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High Pressure Processing of Smallgoods

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Project Summary

The microbial contamination of ready-to-eat products has garnered great concern by the processor and the consumer, and the implementation of novel techniques, alone or in tandem with traditional methods, may help the food industry address this concern in a timely and cost effective manner. One of the potential applications of high pressure processing (HPP) is as an in-package 'cold' pasteurisation step for packaged ready-to-eat meats that may have been contaminated through portioning, slicing, comminuting and/or packaging. Important benefits of this application include improved food safety and extension of the refrigerated shelf life. The objective of this study was to determine the required process criteria (pressure and time parameters) via kinetic inactivation studies and product challenge testing (conducted with four commercially available smallgoods products) to meet a performance criterion of a 4-$\log_{10}$ CFU/g reduction in *Listeria monocytogenes.* Additionally, this study examined the effect of in-package HPP on the refrigerated shelf life of smallgoods (Strassburg, export sausage, low-fat pastrami, and Cajun beef) with regards to sensory attributes and the microbial safety and quality of the product.

The results from inactivation kinetics studies in a broth system, followed by preliminary inoculated pack studies with the four smallgoods products used in this study, indicated that processing at 600 MPa, 20 °C for 180 s would result in a 4-$\log_{10}$ CFU/ml reduction of *L. monocytogenes*. The inoculated pack challenge studies with the four smallgoods products showed that levels of *L. monocytogenes* would remain below detectable levels [reduced from initial level of 4 $\log_{10}$ CFU/g to < -1.4 $\log_{10}$/g (absence in 25 g) after HPP] over at least a 13-week storage period at 4°C for the Cajun beef sample, but other products (Strassburg, export sausage and low-fat pastrami) did have sporadic positive results from selective enrichment procedures. However, enumeration techniques showed that levels remained below detectable limits (<10 CFU/g) for at least 10 weeks. These results suggest that a slightly longer processing time or higher pressure is indicated to assure nondetectable levels if the initial level of *L. monocytogenes* on product is assumed to be 10^4 CFU/g. However, a recently published survey (April 2003) of *L. monocytogenes* in ready-to-eat foods, conducted by the National Food Processors Association (USA), reports that in the 82/9,199 luncheon meat samples which tested positive for *L. monocytogenes*, 81 samples had levels of 0.04-10^3 CFU/g, while only 1 had > 10^3 CFU/g. This indicates that HPP at 600 MPa for 180 s could be a successful in-package pasteurisation method for commercial production of such products. Further evaluation of the effects of various components in the meat products, such as fat level, spices, acids, on the effectiveness of HPP would aid in the development of commercial in-package pasteurisation processes which would ensure the safety and extend the shelf life of refrigerated smallgoods products.

The microbiological analyses of smallgoods products utilized in the shelf life study (Strassburg, low-fat pastrami, export sausage and Cajun beef) showed that HPP (600 MPa, 180 s) was effective in keeping levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds to below the detectable limits (<10 CFU/g), or at low levels throughout the 95 days of storage at 4°C. At no point during the shelf life test did any sample test positive (by enrichment) for *Listeria monocytogenes*, coliforms or *Salmonella* spp. The results indicated that HPP could effectively extend the refrigerated shelf life, with regards to microbial quality, to at least 95 days post-processing, which is about double the current refrigerated shelf life for these commercial products.

Comparison of consumer hedonic ratings for the control (day 7) and corresponding HPP meat samples over the evaluated storage period (97 days) revealed no deterioration in the sensory quality of the Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples used in this study. Comparison of open-ended responses over the evaluated storage period revealed that the majority comments were hedonic in nature, when consumers commented on their like or dislike of the specific test samples. On day 76 and 97 some comment was made about the apparent discoloration around the edges of the Cajun beef meat samples. Such discoloration, which was also present in the export sausage meat samples after 49 days refrigerated storage, was likely to have been caused by vacuum packing rather than HPP treatments. Comparison of consumer hedonic ratings at five time points over an evaluated storage period, provided the statistical power necessary to prove that HPP does not adversely affect the sensory quality of Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples. Moreover, this study revealed that HPP is an effective means of maintaining the organoleptic quality of Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples for an extended refrigerated storage period (98 days).

