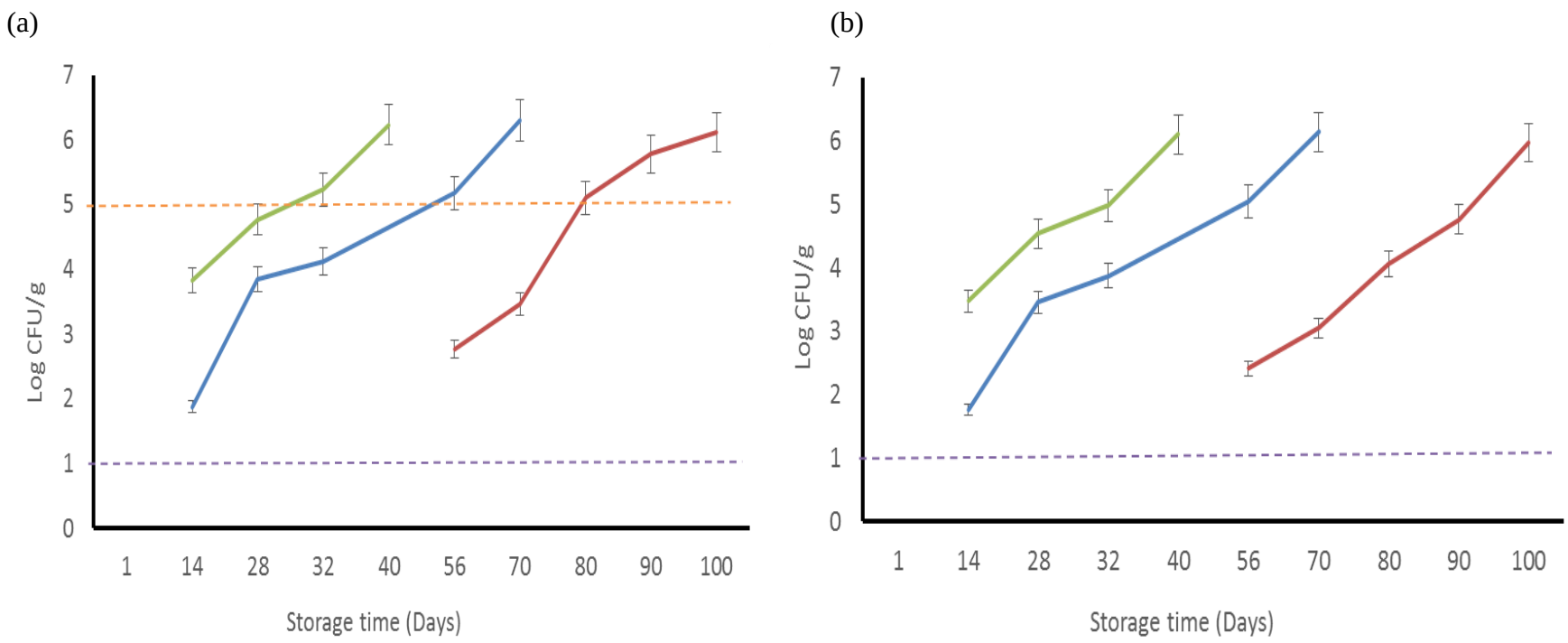

Figure 4.2 Microbiological changes (a) TVC and (b) LAB of Control (—), F-LS/2T (—) and F-LS/1T (—) vacuum packed frankfurters during chilled storage at 4°C. Each point shown is the mean value from two different trials. The dotted lines show the limits of detection (—) or acceptability (—).

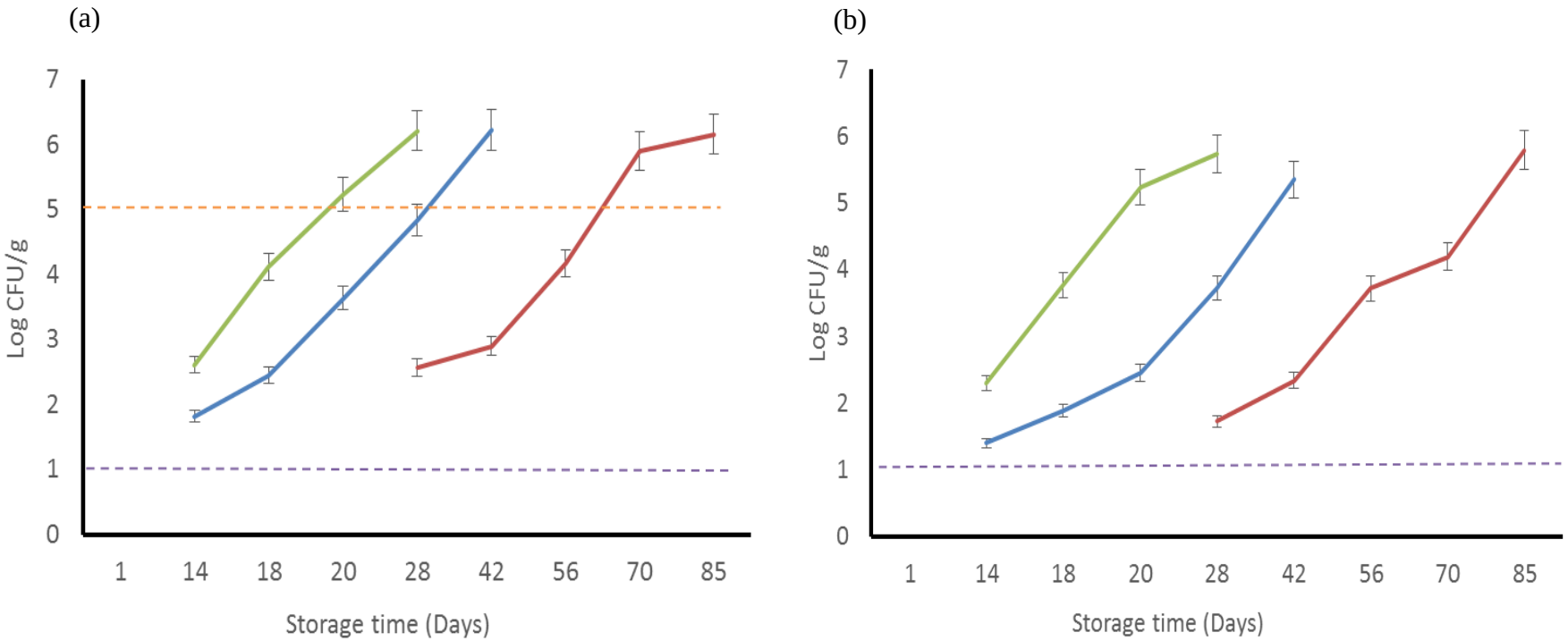

Figure 4.3 - Microbiological changes (a) TVC and (b) LAB of Control (—), H-LS/2T (—) and H-LS/1T (—) vacuum packed cooked ham during chilled storage at 4°C. Each point shown is the mean value from two different trials. The dotted lines show the limits of detection (—) or acceptability (—).

4.4 Conclusion

Throughout the storage, most physicochemical characteristics of frankfurters or cooked ham changed significantly ($P<0.05$). However, regardless of the physicochemical changes, the OSA of the frankfurters or cooked ham was not reduced over storage time. In both processed meat products, independent of the formulation, LAB apparently was the main spoilage micro-organism.

The need for meat processors to reformulate processed meat with lower NaCl levels is an urgent requirement. However, as NaCl is an excellent microbial preservative and enhances microbial safety of meat products, when NaCl levels are reduced a major microbial hurdle is removed. The results found in this study indicated that the optimum combination of HPP and a mix of organic acids Inbac^{TM} compensated for the significant salt reduction and extended ($P<0.05$) the shelf life of low salt frankfurters by 51% and low salt cooked ham by 97% compared to control samples which contained significantly ($P<0.05$) higher NaCl content. These results indicate the potential use of the hurdle approach for improving the shelf-life and safety of low-salt processed meat products.

CHAPTER 5

Comparative study on the acceptability and consumer appeal of commercial products and research optimised low-salt frankfurters and cooked ham manufactured using high pressure processing and organic acids.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

The objective of this study was to assess the acceptability and consumer (n=100) appeal of research optimised low-salt (ROLS) frankfurters or cooked ham manufactured using high pressure processing (HPP) and organic acids as hurdles and compared to research control and gold standard commercially available products. For frankfurters, consumers preferred the firmness and saltiness of the ROLS and research control frankfurters while the flavour and juiciness of commercial frankfurters was preferred. In terms of overall sensory acceptability (OSA), the ROLS frankfurter was liked just as much as the commercial brand frankfurter. For cooked ham, the appearance and firmness of ROLS and research control cooked ham was preferred while the juiciness of the commercial cooked ham was preferred. Consumers did not find significant differences in flavour, saltiness or OSA and the ROLS cooked ham was liked just as much as the commercial brand cooked ham. Overall, these results indicate that the ROLS processed meat products were just as acceptable or better than the gold standard commercially available products confirming the potential of the use of response surface methodology to optimise salt replacer Artisalt™, HPP and organic acids to manufacture consumer accepted low-salt processed meat products with enhanced safety and shelf life.

5.1 Introduction

Consumers are becoming more and more aware of the link between diet and health (Grasso *et al*., 2014). As a result, there is an increasing demand in the meat industry for minimally processed foods which are lower in salt, preservatives, fat and calories, whilst maintaining good-quality products in regards to shelf life, physicochemical, nutritional and sensory characteristics (Weiss *et al*., 2010). The effects of high sodium on blood pressure, and consequently on the risk of cardiovascular disease and various other diseases, have been well documented (Aburto *et al*., 2013; Morgan *et al*., 2001). The general concern in regards to sodium intake from the diet has led to the development of methods to reduce the amount of salt added to products, as well as intensive reformulation of product recipes employing salt replacers to help reduce the quantities of salt used in a range of prepared foods (Doyle and Glass, 2010; Pietrasik and Gaudette, 2014). In Europe, North America and Australia, around 70% of consumed salt comes from processed foods, among which 20% is derived from meat products (Ruusunen and Puolanne, 2005). The main strategies used for salt reduction in processed meat products include product reformulation, compensation by the use of substitutes, use of saltiness enhancers and the use of salt replacers (Kilcast and Angus, 2007). It was also reported that high pressure processing (HPP) can maintain or improve protein functionality where it is desired to reduce the sodium content of processed meats (Mújica,-Paz *et al*,, 2011; Cheftel and Culioli, 1997) and improve safety when applied as one of the hurdles in the hurdle preservation technology (Rodriguez-Calleja *et al*., 2012).

When introducing new technologies in food processing, consumer opinion plays an increasing role (Lyndhurst, 2009). In the past few years, research organisations and social media have been actively working to promote consumer awareness about the newer food processing technologies and associated benefits related to their health and convenience

aspects. Recent reports have indicated positive responses from consumers who are ready to accept the foods that are being processed by novel processing techniques such as HPP (Sorenson *et al*., 2011). Butz *et al*. (2003) surveyed 3000 consumers in France, Germany and UK in relation to their perceptions of HPP and reported that HPP was acceptable to the majority of consumers in France and Germany; however, it was important that the product price does not exceed that of conventional products and that there is also a health benefit.

In our previous chapters (Chapters 2, 3 and 4) sensory accepted ROLS frankfurters and cooked ham were developed through the application of response surface methodology (RSM). The optimum parameters to maximize the OSA of frankfurters were salt replacer Artisalt™ (48%), HPP (580MPa) and Inbac™ (0.3%) while for cooked ham the optimised parameters were salt replacer Artisalt™ (53%), HPP (535MPa) and Inbac™ (0.3%). In those studies, salt was significantly ($P<0.05$) reduced in frankfurters or cooked ham using the salt replacer Artisalt™ to 1.3% or 1.4% total salt, respectively, compared to control frankfurters or cooked ham which contained 2.5% and 2.6% total salt, respectively. From the microbiological point of view, the combined effect of HPP and a mix of organic acids Inbac™ compensated for the significant salt reduction and extended ($P<0.05$) the shelf life of ROLS frankfurters by 51% and ROLS cooked ham by 97% compared to control samples which contained significantly ($P<0.05$) higher salt content. In those studies, sensory accepted ROLS frankfurters and cooked ham with enhanced safety and shelf life were successfully developed using RSM; however, consumer acceptance of these ROLS processed meat products were not investigated.

Many authors who have employed various salt reduction strategies achieved salt reductions between 30-50% and reported that significantly salt reduced meat products were just as acceptable as full salt content products in terms of physicochemical and sensory characteristics (Guardia *et al*., 2008; Skogsberg, 2017; Aliño *et al*., 2009; Dos Santos *et*

al., 2014; Fellendorf *et al*., 2012; Dimitrakopoulou *et al*., 2005; Aaslyng *et al*., 2014). However, none of the studies highlighted above carried out an optimisation process to develop low-salt products using RSM nor did they use the hurdle approach to compensate the loss of microbiological safety due to significant salt reduction nor did carried out a consumer study on the developed low-salt products to confirm acceptability and consumer appeal.

While Guardia *et al*. (2006, 2008), Pietrasik and Gaudette (2014) and Pietrasik *et al*. (2016) carried out consumer studies on research developed low-salt fermented sausages and cooked ham and compared to full salt control samples, they did not compare to commercially available products. Pietrasik *et al*. (2016) used HPP to enhance the quality and shelf life of reduced sodium restructured cooked hams manufactured using modified Potassium Chloride (KCl) as a salt replacer and found that the appearance, flavour, saltiness, texture, juiciness, aftertaste and OSA of the low-salt cooked ham which contained 1.4% Sodium Chloride (NaCl) was significantly less acceptable than the control cooked ham which contained 2.4% NaCl. The lower acceptability of the low salt cooked ham was attributed to the composition of the salt replacer KCl which induced bitterness. The authors also reported no significant differences in consumer acceptability on hams that were subjected to HPP compared to untreated hams indicating that HPP can effectively extend shelf-life of the restructured ham without compromising eating quality. In a different study, Pietrzak *et al*. (2007) reported that HPP did not affect significantly the colour, smell, taste, and consistency of cooked ham which contained low or regular salt.

To the best of our knowledge, there are no consumer studies comparing ROLS processed meat products with enhanced safety and shelf life manufactured using the hurdle approach (combination of HPP (at commercial level) and a mix of organic acids) to research control samples and gold standard commercially available processed meat products. Therefore, the

objective of this study was to assess the acceptability and consumer appeal of previously sensory optimised low-salt frankfurters and cooked ham with enhanced safety and shelf life compared to research control and commercial gold standard frankfurters and cooked ham products available in the Irish market.

5.2 Materials and Methods

5.2.1 Materials

Pork oyster meat (90-95% VL), Pork silverside and pork fat were obtained from Ballyburden meats, Ballincollig, Cork. NaCl, starch, milled wheat, paprika, Sodium caseinate, tomato powder, carmine, Sodium nitrite, Sodium nitrate, Sodium ascorbate and Sodium tripolyphosphate hydrated food grade, Carfosel 990 (Prayon, Belgium) were obtained from All in All ingredients (All in All ingredients Ltd, Dublin). Frankfurter spice and artificial cellulose casings (26mm) were obtained from Fispak (Fispak Ltd, Ireland) and Viscofan (Viscofan, Spain), respectively.

A commercially available salt replacer used in processed meat products Artisalt™ (a mix of Potassium chloride 41%, Ammonium chloride 40% and flavour enhancers - yeast extract, onion and celery 19%) and a commercial antimicrobial Inbac™ (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%,) were obtained from Chemital Ltd (Chemital Ltd, Barcelona, Spain).

5.2.2 Methods

5.2.2.1 Frankfurter manufacture

The frankfurters were manufactured as described in Chapter 3. Briefly, minced pork meat and pork fat were mixed in a bowl chopper with the curing and seasoning ingredients and ice, chopped for 7 minutes, stuffed into 26 mm diameter cellulose casings, hand-linked and cooked at full steam until an internal temperature of 74 °C was achieved. The frankfurters were cooled down in ice water and placed into vacuum pouches, vacuum packed and stored at 4°C in a chill room Research frankfurter treatments and commercial frankfurters used in this study are shown in Table 5.1.

5.2.2.2 Cooked ham manufacture

The cooked ham was manufactured as previously described by in Chapter 2. Briefly, the brine was injected into pork meat to obtain a 10% weight gain, tumbled at 6 rpm for 2 hours, packed into stainless steel moulds and then cooked at full steam (100 °C) until an internal temperature of 74 °C was reached. The cooked hams were cooled down at room temperature, placed into vacuum pouches, vacuum packed and stored at 4 °C in a chill room. Research cooked ham treatments and commercial cooked ham used in this study are shown in Table 5.1.

5.2.2.3 Salt content

Salt content of the ROLS frankfurters, cooked ham and commercial products was determined as described in Chapter 2 and the results are shown in Table 5.1. Briefly, a 1/10 dilution of each sample was made and the dip-in probe of the DiCromat II Salt Analyser was immersed in the filtrate and the percentage of salt in the sample was read in the

instrument display. Each value represents the average of 8 measurements (four samples x two readings).

5.2.2.4 High Pressure Processing

Vacuum-packed frankfurters or cooked ham were HPP according to Table 5.1 at the HPP Tolling facilities (HPP tolling, St. Margaret's, Dublin) using an industrial Hiperbaric 420 litre unit (Burgos, Spain) which uses water as the pressure transmitting medium. The speed of pressurisation was 130 MPa per minute, the speed of depressurisation was instantaneous (~ 1 second) and the holding time was 5 minutes. The temperature of the pressure transmitting medium (water) was 10°C. The initial temperature of the low-salt frankfurters and cooked ham before HPP was 3.4 °C and 2.9ºC respectively, and the final temperature measured after HPP ranged from 6.0 - 6.5 °C.

5.2.2.5 Commercial frankfurters and cooked ham

Leading commercially available frankfurters (Herta™, Germany) and pre sliced cooked ham (Tesco Deli Style ™, Ireland) were purchased in Tesco, Ireland.

Table 5.1 The optimum parameters for the manufacture of research optimised low-salt, research control frankfurters or cooked ham and total salt content of frankfurters and cooked ham including commercial products.

Product		Salt replacement (%)	HPP (MPa)	Inbac™ (%)	Total Salt content (%)
Frankfurters	Research control	0	0.1	0	2.5
	ROLS	48	580	0.3	1.3
	Commercial	N/A	N/A	N/A	2.0
Cooked Ham	Research control	0	0.1	0	2.6
	ROLS	53	535	0.3	1.4
	Commercial	N/A	N/A	N/A	2.3

N/A – Not applicable

5.2.2.6 Sensory analysis

Consumers (students and staff of University College Cork) were recruited via e-mail based on their availability. Consumers (Female n=54, Male n=46) of various age groups, nationalities, occupations and incomes (Table 5.2) participated in this consumer study which took place in the sensory booths at the School of Food and Nutritional Sciences, University College Cork, Ireland. ROLS, research control and commercial brand frankfurters were cut in half and re-heated in a bain-marie at 65ºC. Frankfurter samples were then assigned a three digit random number and served warm on labelled polystyrene plates. ROLS and research control cooked ham samples were sliced at a thickness of 3mm using a slicer (Scharfen G330F, Avery Berkel). Cooked ham samples were assigned a three digit random number and served cold on labelled polystyrene plates. The nine point hedonic scale was used for consumer analysis and the tested attributes were: Appearance (1= unacceptable, 7= excellent), Flavour (1= unacceptable, 7= excellent), Firmness (1= very mushy, 7= very firm), Juiciness (1= very dry, 7= very juicy), Saltiness (1= not salty, 7= extremely salty), and OSA (1= unacceptable, 7= excellent). Rating questions included – How does this compare to your normal brand? (1= no comparison, 6= much better), Would you buy/eat this again? (1= definitely not, 5= definitely) and Which sample did you prefer?

5.2.2.7 Statistical Analysis

Consumer data was analysed by crosstabulation and one way ANOVA using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA). For one way ANOVA, the consumer responses corresponding to each attribute/question were converted to numerical format and significance was assessed using Tukey's test at 5% significance level. Principle component analysis (PCA) was carried out using the Unscrambler software package version 10.3 (CAMO ASA, Trondheim, Norway) with the

X-matrix designated as the various treatments of frankfurters and cooked ham and the Y-matrix designated as the sensory responses.

5.3 Results and Discussion

5.3.1 Consumer demographics

The consumer demographics are shown in Table 5.2. The consumers age ranged between 20-60 years old; with 61 or 39 % of the consumers under or over 40 years old, respectively. While Irish people accounted for 63% of consumers, 37% were of various other nationalities primarily French. In regards to occupation, the majority of consumers were students and researchers. Over half of consumers earned >50k per year with only 11% earning <20k per year. When asked how often do you eat cooked ham, the majority (82%) of consumers chose 'weekly' or more frequently. When asked how often do you eat frankfurters the majority (73%) of consumers chose 'fortnightly' or less frequently while only 27% of consumer stated they eat frankfurters weekly or more often.

Table 5.2 - Consumer demographics (%) and frequency of consumption.

Age range	**%**
20-25	26
26-30	9
31-35	19
36-40	7
41-45	12
46-50	10
51-55	4
56-60	8
60 +	5
Nationality	**%**
Irish	63
French	15
Spanish	4
English	3
Brazilian	3
German	3
Other	9
Occupation	**%**
Student	36
Teacher	7
Researcher	20
Administrator	15
Other	23
Income	**%**
<20k	11
20-50k	35
>50k	54
How often do you eat frankfurters?	**%**
4-5 times per week	5
2-3 times per week	4
Weekly	18
Fortnightly	4
Monthly	58
Very rarely	11
How often do you eat cooked ham?	**%**
Daily	8
4-5 times per week	16
2-3 times per week	26
Weekly	32
Fortnightly	9
Monthly	9

5.3.2 Consumer results for frankfurters

The consumer assessment of the sensory attributes of frankfurters are shown in Table 5.3 while consumer rating of the frankfurters are shown in Figures 5.1a and 5.2a. The statistical analysis of the sensory attributes of frankfurters is shown in Table 5.4. The results for appearance indicated that ≤10% of consumers rated the appearance of the frankfurters (ROLS, research control and commercial) below 'average'. The majority (≥64%) of consumers described frankfurters (ROLS, research control and commercial) as 'good', 'very good' or 'excellent' (Table 5.3). The statistical analysis indicated there were no significant differences on the appearance between ROLS, research control or commercial frankfurters (Table 5.4). For flavour, ≤9% of consumers rated the frankfurters (ROLS, research control and commercial) below 'Average' while the majority of consumers 73% and 68% described the ROLS and research control frankfurters, respectively as 'good' or 'very good'; however, 64% of consumers described the commercial frankfurter as 'very good' or 'excellent' (Table 5.3). The statistical analysis indicated that the flavour of the commercial frankfurters was the most preferred ($P<0.05$) compared to ROLS and research control frankfurters (Table 5.4) and this may be due to the fact that the commercial frankfurters were smoked which may have enhanced flavour acceptability. In regards to firmness, 89% and 72% of consumers described the ROLS and research control frankfurters, respectively, as 'just right', 'slightly firm' or 'firm' compared to 51% of consumers who used these terms to describe the commercial frankfurters. It was also highlighted by 39% of consumers that the commercial frankfurters were 'slightly mushy', 'mushy or 'very mushy' while only 12% and 16% of consumers used these terms to describe the ROLS and research control frankfurters, respectively (Table 5.3). The statistical analysis indicated that the firmness of the ROLS and research control frankfurters were preferred ($P<0.05$) to the commercial frankfurters (Table 5.4). The results for

juiciness showed that the majority (58% and 54%) of consumers described the ROLS and research control frankfurters, respectively, as ‘slightly dry’ or ‘just right’, while the majority of consumers (82%) described the commercial frankfurter as ‘slightly juicy’, ‘juicy’ or ‘very juicy’ (Table 5.3). The statistical analysis indicated that the commercial frankfurter was significantly ($P<0.05$) juicier than the ROLS and research control frankfurters (Table 5.4).

