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

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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.

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 & Strong, 2005), therefore salt reduction in processed meats can be problematic (Pietrasik, 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 & 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 & 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 & 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 & 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 our previous studies (O' Neill *et al*., 2015 & 2018) 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; O' Neill *et al*., 2018; 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^{TM} 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 salt replaced frankfurters and cooked ham from a microbiological and physicochemical point of view".

2. Materials & Methods

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).

2.2 Methods

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 added the curing ingredients, seasonings and half of the ice. 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 (90 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 1.

2.2.2 Ham manufacture

The treatments used for the shelf life analysis are presented in Table 1. The cooked ham was manufactured as previously described by O' Neill *et al.,* (2018). 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 (90 °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. The treatments used for the shelf life analysis are presented in Table 1.

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 1.

2.2.4 Salt content

Salt content was determined as described by O' Neill *et al*., (2018). 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).

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*) & 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).

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).

2.2.7 Texture analysis

Hardness (N) and Springiness (mm) of the cooked hams or frankfurters were determined as previously described by O' Neill *et al*., (2018). 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).

2.2.8 Colour

Colour of ham was determined as previously described by O' Neill *et al.,* (2018) while 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 12 measurements (two independent trials x two samples x three readings per samples).

2.2.9 Sensory evaluation

Sensory analysis was carried out as described by O' Neill *et al*., (2018). 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.

2.2.10 TBARS

Throughout storage, lipid oxidation of frankfurters or cooked ham was measured using the 2-thiobarbituric acid (TBARS) assay (Siu & 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).

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.

3. Results and Discussion

3.1 Proximate composition and salt content of frankfurters and cooked ham

The results for proximate composition in our previous studies (O' Neill *et al*., 2015 & 2018) 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.

3.2 Colour of frankfurters and cooked ham

At day 1, in both low salt frankfurters or cooked ham that were not HPP (F-LS/1T & 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 (Table 2 & 3). These results are in agreement with our previous findings (O' Neill *et al*., 2015 & 2018) 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.

3.3 Texture of frankfurters and cooked ham

While in both frankfurters and cooked ham, initially at day 1, the low salt samples that were not HPP (F-LS/1T & 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 (Table 2 & 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 (O' Neill *et al*., 2015 & 2018) 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 (O' Neill *et al.,* 2018) 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 & 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.

3.4 pH of frankfurters and cooked ham

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 (Table 2 & 3). These results are also in agreement with our previous findings (O' Neill *et al*., 2015 & 2018) 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.

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 & 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 & H-LS/2T) had the highest TBARS values (Fig. 3) compared to control and low-salt samples which were not HPP (F-LS/1T & H-LS/1T). This may be due to the use of HPP in the F-LS/2T & H-LS/2T formulations which has been reported that HPP can accelerate lipid oxidation on HPP meat products (Andres *et al*., 2004; Cheah & 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 & 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 & H/LS/2T) had higher initial TBARS and also the highest TBARS values throughout storage (Fig.3). 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.

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 (data not shown). These results are in agreement with our previous studies (O' Neill *et al*., 2015 & 2018) 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.

3.7 Microbiological Analysis

The microbiological changes for TVC & LAB during chilled storage (4 °C) in all treatments of vacuum packed frankfurters or cooked ham is shown in Figs. 1 & 2. 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 (Fig. 1a).

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 (Fig. 2a). 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 microorganism in all frankfurter and cooked ham treatments was LAB (Figs. 1b & 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 cooked meat products (Korkeala & 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.

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.