Many of the benefits of HPP of foods are indirect. The process does not improve production rates or organoleptic quality of cooked, ready-to-eat meats, however it will extend shelf life, with benefits in restocking frequencies, production size runs, stock levels and pallet utilisation improvements in the warehouses of major retail chains. Extended shelf life has particular benefits in the servicing of overseas markets and may allow sea freight to be used rather than airfreight, with substantial cost savings. Cost estimates conducted utilising Australian food manufacturing information and processing times based on information obtained in this study, estimate processing costs of A$0.05-0.20 per 300 g package.

Introduction

In response to the current consumer demand for convenience foods, the market for sliced ready-to-eat processed meat products has grown. Manufacturing of these types of products involves slicing and packaging operations that take place after thermal treatment and therefore these are operations that have a direct effect on the shelf life and safety of these products (Lopez-Caballero, 1999). The contamination of ready-to-eat products has garnered great concern by the processor and the consumer, and the implementation of novel techniques, alone or in tandem with traditional methods, may help the food industry address this concern in a timely and cost effective manner (Lucore et al., 2000). One of the potential applications of high pressure processing (HPP) is as an in-package 'cold' pasteurisation step for packaged ready-to-eat meats that may have been contaminated through portioning, slicing, comminuting and/or packaging. Important benefits of this application include improved food safety and extension of the refrigerated shelf life. There is little information currently available in the scientific literature about HPP and its ability to reduce pathogenic microflora such as *L. monocytogenes* in packaged, ready-to-eat meats.

HPP is a non-thermal method of food preservation that has attracted much interest in the last couple of decades for its ability to inactivate microorganisms while maintaining the fresh like qualities of many food products. Typically pressures of 300 to 700 MPa are utilised to extend the shelf life and improve the safety of foods (Stewart and Cole, 2001). HPP has several benefits over thermal pasteurisation. Pressure is transmitted instantaneously and uniformly throughout the food, so that it is evenly treated (Smelt, 1998; Kelly, 2000). Pressure, unlike heat, does not disrupt covalent bonds, so that many of the nutrient and flavour compounds of the food are left intact, resulting in a product that often has a superior taste, nutritional value and quality as compared to thermally processed counterparts (Farr, 1990). Several foods are currently available on the international market, including pressurised sliced ham in Spain; guacamole, salsa, juices, ready-to-eat meats and oysters in the USA; jellies in Japan; and juice and fruit smoothie products in several European countries (Grant et al., 2000; Stewart and Cole, 2001). Additionally, seafood products will soon be available in Australia.

Since the 1980's, *Listeria monocytogenes* has emerged as a major foodborne pathogen (Sutherland and Porritt, 1997). *L. monocytogenes* is a widely spread environmental microorganism found in soil, foliage, and faeces of humans and animals. The microorganism frequently enters the human food supply and has been isolated from many foods including milk, cheese, vegetables, fish and other seafood as well as from raw and processed meat and poultry (Grau and Vanderlinde, 1992; Tompkin et al., 1992; Farber and Daley, 1994; Gilbert, 1996). *L. monocytogenes* is able to survive and/or grow in many foods during refrigerated storage (Walker et al., 1990). Although listeriosis occurs relatively infrequently, the ability of *L. monocytogenes* to cause severe illness and death in the young, elderly, pregnant women and in the immunocompromised means it is important to eliminate or reduce numbers in the food supply, particularly in ready-to-eat foods.

L. monocytogenes can tolerate a wide pH range, low water availability and a wide range of temperatures. Growth occurs between pH 4.3-9.6, with all strains growing best at neutral to slightly alkaline pH (Seelinger and Jones, 1986). The microorganism is resistant to high salt concentrations; some strains can tolerate 20% to 30% salt (Seelinger and Jones, 1986). *L. monocytogenes* grows best at 0.97 a_w, but can survive and replicate at 0.90 a_w (Seelinger and Jones, 1986). The temperature limits of growth are from –0.4 to 50°C, with an optimum growth temperature in the range of 30-37°C (Farber and Peterkin, 1991; Walker et al., 1990). Modified atmospheres and vacuum packaging do not significantly affect growth, and *L. monocytogenes* can survive the nitrate levels permissible in foods. *L. monocytogenes* does not survive heating at 60°C for 30 min, or at 72°C for 15 s and therefore is eliminated during correct pasteurisation procedures (Bradshaw et al., 1987; MacDonald and Sutherland, 1993; Seelinger and Jones, 1986). However, manufacturing of many ready-to-eat meat products involves slicing and

packaging operations which take place after thermal treatment and therefore are a crucial factor in the microbial safety and shelf life of the product (Lopez-Caballero et al., 1999).