For saltiness, the majority of consumers (64% and 67%) described as ‘moderately salty’ or ‘just right’ the ROLS and research control frankfurters, respectively, while the majority of consumers (74%) described the commercial frankfurters as ‘quite salty’, ‘very salty’ or ‘extremely salty’ (Table 5.3). The statistical analysis indicated that the commercial frankfurter was perceived to be significantly ($P<0.05$) saltier than the ROLS and research control frankfurters (Table 5.4). Contradictory to these results, the commercial frankfurter had a lower salt content (2.0%) compared to the research control which contained 2.5% salt; however, consumers may have perceived the commercial frankfurters to be saltier as they were juicier ($P<0.05$) which may have increased the saltiness perception. Similar results were found by NIZO researchers (NIZO, 2018) who reported that increasing juiciness in sausages resulted in enhancement of the saltiness perception. Consumers did not find significant differences in the perception of saltiness between the ROLS and research control frankfurters even though the ROLS frankfurter contained 48% less salt and this may be due to the ingredients contained in the salt replacer Artisalt^{TM} including flavour enhancers such as yeast extract, onion and celery.

The results for OSA showed that ≤15% of consumers disliked (extremely, very much or moderately) the frankfurters (ROLS, research control and commercial). The majority of consumers 83%, 79% and 77% liked (moderately, very much or extremely) the ROLS, research control and commercial frankfurters, respectively (Table 5.3) and as a result the

statistical analysis showed that there were no significant differences in OSA between ROLS, research control and commercial frankfurters (Table 5.4).

The consumer results found in this study are in agreement with our previous results (Chapter 3) where the development of sensory accepted optimised low salt frankfurters was carried out by semi trained panellists and no significant differences in appearance, flavour, juiciness, texture, saltiness or OSA between ROLS and research control frankfurters was reported. This was attributed to the calculated ionic strength (IS) of a 50/50 combination of Artisalt^{TM} /NaCl (0.31M) which was similar to that the IS of 2 % NaCl (0.34M) and resulted in the development of ROLS frankfurters without compromising the physiochemical characteristics and sensory acceptability compared to full salt control samples. The salt replacer Artisalt^{TM} also contained flavour enhancers such as yeast extract, onion and celery which may have enhanced saltiness perception. Flavours enhancers have been shown to improve the sensory acceptability of low-salt meat products (Desmond, 2006; Dos Santos *et al*., 2014).

When consumers were asked to compare the frankfurters to their usual brand, 74%, 64% and 62% of consumers stated that the ROLS, research control and commercial frankfurters, respectively, were 'just as good', 'slightly better' or 'much better' than their usual brand (Figure 5.1a). When asked if they would buy or eat this product again, 70%, 58% and 66% of consumers said they probably or definitely would buy or eat again the ROLS, research control and commercial frankfurters, respectively (Figure 5.2a). When asked 'which sample do you prefer', 39% of consumers preferred the ROLS frankfurter, 39% preferred the commercial frankfurter and 22% preferred the research control frankfurter (Figure 5.3a).

The PCA plot (Figure 5.4a) is a graphical representation of the degree of existing correlations between the frankfurter samples, the measured sensory responses and

comparison to usual brand and purchase intent. The plot showed that the ROLS and research control frankfurters were closely related to each other and also to the sensory attribute of firmness. The commercial frankfurters were closely related to flavour, juiciness and saltiness and to a lesser extent they were related to appearance. The plot also indicated that the OSA was closely related to comparison to usual brand; would you buy/eat again? and appearance of the sample.

Overall, the firmness and saltiness of the ROLS and research control frankfurters were preferred by the consumer while the flavour and juiciness of the commercial frankfurters were preferred; however, no significant differences in the appearance, OSA and rating questions; would you buy/eat this again? and comparison to usual brand were noticed between ROLS, research control or commercial frankfurters. It is also important to highlight that consumers did not detect differences in saltiness between the research control and ROLS frankfurters which contained 48% less added salt. These results indicated that the ROLS frankfurters were just as acceptable to consumers as the gold standard commercially available frankfurter confirming the potential of the use of the salt replacer Artisalt™, HPP and a mix of organic acids Inbac™ to manufacture consumer accepted low-salt frankfurters with enhanced safety and shelf life.

Similarly, Guardia *et al*. (2006) reported that from a sensorial point of view it was possible to reduce NaCl in small calibre fermented sausages by 50% using salt replacers (50% KCl or 40% KCl + 10% of Potassium-lactate) and obtain a product acceptable to consumers; however, while consumer acceptability was achieved, compensation for compromising microbial stability due to significant salt reduction was not assessed.

Table 5.3 Consumer hedonic scores (%) on the sensory attributes of frankfurters*

				Appearance			
	Unacceptable	Poor	Fair	Average	Good	Very good	Excellent
ROLS	0	1	5	17	25	42	10
Research control	0	4	6	26	26	32	6
Commercial	0	3	3	17	23	32	22
				Flavour			
	Unacceptable	Poor	Fair	Average	Good	Very good	Excellent
ROLS	1	3	3	12	38	35	8
Research control	0	4	3	20	43	25	5
Commercial	2	5	2	7	25	34	30
				Firmness			
	Very Mushy	Mushy	Slightly Mushy	Just right	Slightly Firm	Firm	Very Firm
ROLS	0	4	8	36	33	20	5
Research control	0	13	3	31	18	23	12
Commercial	2	25	12	13	15	23	10
				Juiciness			
	Very Dry	Dry	Slightly Dry	Just right	Slightly Juicy	Juicy	Very Juicy
ROLS	0	2	24	34	16	23	1
Research control	2	13	25	29	6	24	1
Commercial	0	0	5	13	25	15	42
				Saltiness			
	Not Salty	Slightly Salty	Moderately Salty	Just right	Quite Salty	Very Salty	Extremely Salty
ROLS	5	9	10	54	20	2	0
Research control	6	2	19	48	19	6	0
Commercial	0	0	4	22	32	29	13
				OSA			
	Extremely Dislike	Very Much Dislike	Moderately Dislike	Neither Like nor Dislike	Moderat ely Like	Very Much Like	Extremely Like
ROLS	0	4	4	9	35	40	8
Research control	1	6	6	10	35	31	13
Commercial	2	5	8	8	23	37	17

*Results are expressed as %.

Table 5.4 Consumer hedonic scores for the sensory attributes and comparison to usual brand and purchase intent scores of frankfurters*

	Appearance	Flavour	Firmness	Juiciness	Saltiness	OSA	Comparison to usual brand	Would you buy/eat again?
ROLS	5.32 ± 1.39 [a]	4.61 ± 1.14 [a]	4.97 ± 1.09 [a]	4.1 ± 1.09 [a]	3.81 ± 1.07 [a]	5.27 ± 1.12 [a]	4.1 ± 1.25 [a]	3.67 ± 1.1 [a]
Research control	4.95 ± 1.20 [a]	4.66 ± 1.07 [a]	4.83 ± 1.34 [a]	3.8 ± 1.26 [a]	3.89 ± 1.11 [a]	4.77 ± 1.28 [a]	3.83 ± 1.23 [a]	3.45 ± 1.11 [a]
Commercial	5.44 ± 1.41 [a]	5.78 ± 1.12 [b]	4.45 ± 1.47 [b]	5.89 ± 1.24 [b]	5.27 ± 1.07 [b]	5.22 ± 1.47 [a]	3.71 ± 1.43 [a]	3.61 ± 1.44 [a]

*Values are mean (n=100) ± standard deviation [a, b]. Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

5.3.2 Consumer results for cooked ham

The consumer assessment of the sensory attributes of cooked ham are shown in Table 5.5 while consumer rating of the cooked ham are shown in Figure 5.1b and 5.2b. The statistical analysis of the sensory attributes of cooked ham is shown in Table 5.6. In terms of appearance, 10% of consumers rated the ROLS and research control cooked ham as below 'average' compared to 30% of consumers who described the commercial ham as below 'average'. Furthermore, the majority of consumers (73%) described the ROLS and research control cooked ham samples as 'good', 'very good' or 'excellent' whereas only 43% of consumers used these terms to describe the commercial cooked ham (Table 5.5). The statistical analysis indicated that the commercial cooked ham was significantly ($P<0.05$) less acceptable than the ROLS and research control cooked ham in terms of appearance (Table 5.6). For flavour, ≤16% of consumers rated the cooked ham (ROLS, research control and commercial) as below 'average'. The majority of consumers (74%, 81% and 66%) described the ROLS, research control and commercial cooked ham, respectively, as 'good', 'very good' or 'excellent' (Table 5.5). The statistical analysis indicated that no significant differences in flavour were observed between the cooked ham samples (Table 5.6).

The results for firmness showed that the majority (73% and 83%) of consumers described the ROLS and research control cooked ham, respectively, as 'slightly firm', 'firm', or 'very firm' compared to 24% of consumers who used these terms to describe the commercial cooked ham (Table 5.5). The commercial cooked ham was also described as'slightly mushy' or 'mushy' by 42% of consumers. The statistical analysis indicated that significant differences ($P<0.05$) in firmness were observed between the research control and ROLS cooked ham in comparison to the commercial cooked ham (Table 5.6). In terms of juiciness, the majority (51% and 59%) of consumers described the ROLS and research control cooked ham as 'just right' or 'slightly dry'; however, the majority (70%) of consumers described

the commercial cooked ham as ‘slightly juicy’, ‘juicy’ or ‘very juicy’ (Table 5.5). The statistical analysis indicated that significant differences ($P<0.05$) in juiciness were observed between the research control and ROLS cooked ham in comparison to the commercial cooked ham (Table 5.6).

The saltiness was described to be ‘slightly salty’ ‘just right’ or ‘quite salty’ by the majority of consumers (67%, 78% and 65%) in ROLS, research control and commercial cooked ham, respectively, (Table 5.5) and consequently no significant differences were found between cooked ham samples in this regard (Table 5.6). No significant differences in saltiness between the research control and ROLS frankfurters which contained 53% less added salt may be due to the ingredients contained in the salt replacer Artisalt^{TM} which contains flavour enhancers. The commercial cooked ham had a lower salt content of 2.3% compared to the research control cooked ham which contained 2.6% salt; however, the commercial cooked ham was juicier ($P<0.05$) which may have increased saltiness perception resulting in no significant differences in saltiness between cooked ham samples. The ability of increased juiciness to enhance saltiness perception has been demonstrated by NIZO researchers (2018).

The results for OSA showed that ≤19% of consumers disliked (extremely, very much or moderately) all cooked ham samples (ROLS, research control and commercial). The majority (72%, 70% and 62%) of consumers liked (moderately, very much or extremely) the ROLS, research control and commercial cooked ham, respectively (Table 5.5). The statistical analysis indicated that no significant differences were observed between cooked ham samples in terms of OSA (Table 5.6).

The consumer results found in this study are in agreement with our previous results (Chapter 2) where the development of sensory accepted optimised low salt cooked ham was carried out by semi trained panellists and no significant differences in appearance,

flavour, juiciness, texture, saltiness or OSA between ROLS and research control cooked ham was reported. This was attributed to the calculated ionic strength (IS) of a 50/50 combination of Artisalt^{TM} /NaCl (0.31M) which was similar to that the IS of 2 % NaCl (0.34M) and resulted in the development of ROLS cooked ham without compromising the physiochemical characteristics and sensory acceptability compared to full salt control samples. The salt replacer Artisalt^{TM} also contains flavour enhancers such as yeast extract, onion and celery which may have increased saltiness perception. Flavours enhancers have been shown to improve the sensory acceptability of low-salt meat products (Desmond, 2006; Dos Santos *et al*., 2014).

When asked to compare the cooked ham to their usual brand, the majority (60%, 64% and 52%) of consumers stated that the ROLS, research control and commercial cooked ham, respectively, were 'just as good as', 'slightly better' or 'much better' than their usual brand (Figure 5.1b). When asked if they would buy or eat this product again, 62%, 68% and 58% of consumers said they probably or definitely would buy or eat the ROLS, research control and commercial cooked ham, respectively, again (Figure 5.2b). When asked 'which sample do you prefer', 36% of consumers preferred the ROLS cooked ham, 38% preferred the research control cooked ham and 26% preferred the commercial cooked ham (Figure 5.3b). The PCA plot (Figure 5.4b) is a graphical representation of the degree of existing correlations between the cooked ham samples, the measured sensory responses and comparison to usual brand and purchase intent. The plot showed that the ROLS and research control frankfurters were closely related to each other and also to the sensory attributes of firmness. The commercial frankfurters were closely related to juiciness and saltiness. The sensory attributes flavour, OSA and appearance are closely related to the rating questions; would you but/eat again? and comparison to usual brand and these

attributes were more closely related to the ROLS and research control cooked ham than the commercial cooked ham.

Overall, the appearance and firmness of the ROLS and research control cooked ham was preferred, and the juiciness of the commercial cooked ham was preferred. However, in all cooked ham samples flavour, saltiness, OSA, would you buy/eat this again and comparison to usual brand were not significantly different. The consumers did not detect any differences in saltiness between any of the cooked ham samples even though the ROLS cooked ham contained 53% less added salt compared to the research control cooked ham. These results indicate that the ROLS cooked ham was just as acceptable to consumers as the gold standard commercially available cooked ham confirming the potential of the use of the salt replacer Artisalt^{TM}, HPP and organic acids to produce consumer accepted low-salt cooked ham with enhanced safety and shelf life.

In a recent study Pietrasik *et al*. (2016) used KCl as a salt replacer in the manufacture of reduced sodium cooked hams and then applied HPP to enhance the quality and shelf life. The authors reported that the salt replacement negatively affected the physicochemical characteristics of cooked ham as the low-salt cooked ham (1.4% NaCl) was significantly less acceptable than the control cooked ham (2.4% NaCl) in terms of all sensory attributes (appearance, flavour, saltiness, texture, juiciness, aftertaste and OSA). However, when HPP was applied to these products a significant shelf-life extension was obtained with minimal effects on physicochemical quality. In the present study and the results reported in Chapter 2 there were no significant differences in the physicochemical characteristics between the optimised low salt cooked ham and control samples due to composition of the salt replacer Artisalt^{TM} as the IS of a 50/50 combination of Artisalt^{TM} /NaCl was similar to that the IS of 2 % NaCl and also due to the flavour enhancers contained in the Artisalt^{TM}

which may have masked the bitter taste associated with KCl and increased the saltiness perception resulting in a product with similar acceptability as full salt control samples.

Table 5.5 Consumer hedonic scores (%) on the sensory attributes of cooked ham*

				Appearance			
	Unacceptable	Poor	Fair	Average	Good	Very good	Excellent
ROLS	0	4	6	17	29	35	9
Research control	0	2	8	17	25	36	12
Commercial	1	11	18	27	17	17	9
				Flavour			
	Unacceptable	Poor	Fair	Average	Good	Very good	Excellent
ROLS	0	4	7	15	27	39	8
Research control	0	1	6	12	35	40	6
Commercial	1	8	7	18	26	31	9
				Firmness			
	Very Mushy	Mushy	Slightly Mushy	Just right	Slightly Firm	Firm	Very Firm
ROLS	0	0	11	16	23	36	14
Research control	0	1	0	16	18	47	18
Commercial	0	7	37	32	14	10	0
				Juiciness			
	Very Dry	Dry	Slightly Dry	Just right	Slightly Juicy	Juicy	Very Juicy
ROLS	3	11	24	27	11	15	9
Research control	7	10	23	36	6	15	3
Commercial	0	10	0	20	28	29	13
				Saltiness			
	Not Salty	Slightly Salty	Moderately Salty	Just right	Quite Salty	Very Salty	Extremely Salty
ROLS	9	10	8	37	22	7	7
Research control	8	12	17	40	21	2	0
Commercial	9	11	15	37	13	10	5
				OSA			
	Extremely Dislike	Very Much Dislike	Moderately Dislike	Neither Like nor Dislike	Moderately Like	Very Much Like	Extremely Like
ROLS	3	5	11	9	32	35	5
Research control	0	4	15	11	24	39	7
Commercial	0	7	10	21	28	27	7

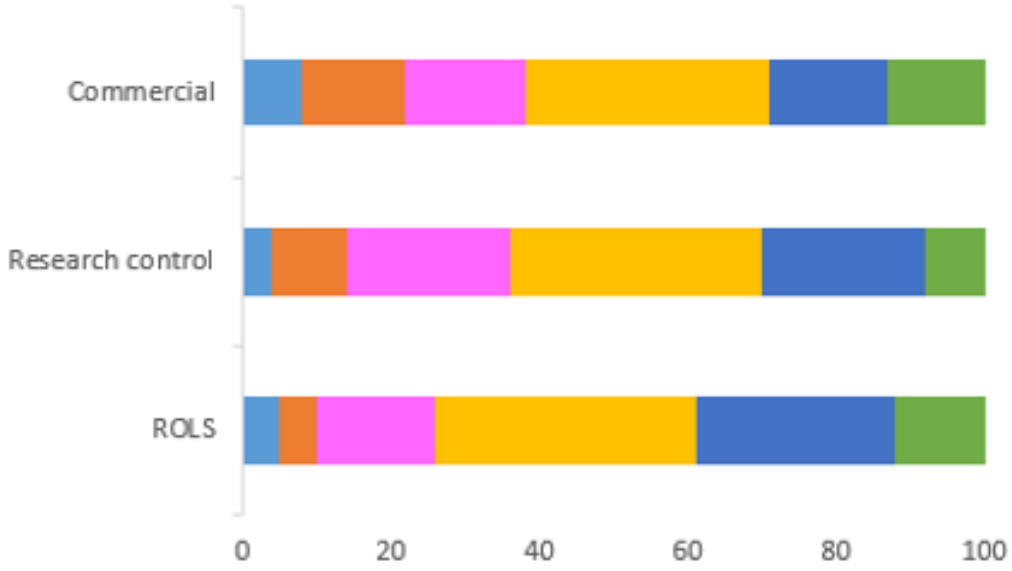
*Results are expressed as %.

Table 5.6 Consumer hedonic scores for the sensory attributes and comparison to usual brand and purchase intent scores of cooked ham*

	Appearance	Flavour	Firmness	Juiciness	Saltiness	OSA	Comparison to usual brand	Would you buy/eat again?
ROLS	5.72 ± 1.21 [a]	4.97 ± 1.27 [a]	5.46 ± 1.21 [a]	4.09 ± 1.14 [a]	4.02 ± 1.56 [a]	4.92 ± 1.42 [a]	3.73 ± 1.25 [a]	3.54 ± 1.2 [a]
Research control	5.81 ± 1.22 [a]	5.31 ± 0.99 [a]	5.68 ± 1.06 [a]	3.89 ± 1.30 [a]	3.58 ± 1.23 [a]	5.02 ± 1.33 [a]	3.90 ± 1.30 [a]	3.72 ± 1.11 [a]
Commercial	4.13 ± 1.40 [b]	4.71 ± 1.1 [a]	3.96 ± 0.99 [b]	5.49 ± 1.18 [b]	3.87 ± 1.54 [a]	4.50 ± 1.42 [a]	3.18 ± 1.13 [a]	3.26 ± 1.20 [a]

*Values are mean (n=100) ± standard deviation [a, b]. Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

(a)



(b)

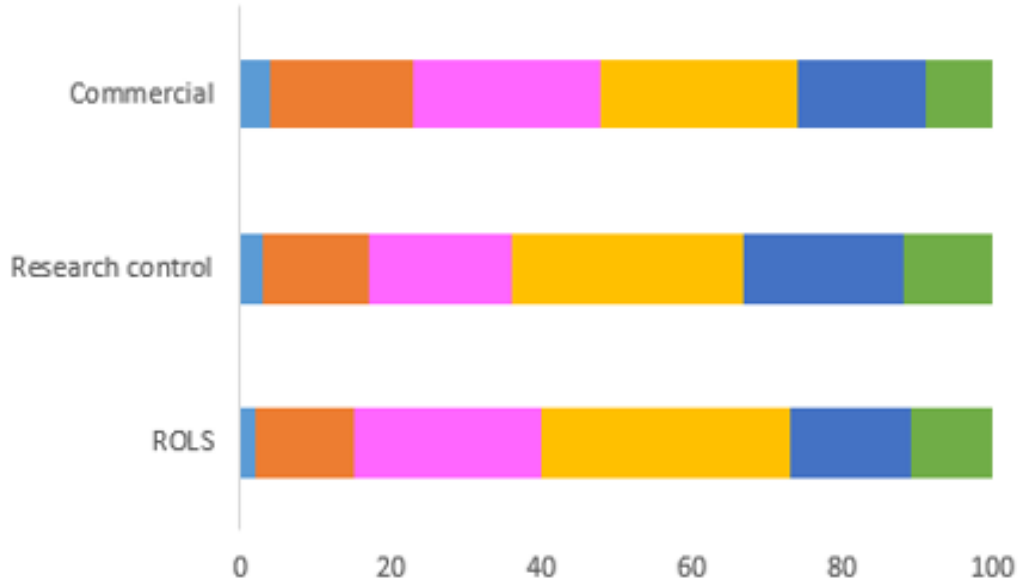
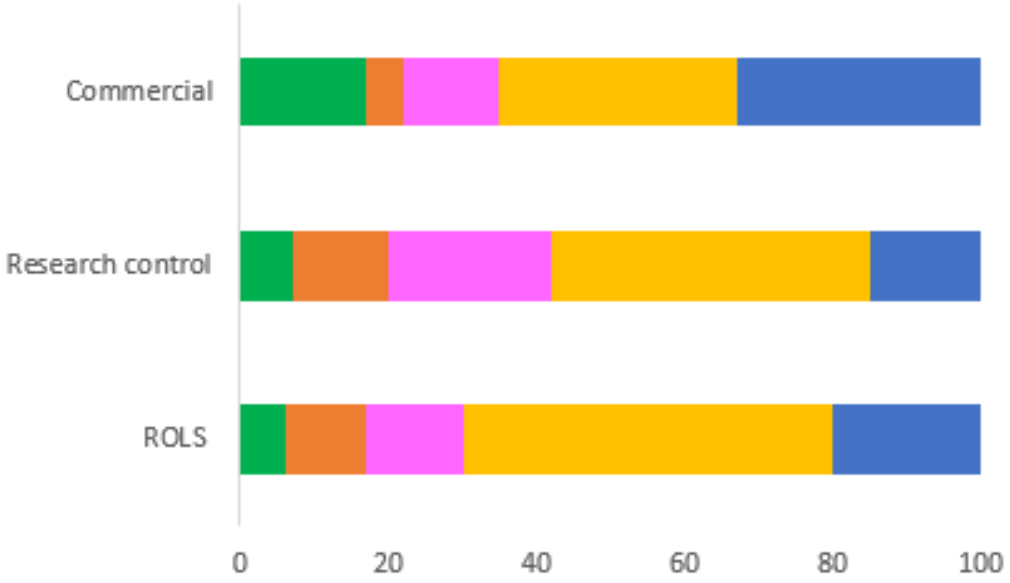


Figure 5.1– Frequency of consumer comparison to usual brand for (a) frankfurters and (b) cooked ham. No comparison (—), much less than (—), slightly less than (—), just as good as (—), slightly better (—), much better (—). Results are expressed as %.