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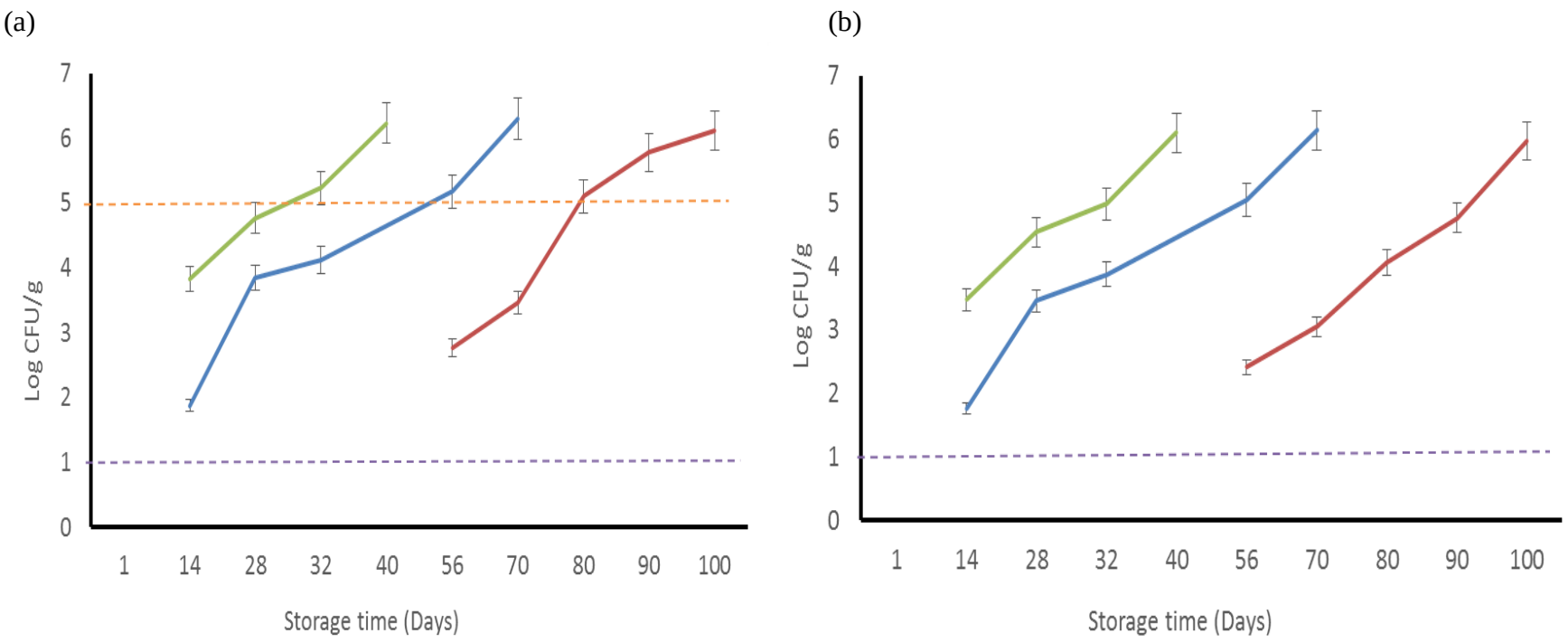


FIGURE 1 Microbiological changes (a) TVC and (b) LAB of Control (—), F-LS/2T (—) and F-LS/1T (—) vacuum packed frankfurters during chilled storage at 4°C. Each point shown is the mean value from two different trials. The dotted lines show the limits of detection (—) or acceptability (—).

(a) (b)