L. monocytogenes is a frequent contaminant of raw materials and therefore can be constantly reintroduced into the manufacturing environment (Gilbert, 1996). *L. monocytogenes* can enter manufacturing plants through soil on workers' shoes and clothing, transport equipment, raw materials (animal or plant based), and healthy human carriers (Pritchard, et al., 1995), therefore it is extremely difficult to exclude from food processing facilities. For example, the incidence of *L. monocytogenes* in raw meat and poultry in a farm environment may be as high as 30-50% (Jay, 1996). Moist refrigerated conditions, often found in processing environments, allow for survival and growth of this microorganism (Venables, 1989). Hygiene within the plant is important in limiting the contamination of processing equipment (Pritchard et al., 1995). The risk of product contamination by *L. monocytogenes* can be reduced but, with current technology, the microorganism cannot be eradicated from the finished product environment (Tompkin et al., 1992). The International Commission on Microbiological Specifications for Foods (ICMSF) has suggested recontamination after cooking is the most common reason for the presence of *L. monocytogenes* in packaged, cooked sausages, such as frankfurters (ICMSF, 2002). If recontamination is assumed to be 10 CFU/g, then an in-package pasteurisation treatment (such as HPP) could be applied as a means to achieve a 4-$\log_{10}$ reduction of *L. monocytogenes* and still meet a performance criterion of [10^{-3} CFU/g ([1 CFU/kg) (ICMSF, 2002).

There are approximately 60 cases of listeriosis in Australia annually, with a mortality rate of 23% (FSANZ, 2002). *L. monocytogenes* has been estimated by the Centers for Disease Control and Prevention to cause 2,493 illnesses, 2,322 hospitalisations and 499 deaths per year in the United States, 99% of which are via consumption of contaminated foods (Mead et al., 1999). Although the number of cases of listeriosis per year is relatively small, *L. monocytogenes* accounts for 28% of estimated food-related deaths in the United States, which is second only to Salmonella (31%). High-risk foods for the susceptible portion of the population include ready-to-eat products that are stored at refrigeration temperatures for long periods of time, conditions that may enable *L. monocytogenes* to grow. Therefore, those individuals in the high-risk segments of the population are advised to avoid certain types of ready-to-eat foods, for example soft cheeses (feta, Brie, Camembert, blue-veined, and Mexican-style cheese) and foods from deli counters and cold cuts, unless thoroughly reheated before eating (FSANZ, 2001).

There have been two significant outbreaks of listeriosis in Australia. The first was in Western Australia in 1990, where there were nine perinatal cases. The outbreak was traced to pate, and resulted in six stillbirths. *L. monocytogenes* was isolated from the pate, and the source of the microorganism was thought to be poorly cleaned equipment (Sutherland and Porritt, 1997). The second outbreak was in Tasmania in 1991, when three people contracted listeriosis after consuming smoked mussels, which had been imported from New Zealand. Counts from leftover mussels showed *L. monocytogenes* contamination of approximately 10^7 CFU/g (Sutherland and Porritt, 1997) . Other cases of listeriosis in Australia have been sporadic, often with unknown food sources (Sutherland and Porritt, 1997).

HPP has been identified as being of special interest for meat products as an in-package pasteurisation process for products that may have been contaminated through portioning, slicing, comminuting and/or packaging operations. Although there are already commercial ready-to-eat meat products on the market in Europe and the US which have been high pressure processed, there is little information available in the scientific literature about HPP and its ability to reduce pathogenic and spoilage microorganisms in packaged, ready-to-eat meats (Lucore et al., 2000) and the subsequent effect on extending the refrigerated shelf life of this type of product. In this study, four ready-to-eat sliced meat products were used, as nominated by two Australian manufacturers of smallgoods products; Strassburg, low-fat pastrami, export sausage and Cajun beef. These products where chosen based on a) their high retail value; b) their potential to support *L. monocytogenes* growth over extended refrigerated storage; c) to cover a range of cured/uncured and comminuted/whole muscle products; and/or d) their sale in the domestic or export market.

Objectives

The target performance criterion for *L. monocytogenes* in a ready-to-eat packaged meat could be set at a 4-$\log_{10}$ reduction by pressure treatment based on published risk assessments (FDA, 2001; ICMSF, 2002). The objective of this study was to determine the required process criteria (pressure and time parameters) via kinetic inactivation studies and product challenge testing to meet the above performance criterion.