(a)



(b)

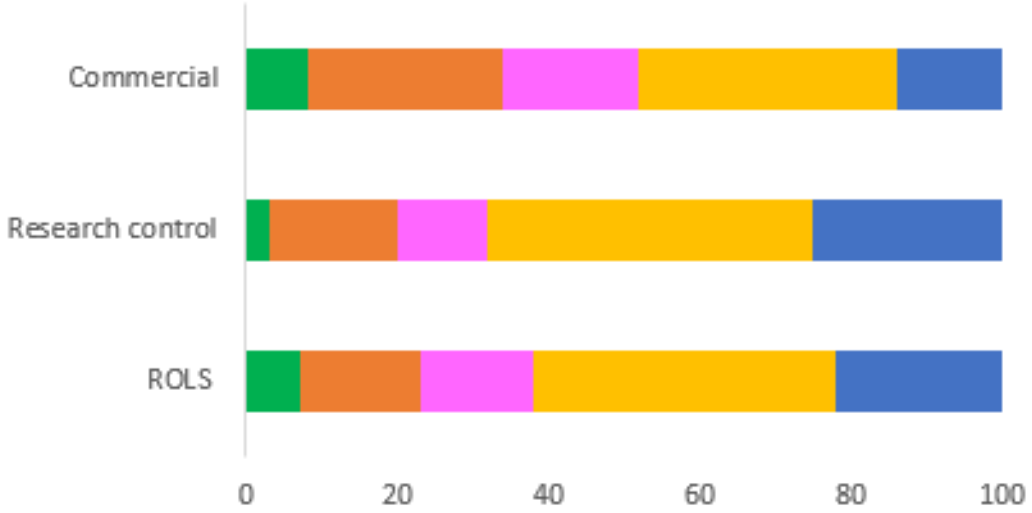


Figure 5.2 - Frequency of consumer purchase intent of (a) frankfurters and (b) cooked ham. Definitely not (—), Probably not (—), Unsure (—), Probably (—), Definitely (—). Results are expressed as %.

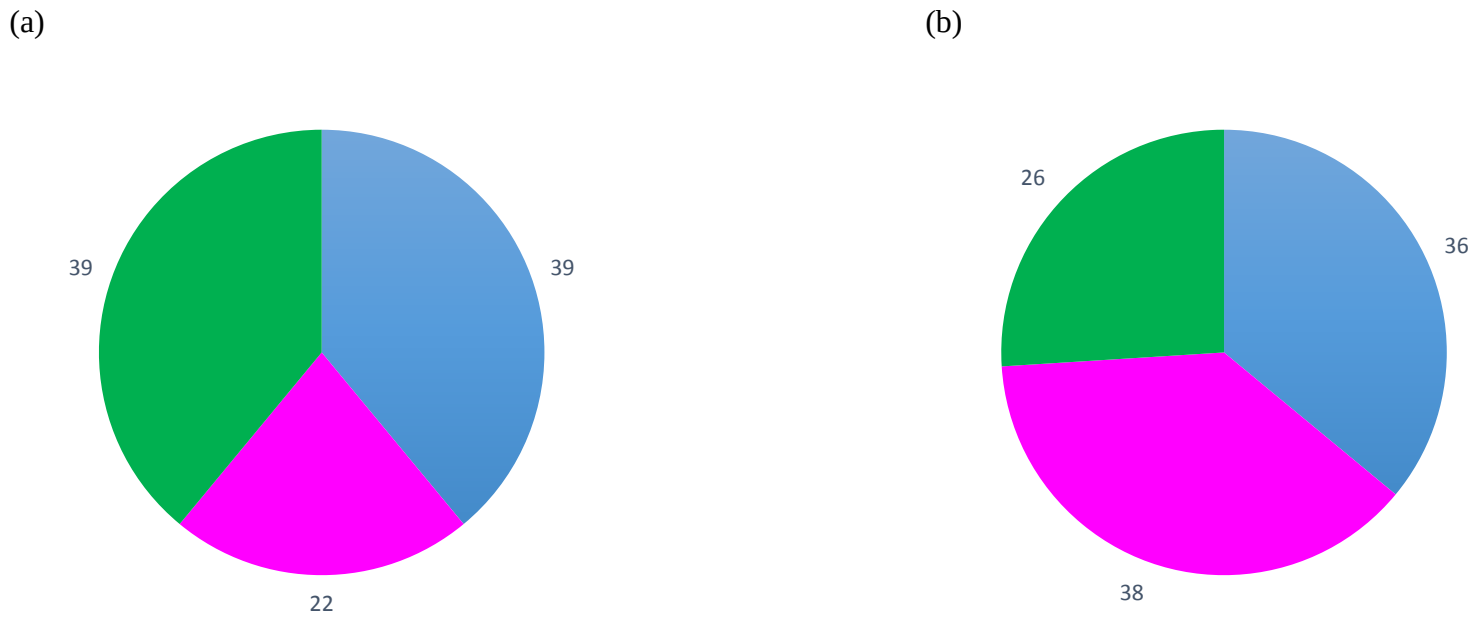
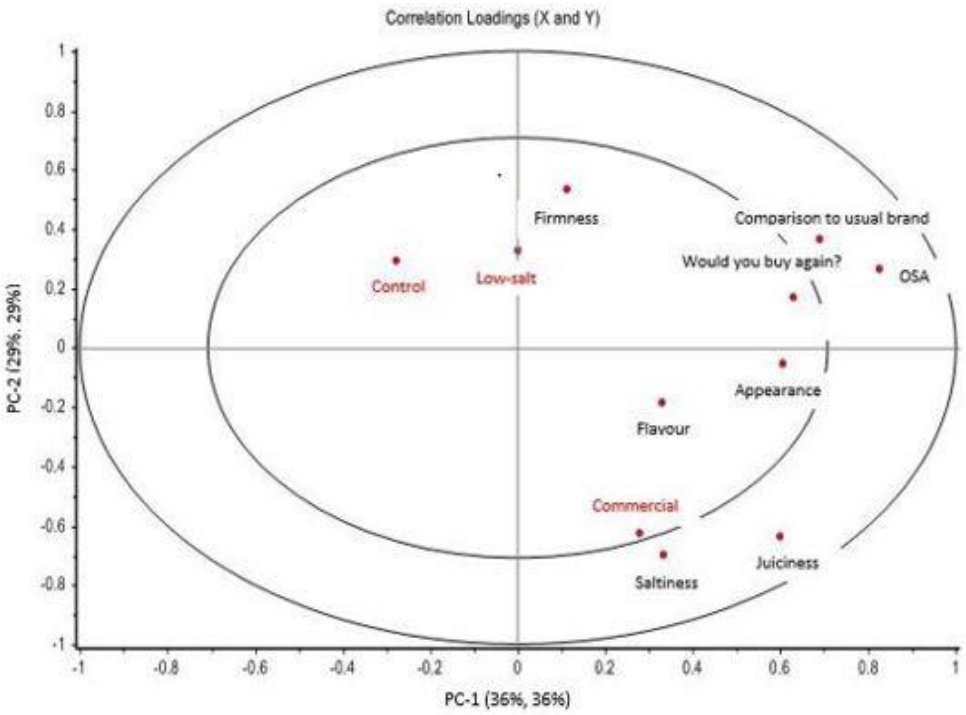


Figure 5.3 – Consumer preference on the Research optimised low-salt (—), Research control (—) and Commercial (—) in (a) frankfurters and (b) cooked ham. Results are expressed as %.

(a)



(b)

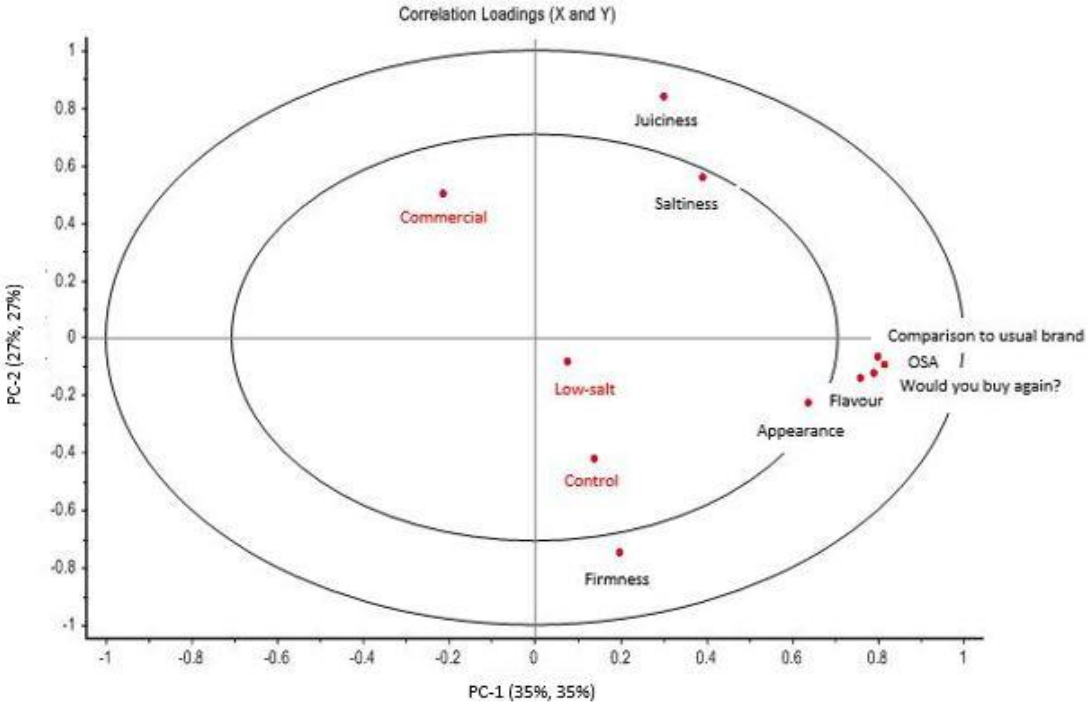


Figure 5.4 – Principle component analysis of (a) frankfurters and (b) cooked ham.

5.4 Conclusion

Salt (NaCl) reduction below the national target levels set by Food Safety Authority of Ireland (FSAI), 2017 for cooked ham (1.6%) and frankfurters (1.5%) can be achieved using the salt replacer Artisalt™, HPP and a mix of organic acids Inbac™ without compromising consumer acceptability and microbial stability. Consumers did not detect differences in the perception of saltiness between the ROLS, research control and commercial frankfurters or cooked hams.

These results indicated that the low-salt processed meat products were just as acceptable or better than the gold standard commercially available products in the Irish market confirming the potential of the use of the salt replacer Artisalt™ and the combined hurdles (HPP and organic acids) to produce consumer accepted low-salt processed meat products with enhanced safety and shelf life. Additionally, as industrial ingredients and processes were used in the development and manufacture of consumer accepted processed meat products with significantly low salt content, the scaling up of the process can easily be achieved. The findings of this study are not just of commercial and processing interest, but also of public health significance.

CHAPTER 6

Improving flavour absorption and shelf life of marinated pork chops through the application of high pressure processing as a hurdle.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

The objective of this study was to determine the efficacy of HPP to accelerate marinade (piri-piri) flavour absorption in pork chops and to study the effects on the physicochemical, sensory and microbiological characteristics during chilled storage. A combination of HPP (300 MPa, 400 MPa or 500 MPa) and a mix of organic acids Inbac™ (0.3%) were used as hurdles to extend the shelf life. The results showed that the level of pressure applied increased the pH, lipid oxidation, toughness and lightness proportionally; however, HPP ≥400 MPa also increased ($P<0.05$) the marinade absorption which enhanced the flavour perception of the marinated pork chops. The piri-piri marinade masked the discolouration caused by HPP and increased ($P<0.05$) the tenderness of the pork chops over storage time. From the microbiological point of view, compared to untreated control samples, the combined effects of HPP at 300, 400 or 500 MPa and Inbac™ (0.3%) extended ($P<0.05$) the shelf-life by 16, 22 and 29 days, respectively. The results highlighted the potential of a combined effect of HPP and antimicrobial Inbac™ to accelerate flavour absorption and significantly extend the shelf life of marinated pork chops.

6.1 Introduction

Marinade technology has been used in the meat industry for several decades. The role and perception of marinades has evolved from flavouring and tenderising to enhancing yield and quality of meats. Marinades are also applied to meat products for preservation and to improve colour (Yusop *et al*., 2011). Marination is a common method for infusing meat with savoury ingredients to improve flavour, texture and juiciness (Toledo, 2001). Consumers generally incorporate marinades into meat via immersion which consists of immersing the meat in a liquid marinade and allowing penetration of the marinade on the meat through diffusion over time. However, dry/paste marinating is also a common method of marinate delivery with consumers; Nevertheless, injection processing and tumbling/massaging are marination processes more commonly used in the meat industry (Yusop *et al*., 2011). Based on their functionality marinade ingredients are classified into two categories, Toledo (2007): 1) Ingredients that affect the water-binding or textural properties, and condition the meat to bind water via ionic strength and pH such as water, salt, phosphates, organic acids, hydrocolloids, protein isolates, curing aids and enzymes and 2) ingredients which affect the consumer appeal and the eating quality of marinated meat products such as herbs and spices, flavour extracts and sweeteners.

The demand for value added meat products continues to increase in the market place and an increase in the range of commercially available marinade products was reported (Hall *et al*., 2008; Yusop *et al*., 2011) and flavour components such as barbeque and piri-piri marinade are in high consumer demand (Nachay, 2011). Ethnic marinades and ethnic flavour-marinated meat products are very popular due to the increased demand for such products by consumers who are more adventurous, demand products possessing more authenticity and desire a more flavourful experience when eating meat (Yusop *et al*., 2009a; Yusop *et al*., 2009b; Yusop *et al*., 2010). Marinades can increase the sensory

acceptability of meat products by enhancing flavour (Yusop *et al*., 2011). Kim *et al.* (2010) found that pork marinated with garlic and onion juice had significantly higher ($P<0.05$) flavour attributes than control samples which were unmarinated. Previous studies reported the tenderising effect of acidic marinades (i.e. organic acids) on beef and chicken (Aktas *et al*., 2003; Berge *et al*., 2001; Burke and Monahan, 2003; Lewis and Purslow, 1991; Oreskovich *et al*., 1992; Bowkler *et al*., 2010; Birk *et al*., 2010). Traditional methods for impregnating flavour such as tumbling, vacuum impregnation are time consuming and in the process the product can be contaminated and spoiled. The growing consumer demand for high quality, minimally processed, easy to store and prepare, additive-free and microbiologically safe meat food products has created a need for new food processing methods.

HPP is a relatively new technology gaining importance in the food industry because of its advantage of inactivating microorganisms and enzymes at ambient or low temperatures without affecting the nutritional properties of food (Indrawati *et al*., 2003); however, pressure levels >300 MPa can negatively affect some other important product qualities, such as tenderness, colour and lipid oxidation (Cheftel and Culioli, 1997). Synergetic effects on microbial inactivation of HPP when used in combination with organic acids, antimicrobial peptides, the lactoperoxidase system, and phenolic compounds have been reported in the literature (Rodriguez-Calleja *et al*., 2012; Cheftel and Culioli, 1997; Mañas and Pagán, 2005; Raso and Barbosa-Canovas, 2003).

Several authors have reported that HPP primarily affects the physicochemical properties of raw/uncooked meat products and has minimum effects on cooked products (Considine *et al*., 2008; Neto *et al*., 2015; Bansal *et al*., 2015). While many studies report the ability of HPP to increase the safety and shelf life of meat products (Kruk *et al.*, 2011; Garriga *et al.*, 2004; Wang *et al.*, 2015; Karlowski *et al.*, 2010), many authors also report the negative

impact of HPP on the colour (Rodriguez calleja *et al*,. 2012; Karlowski *et al.*, 2010; Bajovic *et al.*, 2012), texture (Sun and Holley, 2010; Mc Ardle *et al.*, 2011) and lipid oxidation (Kruk *et al*., 2011; Medina-Meza *et al*., 2014; He *et al*., 2012). Such altered physicochemical characteristics may have a negative effect on the sensory acceptability of HPP meat; however, marinades may be able not only to mask the physicochemical changes such as colour and improve the tenderness but also increase sensory acceptability by enhancing flavour of the marinated meat products.

Shelf life is the period of time during which a food retains acceptable characteristics of flavour, colour, aroma, texture, nutritional value, and safety under defined environmental conditions (Lee *et al*., 2009). Kruk *et al*. (2011) used HPP at 300-600 MPa for 5 mins to extend the shelf life of raw chicken breast fillets and found that HPP at 600 MPa for 5 mins inactivated all microorganisms below delectable levels and improved shelf-life for 7–14 days; however lipid oxidation, lightness and shear force (SF) were significantly increased. Rodriguez-Calleja *et al*., (2012) demonstrated the strongly potential synergetic interaction of HPP (300 MPa for 5 mins) and a mix of organic acids as hurdles extending the shelf-life of skinless chicken breast fillets up to 4 weeks and concluded that the combined effect of the antimicrobial edible coating Articoat™ and HPP was more effective than either treatment alone.

Wang *et al.* (2015) examined the effects of HPP (350-600 MPa for 3 mins) on the quality and shelf life of honey garlic marinated pork chops and concluded that the marinade partially masked meat discolouration due to HPP, the pH of HPP marinated pork chops was higher ($P<0.05$) than the control pork chops and HPP of 450 MPa or higher for 3 mins can extend the shelf life from 10 days to 31 days with minimal effects on meat quality. Kingsley *et al.* (2015) found that a combination of Sriracha® hot sauce flavouring and HPP at 600 MPa for 5 mins yielded a raw oyster with improved sensory quality in regards to flavour

and also lower bacterial counts (4 log) over 10 days of refrigerated storage. As outlined above, there are limited number of studies using the combined effects of HPP and a mix of organic acids to enhance flavour uptake and shelf life enhancement. Moreover, most of the studies that applied HPP to enhance safety and shelf life reported in the literature were carried out using lab scale HPP (Vercammen *et al*., 2011; Rodriguez-calleja *et al*., 2012; O'Flynn *et al*., 2014; Crehan *et al*., 2000; Andres *et al*., 2004; Han *et al*., 2011; Cava *et al*., 2009) with a few studies reported using industrial HPP units for treating meat products. (Garriga *et al*., 2004; Jofre *et al.*, 2009; Marcos *et al.*, 2007);

In the present study an industrial scale HPP unit and commercially available organic acids were used to treat marinated pork chops which have the advantage of easily scaling up. HPP and cooking of products in their final packaging also result in an extremely convenient product for consumer use. Other technologies such as hydrodynamic pressure (HDP) have been used to increase marinade absorption. Bowkler *et al*. (2010) applied HDP to turkey breasts before marination via tumbling in a brine consisting of water, salt, and phosphate and found that HDP enhanced the marinade absorption, increased processing yield, which resulted in improved tenderness; however, to the best of our knowledge, there are no studies which have been carried out examining the ability of HPP to accelerate the marinade (e.g. piri-piri sauce) absorption in pork chops, and their subsequent physicochemical analysis during chilled storage. Hence, the objectives of this research were to determine the efficacy of HPP to accelerate the marinade (e.g. piri-piri marinade) absorption in pork chops and investigate the effects of a combination of HPP and a commercially available mix of organic acids on the physicochemical, sensory and microbiological quality of marinated pork chops during chilled storage at 4°C.

6.2 Materials and Methods

6.2.1 Materials

Pork loins were obtained from a local meat processor (Ballyburden, Ballincollig, Cork). Piri-Piri marinade (Rapeseed oil 60%, Spices and flavourings 36% (chilli, garlic, jalapeno, black pepper, onion, paprika, lovage root, fenugreek seed, bird clover, onion leek, coriander, turmeric, ginger, cumin seed, fennel, sugar, grapefruit, passion fruit, papaya, mango, palm fat) and Salt 4%) was obtained from Oliver Carty (Athlone, Co. Roscommon, Ireland). A commercial antimicrobial mix of organic acids Inbac^{TM} (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%) was obtained from Chemital (Chemital Ltd, Barcelona, Spain).

6.2.2 Methods

6.2.2.1 Marination of pork chops

The pork loins were cut into 3 cm chops including the fat ring, weighed and placed in a combivac vacuum pouch (20 polyamide/70 polyethylene bags (Alcom, Campogalliano, Italy) and piri-piri marinade which contained Inbac™ (0.3%) at a weight ratio 80:20 (Pork chop:marinade) was added and then vacuum packed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, German). Marinated control samples were stored in a chill room at 4°C for the duration of the shelf life. For samples requiring HPP (300 MPa, 400 MPa or 500 MPa), marinated pork chop samples were HPP (as outlined in section 6.2.2.2) before storage in a chill room at 4°C for the duration of the shelf life.

6.2.2.2 High Pressure Processing

Vacuum-packed marinated pork chops were HPP using an industrial Hiperbaric 420 litre unit (Burgos, Spain) at the HPP Tolling facilities (HPP tolling, St. Margaret's, Dublin) using water as the pressure transmitting medium. The speed of pressurisation was 130 MPa per minute, the speed of depressurisation was instantaneous (~ 1 second) and the holding time was 3 minutes. The temperature of the pressure transmitting medium (water) was 10°C. The initial temperature of the raw marinated pork chops before HPP was 3.4 °C and the final temperature measured after HPP was 6.5 °C.