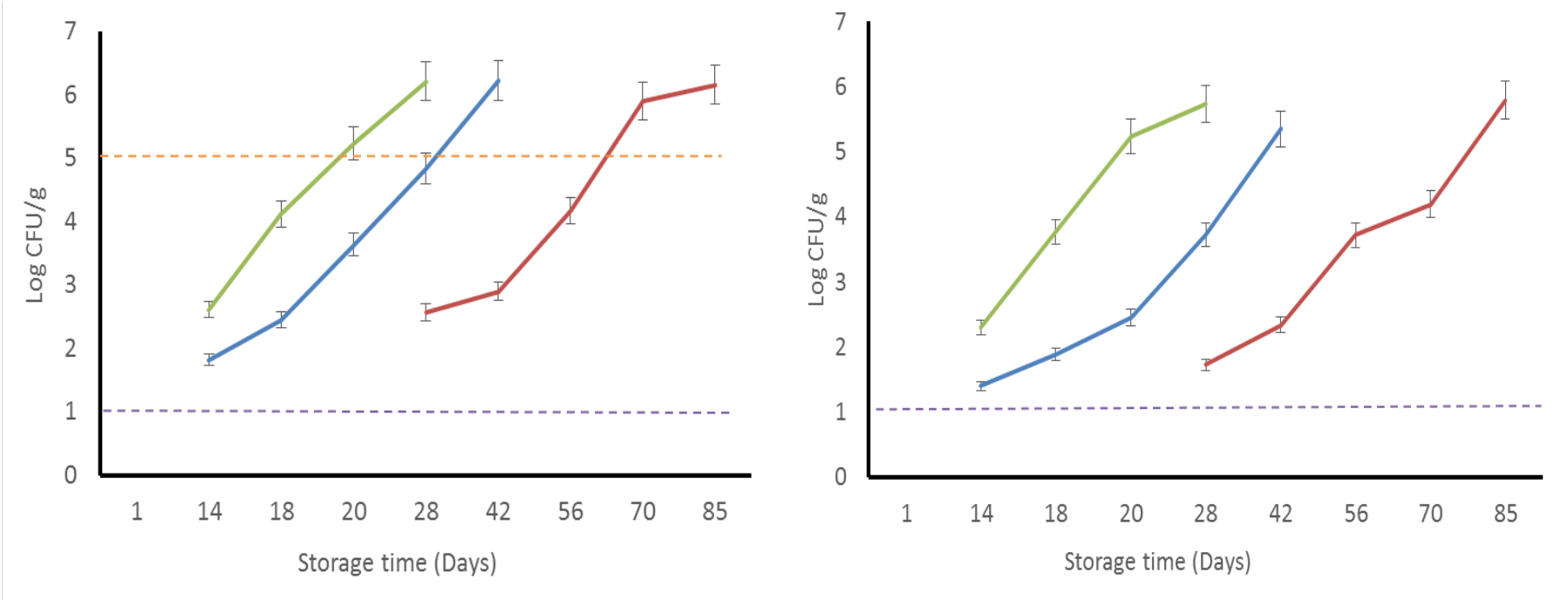


FIGURE 2 - Microbiological changes (a) TVC and (b) LAB of Control (—), H-LS/2T (—) and H-LS/1T (—) vacuum packed cooked ham during chilled storage at 4°C. Each point shown is the mean value from two different trials. The dotted lines show the limits of detection (—) or acceptability (—).

(a) (b)

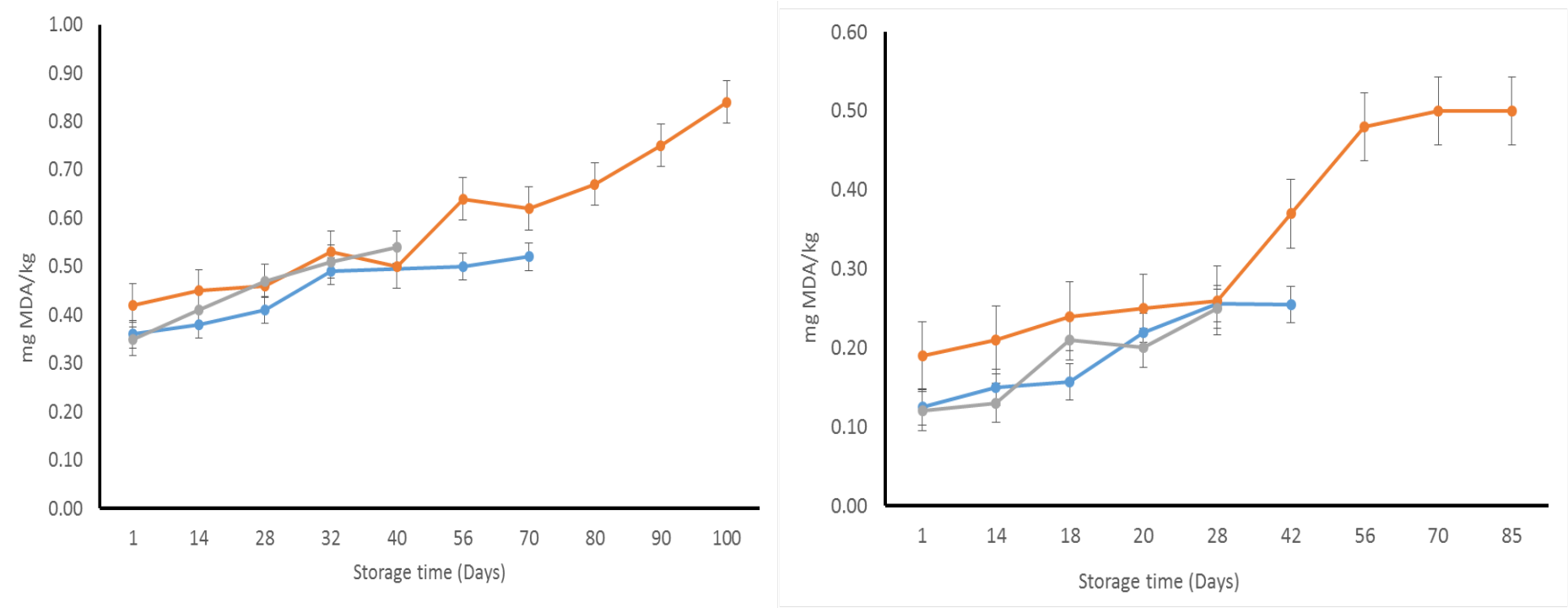


FIGURE 3 – Lipid oxidation (TBARS) of (a) vacuum packed frankfurters; control (—),F-LS/2T (—) and F-LS/1T (—) and (b) vacuum packed ham; control (—), H-LS/2T (—) and H-LS/1T (—) during chilled storage at 4°C. Each point shown is the mean value from two different trials.

TABLE 1 Frankfurter and cooked ham treatments.*

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

TABLE 2 Physicochemical changes of frankfurters during storage at 4°C*

*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.

	Day 1	Day 14	Day 28	Day 32	Day 40	Day 56	Day 70	Day 80
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}	/	/	/	/
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}	/	/	/	/
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								
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								
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}	/	/	/	/

TABLE 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
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}	/	/	/	/
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}	/	/	/	/
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								
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								
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.

TABLE 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.