Additionally, this study examined the effect of in-package HPP on the refrigerated shelf life of smallgoods with regards to sensory attributes and the microbial safety and quality of the product.

Materials and Methods

Listeria monocytogenes strains

Nine *L. monocytogenes* strains isolated from a variety of sources including processed meats were selected for resistance testing to HPP (Table 1). Each strain was initially identified as *L. monocytogenes* using colony morphology on tryptone soya agar (TSA), Gram stain, catalase production and a positive CAMP test. Final confirmation was obtained using Listeria API strips (BioMerieux, France).

Table 1: *Listeria monocytogenes* strains and origins

Culture Collection Source	Strain number	Original Isolation Source
FRRB	2472	ATCC strain Scott A (clinical specimen)
FRRB	2542	Salami
FRRB	2655	Chicken feathers
FRRB	2657	Chicken skin
FRRW	2340	Salad with pasta, cheese & ham/bacon
FRRW	2341	Salad with pasta, cheese & ham/bacon
FRRW	2343	Salad with pasta, cheese & ham/bacon
FRRW	2342	Ham
FRRW	2345	Ham

W= Werribee, VIC Food Science Australia culture collection
B= North Ryde, NSW Food Science Australia culture collection

Culture storage

The isolates were stored as glycerol stocks. Each of the nine strains was streaked onto TSA and incubated overnight at 37°C. Several colonies were collected with a loop and immersed into a sterile 1.7 ml CryoTube™ Vial (Nalge Nunc International, Denmark) containing 0.9 ml sterile tryptone soya broth (TSB). The tubes were vortexed, and then 0.5 ml of sterile 40% (v/v) glycerol (Sigma, Australia) was added. The tubes were vortexed and then immediately placed in the -80°C freezer for long-term storage.

Characteristics of *L. monocytogenes* growth

Growth curves were obtained for all nine strains at 15°C in TSB (pH 6.6, adjusted with 1 M HCl) to determine the time needed for the cells to reach the stationary phase of growth. A small loopful of glycerol stock was inoculated into 10 ml TSB and incubated for 18 h at 37°C. The overnight culture was diluted with 0.1% peptone in order to inoculate 100 ml TSB to a final concentration of 1×10^3 CFU/ml. The cultures were incubated at 15°C in a refrigerated shaking waterbath (45 strokes/min, Lauda, Germany).

One ml of culture was removed aseptically at 22 time points ranging from 0 to 82 hours of incubation (after thorough shaking). The sample was placed in a plastic cuvette, and the absorbance at 600 nm determined using a Smart Spec 3000™ (BioRad, Australia).

High pressure screening of *L. monocytogenes* strains

Strains were screened in groups of three. Experiments were performed twice, on separate days with two replicates each day. Cells were resuscitated from glycerol stock by incubating in 10 ml TSB (pH 6.6) for

18 h at 37°C. One hundred µl was inoculated into pre-chilled TSB, and incubated for 72 h at 15°C in a shaking waterbath (45 stroke/min).

Stationary phase cells of each strain were diluted in fresh TSB to obtain a final concentration of 10^6 CFU/ml. Five ml of 10^6 CFU/ml diluted culture was dispensed into the sterile sample tubes (transfer plastic pipettes, Copan, Italy). The tubes were heat sealed and placed into plastic bags (Cryovac, Australia) with 5000 ppm peroxyacetic acid (to act as a disinfectant in case a tube failed) and heat sealed just prior to pressure treatment.

The cells were treated at 600 MPa for 0, 10, 20, 30, 40 and 60 s in a 2 L high-pressure unit (Avure Technologies, USA). The time to reach 600 MPa (come-up time) was approximately 10 s and pressure release after the indicated hold times was < 2 s. All HPP was conducted at ambient temperature (approximately 20°C). Immediately after pressure treatment the cultures were decimally diluted (0.1% peptone) and plated on tryptone soya yeast extract agar (TSYEA) and incubated at 37°C for 48 h before enumeration.

Smallgoods utilised in the inoculated challenge studies and shelf life tests.