6.2.2.3 Cooking

Vacuum-packed marinated pork chops were cooked at full steam (100 °C) in a Zanussi oven pH(Zanussi Professional, Italy) and temperature monitored using a thermocouple data logger (Omega Engineering Ltd., Manchester, UK) inserted into the coldest point of the marinated pork chops until an internal temperature of 74 °C was reached. The samples were then cooled down at room temperature before analysis was carried out.

6.2.2.4 Marinade absorption

The initial weight of raw unmarinated pork chops was recorded. Samples were then marinated as described in Section 6.2.2.1 and after 24 hours storage at 4°C untreated and HPP samples were placed on an elevated stainless steel wire rack for 5 mins to allow dripping of the excess marinade and then re-weighed. Calculation for marinade absorption was as follows;

% marinade absorption = (weight after 24 hours marination – initial unmarinated weight) / (initial unmarinated weight) * 100.

Each value represents the average of 8 measurements (two independent trials x four samples).

6.2.2.5 Cook loss

The cook loss of both untreated control and HPP marinated pork chops was determined on Day 1. Briefly, the initial weight of the raw marinated pork chops was recorded after samples had been placed on an elevated stainless steel wire rack for 5 mins. After cooking the samples were re-weighed and cook loss calculated as follows:

% cook loss = (cooked weight – initial raw weight) / (initial raw weight) * 100

Each value represents the average of 8 measurements (two independent trials x four samples).

6.2.2.6 Compositional analysis

To obtain a representative sample for proximal composition analysis marinated pork chops, the outer layer of fat was removed after cooking and then the meat was homogenised for 1 min in a Buchi™ mixer B-400 (Büchi Labortechnik, Switzerland). Proximate composition (fat, moisture, protein and ash) of cooked marinated pork chops was determined on Day 1 using the methods previously described in Chapter 2. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

6.2.2.7 Microbiological analysis

Microbiological analysis of the raw marinated pork chops was carried out throughout storage at 4 °C. Briefly, 10 g of the surface of the raw marinated pork chop was weighed aseptically into a stomacher bag in a vertical laminar-flow cabinet and a primary 10-fold dilution was performed by the addition of 90 ml of sterile maximum recovery diluent (MRD) (Oxoid, Basingstoke, U.K.), stomached (Steward Stomacher 400 Lab Blender, London, UK) for 3 min and homogenates were 10-fold serially diluted using MRD solution. For the enumeration of total viable counts (TVC) 1 ml of each appropriate dilution was inoculated on duplicated plates in the centre of compact dry-total count plates (20 cm^2) (Nissui Pharmaceutical, Co. Ltd., Japan) following incubation at 37 °C for 48 hours. Lactic acid bacteria (LAB) was determined on overlaid de Man Rogosa Sharpe medium (Oxoid); 1ml of each appropriate dilution was inoculated on duplicated plates and incubated at 30 °C for 48 hours. *Escherichia coli* (*E. Coli*) and total coliforms were determined using Compact Dry EC plates (Nissui Pharmaceutical, Japan) to which 1ml of each appropriate dilution was inoculated on duplicated plates (20 cm^2) and incubated at 37 ºC for 24 hours. At the start and the end of the shelf life, marinated pork chops were tested also for the presence or absence of *Salmonella* spp. in Compact dry SL plates (Nissui Pharmaceutical, Co. Ltd., Japan). For this, pre-enrichment process was carried out by weighing 25 g of sample into a sterile filter stomacher bag and then 225 ml of Buffered Peptone water (Oxoid) was added and homogenised with a stomacher for 1 min and incubated at 37 °C for 24 hr. The bag was taken from the incubator and 0.1 ml of enriched specimen was then dropped gently on the sheet 1 cm from the edge of the plate. After inoculation of the enriched culture, 1 mL of sterilized water was dropped at the opposite point were the specimen was dropped. The sterilised water diffused automatically and the sheet was wetted uniformly. The inoculated compact dry SL plates were incubated at 42 °C for 24

hrs. All results (except *Salmonella*) were expressed as $\log_{10}$ colony-forming units (CFU/g). Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

6.2.2.8 pH

The pH of raw and cooked untreated and HPP marinated pork chops was measured using a digital pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the sample. The pH was measured throughout the shelf life. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

6.2.2.9 Warner-Bratzler Shear force

Warner-Bratzler Shear force (WBSF) was measured according to the method outlined by Shackelford *et al.* (1991). Briefly, the 3 cm thick marinated pork chops were cooked as described in Section 2.2.3 to an internal temperature of 74 °C and then cooled at room temperature (20 °C). Four cylinders of a 1.27 cm diameter were obtained from each pork chop parallel to the muscle fibre direction using a corer. The pork steak cylinders were sheared using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK) attached with a Warner Bratzler V-shaped shearing device at a crosshead speed of 4 mm/s. The WBSF of the untreated and HPP marinated pork chops were determined throughout the shelf life. Each value represents the average of 8 measurements (two independent trials x four samples).

6.2.2.10 Colour

The colour of the surface of the raw and cooked marinated pork chop was measured as described in Chapter 2. CIE L*, a* and b* values (Lightness, redness and yellowness, respectively) during chilled storage at 4°C are reported and each value represents the average of 12 measurements (two independent trials x two samples x three readings).

6.2.2.11 Sensory evaluation

A 25 member semi-trained taste panel was used to evaluate the cooked untreated and HPP marinated pork chops over two sessions using a 9-point hedonic scale. The panellists were recruited from staff and postgraduate students at the School of Food and Nutritional Sciences, University College Cork and chosen based on their experience in the sensory analysis of meat products and on their availability. The panellists have partaken in sensory analysis of meat products on numerous occasions and were familiar with the sensory terminology.

Vacuum pouches containing the raw marinated pork chops were labelled with a three digit random number and panellists evaluated the appearance of the vacuum packed untreated and HPP raw marinated pork chops before sensory evaluation of the cooked samples. Samples for cooked sensory analysis were then labelled with the corresponding three digit random number and cooked as described in Section 2.2.3 before being removed from the packaging and served warm (~60 °C) on labelled polystyrene plates. The tested attributes were: Liking of Appearance (raw), Liking of Appearance (cooked), Liking of Texture, Liking of Flavour, Juiciness, Tenderness, and Overall sensory acceptability (OSA).

To ensure that all samples were safe for consumption, microbiological analysis was carried out before each sensory test. Sensory analysis was carried out at day 1 and at the time when

samples reached Log 5 CFU/g of sample which indicated end of shelf life based on the microbiological limit for TVC (< $5x10^6$ CFU/g of product) as outlined in Section 3.3. For control samples (0.1 MPa), sensory analysis was carried out on day 8 while that for samples HPP at 300, 400 or 500 MPa was carried out on day 13, 29 or 34, respectively.

6.2.2.12 Lipid oxidation

Lipid oxidation of the raw marinated pork chops was measured using the 2-thiobarbituric acid (TBA) assay (Siu and Draper, 1978). The malondialdehyde (MDA) content was calculated using an extinction coefficient of 1.56 × 105 L mol^{-1} cm^{-1}. The lipid oxidation was measured throughout the shelf life and results were expressed as 2-thiobarbituric acid-reactive substances (TBARS) in mg MDA/kg sample. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

6.2.2.13 Statistical analysis

All physicochemical results (marinade absorption, proximate composition, cook loss, colour, texture, TBARS and pH and sensory) were tested using one way ANOVA, sensory data was also analysed using t-test analysis and significance assessed using Tukey's test at 5% significance level using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA). Two independent trials were carried out and all analysis was carried out at least in duplicate.

6.3 Results and Discussion

6.3.1 Marinade absorption

The results showed that marinade absorption/yield of the pork chops HPP at 300 MPa did not increase significantly compared to control untreated samples; however, an increased ($P<0.05$) marinade absorption was noticed when the marinated pork chops were HPP at pressures ≥400 MPa (Table 6.1). It was reported that marinades diffuse from the meat surface into the interior of the meat due to the gradient formed from the higher concentration of marinade to the lower concentration of fluid in the interior of the meat (Yusop *et al*., 2011) and apparently the increased HPP levels may have accelerated this diffusion process. Previous studies reported that injection of a marinade consisting of salt, tripolyphosphate and bicarbonate can increase the yield and tenderness of pork loin (Sheard and Tali, 2004); however; to the best of our knowledge, there are no studies on the ability of HPP to increase the marinade (e.g. piri-piri) absorption of flavour components via immersion.

6.3.2 Cook loss

The results indicated that while marinated pork chops HPP at 400 MPa or 500 MPa had lower cook loss values compared to untreated control and marinated pork chops that were HPP at 300 MPa, these differences were not statistically significant (Table 6.1). These lower cook loss values may be due to the fact marinated pork chops HPP at ≥400 MPa had a higher ($P<0.05$) marinade absorption which may have increased the water holding capacity (WHC) and limited the moisture lost from the surface during cooking. Conversely, HPP has been shown to significantly decrease the cook loss on meat products such as

chicken and pork when HPP at 300 MPa for 5 mins or 215 MPa for 5 mins, respectively (Rodriguez calleja *et al*., 2012; Souza *et al*., 2011).

6.3.3 Compositional analysis

The results for proximate composition showed that HPP did not significantly affect the moisture, protein, fat or ash content of the cooked marinated pork chops (Table 6.1) which correlated with the cook loss results which were not significantly different. Conversely, Kruk *et al.* (2011) reported HPP increased significantly the moisture content of cooked chicken breast fillets when chicken breast fillets were HPP at 300, 450 or 600 MPa for 5 mins and this moisture increase was attributed to the significant cook loss differences.

Table 6.1 Physicochemical changes of marinated pork chops*

HPP (MPa)	**Marinade absorption** (%)	**Cook Loss** (%)	**Moisture** (%)	**Protein** (%)	**Fat** (%)	**Ash** (%)
0.1	1.95 ± 0.22 [a]	19.00 ± 1.04 [a]	63.37 ± 1.03 [a]	28.15 ± 0.49 [a]	4.42 ± 1.35 [a]	1.54 ± 0.11 [a]
300	2.16 ± 0.19 [ab]	19.10 ± 1.56 [a]	63.50 ± 2.04 [a]	28.31 ± 0.82 [a]	4.78 ± 1.47 [a]	1.59 ± 0.04 [a]
400	2.63 ± 0.31 [bc]	17.75 ± 1.04 [a]	62.85 ± 2.19 [a]	28.51 ± 1.22 [a]	4.9 ± 1.82 [a]	1.56 ± 0.04 [a]
500	2.9 ± 0.48 [c]	17.38 ± 1.09 [a]	63.29 ± 1.04 [a]	29.11 ± 0.38 [a]	4.02 ± 1.92 [a]	1.58 ± 0.34 [a]

*Values are Mean ± standard deviation. [a,b,c] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

6.3.4 Colour

At day 1, HPP raw marinated pork chops had significantly ($P<0.05$) higher CIE L-, and b-values (lightness and yellowness, respectively) and the lowest a-values (redness) compared to control untreated raw marinated pork chops (0.1 MPa) (Table 6.2). Untreated control samples were the darkest ($P<0.05$) with lightness increasing ($P<0.05$) proportionally as the pressure level applied increased with raw marinated pork chops HPP at 500 MPa showing the highest ($P<0.05$) lightness. Colour changes in muscle food products after HPP have been reported that may be related to the denaturation of the myofibrillar and sarcoplasmic proteins (Zhou *et al*, 2010; Ma and Ledward, 2013). Similar results have been reported by Carlez *et al*. (1995) who suggested that fresh meat discolouration after HPP at 200-350 MPa is due to a "whitening" effect (increase in L* values) caused by globin denaturation, haem release or displacement or by oxidation of ferrous myoglobin to ferric metmyoglobin when fresh meat is HPP at pressures ≥400 MPa. Goutefongea *et al*., (1995) also suggested discolouration due to HPP occurs as a results of protein coagulation which would affect sample structure and surface properties. Kruk *et al*. (2011) HPP raw chicken breast fillets at 300, 450 or 600 MPa for 5 mins and found that the lightness and yellowness increased significantly and this increase was proportional to the pressure level applied which is in agreement with our findings that the increased lightness depended on the pressure level applied.

During storage, the lightness of both raw untreated control and raw marinated pork chops HPP decreased ($P<0.05$) significantly and these significant changes were noticed on day 11 for raw untreated control marinated pork chops or on day 16, 23 or 30 for samples that were HPP at 300, 400 or 500 MPa, respectively. The decreased lightness may be due to the presence of oxidised products of meat pigments which have a brown and darker colour (Wettasinghe and Shahidi, 1997). It was also reported that the enzymatic systems can also

be affected by HPP and this could explain the progressive accumulation of metmyoglobin content during storage (Jung *et al*., 2003). Regarding the redness and yellowness, these colour parameters did not change significantly during storage in untreated control or HPP samples and this may be due to presence on the surface of the meat of the piri-piri marinade which is highly pigmented with L*, a* and b* values of 26.86, 17.68 and, 54.28, respectively. Throughout storage, the colour differences observed on day 1 among raw untreated control samples and HPP samples (higher lightness, higher yellowness and decreased redness in HPP samples) were also observed during chilled storage.

After cooking, untreated and HPP marinated pork chops became significantly ($P<0.05$) darker, less red and less yellow (Table 6.3). The decrease in lightness may be due to the denaturation of myoglobin as the cooked pigment is denatured metmyoglobin which is darker in colour (Boles and Pegg, 1999). Decreased redness and yellowness may be due to loss of the red and yellow marinade pigments due to cook loss. Compared to cooked untreated control samples, greater colour changes were noticed on cooked HPP marinated pork chops which may be due to the fact that when marinated pork chops were HPP, HPP may have caused denaturation of proteins before cooking and that heat treatment may have caused further protein denaturation compare to untreated control marinated pork chops which were just heat treated and apparently resulting in less protein denaturation and therefore a less colour change compared to HPP marinated pork chops. It was reported that the effects of pressure on proteins may be reversible or irreversible, similar to the effects of increasing temperatures on the denaturation of proteins (Balny and Masson, 1993). Reversible effects are generally observed at pressures less than 200 MPa, whereas irreversible effects occur at pressures greater than 300 MPa. Unlike thermal denaturation and unfolding induced by denaturing agents, volume determinations and limited changes

in heat capacity indicate that pressure-induced denaturation corresponds to partial unfolding of the protein (Balny and Masson, 1993)

On day 1, independent of the pressure level applied cooked HPP marinated pork chops were lighter ($P<0.05$) than untreated control samples; however, no significant differences between samples were observed in regards to redness and yellowness, and this may be due to the presence of the marinade on the surface of the marinated cooked pork chops. During storage, the lightness of both cooked untreated marinated pork chops and HPP cooked marinated pork chops decreased ($P<0.05$) significantly and these significant changes were noticed on day 11 for cooked untreated marinated pork chops or on day 23, 16 or 23 for samples that were HPP at 300, 400 or 500 MPa, respectively. This decrease in lightness over storage time also may be due to oxidised products of meat pigments which have a brown and darker colour (Wettasinghe and Shahidi, 1997). Similar to raw marinated pork chops, the redness and yellowness did not change over storage time in both untreated or HPP cooked marinated pork chops and this may be due to presence on the surface of the meat of the piri-piri marinade which is highly pigmented. Throughout storage, the colour differences observed on day 1 among cooked untreated control samples and HPP samples (higher lightness in HPP samples) was also observed during storage.

6.3.5 Texture

Results on day 1 showed that as the pressure level increased, the WBSF of the cooked marinated pork chops increased ($P<0.05$) proportionally compared to untreated control marinated pork chops which had the lowest WBSF and that the marinated pork chops that were HPP at 500MPa had the highest WBSF indicating that these were the toughest samples (Table 6.3). It was reported that pressures up to 1000 MPa can influence meat

protein conformation and induce protein denaturation, aggregation or gelation which can result in meat becoming either tenderised or toughened and these outcomes depend on the meat protein system, the temperature used, the pressure applied and the holding time (Sun and Holley, 2010). The results found in this study are in agreement with the findings of Kruk *et al*. (2011) and Zamri *et al*. (2006) who found that hardness in chicken breast fillets increased proportionally with increasing pressure levels up to 600 MPa while McArdle *et al.* (2011), Ma and Ledward (2004) reported higher WBSF and hardness values in beef HPP at 600 MPa than in beef treated at 400 MPa. Similarly, Rodrigues *et al*. (2016) reported that marinated beef HPP at 300, 450 or 600 MPa increased significantly the WBSF as the pressure level increased and that samples HPP at 600 MPa resulted in the toughest samples compared to the other treatments. The increased toughness with pressure has been attributed to an increasing incidence of sarcomeres, in which thick filaments have been compressed onto the Z-line, thus removing the I-band as a zone of weakness (Macfarlane *et al*., 1980).

Throughout storage time, in both untreated control and HPP marinated pork chops, WBSF values decreased significantly ($P<0.05$) resulting in marinated pork chops becoming more tender. The decrease in untreated control marinated pork chops was noticed after 11 days and on day 16, 23 or 11 for marinated pork chops that were HPP at 300, 400 or 500 MPa, respectively. While at day 1, significantly ($P<0.05$) tougher samples were noticed in marinated pork chops that were HPP; however, at day 7 onwards until the end of their respective shelf life, there were no significant differences in toughness between untreated control and HPP marinated pork chops which suggested the ability of higher pressure levels (HPP ≥400 MPa) to not only accelerate marinade absorption but also resulted in an increased rate of tenderisation as the 500 MPa marinated pork chops were more tender ($P<0.05$) at day 44 compared to control untreated samples at day 1 or throughout storage.

These results highlight the potential of the combination of marinades and HPP to tenderise meat which has become tougher immediately due to the application of HPP. Many authors have demonstrated the ability of marinades to tenderise meat products such as beef, chicken and pork (Aktas *et al*., 2003; Berge *et al*., 2001; Burke and Monahan, 2003; Lewis and Purslow, 1991; Oreskovich *et al*., 1992, Bowkler *et al*., 2010; Birk *et al*. 2010, Burke and Monahan 2003; Wang *et al* 2015). Similar to our results, Rodrigues *et al.* (2016) reported that the WBSF decreased ($P<0.05$) during storage in low-salt beef marinated in citric acid that were HPP at 600 MPa which may be due to tenderising effect of the marinade. Conversely, Souza et al (2011) examined the tenderness of pork meat HPP at 215 MPa over time and found that WBSF remained relatively unchanged; however, this study did not include the addition of marinades. The tenderisation of meat using marinades was attributed to marinade uptake by muscle proteins and also to solubilisation of collagen (Burke and Monahan, 2003).

6.3.6 pH

The results for pH showed that in raw marinated pork chops, the level of HPP increased the pH proportionally as untreated control samples had the lowest ($P<0.05$) pH values and 500 MPa samples had the highest ($P<0.05$) pH values (Table 6.2). The pH of the piri-piri marinade was 4.4 and due to the higher marinade absorption in samples which were HPP at 400 or 500 MPa it would be expected that these samples would also have a lower ($P<0.05$) pH compared to untreated control and 300 MPa marinated pork chops which had lower ($P<0.05$) marinade absorption; however, independent of the pressure applied HPP increased the pH of the marinated pork chops regardless of the level of marinade absorption.. Increase in pH after HPP has been attributed to a decrease in available acidic

groups in the meat as a result of conformational changes associated with protein denaturation (McArdle *et al*., 2010; Angsupanich and Ledward, 1998). Rodriguez-Calleja et al. (2012) found that the pH values of chicken HPP at 300 MPa for 5 mins were significantly higher than control samples and Wang *et al*. (2015) also found that the pH was higher ($P<0.05$) in honey garlic pork chops treated at 450-600 MPa for 3 mins compared to control samples. Similar results on increased pH on muscle food products were reported by McArdle *et al.* (2011) and Cruz-Romero *et al*. (2007; 2008a; 2008b).

Throughout storage time, independent of the treatment applied to raw marinated pork chops, the pH decreased ($P<0.05$) which may have been due to the production of lactic acid through LAB metabolism (Farber, 1991). In general, the pH decrease occurred when LAB reached ~Log 4 CFU/g of sample. These results are in agreement with the findings of Kruk *et al*. (2011) who observed significant ($P<0.05$) reductions in pH values throughout the storage period for raw chicken breast fillets.

After cooking of marinated pork chops, the pH increased ($P<0.05$) in all treatments; however, the increase was not significantly different between untreated or HPP marinated pork chops at day 1 or throughout storage time (Table 6.3). Increase in pH due to cooking may be due to the decreased number of acidic groups in muscle proteins as proteins unfold (Hamm and Deatherage, 1960). In the HPP marinated pork chops, the effects of the combined application of cooking and HPP were not additive in regards to increasing of pH and therefore no significant differences were observed compared to untreated control samples which were cooked but not HPP. This may be due to increased severity of the cooking process in comparison to the milder process of HPP. A similar effect on pH was also observed in HPP cooked beef muscle compared to untreated cooked samples (Ma and Ledward, 2004).