In this study, four ready-to-eat sliced commercial meat products were used, as nominated by two Australian manufacturers of smallgoods products; Strassburg, low-fat pastrami, export sausage and Cajun beef. The Strassburg is a cooked, cured comminuted beef product (typical moisture 66%, NaCl 2%, sodium nitrite/nitrate 63 ppm); the low-fat pastrami product is a cooked, cured whole beef muscle product (typical moisture 73%, NaCl 1.85%, sodium nitrite/nitrate 33 ppm); the export sausage is a cooked, cured comminuted beef product produced entirely for export to the Asian market (typical moisture 52%, NaCl 1.85%, sodium nitrite/nitrate 73 ppm) and the Cajun beef is a cooked, uncured whole beef muscle encrusted with spices product (typical moisture 73%, NaCl 3.6%, sodium nitrite/nitrate 57 ppm).

Determination of effect of NaCl on pressure inactivation of *L. monocytogenes* 2542

Since *L. monocytogenes* strain 2542 (originally isolated from salami) was the most pressure resistant of the nine strains tested, it was selected for further screening experiments to determine the effect of NaCl on resistance to HPP. NaCl levels were chosen, based on the NaCl levels of 4 products to be used in the inoculated challenge studies and shelf life studies, which had a range of NaCl concentrations of 1.85% - 3.6%. Cell cultures were resuscitated from glycerol stocks as described previously. Stationary phase cells (500 µl) were inoculated into fresh TSB (9.5 ml) amended with NaCl [no salt added (0.5% NaCl w/v), 1.85% (w/v) NaCl and 3.6% (w/v) NaCl, all pH 6.3 adjusted with HCl] to obtain cell levels of approximately 10^8 CFU/ml. Samples were transferred from these three cell suspensions (0.5 ml) to 50 ml TSB, amended as above, to obtain cell suspensions with levels of approximately 10^6 CFU/ml. The tubes were sealed by heating in a flame and crimping with pliers. The tubes were placed into plastic bags (Barrier Packaging, Cryovac, Australia) filled with 5000 ppm peroxyacetic acid and heat sealed immediately prior to pressure treatment. The cells were treated at 600 MPa for 0, 40, 60, 90 and 120 s. Pressure treatment and subsequent enumeration were conducted as described previously, with two replicates.

L. monocytogenes Challenge Studies with Smallgoods

TSB (10 ml) was separately inoculated with one loopful 2472, 2542, 2345, 2343, 2655 from glycerol stocks stored at –80°C. After incubation at 37°C for 18 h, 100 µl of culture was inoculated in 50 ml TSB (pH 6.3, adjusted with 1 M HCl) and incubated at 15°C for 72 h. Each strain (100 µl) was added to the same 9.5 ml of 0.1% peptone to obtain a cocktail with approximately 10^8 CFU/ml. The cocktail was further diluted in 0.1% peptone so that 10 µl contained approximately 10^4 CFU/ml.

Low-fat pastrami, Strassburg, Cajun beef and export sausage (~25 g) were placed in Cryovac Barrier packaging (Fawkner, Vic, Australia; OTR < 5cc/m^2/24h/atm @ 23°C/75% RH) and weights were recorded on the bag. The meats were inoculated with approximately 250 µl of the *L. monocytogenes* cocktail (10 µl/g meat) to achieve a final concentration of approximately 10^4 CFU/g. The inoculum was spread over the surface of the meat and the samples were massaged by hand for 30 s. The bags were vacuum

packaged (Multivac, Sepp Haggenmuller GmbH and Co, Wolfeitschwenden, Germany), and the meats were HPP at 600 MPa for 180 s as described above. The meats were stored at 4°C until needed.

Chemical analyses of Smallgoods Used in Challenge Studies

The water activity and pH of the uninoculated untreated meat samples were measured on the initial day of the trial. The pH was measured with a Beckman Coulter pH meter (model 390, Fullerton, CA, USA), using a surface probe. The water activity was determined using an Aqualab CX-3 water activity meter (Graintec, Australia). Both were measured on duplicate samples.

Enumeration and enrichment for *L. monocytogenes* of smallgoods used in challenge studies

Both enumeration and enrichment for *L. monocytogenes* were conducted on a) uninoculated, untreated meat samples, b) inoculated, untreated meat samples and c) inoculated HPP meat samples after one day of storage at 4°C post-HPP treatment. Subsequent sampling over the 13-week storage period was conducted on inoculated HPP meat samples only.