Table 6.2 Changes in colour and pH during chilled storage of raw untreated and HPP marinated pork chops*

	Treatment (MPa)	Day 1	Day 7	Day 9	Day 11	Day 16	Day 23	Day 30	Day 37	Day 44
Lightness (L*)	0.1	55.06 ± 2.68 ^{aA}	54.77 ± 1.40 ^{aA}	54.03 ± 1.42 ^{aAB}	51.97 ± 1.21 ^{aBC}	51.29 ± 2.34 ^{aC}	/	/	/	/
	300	63.64 ± 3.05 ^{bA}	63.20 ± 2.25 ^{bAB}	63.01 ± 1.48 ^{bAB}	62.89 ± 2.85 ^{bAB}	61.93 ± 1.81 ^{bBC}	58.11 ± 2.41 ^{aC}	58.01 ± 2.31 ^{aC}	/	/
	400	66.79 ± 1.83 ^{cA}	65.75 ± 3.27 ^{bcA}	64.95 ± 2.55 ^{bcA}	63.69 ± 1.45 ^{bcAB}	63.59 ± 3.85 ^{bcAB}	60.02 ± 3.29 ^{aBC}	58.69 ± 2.74 ^{aC}	58.62 ± 2.70 ^{aC}	/
	500	68.18 ± 2.39 ^{cA}	67.11 ± 1.62 ^{cA}	66.95 ± 2.06 ^{cAB}	66.54 ± 2.81 ^{cAB}	65.51 ± 2.99 ^{cAB}	64.02 ± 2.53 ^{bAB}	62.53 ± 2.15 ^{bB}	62.51 ± 1.16 ^{bB}	59.92 ± 1.77 ^{B}
Redness (a*)	0.1	14.12 ± 1.95 ^{aA}	13.71 ± 3.40 ^{aA}	14.36 ± 2.96 ^{aA}	13.23 ± 2.79 ^{aA}	13.03 ± 1.30 ^{aA}	/	/	/	/
	300	11.58 ± 1.01 ^{bA}	10.94 ± 1.58 ^{bA}	11.45 ± 1.86 ^{bA}	10.23 ± 2.72 ^{bA}	10.37 ± 1.34 ^{bA}	10.34 ± 1.09 ^{aA}		/	/
	400	11.30 ± 1.45 ^{bA}	10.98 ± 1.14 ^{bA}	11.46 ± 1.73 ^{bA}	10.37 ± 0.92 ^{bA}	10.81 ± 1.38 ^{bA}	10.54 ± 1.44 ^{aA}	11.19 ± 1.75 ^{aA}	10.87± 2.05 ^{aA}	/
	500	10.65 ± 1.37 ^{bA}	11.04 ± 2.01 ^{bA}	10.87 ± 1.81 ^{bA}	10.64 ± 2.14 ^{bA}	10.57 ± 1.20 ^{bA}	10.48 ± 0.88 ^{aA}	10.69 ± 1.73 ^{aA}	10.22 ± 2.26 ^{aA}	10.17 ± 1.69 ^{A}
Yellowness (b*)	0.1	26.74 ± 2.67 ^{aA}	24.41 ± 3.86 ^{aA}	25.1 ± 2.41 ^{aA}	27.09 ± 4.24 ^{aA}	25.95 ± 3.67 ^{aA}	/	/	/	/
	300	36.54 ± 2.40 ^{bA}	34.21 ± 3.51 ^{bA}	37.54 ± 2.47 ^{bA}	38.31 ± 3.95 ^{bA}	38.44 ± 2.89 ^{bA}	38.08 ± 4.03 ^{aA}	38.39 ± 2.47 ^{aA}	/	/
	400	34.21 ± 4.47 ^{bA}	32.59 ± 4.74 ^{bA}	33.58 ± 3.57 ^{bA}	34.57 ± 4.01 ^{bA}	37.63 ± 2.94 ^{bA}	38.13 ± 4.92 ^{aA}	37.76 ± 3.93 ^{aA}	37.44 ± 2.84 ^{aA}	/
	500	37.36 ± 3.35 ^{bA}	35.00 ± 2.77 ^{bA}	37.3 ± 4.84 ^{bA}	37.94 ± 3.68 ^{bA}	37.08 ± 3.12 ^{bA}	37.09 ± 4.09 ^{aA}	37.76 ± 3.12 ^{aA}	35.14 ± 3.47 ^{aA}	37.85 ± 2.97 ^{A}
pH	0.1	5.59 ± 0.02 ^{aA}	5.48 ± 0.04 ^{aB}	5.46 ± 0.05 ^{aB}	5.40 ± 0.08 ^{aB}	5.28 ± 0.07 ^{aC}	/	/	/	/
	300	5.68 ± 0.03 ^{abA}	5.65 ± 0.07 ^{bA}	5.53 ± 0.05 ^{bB}	5.52 ± 0.05 ^{bB}	5.50 ± 0.02 ^{bB}	5.46 ± 0.08 ^{aB}	5.37 ± 0.04 ^{aC}	/	/
	400	5.71 ± 0.16 ^{bA}	5.68 ± 0.08 ^{bcA}	5.67 ± 0.06 ^{cA}	5.62 ± 0.06 ^{cAB}	5.65 ± 0.05 ^{cAB}	5.59 ± 0.10 ^{abAB}	5.50 ± 0.02 ^{bBC}	5.38 ± 0.08 ^{aC}	/
	500	5.73 ± 0.06 ^{bA}	5.75 ± 0.05 ^{cA}	5.69 ± 0.04 ^{cA}	5.68 ± 0.04 ^{cA}	5.69 ± 0.07 ^{cA}	5.65 ± 0.02 ^{bA}	5.48 ± 0.02 ^{bB}	5.44 ± 0.07 ^{aB}	5.34 ± 0.08 ^{C}

*Values are Mean ± standard deviation $^{a, b, c}$ Different superscripts in the same column indicate significant difference (P<0.05) between different treatments.

$^{A, B, C}$ Different superscripts in the same row indicate significant difference (P<0.05) in the same treatment over time.

(/) indicates analysis was not determined on this day as end of shelf life was reached.

Table 6.3 Changes in colour, pH and hardness over the shelf life of cooked marinated pork chops*

	Treatment (MPa)	Day 1	Day 7	Day 9	Day 11	Day 16	Day 23	Day 30	Day 37	Day 44
Lightness (L*)	0.1	51.74 ± 1.99 ^{aA}	52.20 ± 3.37 ^{aA}	51.48 ± 3.52 ^{aA}	47.44 ± 2.27 ^{aB}	47.15 ± 4.11 ^{aB}	/	/	/	/
	300	58.82 ± 2.00 ^{bA}	58.61 ± 3.89 ^{bA}	58.91 ± 3.55 ^{bA}	58.43 ± 3.37 ^{bA}	57.27 ± 4.03 ^{bAB}	54.80 ± 2.57 ^{aB}	53.80 ± 2.84 ^{aB}	/	/
	400	59.62 ± 3.45 ^{bA}	61.30 ± 3.01 ^{bA}	58.98 ± 2.87 ^{bA}	58.25 ± 2.91 ^{bAB}	54.47 ± 2.64 ^{bBC}	54.59 ± 1.78 ^{aBC}	53.44 ± 1.25 ^{aC}	53.34 ± 3.24 ^{aC}	/
	500	60.99 ± 3.08 ^{bA}	61.52 ± 3.29 ^{bA}	60.33 ± 3.17 ^{bAB}	60.97 ± 3.81 ^{bA}	55.73 ± 2.13 ^{bC}	56.26 ± 1.66 ^{aBC}	55.92 ± 3.04 ^{aC}	55.70 ± 1.94 ^{aC}	54.75 ± 2.85 ^{C}
Redness (a*)	0.1	8.96 ± 1.73 ^{aA}	8.89 ± 1.16 ^{aA}	8.26 ± 1.43 ^{aA}	8.92 ± 1.18 ^{aA}	8.67 ± 1.59 ^{aA}	/	/	/	/
	300	8.66 ± 2.18 ^{aA}	8.76 ± 1.35 ^{aA}	8.45 ± 2.14 ^{aA}	8.81 ± 1.36 ^{aA}	8.67 ± 1.24 ^{aA}	8.78 ± 1.18 ^{aA}	8.91 ± 1.20 ^{aA}	/	/
	400	8.67 ± 1.69 ^{aA}	9.14 ± 1.47 ^{aA}	8.03 ± 1.62 ^{aA}	8.54 ±1.87 ^{aA}	8.64 ± 1.04 ^{aA}	9.10 ± 1.39 ^{aA}	9.04 ± 1.93 ^{aA}	8.49 ± 2.03 ^{aA}	/
	500	8.38 ± 2.31 ^{aA}	8.28 ± 1.74 ^{aA}	8.07 ± 1.80 ^{aA}	8.71 ± 2.14 ^{aA}	8.33 ± 1.20 ^{aA}	9.06 ± 1.90 ^{aA}	8.64 ± 2.33 ^{aA}	8.27 ± 1.77 ^{aA}	8.19 ± 2.12 ^{A}
Yellowness (b*)	0.1	30.83 ± 3.35 ^{aA}	30.59 ± 3.97 ^{aA}	32.07 ± 3.02 ^{aA}	31.19 ± 2.64 ^{aA}	32.77 ± 3.02 ^{aA}	/	/	/	/
	300	30.42 ± 3.73 ^{aA}	28.52 ± 2.82 ^{aA}	30.87 ± 2.98 ^{aA}	29.62 ± 2.71 ^{aA}	30.76 ± 3.34 ^{aA}	30.04 ± 2.82 ^{aA}	30.24 ± 3.51 ^{aA}	/	/
	400	31.06 ± 3.43 ^{aA}	31.65 ± 2.47 ^{aA}	31.49 ± 2.67 ^{aA}	30.57 ± 3.54 ^{aA}	31.44 ± 3.44 ^{aA}	33.65 ± 3.24 ^{aA}	32.06 ± 2.47 ^{aA}	32.54 ± 3.84 ^{aA}	/
	500	30.12 ± 2.35 ^{aA}	31.82 ± 4.48 ^{aA}	34.16 ± 3.98 ^{aA}	32.02 ± 4.39 ^{aA}	30.06 ± 2.32 ^{aA}	32.94 ± 3.45 ^{aA}	33.24 ± 3.45 ^{aA}	33.94 ± 2.48 ^{aA}	33.28 ± 3.41 ^{A}
pH	0.1	5.86 ± 0.06 ^{aA}	5.87 ± 0.04 ^{aA}	5.83 ± 0.07 ^{aA}	5.79 ± 0.09 ^{aA}	5.78 ± 0.08 ^{aA}	/	/	/	/
	300	5.87 ± 0.07 ^{aA}	5.83 ± 0.06 ^{aA}	5.79 ± 0.08 ^{aA}	5.80 ± 0.06 ^{aA}	5.84 ± 0.07 ^{aA}	5.79 ± 0.06 ^{aA}	5.80 ± 0.05 ^{aA}	/	/
	400	5.91 ± 0.06 ^{aA}	5.86 ± 0.04 ^{aA}	5.85 ± 0.04 ^{aA}	5.85 ± 0.06 ^{aA}	5.87 ± 0.05 ^{aA}	5.84 ± 0.06 ^{aA}	5.85 ± 0.08 ^{aA}	5.84 ± 0.05 ^{aA}	/
	500	5.90 ± 0.07 ^{aA}	5.83 ± 0.04 ^{aA}	5.79 ± 0.05 ^{aA}	5.83 ± 0.07 ^{aA}	5.83 ± 0.05 ^{aA}	5.80 ± 0.05 ^{aA}	5.84 ± 0.06 ^{aA}	5.87 ± 0.05 ^{aA}	5.79 ± 0.07 ^{A}
Shear force (N)	0.1	14.71 ± 2.82 ^{aA}	14.25 ± 3.01 ^{aAB}	14.84 ± 1.67 ^{aA}	12.66 ± 1.59 ^{aAB}	12.07 ± 1.96 ^{aB}	/	/	/	/
	300	15.08 ± 2.37 ^{abA}	15.63 ± 1.97 ^{aA}	16.39 ± 2.23 ^{aA}	14.82 ± 2.06 ^{aA}	12.46 ± 1.52 ^{aB}	12.17 ± 1.43 ^{aB}	11.67 ± 1.38 ^{aB}	/	/
	400	16.52 ± 3.02 ^{abA}	14.00 ± 2.89 ^{aAB}	14.68 ± 2.23 ^{aAB}	14.30 ± 2.75 ^{aAB}	13.94 ± 2.62 ^{aAB}	11.96 ± 1.90 ^{aB}	11.89 ± 2.26 ^{aB}	12.04 ± 1.87 ^{aB}	/
	500	18.83 ± 2.16 ^{bA}	15.82 ± 3.64 ^{aAB}	15.50 ± 1.77 ^{aAB}	14.05 ± 3.43 ^{aBC}	14.15 ± 3.61 ^{aBC}	12.91 ± 2.90 ^{aBC}	11.21 ± 2.65 ^{aC}	12.32 ± 2.16 ^{aBC}	11.04 ± 2.57 ^{C}

*Values are Mean ± standard deviation $^{a, b}$ Different superscripts in the same column indicate significant difference (P<0.05) between different treatments.

$^{A, B, C}$ Different superscripts in the same row indicate significant difference (P<0.05) in the same treatment over time.

(/) indicates analysis was not determined on this day as end of shelf life was reached.

6.3.7 Lipid oxidation

From the sensory point of view, lipid oxidation cause rancidity problems which are considered unpleasant for consumers (Jeremiah, 2001) Lipid oxidation also results in off odours, off-flavour development, drip losses, discolouration, loss of nutrient value, decrease in shelf life, and the accumulation of toxic compounds, which may be detrimental to the health of consumers (Chaijan, 2008; Mapiye *et al*., 2012; Richards *et al*., 2002). The results for TBARS showed that HPP increased ($P<0.05$) the lipid oxidation of the marinated pork chops and this increase was proportional to the HPP level applied as the control untreated marinated pork chops had the lowest TBARS values and that the marinated pork chops HPP at 500 MPa had the highest TBARS values (Figure 6.1). These results are in agreement with Cheah and Ledward (1996, 1997) who reported that the effect of HPP on the oxidative stability of lipids in pork meat depends on the applied pressure with a value between 300 and 400 MPa constituting the critical pressure to accelerate lipid oxidation. Similarly, it was reported that the pressure level and holding time increased the extent of lipid oxidation in meat products such as dry-cured Iberian ham, pork loin, chicken breast fillets and pork (Cava *et al*., 2002; Kruk *et al*., 2011; Souza *et al*., 2011). Increased rates of lipid oxidation due to HPP has been attributed to pressure-induced protein denaturation which leads to the release of free-radicals catalysing oxidation (Cheftel and Culioli, 1997) and also has been attributed to the release of metal ions from iron complexes promoting auto-oxidation of lipids in HPP meat and also due to membrane damage (Angsupanich and Ledward, 1998; Cheah and Ledward, 1996; Cheah and Ledward. 1997; Chevalier *et al*. 2001).

Over storage time, the TBARS values increased significantly ($P<0.05$) in untreated control and HPP marinated pork chops (Figure 6.2). At the end of their respective shelf life, the TBARS differences observed on day 1 between untreated control and HPP marinated pork

chops (TBARS increased as HPP level increased) were similar. However, throughout storage, TBARS values in all samples were below the maximum acceptable limit for TBARS of 1 mg/kg (Warriss, 2000) which is regarded as the limit beyond which meat products will normally develop objectionable odours/tastes. In agreement with the results found in this study Rodrigues *et al*. (2016) and Grossi *et al* (2014) reported significantly increased TBARS values during storage of marinated beef or brine injected pork meat that were HPP at 600 MPa.

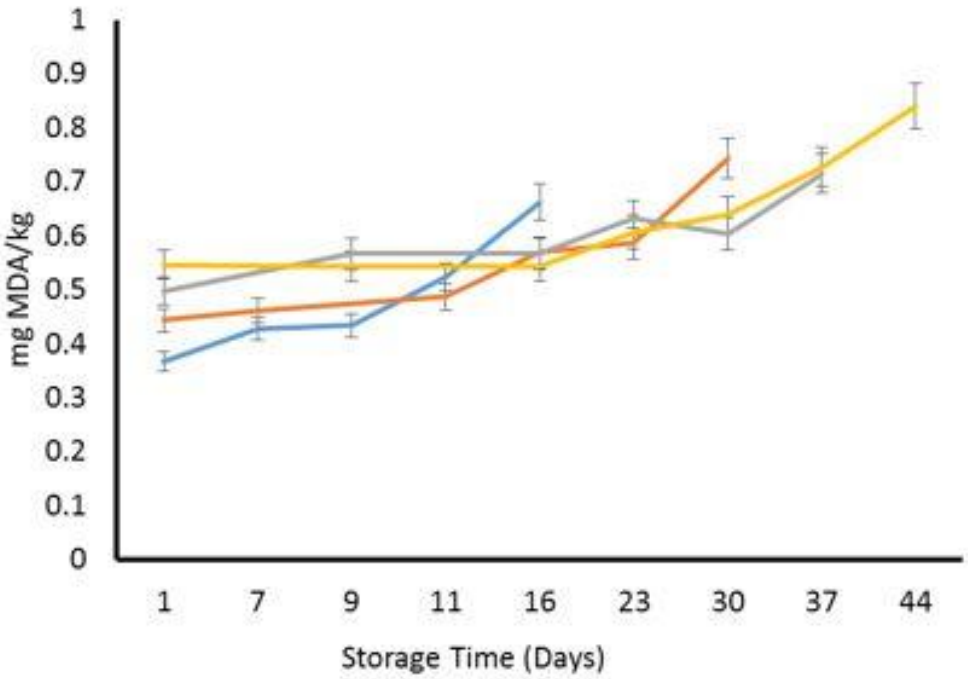

Figure 6.1 – Lipid oxidation (TBARS) changes during chilled storage at 4°C of control raw marinated pork chops (0.1 MPa) (—) or raw marinated pork chops HPP at 300 MPa (—), 400 MPa (—) or 500 MPa (—) for 3 mins. Each point shown is the mean value from two different trials.

6.3.8 Sensory Analysis

The results for sensory analysis showed that at day 1 there were no significant differences between untreated control or HPP marinated pork chops in terms of appearance (raw in packaging), appearance (cooked), juiciness or OSA; however, significant differences (P<0.05) were observed in flavour, texture and tenderness (Table 6.4). In terms of flavour, the control untreated marinated pork chop was the least (P<0.05) preferred and the 500 MPa sample was the most (P<0.05) preferred which may be attributed to HPP's ability to increase the marinade absorption proportionally which in turn may have improved the flavour of the cooked marinated pork chops. The higher flavour acceptability may also be due to the fact that marinated raw pork chops HPP at 400 or 500 MPa had lower cook loss values which may have also resulted in more marinade being retained on the surface of the pork chops compared to control untreated and 300 MPa marinated raw pork chop samples (Table 6.1)

Regarding the texture of the marinated pork chops, at day 1, there were no significant differences in the texture acceptability of control untreated and marinated pork chops HPP at 300 MPa ; however, marinated pork chops that were HPP at 400 MPa or 500 MPa were the least (P<0.05) preferred compared to untreated control or samples that were HPP at 300 MPa Similarly to the WBSF values, untreated control samples had the lowest WBSF values and were therefore the most tender; however, WBSF values increasing as samples becoming tougher (P<0.05) as the pressure level of HPP increased (Table 6.3). The ability of HPP to increase the toughness of post rigor meat has been well documented (Kruk *et al*., 2011; Ma and Ledward, 2004; Del Olmo *et al.*, 2010; Zamri e*t al*., 2006; Jung *et al.*, 2000; Grossi *et al.*, 2014). Interestingly, regardless of the ability of HPP to initially increase toughness and decrease liking of texture in marinated pork chops HPP at 400 or 500 MPa; these samples were preferred in regards to flavour which ultimately resulted in no

significant differences on day 1in the OSA of the untreated control and marinated pork chops that were HPP. This suggests that the use of HPP to accelerate marinade absorption and improve flavour can compensate for the negative effects on texture.

Over storage time, there were no significant differences in terms of appearance (raw in packaging), appearance (cooked), flavour, juiciness or OSA in untreated control and HPP marinated pork chops; however, marinated pork chop samples that were HPP at ≥400 MPa became more tender ($P<0.05$) and as a result the liking of texture increased ($P<0.05$). These results are in agreement with findings on the instrumental WBSF results which decreased significantly over time, subsequently increasing tenderness. Despite increased tenderness in marinated pork chops that were HPP at 400 or 500 MPa, no significant differences on the OSA were observed over storage time. While the tenderness and liking of texture also increased over storage time in untreated control and marinated pork chops that were HPP at 300 MPa; however, these differences were not statistically significant which may be due to a lower ($P<0.05$) marinade absorption compared to marinated pork chops that were HPP at 400 or 500 MPa and According to Burke and Monahan, (2003), marination has been reported to increase tenderness due to marinade uptake by muscle proteins and through solubilisation of collagen

Conversely, Diaz *et al.* (2008) reported that sensory spoilage preceded microbiological spoilage of sous vide pork loin and this loss of acceptance was mainly due to the deterioration of meaty flavour and odour, although the loss of appearance, juiciness and toughness also contributed. In that case, the sensory analysis was the most effective method for determining the shelf life of the sous vide pork. While the shelf life in the study reported by Diaz *et al.* (2008) concluded that the sensory acceptability of pork loin decreased ($P<0.05$) over storage time, this study did not include marinades which are known to improve the sensory acceptability of meat products nor did it include HPP.

Cheftel and Culioli (1997) suggested that HPP of fresh meat causes drastic changes, especially in redness, and thus cannot be suitable of practical applications. Souza *et al*. (2011) also stated that consumers' purchasing preferences are highly based on fresh meat colour and HPP treatment caused meat to appear lighter meaning that more work is needed to investigate meat colour preservation. However, in the current study the addition of the piri-piri marinade masked the significant colour changes of the raw pork meat after HPP as there were no significant differences in the sensory attribute of appearance (vacuum packaged raw marinated pork chops) between samples that were HPP and untreated control samples even though instrumental colour results showed a significant ($P<0.05$) increase in lightness and yellowness and a decrease in redness in raw marinated pork chops that were HPP. These results indicate the potential of marinades to mask the whitening effect/discolouration of HPP on raw meat which can decrease consumer acceptability. Similarly, Wang *et al.* (2015) concluded that the application of honey garlic marinade partially masked meat discolouration due to the application of HPP up to 600 MPa.

In the present study, as TBARS values were below the acceptability limits throughout storage and sensory acceptability did not change significantly; the end of shelf life for all marinated pork chop samples was determined based on the recommended microbiological limits for raw meat products.

Table 6.4 Sensory changes over the shelf life of cooked marinated pork chops*

Sensory Attribute	Treatment (MPa)	Day 1	End of shelf life
Appearance (raw in packaging)	0.1	6.72 ± 1.24 ^{aA}	6.46 ± 1.33 ^{aA}
	300	6.78 ± 1.39 ^{aA}	6.51 ± 1.08 ^{aA}
	400	6.54 ± 1.15 ^{aA}	6.34 ± 1.41 ^{aA}
	500	6.70 ± 1.42 ^{aA}	6.52 ± 1.27 ^{aA}
Appearance (cooked)	0.1	7.48 ± 0.59 ^{aA}	7.25 ± 0.61^{aA}
	300	7.25 ± 0.45 ^{aA}	7.42 ± 0.62 ^{aA}
	400	7.22 ± 1.24 ^{aA}	7.14 ± 0.67 ^{aA}
	500	7.35 ± 0.66 ^{aA}	7.15 ± 0.84 ^{aA}
Flavour	0.1	6.08 ± 1.56 ^{aA}	6.24 ± 1.65 ^{aA}
	300	6.78 ± 1.63 ^{abA}	6.36 ± 1.37 ^{aA}
	400	6.91 ± 1.22 ^{abA}	6.94 ± 0.91 ^{abA}
	500	7.54 ± 1.05 ^{bA}	7.61 ± 0.77 ^{bA}
Texture	0.1	6.30 ± 0.90 ^{aA}	7.03 ± 0.89 ^{aA}
	300	5.84 ± 1.16 ^{aA}	6.12 ± 1.44 ^{aA}
	400	3.99 ± 0.78 ^{bA}	6.01 ± 0.92 ^{aB}
	500	3.82 ± 0.84 ^{bA}	6.10 ± 1.11 ^{aB}
Juiciness	0.1	5.82 ± 0.90 ^{aA}	5.86 ± 1.15 ^{aA}
	300	5.33 ± 2.25 ^{aA}	5.68 ± 0.81 ^{aA}
	400	5.59 ± 1.74 ^{aA}	5.86 ± 1.03 ^{aA}
	500	5.49 ± 1.81 ^{aA}	5.62 ± 0.89 ^{aA}
Tenderness	0.1	6.44 ± 0.85 ^{aA}	6.73 ± 1.14 ^{aA}
	300	6.29 ± 0.99 ^{aA}	6.63 ± 1.24 ^{aA}
	400	4.34 ± 0.97 ^{bA}	6.37 ± 0.91 ^{aB}
	500	4.45 ± 1.10 ^{bA}	6.26 ± 1.22 ^{aB}
OSA	0.1	6.84 ± 1.18 ^{aA}	6.63 ± 0.64 ^{aA}
	300	6.83 ± 1.28 ^{aA}	6.85 ± 1.07 ^{aA}
	400	7.08 ± 1.32 ^{aA}	6.93 ± 1.20 ^{aA}
	500	6.78 ± 1.25^{aA}	6.46 ± 0.94 ^{aA}

*Values are Mean. $^{a, b}$ Different superscripts in the same column indicate significant difference ($P < 0.05$) between different treatments.