Sample packages were aseptically opened and were diluted 1:10 (w/w) using sterile 0.1% peptone diluent. The packages were heat sealed and stomached for 2 minutes (Colworth stomacher model 400, Seward Laboratories, London). One ml of sample was spread over three plates each of Oxford agar and TSAYE. If necessary, serial dilutions were performed with 0.1% peptone diluent and plated onto Oxford agar and TSAYE. The plates were incubated at 37°C for 48 h. Duplicate samples were analysed at each pull time.

For selective enrichment, the sample packages were sprayed with 1% sodium hypochlorite and were held for 10 min until dry. The packages were aseptically opened and sterile half Fraser broth was aseptically added directly into the package to give a 1:10 dilution. The packages were heat sealed, hand massaged for 10 s and incubated at 30°C for 24 h. The packages were opened and 100 µl was transferred to 10 ml of sterile Fraser broth and incubated for 48 h at 37°C. One loopful of broth from both the packages containing the samples with half Fraser and Fraser broth were streaked onto Oxford agar and incubated for 48 h at 37°C. Any colonies that appeared on the plates were confirmed as *L. monocytogenes* by assessing colony morphology on TSYEA, Gram stain, catalase production and CAMP test.

Challenge study testing schedule

HPP of all products was conducted on day 0. Microbiological testing was conducted on day 1, day 3, day 7, week 4, week 6, week 10, and week 13.

High pressure processing (HPP) of smallgoods for shelf life study

Four types of small goods products (Cajun beef, export sausage, Strassburg and low-fat pastrami) were obtained from two Australian manufacturers, from their typical manufacturing line. All of the meats were received pre-sliced. The low-fat pastrami and Strassburg products were in retail packs of 100 and 125 g and were held at 4°C prior to processing. Due to manufacturing schedules, the export sausage (received frozen) and Cajun beef were received in 1 kg bulk packs and were held at –20°C for 18 days before the study was initiated. Prior to HPP, the export sausage and Cajun beef were distributed into 500 g lots (for sensory testing) and 200 g lots for (chemical and microbiological analyses) in Cryovac barrier packaging (Fawkner, Vic, Australia), and vacuum packaged using a Web-o-matic Easypack system (Maschinefabrick, Bochum, Germany). The products were then pressure treated at 600 MPa for 180 s at ambient temperature (ca. 20°C) utilizing a 35 L high pressure unit (Avure Inc., Seattle, Washington) in the Food Science Australia Werribee pilot plant. Immediately after pressure treatment the samples were held at 4°C, then shipped via refrigerated truck to Food Science Australia's North Ryde Facility for analyses over the shelf life period of up to 98 days storage at 4°C (last sensory evaluation scheduled for 5 June 2003).

Shelf life testing schedule

HPP of all products was conducted on day 0. Microbiological and sensory analyses were scheduled as follows:

Microbiological analyses	Consumer Acceptance Testing
day 4	day 7
day 11	day 14
day 46	day 49
day 74	day 77
day 95	day 98

Chemical analyses of smallgoods in shelf life study

The water activity and pH of the products was monitored over the shelf life study. The water activity was determined using an Aqualab CX-3 water activity meter (Graintec, Australia). The pH was measured with a Beckman Coulter pH meter (model 390, Fullerton, CA, USA), using a surface probe. Duplicate samples were tested.

Microbiological analyses of smallgoods in shelf life study

Microbiological counts

One packet of each sample was removed from storage at 4°C. The packet was sprayed with 70% ethanol and cut open using sterile scissors. Sterile tongs were used to remove one or two slices of meat (~20 to 30 g) and placed in a stomacher bag. Sterile 0.1% peptone diluent was added to achieve a one in ten dilution. The bag was heat sealed and stomached for 2 min (Colworth stomacher model 400, Seward Laboratories, London). Two ml of sample was aseptically removed from the stomacher bag and was spread equally over 6 plates of each type of media required (Table 2). Microbiological testing was conducted to determine total aerobic plate count, total anaerobic plate count, and the presence of *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, *Brochothrix thermospacta*, and yeast and moulds. The plates were incubated as per Table 2 and counted. All product samples were tested in duplicate, except for the first and second (week 2) pull, from which microbiological analyses were conducted on only one sample of each product. All media was obtained from Oxoid (Basingstoke, England).