$^{A, B}$ Different superscripts in the same row indicate significant difference ($P<0.05$) in the same treatment over time.

6.3.9 Microbiological Analysis

The microbiological changes for TVC and LAB during vacuum packed chilled storage at 4°C in untreated control and marinated pork chops that were HPP is shown in Figure 6.2. The following recommended microbiological limits are applied for fresh meat products: Aerobic plate counts < $5x10^6$ CFU/g of product; *E. coli* < 10 CFU/g of product; LAB < 10^9 CFU/g of product, *Salmonella*: absent in 25 g of product (FSAI, 2015). For this study, the recommended microbiological limits of acceptability for the raw marinated pork chops were set as above with reference to TVC, *E. coli* and *Salmonella.* The initial microbiological quality of the untreated marinated pork chops were of good quality (Figure 6.1). After HPP the marinated raw pork meat samples were below the limit of detection <10 CFU/g, *E. coli* < 10 CFU/g and absence of *Salmonella* in 25 g of sample and untreated control samples (0.1 MPa) had a TVC of 2 log (CFU/g), *E. coli* < 10 CFU/g and absence of *Salmonella* in 25 g of sample. Throughout storage *Salmonella* and *E.Coli* remained absent in all samples.

For untreated control raw marinated pork chop samples which contained 0.3% Inbac^{TM}, the limit of acceptability in terms of TVC was reached after 14 days of storage while the limit of acceptability for raw marinated pork chop samples that were HPP at 300, 400 or 500 MPa and contained 0.3% Inbac^{TM} was reached after 30, 36 or 43 days, respectively, indicating that the shelf life significantly increased by 114, 157 and 207 % when the raw marinated pork chops were HPP at 300, 400 or 500 MPa, respectively compared to untreated control samples (Figure 6.2a). Apparently, for untreated control and marinated raw pork chops that were HPP, the main spoilage microorganism was LAB (Figure 6.2b) which increased significantly (P<0.05) over storage time at a rate similar to TVC. It is well known that LAB is the major group associated with spoilage of refrigerated vacuum or

modified atmosphere packed meat products (Korkeala and Björkroth, 1997) and vacuum packed HPP meat products (Pietrasik *et al*., 2017; Yanqing *et al*., 2009).

These results are in agreement with the results reported by Kruk *et al.* (2011) who found that HPP at 600 MPa for 5 mins reduced the total bacterial count by 6–8 log (CFU/g) improving shelf-life for 7–14 days in raw chicken breast fillets. Similarly, Rodriquez-Calleja *et al.* (2012) reported that a combination of HPP 300 MPa for 5 mins and an edible antimicrobial coating Articoat™ reduced the bacterial load on raw chicken breast fillets below the detection limit and the shelf life of skinless chicken fillets was extended up to four weeks with LAB constituting the main spoilage micro-organism. Garriga *et al*. (2004) also reported that HPP at 600MPa for 6 mins of vacuum-packed marinated beef loin samples reduced at least 4 log cycle for aerobic, psycrothrophic, and LAB counts and that *E. coli* and *Staphylococcus aureus* were kept below the detection limit (<10 or $<10^2$ CFU/g), respectively, during the chilled storage for 120 days. Wang *et al.* (2015) reported that HPP at pressures ≥450 MPa for 3 min significantly extend the shelf life of honey garlic marinated pork chops from 10 days to 31 days based on results for TVC.

The results presented in this study indicated that a combined effect of HPP and Inbac™ extended the shelf life of marinated pork chops and that the shelf life extension depended on the pressure level applied. The results presented in this study also indicated the effectiveness of the combined effect of HPP and a mix of organic acids not only in improving the safety and shelf life of marinated pork chops but also, the effectiveness of HPP at pressures ≥ 400 MPa in accelerating the marinade absorption of pork chops which in turn improved the flavour, masked the discolouration caused by HPP and improved the texture of the marinated pork chops over storage time.

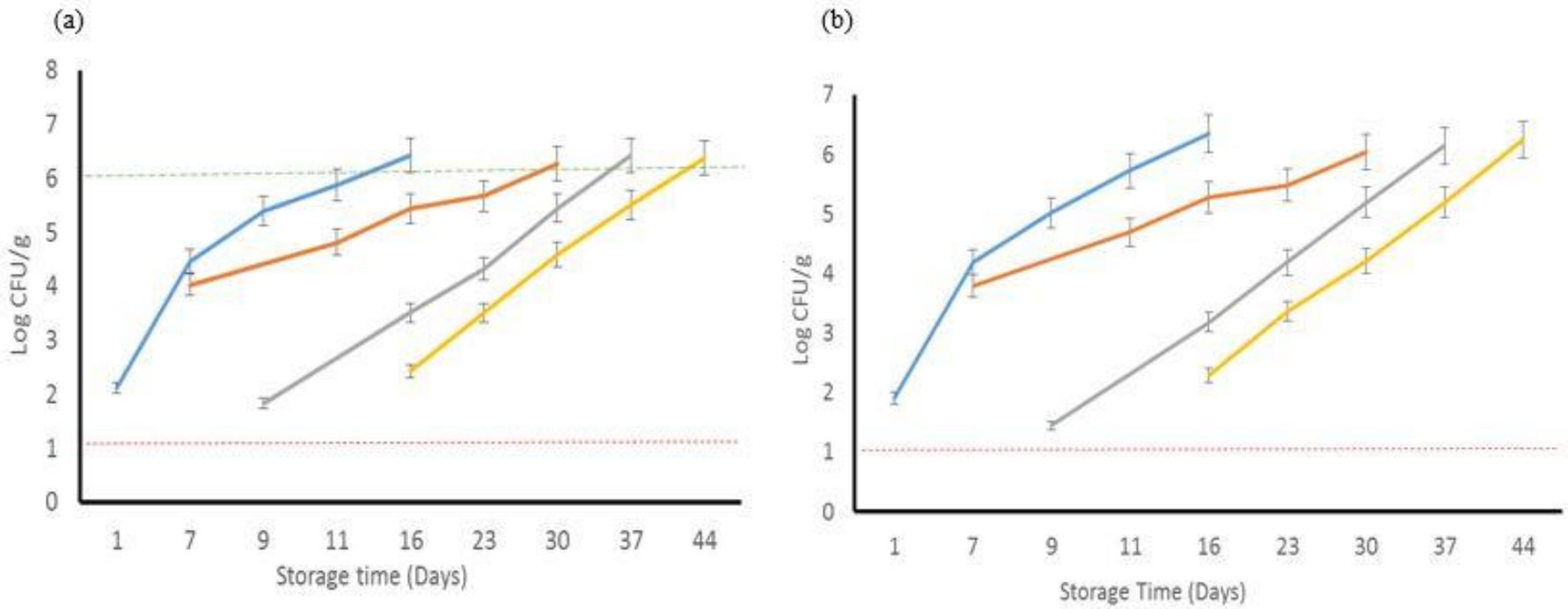

Figure 6.2 - Microbiological changes of (a) Total viable count and (b) Lactic acid bacteria during chilled storage at 4°C of control raw marinated pork chops (0.1 MPa) (—) or or raw marinated pork chops HPP at 300 MPa (—), 400 MPa (—) or 500 MPa (—) for 3 mins. Each point shown is the mean value from two different trials. The dotted lines show the limits of detection (—) and acceptability (—).

6.4 Conclusion

Pressures higher than 400 MPa were required to significantly accelerate ($P<0.05$) the piri-piri marinade absorption in pork chops and improve the flavour acceptability; therefore compensating in terms of OSA for the negative textural effects caused by HPP. Throughout storage, marinated pork chops that were HPP at pressures ≥400 MPa became more tender ($P<0.05$); however, the OSA did not change. A symbiotic effect between HPP and the piri-piri marinade was observed as HPP increased marinade absorption and in turn the marinade increased the flavour acceptability and the tenderness of the marinated pork chops over storage time. The piri-piri marinade also masked the whitening effect on raw pork due to HPP which can decrease consumer acceptability.

The results found in this study indicated that the combination of HPP and antimicrobial Inbac™ increased the safety and shelf life of piri-piri marinated pork chops and that the shelf-life compared to control untreated samples was increased proportionally to the pressure level applied resulting in a shelf life extension of 16, 22 or 29 days, for samples that were HPP at 300, 400 or 500 MPa, respectively. LAB apparently was the main spoilage micro-organism.

Herein we have demonstrated that this relatively novel processing method can improve the flavour of marinated pork chops by accelerating the marinade absorption and in combination with the commercial antimicrobial Inbac™ can extend significantly the shelf life of marinated pork chops without compromising the physicochemical or sensory quality of the pork meat. The extended shelf life can enhance sustainability by reducing food waste of these meat products and also offers potential benefits to meat processors, retail food service suppliers and consumers.

CHAPTER 7

Comparative effect of different cooking methods on the physicochemical and sensory characteristics of high pressure processed marinated pork chops.

Ciara M. O' Neill, Malco C. Cruz-Romero, Geraldine Duffy, Joseph P. Kerry

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Abstract

The objective of this study was to assess the effect of griddle and steam cooking on the physicochemical and sensory characteristics of high pressure processed (HPP) piri-piri marinated pork chops (MPC). Raw MPC that were HPP at 400 MPa had higher ($P<0.05$) marinade absorption compared to untreated samples. After cooking, griddled MPC were significantly ($P<0.05$) darker, less red, less yellow, tougher and had higher cook loss compared to steam cooked samples. The appearance of the griddled MPC was preferred while the texture, tenderness, juiciness and overall sensory acceptability (OSA) were preferred in steam cooked MPC. The increased marinade absorption in MPC that were HPP modified the fatty acid composition resulting in increased ($P<0.05$) levels of oleic acid (C18:1c). Steam cooked MPC had a lower ($P<0.05$) n-6: n-3 PUFA ratio and were preferred by the sensory panel compared to griddled MPC. Overall, from the cooking methods assessed steam cooking was the best cooking method for untreated and MPC that were HPP.

Industrial Relevance

Processed meat manufacturers are constantly looking for new ways to increase yield, safety and shelf life of meat products. While high pressure processing (HPP) of raw meat has been shown to increase the safety and shelf life of these products; however, negative effects on the physicochemical characteristics of raw meat products have been reported. For example, HPP of raw meat products causes a whitening effect which may negatively affect consumers' acceptance of these products. In this study, we used a novel approach (a combination of HPP, marinade and a mix of organic acids Inbac^{TM}) which showed great potential not only for enhancing the yield of marinated pork chops but also enhancement of the sensory properties, safety and shelf life and particularly the piri-piri marinade masked the discoloration of raw pork meat caused by HPP. This study also provides consumers, retailers and caterers with information on how to best prepare HPP meat products and showed that steam cooked HPP marinated pork chops had the best physicochemical and sensory characteristics compared to griddled marinated pork chops.

7.1 Introduction

Pork is currently the most widely consumed meat in the world followed by poultry, beef, and mutton (Worldwatch Institute, 2018) and the global demand for pork meat continuing to rise. (Bord Bia, 2018). The consumer demand for convenience is a driving force within the meat industry (Bord Bia, 2011) and an increase in the range of commercially available marinated products was reported (Hall *et al*., 2008; Yusop *et al*., 2011). HPP is gaining importance in the food industry because of its advantage of inactivating microorganisms and enzymes at ambient or low temperatures without affecting the nutritional properties of food (Indrawati *et al*., 2003). However, pressure levels applied at commercial levels in raw meat products have been reported to denature protein, increase lipid oxidation and induce colour and texture changes (Yagiz *et al*., 2009). Our previous study (Chapter 6), evaluated the ability of HPP to accelerate marinade absorption and improve flavour of MPC and the results showed that HPP at 400 and 500 MPa combined with Inbac^{TM} (0.3%) accelerated (P<0.05) the marinade absorption of raw piri-piri pork chops and enhanced the flavour acceptability and also extended the shelf life significantly; however, immediately after HPP at 500 MPa the MPC were tougher (P<0.05) than untreated control samples or MPC that were HPP at 300 or 400 MPa. HPP at 400MPa was apparently the best pressure level at which significantly lower changes on the physicochemical characteristics of MPC were obtained with an enhanced safety and shelf life.

As meat is usually cooked before consumption it is important to understand the physicochemical and sensorial characteristics of meat products that were HPP and cooked before consumption. Cooking of meat is essential to achieve a palatable and safe product (Tornberg, 2005) as it enhances flavour and tenderness, inactivates pathogenic microorganisms (Broncano *et al*., 2009; Rodríguez-Estrada *et al*., 1997), denature proteins and increases the digestibility and bioavailability of nutrients (Davey and Gilbert, 1974;

Meade *et al*., 2005). The physical properties and quality of cooked meat are strongly affected by the degree of protein denaturation as a result of the type of heat treatment applied, the temperature and length of time of cooking (Ishiwatari *et al*., 2013). Many studies have shown that protein denaturation due to cooking causes structural changes in meat and affects its physical properties such as water-holding capacity, texture, and colour (Bendall and Restall, 1983; Palka and Daun, 1999; Tornberg, 2005; Garcia-Segovia *et al*., 2007) and as a result all sensory attributes can be influenced by changes in the cooking technique (Bejerholm and Aaslyng, 2004).

The most common methods of cooking meat includes roasting, boiling, grilling, broiling, frying, braising, steaming, griddling, poaching, microwaving, baking, poaching, barbequing, *sous*vide and *confit* (AMSA, 2018; Sobral *et al*., 2018). The three main factors that differ among various cooking techniques are the temperature on the surface of the meat, the temperature profile through the meat and the method of heat transfer (convection or conduction by contact, air or steam) (Bejerholm and Aaslyng, 2004). Time also plays an important role in the characteristics of cooked muscle-based food products (Sobral *et al*. 2018). Steam cooking is a widely used, convenient and healthy cooking method as the typical characteristics of colour, flavour, texture, palatability and nutrients are retained (Kahlon *et al*., 2008). Steaming relies on cooking with steam heat resulting from boiling water. The meat has direct contact only with steam which contributes to the moist texture of steam cooked meat (Sobral *et al*. 2018). Air convection is often coupled with steam injection in the oven chamber to improve meat tenderness and to reduce cooking losses (Murphy *et al*., 2001). Griddle cooking is gaining popularity in meat research, especially in industry settings. The griddle cooks meat through conduction heating as the heat is transferred directly from the hot griddle surface to the meat (Yancey *et al*., 2011).

It is well known that different cooking techniques result in different eating qualities of meat products (Fjelkner-Modig, 1986; Heymann *et al*., 1990; Wood *et al*., 1995). Cooking method can also alter the fatty acid composition in meat products (Badiani *et al*., 2004; Maranesi *et al*., 2005; Sarriés *et al*., 2009) due to increased cook loss or due to oxidation (Weber *et al*., 2008).

Dreeling *et al*. (2000) examined the effect of various cooking methods (grilling, frying, griddling, roasting or deep fat frying) on the quality of low-fat beef burgers and found that the cooking method significantly affected the cook loss with deep fat frying and grilling resulting in the highest cooking losses and deep fat frying also resulted in beef burgers with he lowest moisture content. The sensory characteristics of overall sensory acceptability (OSA), tenderness, flavour, appearance, texture and juiciness were significantly affected by the cooking method and griddling was the most acceptable cooking method in terms of OSA. Latif (2010) concluded that the most suitable cooking methods for marinated chicken breast meats were roasting and boiling as they reduced the cook loss compared to microwaving and frying; however, griddling and steaming were not investigated in this study. Barbanti and Pasquini, (2005) reported that marination, followed by air-steam cooking is the best combination to obtain the most tender chicken breast slices.

While there are studies that assessed the effects of various cooking methods on the physicochemical and sensory characteristics of marinated muscle-based food products (Barbanti and Pasquini, 2005; Latif , 2010; Kim *et al.*, 2008; Dhanda *et al.*, 2006), to the best of our knowledge, there are no studies investigating the effects of different cooking methods (griddle and steam cooking) on the physicochemical characteristics of MPC that were HPP; therefore the objective of this study was to assess the effects of different cooking methods (e.g. griddle and steam cooking) on the physicochemical and sensory characteristics of MPC that were HPP.

7.2 Materials and Methods

7.2.1 Materials

Pork loins were obtained from a local meat processor (Ballyburden, Ballincollig, Cork). Piri-Piri marinade (Rapeseed oil 60%, Spices and flavourings 36% (chilli, garlic, jalapeno, black pepper, onion, paprika, lovage root, fenugreek seed, bird clover, onion leek, coriander, turmeric, ginger, cumin seed, fennel, sugar, grapefruit, passion fruit, papaya, mango, palm fat) and Salt 4%) was obtained from Oliver Carty (Athlone, Co. Roscommon, Ireland). A commercial antimicrobial mix of organic acids Inbac^{TM} (a mix of Sodium acetate 43%, Malic acid 7%, emulsifier-mono and diglycerides of fatty acids and technological coadjuvants; anticaking agents, calcium phosphate, magnesium carbonate and silicon dioxide ~50%) was obtained from Chemital (Chemital Ltd, Barcelona, Spain).

7.2.2 Methods

7.2.2.1 Marination of pork chops

The pork loins were cut into 3cm chops including the fat ring, weighed and placed in a combivac vacuum pouch (20 polyamide/70 polyethylene bags (Alcom, Campogalliano, Italy) and piri-piri marinade which contained Inbac™ (0.3%) at a weight ratio 80:20 (Pork chop:marinade) was added and then vacuum packed using a Webomatic vacuum packaging system (Werner Bonk, type D463, Bochum, German). Marinated untreated control samples were stored in a chill room at 4 °C for 24 hrs before cooking. For samples requiring HPP (400 MPa), MPC were HPP (as outlined in section 7.2.2.2) before storage in a chill room at 4 °C for 24 hrs before cooking.

7.2.2.2 High Pressure Processing

Vacuum-packed pork chops that were marinated for 24 hr were HPP using an industrial Hiperbaric 420 litre unit (Burgos, Spain) at the HPP Tolling facilities (HPP tolling, St. Margaret's, Dublin) using water as the pressure transmitting medium. The speed of pressurisation was 130 MPa per minute, the speed of depressurisation was instantaneous (~ 1 second) and the holding time was 3 minutes. The water inlet temperature was 10°C. Before HPP, the initial temperature of the surface of the vacuum packaged MPC was 3.6 °C and after HPP the temperature on the surface of the meat was ~6.5 °C and this was measured using a hand held temperature probe (Monika, United Kingdom).

7.2.2.3 Marinade absorption

The initial weight of raw unmarinated pork chops was recorded. Samples were then marinated as described in Section 7.2.2.1 and after 24 hrs storage at 4°C untreated and HPP samples were placed on an elevated stainless steel wire rack for 5 mins turned half way through and then re-weighed. Calculation for marinade absorption was as follows;

% marinade absorption = (weight after 24 hours marination – initial unmarinated weight) / (initial unmarinated weight) * 100.

Each value represents the average of 8 measurements (two independent trials x four samples).

7.2.2.4 Cooking

MPC were either steam cooked or griddled. For steam cooked, vacuum-packed MPC were cooked at full steam (100 °C) in a Zanussi oven (Zanussi Professional, Italy) and

temperature monitored using a thermocouple data logger (Omega Engineering Ltd., Manchester, UK) inserted into the coldest point of the MPC until an internal temperature of 74 °C was reached. For griddling, MPC were removed from the vacuum pouch and placed on a Gico grill plate, Model 90185 (Gico, Italy), turned half way through and temperature monitored using the thermocouple data logger which was inserted into the coldest point of the MPC until an internal temperature of 74 °C was reached. The samples were then cooled down at room temperature before analysis was carried out.

7.2.2.5 Cook loss

The cook loss of both untreated MPC and MPC that were HPP was determined after griddle or steam cooking. Briefly, the initial weight of the raw MPC was recorded after samples had been placed on an elevated stainless steel wire rack for 5 mins. After cooking, the samples were re-weighed and calculated as follows:

% cook loss = (cooked weight – initial raw weight) / (initial raw weight) * 100

Each value represents the average of 8 measurements (two independent trials x four samples).

7.2.2.6 Compositional analysis

To obtain a representative sample for proximal composition analysis of cooked MPC the outer layer of fat was removed and the meat was homogenised for 20 seconds in a Buchi™ mixer B-400 (Büchi Labortechnik, Switzerland). Proximate composition (fat, moisture, protein and ash) of cooked MPC was determined using the methods previously described

in Chapter 2. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

7.2.2.7 pH

The pH of cooked untreated MPC and MPC that were HPP was measured using a digital pH metre (Mettler-Toledo GmbH, Schwerzenbach, Switzerland) by inserting the glass probe directly into the sample. Each value represents the average of 8 measurements (two independent trials x two samples x two readings).

7.2.2.8 Warner-Bratzler Shear force

Warner-Bratzler Shear force (WBSF) was measured according to the method outlined by Shackelford *et al.* (1991). Briefly, the 3 cm thick MPC were cooked as described in Section 7.2.2.4 to an internal temperature of 74 °C and then cooled at room temperature (20 °C). Four cylinders of a 1.27 cm diameter were obtained from each cooked pork chop parallel to the muscle fibre direction using a corer. The pork steak cylinders were sheared using a Texture Analyser TA-XT2 (Stable Micro Systems, Surrey, UK) attached with a Warner Bratzler V-shaped shearing device at a crosshead speed of 4 mm/s. Each value represents the average of 8 measurements (two independent trials x four samples).

7.2.2.9 Colour

The colour of the surface of the cooked MPC was measured as described in Chapter 2. CIE L*, a* and b* values (Lightness, redness and yellowness, respectively) are reported and

each value represents the average of 12 measurements (two independent trials x two samples x three readings).

7.2.2.10 Fatty acid analysis

7.2.2.10.1 Lipid extraction, Transesterification and Gas chromatography

Total lipids for fatty acid analysis were extracted using the method described by Bligh and Dyer (1959). Briefly, 1.5g of MPC was homogenised in methanol and lipid was extracted from 0.6g of homogenate using water, methanol and chloroform as the extracting solvents. Following phase separation, the lower chloroform layers were dried under nitrogen prior to transesterification. The lipid fractions were trans-esterified to fatty acid methyl esters (FAME's) according to the procedure described by Slover and Lanza (1979). FAME's were separated using a Varian 3800 gas chromatograph (Varian, Walnut Creek, CA, USA) using a WCOT fused silica capillary column (Varian CP-SIL 88 Tailor Made FAME, 60 m x 0.25 mm i.d. x 0.20 µm film thickness) and a flame ionisation detector. Helium was used as the carrier gas at a pressure of 30 psi. The injection volumes and split ratios for FAME's were 1 µl and 1:2 split, respectively. Individual fatty acids were identified by comparing relative retention times with pure FAME standards (Supleco 37 component FAME mix, Sigma-Aldrich Ireland Ltd., Vale Road, Arklow, Wicklow, Ireland). Each value is the average of 8 measurements (two independent trials x four samples).

7.2.2.11 Sensory evaluation

A 25 member semi-trained taste panel was used to evaluate the cooked untreated MPC and MPC that were HPP over two separate sessions using a 9-point hedonic scale. The panellists were recruited from staff and postgraduate students at the School of Food and Nutritional Sciences, University College Cork and chosen based on their experience in the sensory analysis of meat products and on their availability. The panellists have partaken in sensory analysis of meat products on numerous occasions and were familiar with the sensory terminology.

Samples were labelled with a three digit random number, steam or griddled cooked as described in Section 2.2.3 and served warm (50 °C) on labelled polystyrene plates. The tested attributes were: Liking of Appearance (1=Extremely dislike, 9=Extremely like), Liking of Texture (1=Extremely dislike, 9=Extremely like), Liking of Flavour (1=Extremely dislike, 9=Extremely like), Juiciness (1=Very dry, 9=Very juicy), Tenderness (1-Extremely tough, 9= Extremely tender), Off-flavour (1= Imperceptible, 9=Extremely pronounced) and Overall sensory acceptability (OSA) (1=Extremely dislike, 9=Extremely like).

7.2.2.12 Statistical analysis

Colour, texture, cook loss, marinade absorption, proximate composition, pH, fatty acid composition and sensory data were tested using one way ANOVA and significance assessed using Tukey's test at 5% significance level using SPSS software package (SPSS for Windows, version 21 IBM Corp., Armonk, NY, USA).

7.3 Results and Discussion

7.3.1 Marinade absorption and Cook loss

The results showed that marinade absorption/yield of the pork chops increased ($P<0.05$) when MPC were HPP at 400 MPa compared to untreated MPC samples (Table 7.1). It was reported that marinades diffuse from the meat surface into the interior of the meat due to the gradient formed from the higher concentration of marinade to the lower concentration of fluid in the interior of the meat (Yusop *et al*., 2011) and apparently HPP may have accelerated this process.

The cook loss was significantly higher ($P<0.05$) in griddled MPC compared to steam cooked MPC independent of whether HPP was applied or not (Table 7.1). The higher cook loss in samples that were cooked using the griddle may be due to the longer cooking time as this cooking method is by contact between the hot surface and the MPC and the main method of heat transfer is *via* conduction. It was reported that different cooking methods have significant effects on the physicochemical changes due to the cook loss of meat products that affect the cooking times, temperatures and methods of heat transfer (Ishiwatari *et al*., 2013; Bejerholm and Aaslyng, 2004). Alfaia *et al.* (2010) and Utama *et al.* (2018) reported increased cook loss when beef was oven roasted rather than boiled due to prolonged heat exposure duration. In this study, independent of the cooking method used (Griddle or steam cooking) MPC that were HPP at 400 MPa had lower cook loss values than untreated MPC samples; however, these differences were not statistically significant. Similar results on the decrease of cook loss in HPP chicken and pork have been reported (Rodriguez Calleja *et al*., 2012; Souza *et al*., 2011).

7.3.2 Compositonal analysis and pH

The results for proximate composition of untreated MPC or MPC that were HPP and cooked using griddle or steam cooking are shown in Table 7.1. The results indicated that the cooking method did not affect significantly the protein and ash content of untreated MPC or MPC that were HPP; however, the moisture content was significantly lower ($P<0.05$) when MPC were cooked using the griddle method compared to steam cooked MPC independent of whether HPP was applied or not and this may be attributed to the significantly ($P<0.05$) higher cook loss in samples that were cooked using the griddle (Table 7.1). The fat content of untreated or HPP steam cooked MPC was higher compared to griddled untreated or MPC that were HPP which may be due to the lower ($P<0.05$) cook loss; however, these differences were not statistically significant. Similarly, Dreeling *et al.* (2000) reported that cooking method (griddling, grilling, frying, deep fat frying or roasting) did not significantly affect the protein or fat content in beef burgers. Conversely, the authors reported that griddled burgers had the lowest cook loss and highest moisture content.

The results also showed that independent of the cooking method used no significant differences in the pH were observed on untreated MPC or MPC that were HPP (Table 7.1). It was reported that HPP causes an increase in pH in raw meat (Rodriguez-Calleja *et al*. 2012; Wang *et al*., 2015; Cruz-Romero *et al*., 2007) which usually occurs due to the decrease in available acidic groups in the meat as a result of conformational changes associated with protein denaturation (McArdle *et al*., 2011) while the increase in pH due to cooking may be due to decreases in the number of acidic groups in muscle proteins as proteins unfold (Hamm and Deatherage, 1960). Regarding the increase of pH on cooked MPC that were HPP, apparently the combined effects of cooking (heat treatment) and HPP were not additive as the pH was not significantly different compared to the pH of cooked untreated MPC. This may be due to the increased severity of the cooking processes in

comparison to the milder non-thermal process (HPP). A similar effect on pH in cooked beef muscle was reported by Ma and Ledward, (2004) as the combined effects of cooking and HPP were not additive and the pH of the HPP beef meat was not significantly different compared to the pH of cooked untreated beef.

Table 7.1 Physicochemical characteristics of untreated and high pressure processed marinated pork chops cooked using steam cooking or griddle*

Treatment	Cook Loss	Marinade absorption**	Moisture	Protein	Fat	Ash	pH
Pressure (MPa)/cooking method	(%)	(%)	(%)	(%)	(%)	(%)	
0.1/steam	16.93 ± 0.97 [a]	1.88 ± 0.14 [a]	64.11 ± 1.80 [a]	29.08 ± 1.57 [a]	4.73 ± 0.66 [a]	1.39 ± 0.04 [a]	5.86 ± 0.11 [a]
0.1/griddle	19.73 ± 0.94 [b]	1.95 ± 0.19 [a]	61.87 ± 1.54 [b]	29.37 ± 0.80 [a]	4.13 ± 0.20 [a]	1.43 ± 0.08 [a]	5.87 ± 0.05 [a]
400/steam	16.67 ± 1.07 [a]	2.68 ± 0.23 [b]	65.23 ± 1.13 [a]	30.31 ± 1.52 [a]	4.63 ± 0.56 [a]	1.46 ± 0.08 [a]	5.91 ± 0.06 [a]
400/griddle	18.88 ± 1.28 [b]	2.65 ± 0.27 [b]	61.90 ± 1.13 [b]	30.18 ± 1.36 [a]	4.07 ± 0.12 [a]	1.47 ± 0.06 [a]	5.90 ± 0.07 [a]

*Values are Mean ± standard deviation. [a,b] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments. **Analysis carried out before cooking.

7.3.3 Colour

The results showed that the untreated MPC or MPC that were HPP and steam cooked were significantly ($P<0.05$) redder and yellower compared to untreated MPC or MPC that were HPP and cooked using the griddle method which may be due to the presence of the highly pigmented piri-piri marinade (CIE L*, a* and b* values of 26.86, 17.68 and 54.28, respectively) on the surface of the meat (Table 7.2). In regards to lightness, griddle cooked MPC (untreated and HPP) were significantly darker ($P<0.05$) than steam cooked MPC (untreated and HPP) (Table 7.2). This increased darkness may be due to the fact that the in griddled MPC, Maillard reaction, a nonenzymatic chemical reaction between amino acids and reducing sugars which causes browning (Tamana and Mahood, 2015) has taken place. This reaction occur upon heating meat because meat, a proteinaceous material also contains a small amount of carbohydrates, primarily those originating from glycogen and nucleotides (Schmidt, 1988). Humidity has been reported to influence colour development of cooked meat as high humidity will prevent Maillard reactions from taking place (Bejerholm and Aaslyng, 2004) and this may be the reason why steam cooked MPC were significantly lighter compared to griddled MPC. The results found in this study are in agreement with the findings of Chao *et al*. (2009), Delgado-Andrade *et al*. (2010) and Hull *et al*. (2012) who reported that dry heat cooking methods such as frying and grilling increased Maillard reaction compared to moist heat methods such as boiling. Colour changes due to cooking may be due to the denaturation of myoglobin as the cooked pigment is denatured metmyoglobin which is darker in colour (Boles and Pegg, 1999). Colour changes in muscle-based food products after HPP have been reported that may be related to the denaturation of the myofibrillar and sarcoplasmic proteins (Zhou *et al*., 2010; Ma and Ledward, 2013).

Interestingly, MPC that were HPP at 400 MPa and steam cooked resulted in lighter ($P<0.05$) MPC compared to steam cooked untreated MPC samples; however, this effect was not observed in the griddled MPC and no significant differences between griddled MPC that were HPP and griddled untreated MPC were found. This effect in steam cooked MPC may be due to the fact that when MPC were HPP, it may have increased lightness due to the denaturation of proteins before cooking and then the cooking process may have resulted in further protein denaturation compared to untreated MPC that were cooked but not HPP and apparently resulting in lesser degree of protein denaturation and therefore a lesser lightness change. The reason this effect was not evident in griddled MPC may be due to the severity of the griddling cooking process which employs direct contact heat at higher temperature for a longer duration to reach the required internal temperature inducing Maillard reaction, compared to a milder treatment such as steam cooking.

7.3.4 Texture

The results of the WBSF showed that griddled MPC were significantly harder ($P<0.05$) than steam cooked samples (Table 7.2) regardless of whether samples were HPP or not. Higher WBSF values for griddled MPC may be due to the higher cook loss and subsequent lower moisture and fat content (Table 7.1) as higher fat content has been directly linked to increased tenderness (Hand *et al*., 1987; Tobin *et al*., 2012; Fellendorf *et al*., 2015). The higher cook loss may have resulted from a longer cooking time due to the heat transfer by conduction used in the griddle method. Similarly, Yancey *et al.* (2011) reported that beef steaks which were griddle cooked were tougher compared to beef steaks which were grilled or oven cooked. Myofibrillar and connective tissue proteins (collagen and elastin) affect the toughness of muscle tissues and during heating, these proteins are denatured, causing

destruction of cell membranes, shrinkage of fibres, aggregation, and gelling of myofibrillar and sarcoplasmic proteins, and shrinkage and solubilisation of connective tissue (Tornberg 2005; Yu *et al*., 2017).

It was reported that HPP increased the hardness of muscle foods (Kruk *et al*., 2011; Zamri, *et al*., 2006; Rodrigues *et al*., 2016; Macfarlane *et al*., 1980). The increased toughness with pressure has been attributed to an increasing incidence of sarcomeres, in which thick filaments have been compressed onto the Z-line, thus removing the I-band as a zone of weakness (Macfarlane *et al*., 1980). However, in this study the significant differences ($P<0.05$) on hardness were observed in the cooking methods applied and the application of HPP apparently did not have an additive effect in terms of increasing hardness of the MPC. This may be due to the severity of the thermal cooking process and its increased ability to denature proteins compared to HPP which is a milder non-thermal process.

Table 7.2 Colour and textural analysis of untreated and high pressure processed marinated pork chops cooked using steam cooking or griddle*

Treatment	**Lightness**	**Redness**	**Yellowness**	**WBSF**
Pressure (MPa)/cooking method	(L*)	(a*)	(b*)	(N)
0.1/steam	52.41 ± 2.77 [a]	9.30 ± 0.92 [a]	33.78 ± 2.16 [a]	15.93 ± 1.87 [a]
0.1/griddle	45.58 ± 2.58 [c]	7.45 ± 0.84 [b]	29.07 ± 1.88 [b]	21.52 ± 2.35 [b]
400/steam	59.34 ± 3.43 [b]	9.33 ± 1.50 [a]	35.80 ± 3.59 [a]	16.44 ± 2.06 [a]
400/griddle	47.64 ± 2.06 [c]	7.93 ± 0.59 [b]	29.74 ± 2.35 [b]	21.83 ± 2.51 [b]

*Values are Mean ± standard deviation. [a,b,c] Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

7.3.5 Sensory analysis

The sensory analysis results are shown in Table 7.3. Griddled MPC were significantly (P<0.05) more acceptable in terms of appearance compared to steam cooked samples which may be due to chargrilled effect and darker colour on the surface of the meat as a result of Maillard reaction. Similarly, Dreeling *et al.* (2000) reported that grilling and griddling were the most preferred cooking method in terms of appearance compared to roasting and frying.

Steam cooked MPC (untreated and HPP) were preferred (P<0.05) in regards to texture, tenderness, juiciness and OSA compared to griddled MPC (untreated and HPP) which may be attributed to the lower cook loss and subsequent higher fat and moisture content. The results of the sensory analysis are also in agreement with the instrumental texture results (Table 7.2) which showed that steam cooked MPC (untreated or HPP) were tenderer (P<0.05) than griddled MPC (untreated or HPP). Barbanti and Pasquini. (2005) suggested that marination process followed by air-steam cooking was the best combination to obtain the most tender chicken breast slices. Although the differences were not statistically significant, griddled and steam cooked MPC which were HPP at 400 MPa had higher flavour acceptability than untreated MPC which may be due to higher (P<0.05) marinade absorption (Table 7.1). Similar to our results, Dreeling *et al.* (2000) concluded that the sensory characteristics of OSA, tenderness, appearance, texture and juiciness were significantly affected by the cooking method applied (grilling, frying, griddling, roasting or deep fat frying) and griddling was the most acceptable cooking method in terms of OSA; however, steam cooking was not investigated in this study.

HPP can increase the toughness of post rigor meat (Ma and Ledward, 2004; Del Olmo *et al.*, 2010; Jung *et al*., 2000; Grossi *et al*., 2014); however, our results indicated that cooking method of the MPC had a more significant effect on the textural and sensory characteristics

of MPC than HPP which may be due to the severity of the cooking process compared to HPP which is a non-thermal and milder process. Furthermore, the sensory results showed that steam cooking resulted in a more acceptable MPC in terms of texture and OSA compared to griddled MPC. Steam cooked MPC were also cooked in their final packaging resulting in a more convenient product for consumer use compared to griddled MPC.

Table 7.3 Sensory analysis of untreated and high pressure processed marinated pork chops cooked by steam cooking or griddle*

Treatment	**Sensory Attributes**						
Pressure (MPa)/cooking method	Appearance	Texture	Flavour	Tenderness	Juiciness	Off-flavour	OSA
0.1/steam	5.93 ± 1.88 [a]	6.68 ± 1.47 [a]	6.31 ± 1.56 [a]	5.90 ± 1.28 [a]	5.93 ± 1.88 [a]	1.96 ± 1.78 [a]	7.05 ± 1.34 [a]
0.1/griddle	6.68 ± 1.23 [b]	5.13 ± 1.27 [b]	6.68 ± 1.40 [a]	4.41 ± 0.94 [b]	4.87 ± 1.23 [b]	2.23 ± 1.84 [a]	6.12 ± 1.09 [b]
400/steam	5.65 ± 1.07 [a]	6.41 ± 1.29 [a]	6.83 ± 1.14 [a]	5.82 ± 1.44 [a]	5.94 ± 1.52 [a]	1.73 ± 1.30 [a]	6.99 ± 1.02 [a]
400/griddle	7.14 ± 1.17 [b]	5.67 ± 1.32 [b]	7.03 ± 1.45 [a]	4.17 ± 1.12 [b]	5.00 ± 1.19 [b]	2.43 ± 1.89 [a]	6.02 ± 1.25 [b]

*Values are Mean ± standard deviation. [a, b]. Different superscripts in the same column indicate significant difference ($P<0.05$) between treatments.

7.3.6 Fatty acid composition

The fatty acid composition of the untreated MPC, MPC that were HPP and the piri-piri marinade is presented in Table 7.4. The results showed that in the piri-piri marinade 15 fatty acid were present and in the untreated and MPC that were HPP 28 fatty acids were present. The main fatty acids detected in marinade were C16 (Palmitic acid- SFA), C18:1c (Oleic acid- MUFA) and C18:2c (Linoleic acid- PUFA) which accounted for 83% of the total fatty acid composition. In the MPC, the main fatty acids present were typical for pork and consisted of C16 (Palmitic acid- SFA), C18 (Stearic acid - SFA), C18:1 (Oleic acid - MUFA), C18:2 (Linoleic acid- PUFA) which accounted for ~85% of the total fatty acids in all MPC. Fatty acid composition of meat is of major importance for consumers due to their importance for meat quality and nutritional value (Wood *et al*., 2004).

HPP is a very mild process in terms of its effect on fatty acids (Yagiz *et al*., 2009). Independent of the cooking methods used, the results showed that in general, there were no significant differences in SFA and PUFA in MPC that were HPP compared to untreated marinated samples; however, the MPC that were HPP had significantly ($P<0.05$) higher C18:1c (Oleic acid – MUFA) which may be due to the higher marinade absorption by the pork chops as Oleic acid is the main fatty acid present in the piri-piri marinade. Similarly, previous studies have indicated that HPP of salmon, beef, goat and oysters up to 800 MPa had no significant effect on their overall fatty acid composition (Cruz-Romero *et al*., 2008; McArdle *et al.*, 2010, 2011; Yagiz *et al*., 2009; He *et al*., 2012); however, these studies did not include the addition of marinade. Kruk *et al*. (2014) reported that HPP at 300 MPa did not affect the fatty acid composition of chicken breast meat; however, the addition of olive oil and soya sauce in combination with HPP at 300 MPa significantly changed the fatty acid composition which may be due to composition of these marinades. Interestingly, when olive oil was added to chicken breast fillets and HPP at 600 MPa, the fatty acid composition

was modified further and increased MUFA's obtained which may have been due to the ability of HPP to increase marinade absorption; however, the authors did not determine marinade absorption nor did they determine the fatty acid composition of the marinades.

It was reported that cooking methods such as frying or deep fat frying can affect the fatty acid composition due to the addition of oils or fats (Broncano *et al*., 2009). The cooking methods applied in this study did not change significantly the fatty acid composition; however, the MPC that were steam cooked had a lower ($P<0.05$) n-6/n-3 ratio compared to griddled samples. Typical n-6:n-3 PUFA ratios in pork meat were reported to be 6.30 (Shortle, 2016) which is similar to the results found for griddled MPC. A lower n-6/n-3 ratio was reported to increase the ratio of these groups of fatty acids and have increased health benefits including the prevention of cardiovascular, cardiometabolic, and other chronic diseases as well as the reduction of inflammation (Siscovick *et al*., 2017; Chen *et al*., 2015; Sanders, 2014). The health attributes of n-3 PUFA is due to the direct effects of α-linolenic acid (ALA), which cannot be synthesized by humans, or the conversion of ALA to eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and/or the decrease in the n-6:n-3 PUFA ratio (Domenichiello *et al*., 2015).

Table 7.4 Fatty acid composition of untreated and high pressure processed marinated pork chops cooked by steam cooking or griddle*

Fatty acid	Piri-piri Marinade	0.1MPa/ griddle	0.1 MPa/ steam	400 MPa/ griddle	400 MPa/ steam
Lauric acid (C12:0)	ND	0.14 ± 0.02 [a]	0.16 ± 0.03 [a]	0.12 ± 0.04 [a]	0.10 ± 0.00 [a]
Myristic acid (C14:0)	0.14 ± 0.01	1.36 ± 0.03 [ab]	1.57 ± 0.24 [b]	1.08 ± 0.07 [a]	1.14 ± 0.04 [a]
Palmitic acid (C16:0)	6.73 ± 0.16	24.54 ± 0.66 [a]	25.30 ± 3.33 [a]	23.34 ± 0.69 [a]	22.31± 0.49 [a]
Heptadecanoic acid (C17:0)	ND	0.32 ± 0.01 [a]	0.45 ± 0.11 [a]	0.34 ± 0.02 [a]	0.31 ± 0.02 [a]
Stearic acid (C18:0)	1.07 ± 0.37	13.05 ± 0.92 [a]	13.27 ± 2.33 [a]	12.62 ± 0.97 [a]	12.73 ± 0.67 [a]
Arachidic acid (C20:0)	0.50 ± 0.02	ND	ND	ND	ND
Heneicosanoic acid (C21:0)	ND	0.17 ± 0.03 [a]	0.18 ± 0.06 [a]	0.28 ± 0.03 [b]	0.19 ± 0.02 [ab]
Behenic acid (C22:0)	ND	0.68 ± 0.26 [a]	0.59 ± 0.22 [a]	0.35 ± 0.02 [a]	0.78 ± 0.08 [a]
Tricosanoic acid (C23:0)	ND	0.55 ± 0.31 [a]	0.42 ± 0.20 [a]	0.46 ± 0.03 [a]	0.63 ± 0.14 [a]
Lignoceric acid (C24:0)	ND	0.81 ± 0.08 [a]	0.50 ± 0.22 [a]	0.34 ± 0.03 [a]	0.62 ± 0.32 [a]
Palmitoleic acid (C16:1 (n-7))	0.25 ± 0.02	1.42 ± 0.10 [a]	1.70 ± 0.26 [a]	1.38 ± 0.13 [a]	1.16 ± 0.31 [a]
Cis-10 Heptadecenoic acid (C17:1c (n-7))	ND	0.13 ± 0.01 [a]	0.19 ± 0.02 [b]	0.15 ± 0.03 [ab]	0.12 ± 0.03 [a]
Elaidic acid (C18:1t (n-7))	0.16 ± 0.05	3.38 ± 1.10 [a]	1.18 ± 1.12 [b]	1.46 ± 0.11 [b]	2.24 ± 0.08 [ab]
Oleic acid (C18:1c (n-9))	56.32 ± 0.94	29.58 ± 0.38 [a]	31.00 ± 2.12 [ab]	34.85 ± 1.20 [c]	33.20 ± 0.75 [bc]
Cis-11 Eicosenoic acid (C20:1c (n-9))	8.27 ± 0.20	1.51 ± 0.05 [a]	1.49 ± 0.05 [a]	1.29 ± 0.34 [a]	0.99 ± 0.29 [a]
Erucic acid (C22:1c (n-9))	0.26 ± 0.09	0.34 ± 0.03 [a]	0.32 ± 0.07 [a]	0.40 ± 0.08 [a]	0.35 ± 0.04 [a]
Nervonic acid (C24:1c (n-9))	ND	0.27 ± 0.02 [a]	0.41 ± 0.17 [a]	0.44 ± 0.11 [a]	0.46 ± 0.15 [a]
Linoelaidic acid (C18:2t (n-6))	ND	1.00 ± 0.07 [a]	1.27 ± 0.40 [a]	1.11 ± 0.02 [a]	1.10 ± 0.28 [a]
Linoleic acid (C18:2c (n-6))	19.15 ± 0.56	14.94 ± 0.50 [a]	13.93 ± 3.04 [a]	15.58 ± 0.63 [a]	15.14 ± 1.01 [a]

G - Linolenic acid (C18:3c (n-3))	1.25 ± 0.07	0.60 ± 0.02 [a]	0.55 ± 0.03 [a]	0.61 ± 0.08 [a]	0.47 ± 0.09 [a]
A - Linolenic acid (C18:3c (n-6))	0.39 ± 0.07	0.36 ± 0.02 [a]	0.34 ± 0.04 [a]	0.30 ± 0.02 [a]	0.32 ± 0.01 [a]
Cis-11,14 Eicosenoic acid (C20:2 (n-6))	0.09 ± 0.01	0.68 ± 0.06 [a]	0.63 ± 0.10 [a]	0.87 ± 0.10 [a]	0.64 ± 0.18 [a]
Cis-8,11,14 Eicosatrienoic acid (C20:3c (n-3))	0.13 ± 0.03	1.70 ± 0.47 [a]	2.38 ± 1.07 [a]	1.75 ± 0.60 [a]	1.72 ± 0.18 [a]
Cis-11,14,17 Eicosatrienoic acid (C20:3c (n-6))	0.31 ± 0.02	0.63 ± 0.19 [a]	0.51 ± 0.28 [a]	0.57 ± 0.02 [a]	0.71 ± 0.18 [a]
Arachidonic acid (C20:4 (n-6))	ND	0.43 ± 0.12 [a]	0.36 ± 0.13 [a]	0.34 ± 0.03 [a]	0.36 ± 0.16 [a]
Eicosapentaenoic acid (C20:5 (n-3))	ND	0.19 ± 0.10 [a]	0.24 ± 0.04 [a]	0.15 ± 0.11 [a]	0.42 ± 0.18 [a]
Cis-13,16 Docosadienoic acid (C22:2 (n-6))	ND	0.45 ± 0.20 [a]	0.45 ± 0.18 [a]	0.41 ± 0.10 [a]	0.50 ± 0.07 [a]
Docosahexaenoic acid (C22:6 (n-3))	ND	0.50 ± 0.19 [a]	0.42 ± 0.13 [a]	0.43 ± 0.10 [a]	0.78 ± 0.18 [a]
ΣSFA	**8.44**	**41.62 ± 1.49 [a]**	**42.61 ± 2.64 [a]**	**38.73 ± 1.09 [a]**	**39.31 ± 1.87 [a]**
ΣMUFA	**65.26**	**36.63 ± 3.16 [a]**	**36.29 ± 1.71 [a]**	**39.97 ± 2.87 [a]**	**38.52 ± 2.14 [a]**
ΣPUFA	**21.32**	**21.48 ± 1.36 [a]**	**21.08 ± 1.35 [a]**	**22.12 ± 1.27 [a]**	**22.16 ± 1.84 [a]**
Σn-3	**1.38**	**2.99 ± 1.07 [ab]**	**3.59 ± 0.44 [a]**	**2.77 ± 0.64 [b]**	**3.39 ± 0.49 [a]**
Σn-6	**19.94**	**18.49 ± 0.80 [a]**	**17.49 ± 0.78 [b]**	**19.18 ± 1.25 [a]**	**17.44 ± 1.24 [b]**
n-6/n-3	**14.45**	**6.18 ± 0.82 [a]**	**4.87 ± 1.12 [b]**	**6.92 ± 1.34 [a]**	**5.14 ± 0.74 [b]**

*Values are mean ± standard deviation [a, b, c]. Different superscripts in the same row indicate significant difference ($P<0.05$) between treatments. Fatty acids are % of total. ND = Not detected.

7.4 Conclusion

While HPP at 400 MPa accelerated ($P<0.05$) marinade absorption in raw MPC, it had minimal effects on the quality of the cooked MPC. The acceleration of marinade absorption on the pork chops by HPP apparently modified the fatty acid composition of the MPC and significantly ($P<0.05$) increased the level of oleic acid which was the main fatty acid present in the piri-piri marinade.

The cooking methods applied (Steam or griddle cooking) had a significant effect on the physicochemical and sensory quality of cooked MPC. Griddled MPC were preferred in terms of appearance; however, steam cooking resulted in better quality MPC in terms of physicochemical (cook loss, moisture content, WBSF and n-6: n-3 PUFA ratio) and sensory (texture, tenderness, juiciness and OSA) characteristics.

Overall, the results showed that from the cooking methods assessed steam cooking was the best cooking method for MPC that were HPP and provided an advantage as it can be cooked vacuum-packed resulting in a convenient product for consumer use and extended shelf life of the MPC due to the hurdle approach used (combined effect of HPP and antimicrobial Inbac^{TM}) found in our previous study.

CHAPTER 8 - General Discussion, Conclusion and Future Work.

8.1 General Discussion

Concerns associated with processed meat consumption and human health has prompted much research into the development of healthier processed meat products specifically in regards to salt reduction (Doyle and Glass, 2010; Pietrasik and Gaudette, 2014). The link between high salt intake and cardiovascular disease has been well established and as a result regulatory agencies such as the FSAI have set national targets for salt reduction in meat products. (Aburto *et al*., 2013; Morgan *et al*., 2001; Desmond, 2006).

In Chapter 1, assessment of the literature was carried out, a full background of this area of research was provided and ultimately the potential of salt replacers and high pressure processing (HPP) for salt reduction and enhancement of safety and shelf life with minimum effects on food quality was highlighted. In the initial experimental part of this study (Chapters 2 and 3), a novel approach which showed great potential for optimising salt reduction (NaCl) in frankfurters and cooked ham was used. Response surface methodology was applied in order to determine the best combination of Salt replacer Artisalt^{TM}, HPP and a mix of organic acid Inbac^{TM} to produce frankfurters and cooked ham with significantly low-salt content without compromising the physiochemical characteristics and sensory acceptability. When NaCl was partially replaced (50%) with salt replacer Artisalt^{TM}; quality parameters such as pH, cook loss, emulsion stability, colour, texture or sensory attributes of frankfurters were not significantly affected compared to full salt control samples, while quality parameters such as pH, cook loss, sliceability, expressible moisture, colour, texture or sensory attributes of cooked ham were not significantly affected compared to full salt control cooked ham. However, in both products when added NaCl was fully replaced (100%) with salt replacer Artisalt^{TM} all physicochemical quality parameters were significantly ($P<0.05$) affected and the obtained frankfurters and cooked ham were significantly less acceptable by the sensory panel. The non-significant effect on

the quality parameters of the processed meat products when 50% of NaCl was replaced with salt replacer Artisalt™ was attributed to the calculated ionic strength (IS) of a 50/50 combination of Artisalt™ /Sodium Chloride (NaCl) (0.31M) which was similar to that the IS of 2 % NaCl (0.34M) and the use of salt replacer Artisalt ™ resulted in the development of optimised low-salt products without compromising the physiochemical characteristics and sensory acceptability associated with these type of products. Due to the fact that the salt replacer Artisalt™ also contained flavour enhancers the saltiness perception was increased.

The optimisation process was initially carried out based on maximising the texture (hardness) and the sensory attributes (flavour, saltiness and overall sensory acceptability (OSA)). While the attributes of hardness, flavour and saltiness were predicted by the models; a higher level of salt replacement and HPP was achieved when product optimisation was carried out based on OSA which subsequently produced a product with lower salt content and increased safety due to the use of a higher level of HPP in combination with the antimicrobial organic acids; therefore, production of the optimised processed meat products was carried out based on maximising the OSA. The results of the optimisation process based on the OSA showed that in frankfurters it was possible to reduce 48% of the added salt (NaCl) in combination with HPP at 580 MPa and 0.3% Inbac™, and in cooked ham it was possible to reduce 53% of the added salt (NaCl) in combination with HPP at 535 MPa and 0.3% Inbac™.

The resulting total salt contents in the optimised low salt frankfurters or cooked ham were 1.3% or 1.4%, respectively, therefore salt reduction below the national target levels set by Food Safety Authority of Ireland (FSAI) in 2017 for cooked ham (1.6%) and frankfurters (1.5%) was achieved using the salt replacer Artisalt™, HPP and a mix of organic acids Inbac™ without compromising on OSA. It was expected that the hurdle technology (HPP

and Inbac^{TM}) applied in the manufacture of frankfurters or cooked ham through product optimisation process would compensate the safety and shelf life due to the significant salt reduction and decreased preservative effect of NaCl.

When salt is significantly reduced, the preservative effects of salt can also be reduced and as a result processed meat manufacturers are constantly looking for new ways to reduce salt levels without compromising food safety and shelf-life. In Chapter 4, the efficacy of a combination of optimum levels of HPP and Inbac^{TM} as hurdles to extend the shelf life of previously optimised sensory accepted frankfurters and cooked ham with significantly (P<0.05) lower salt content was assessed. Throughout storage, physicochemical (colour, texture, pH, lipid oxidation), microbiological (Total viable count (TVC), *Lactic acid bacteria* (LAB) *E. Coli* and *Salmonella*) and sensory analysis was carried out. The following recommended microbiological limits are applied for cook-chill products examined at the point of consumption before reheating or cooking is applied: Aerobic plate counts < $5x10^5$ CFU/g of product; E. coli< 10 CFU/g of product; LAB < 10^9 CFU/g of product, Salmonella: absent in 25 g of product (FSAI, 2014). For this study, the recommended microbiological limits of acceptability for the frankfurters and cooked ham were set as above with reference to TVC, *E. coli* and *Salmonella.* While most of the physicochemical characteristics of frankfurters or cooked ham changed significantly (P<0.05) during storage time; however, the OSA of the frankfurters or cooked ham was not reduced over storage time and TBARS values were below the acceptability limits therefore the end of shelf life for all frankfurter and cooked ham formulations was determined based on the recommended microbiological limits for cook-chill product in terms of TVC.

The results showed that the optimum levels of hurdles (HPP and Inbac™) applied in the manufacture of low-salt processed meat products extended (P<0.05) the shelf-life by 51% or 97% of low-salt frankfurters or low-salt cooked ham, respectively, compared to control

samples which contained full salt content and the preservative effects of salt. These results highlighted the potential of the hurdle strategy for extending the safety and shelf-life of low-salt processed meat products. To the best of our knowledge, the application of hurdle technology (a combination of HPP and a mix of organic acids) to compensate the microbiological stability, safety and shelf-life in processed meat products due to significant salt reduction is novel and has not been previously reported in the literature. This opens the opportunity to apply this technology for shelf life and safety enhancement in salt reduced processed meat products.

In Chapter 5, a comparative study of commercial products and research optimised low-salt frankfurters and cooked ham was carried out in order to confirm acceptance and consumer (n=100) appeal of these low-salt processed meat products with enhanced safety and shelf life in comparison to gold standard commercially available products. The results showed that the commercial brand frankfurter was the most preferred in terms of flavour and juiciness; however, the firmness and saltiness of the research optimised low salt and research control frankfurters was preferred. Overall, the research optimised low-salt frankfurter was liked just as much as the commercial brand frankfurter. For cooked ham, while the research optimised low-salt and research control cooked ham was preferred to the commercial brand cooked ham in terms of appearance and firmness, the commercial brand cooked ham was preferred in terms of juiciness. Furthermore, there were no significant differences in flavour or saltiness between any of the cooked ham samples. Overall, the research optimised low-salt cooked ham was liked just as much as the commercial brand cooked ham. Consumers also did not detect differences in saltiness between the research optimised low-salt and research control products despite the significant salt reduction which was due to the inclusion of the salt replacer Artisalt™ which contains flavour enhancers such as yeast extract, onion and celery. These results

indicated that the low-salt processed meat products were just as acceptable or better than the gold standard commercially available products in the Irish market confirming the potential of the use of the salt replacer Artisalt^{TM} and the combined hurdles (HPP and organic acids) to produce consumer accepted low-salt processed meat products with enhanced safety and shelf life.

The novel approach used showed great potential for significantly reducing salt in frankfurters and cooked ham without compromising the safety and shelf life. The results presented in Chapters 2-5 resulted in the successful development of consumer accepted ready to eat convenience processed meat products which not only contained significantly lower salt content and enhanced safety and shelf-life but also with similar physicochemical and sensory characteristics to control samples which contained significantly higher amount of salt.

In Chapter 6, a novel approach was used which showed great potential for flavour enhancement of marinated fresh meat. It was reported that the demand for value added meat products continues to rise and an increase in the range of commercially available marinade products (Hall *et al*., 2008; Yusop *et al*., 2011), as a result flavour components such as barbeque and piri-piri marinade are in high consumer demand (Nachay, 2011). HPP has not been previously applied as a methodology to accelerate marinade absorption and improve flavour of value added meat products and is therefore a novel application. The ability of HPP and Inbac^{TM} to accelerate piri-piri marinade absorption and extend the shelf life of pork chops was examined in Chapter 6. Throughout storage, physicochemical (colour, texture, pH, lipid oxidation), microbiological (Total viable count (TVC), Lactic acid bacteria (LAB) *E. Coli* and *Salmonella*) and sensory analysis was carried out. The following recommended microbiological limits were applied for fresh meat products: Aerobic plate counts < $5x10^6$ CFU/g of product; *E. coli*< 10 CFU/g of product; LAB < 10^9

CFU/g of product, *Salmonella*: absent in 25 g of product (FSAI, 2015). For this study, the recommended microbiological limits of acceptability for the raw marinated pork chops were set as above with reference to TVC, *E. coli* and *Salmonella.* The results showed that the various levels of pressure applied (300, 400 and 500 MPa) increased the pH, lipid oxidation, toughness and lightness proportionally; however, HPP ≥400 MPa also increased ($P<0.05$) the marinade absorption which enhanced the flavour perception of the marinated pork chops.

A symbiotic effect between HPP and the piri-piri marinade was observed as HPP increased marinade absorption and in turn the marinade increased the flavour acceptability. The piri-piri marinade increased the tenderness of the marinated pork chops over storage time and also masked the whitening effect caused by HPP. HPP can increase toughness of post rigor meat (Kruk *et al*., 2011; Zamri *et al*., 2006; McArdle *et al.*, 2011; Ma and Ledward, 2004). Cheftel and Culioli (1997) also suggested that HPP of fresh meat causes drastic changes in colour and thus cannot be suitable of commercial applications as consumers' purchasing preferences are highly based on fresh meat colour (Souza *et al*., 2011); however, the results found in this study suggested that the use of marinades to mask discoloration and increase tenderness may overcome these issues resulting in marinated HPP meat products that are accepted by consumers.

During storage, physiochemical changes occurred; however, the OSA did not change significantly; therefore, the end of shelf life for all marinated pork chop samples was determined based on the recommended microbiological limits in terms of TVC for raw meat products. From the microbiological point of view, ($P<0.05$) the shelf-life of marinated pork chops which contained Inbac™ (0.3%) was extended by 16, 22 and 29 days compared to untreated control samples when marinated pork chops were HPP at 300, 400 or 500 MPa, respectively. The results of this study highlighted the potential of a combined effect of HPP

and antimicrobial Inbac™ to accelerate flavour absorption and significantly extend the shelf life of marinated pork chops.

Overall, in this study, we used a novel approach (a combination of HPP, marinade and a mix of organic acids Inbac™) which showed great potential not only for enhancing the yield of marinated pork chops but also enhancement of the sensory properties, safety and shelf life and particularly the piri-piri marinade masked the discoloration of raw pork meat caused by HPP and also increased the tenderness over storage time.

Currently, very little attention has been given to the comparative physicochemical and sensory properties of cooked HPP meat products and as a result consumers do not have information available on how to best prepare HPP meat products to retain the maximum nutritional quality and physicochemical and sensory characteristics when cooking HPP meat products. Therefore, it was deemed important to determine the physicochemical changes in marinated pork chops that were HPP and cooked using common cooking methods (e.g. steam or griddle cooking). In Chapter 7, the effects of these different cooking methods (griddle and steam cooking) on the physiochemical and sensory characteristics of marinated pork chops was investigated. According to the results found in Chapter 6, HPP at 400MPa was apparently the best pressure level at which significantly lower changes on the texture of marinated pork chops were obtained with a significantly enhanced safety and shelf life compared to untreated control samples or marinated pork chops that were HPP at 300 or 500 MPa. Therefore, 400 MPa was the chosen pressure level to be applied in this subsequent study. The results showed that griddled marinated pork chops had higher ($P<0.05$) cook loss and lower moisture content and were significantly ($P<0.05$) darker, less red, less yellow, tougher compared to steam cooked samples. The sensory analysis indicated that the appearance of the griddled marinated pork chops was preferred while the texture, tenderness, juiciness and OSA were preferred in steam cooked marinated pork

chops. Apparently, the increased marinade absorption in HPP marinated pork chops modified the fatty acid composition resulting in increased ($P<0.05$) levels of Oleic acid (C18:1c) which was the main fatty acid present in the piri-piri marinade. Overall, the results showed that the cooking method applied significantly affected the physicochemical and sensory quality of marinated pork chops that were HPP. Steam cooking resulted in marinated pork chops with improved physicochemical characteristics, lower n-6: n-3 PUFA ratio which indicates improved health benefits, and were also preferred by the sensory panel compared to griddled marinated pork chops. Another advantage was that when steam cooking was used the marinated pork chops were cooked in their final packaging resulting in an extremely convenient product for consumer use. Overall, from the cooking methods assessed steam cooking was the best cooking method for untreated and marinated pork chops that were HPP.

In Chapter 6, the best combination of HPP and Inbac™ to accelerate marinade absorption and extend the shelf life of added value raw meat products with minimal physicochemical changes was determined and in Chapter 7 the most suitable cooking method for marinated meat products that were HPP was determined.

In all experimental chapters of this thesis, a commercially available salt replacer (Chapters 2-5) and antimicrobial (Chapters 2-7) were used and in Chapters 4-7 an industrial scale HPP unit was used to treat meat products which has an advantage as the scaling up of the process can easily be achieved.

The cost of the HPP at industrial scale was estimated and it varied for each different product. The cost of HPP was calculated using the formula;
Cost of cycle / kg of product per cycle = cost per kg of product

For cooked ham, it was estimated that 200kg of product could be HPP in one cycle (€90). 90/200 = 45 cent per kg.

For frankfurters, it was estimated that 220kg could be HPP in one cycle (€90) 90/220 = 40 cent per kg.

For marinated pork chops, it was estimated that 250kg could be HPP in one cycle (€90)

90/250 = 36 cent per kg.

Although HPP will slightly increase the cost of production, the ability of HPP to significantly increase the safety and shelf life of these meat products (as demonstrated in this thesis) can also reduce food waste of these products which will enhance sustainable food production and possibly reduce loss of company profits due to food waste reduction.

As outlined above, the findings of this thesis are not just of commercial and food processing interest with potential benefits to meat processors, retail food service suppliers, caterers and consumers, but they are also of public health significance as processed meat products with significantly lower salt and enhanced safety and shelf life were manufactured.

8.2 General Conclusions

- Response Surface Methodology is a powerful tool that can be successfully used to develop low-salt processed meat products. (Chapter 2 and 3).
- The optimised formulation to obtain cooked ham with significantly lower salt content without compromising physicochemical or sensory quality; Salt replacer Artisalt™ (53%), HPP (535 MPa) and Inbac™ (0.3%). (Chapter 2)
- The optimised formulation to obtain frankfurters with significantly lower salt content without compromising physicochemical or sensory quality were: Salt replacer Artisalt™ (48%), HPP (580 MPa) and Inbac™ (0.3%). (Chapter 3).
- The total salt content of the optimised low-salt cooked ham and frankfurters were below the national salt reduction targets set by FSAI in 2017 for frankfurters and cooked ham which are 1.6% and 1.5% salt, respectively. (Chapter 2 and 3).
- The hurdle technology (HPP and Inbac™) applied in the manufacture of frankfurters or cooked ham through the product optimisation process compensated in terms of safety and shelf life for the significant salt reduction and the preservative effects of salt (Chapter 4).
- Consumers did not detect differences in saltiness between control and optimised low-salt frankfurters or cooked ham despite the significant salt reduction. The optimised low-salt processed meat products were liked just as much as the commercially available products. (Chapter 5).
- Pressures higher than 400 MPa were required to significantly accelerate the piri-piri marinade absorption in pork chops and improve the flavour acceptability of marinated pork chops. (Chapter 6).
- Over storage time, the piri-piri marinade increased the tenderness of the marinated pork chops which were initially tougher due to HPP. (Chapter 6).

- The piri-piri marinade masked the whitening effect on raw pork due to HPP which can decrease consumer acceptability (Chapter 6).
- The combination of HPP and Inbac^{TM} extended the shelf-life of marinated pork chops proportionally to the pressure level applied resulting in a maximum shelf life extension of 29 days compared to control marinated pork chops when HPP at 500 MPa was applied (Chapter 6).
- The acceleration of marinade absorption by HPP modified the fatty acid composition and increased the level of Oleic acid significantly as this was the main fatty acid present in the marinade. (Chapter 7).
- Steam cooking was the best cooking method for untreated and marinated pork chops that were HPP (Chapter 7).

8.3 Future Work

This thesis provides a framework upon which future research on the application of HPP in processed and fresh meat products can be based. Some studies which could be conducted in future are as follows;

- The application of response surface methodology to reduce not only salt but also fat content in processed meat products.
- Investigation of commercially available marinades to mask the discolouration caused by HPP in fresh meat products increasing consumer acceptability of HPP meat products.
- Assessment of a wider range of commonly used household cooking methods (e.g. grilling, oven cooking, microwaving and frying) on the physicochemical and sensory quality of HPP meat products.
- Investigation into various types of marinades and their ability to increase the tenderness of HPP meat during storage which is tougher immediately after HPP.
- The application of response surface methodology to optimise salt replacer Artisalt™, HPP and Inbac™ and significantly reduce salt in other processed meat products.
- Investigate the use of HPP to accelerate a wide range of commercially available marinade flavours absorption, increase the yield and improve the flavour acceptability in marinated meat products.
- Study of the growth of specific spoilage microorganisms during storage of HPP marinated meat products to understand the shelf life of these products.
- Determination of kinetics of destruction of different pathogens present in raw meat products to ensure that marinated meat products that are HPP are safe for human consumption.

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