Table 2. Medium and incubation conditions for microbial analyses

Media	Target microorganism	Incubation conditions
Standard plate count agar (SPCA)	Total aerobic plate count	96 h @ 25°C
De Man, Rogosa, Sharpe agar (MRSA)	*Lactobacillus* spp	48 h @ 30°C
Listeria selective agar	*Listeria* spp	48 h @ 37°C
Baird-Parker agar	*Staphylococcus* spp	48 h @ 37°C
Eosin Methylene Blue agar (EMB)	Coliforms	24 h @ 37°C
Brain Heart Infusion agar (BHIA)	Anaerobic plate count	72 h @ 30°C in anaerobic jar (Oxoid)
Streptomycin Sulphate Thallous Acetate Actidione agar (STAA)	*Brochothrix thermosphacta*	48 h @ 30°C
Dichloran Rose-Bengal Chloramphenicol agar (DRBC)	Yeasts and moulds	5 days @ 25°C

Microbiological enrichments

Coliform enrichment: 100 µl of sample (from stomached product samples as described previously) was added to 10 ml of Lauryl Tryptose (LT) broth and incubated at 37^{o}C for 48 h. If there was any gas formation, the broth was streaked onto Eosin Methylene Blue agar (EMB) to confirm the presence of coliforms.

Salmonella enrichment: 1 ml of sample (from stomached products samples as described previously) was added to 9 ml of buffered peptone water (BPW) and incubated at 37^{o}C for 16 h. Subsequently, 100 µl were transferred into10 ml of Rapport-Vassiliadis (RV) broth and subsequently incubated at 42^{o}C for 24 h, after which the RV broth was streaked onto Xylose lysine desoxycholate (XLD) agar to confirm the presence of *Salmonella* spp.

Listeria enrichment: 1 ml of sample was added to 9 ml of half Fraser broth and incubated for 24 h at 30^{o}C. Subsequently, 100 µl was transferred to 10 ml of Fraser broth and incubated for an additional 48 h at 37^{o}C. If any growth was observed, the both the half Fraser and Fraser broth was streaked onto Oxford agar plates to confirm the presence of *Listeria* spp.

Consumer acceptance evaluation of smallgoods in shelf life study

Consumer acceptance testing was conducted with approximately 40 consumers who currently consume cold ready-to-eat meat products participating at baseline 7, 14, 49, 77 and 98 days.

Consumers were asked to assess the products and indicate their liking for the appearance, aroma, flavour, texture, aftertaste and overall acceptability using the 9-point hedonic scale shown below:

*e.g. How much do you like the **appearance** of this sample?*

☐	☐	☐	☐	☐	☐	☐	☐	☐
dislike extremely	dislike very much	dislike moderately	dislike slightly	neither like nor dislike	like slightly	like moderately	like very much	like extremely
[1]	[2]	[3]	[4]	[5]	[6]	[7]	[8]	[9]

Additionally, consumers were asked to indicate how likely they would be to buy the sample using the 5-point purchase intent scale:

*e.g. How likely would you be to **purchase** this sample (if the price was right):*

☐	☐	☐	☐	☐
Definitely would NOT buy	Probably would NOT buy	Maybe would / maybe wouldn't	Probably WOULD buy	Definitely WOULD buy
[1]	[2]	[3]	[4]	[5]

All sensory testing took place in the sensory laboratory at Food Science Australia's Sydney facility according to International Standards on Sensory Analysis (ISO 6658:1985).

Hedonic sensory data were analysed using one-way analysis of variance (ANOVA) and Duncan's multiple comparison tests for means separation to determine differences between hedonic ratings of the control (day 7) and HPP meat samples at the evaluated time points (day 7, 14, 49, 77 and 98 days). Likewise, one-way ANOVA and Duncan's multiple comparison tests were used to determine differences between hedonic ratings of individual HPP meat samples over the evaluated storage period (98 days).

Purchase intent data were analysed using the Chi-square statistic to determine differences between purchase intent ratings for the control (day 7) and HPP meat samples at the evaluated time points (day 7, 14, 49, 77 and 98 days). Significant differences in purchase interest were further analysed using one-way ANOVA and Duncan's multiple comparison tests for means separation. Likewise, Chi-square, one-way ANOVA and Duncan's multiple comparison tests were used to determine differences between purchase intent ratings for individual HPP meat samples over the evaluated storage period (98 days).

Results

Characteristics of *L. monocytogenes* growth

The growth curves for each of the nine *L. monocytogenes* strains studied showed that stationary phase was reached within 72 h at 15°C (Figure 1). These parameters were used to generate cells for screening resistance/sensitivity to pressure at 600 MPa.

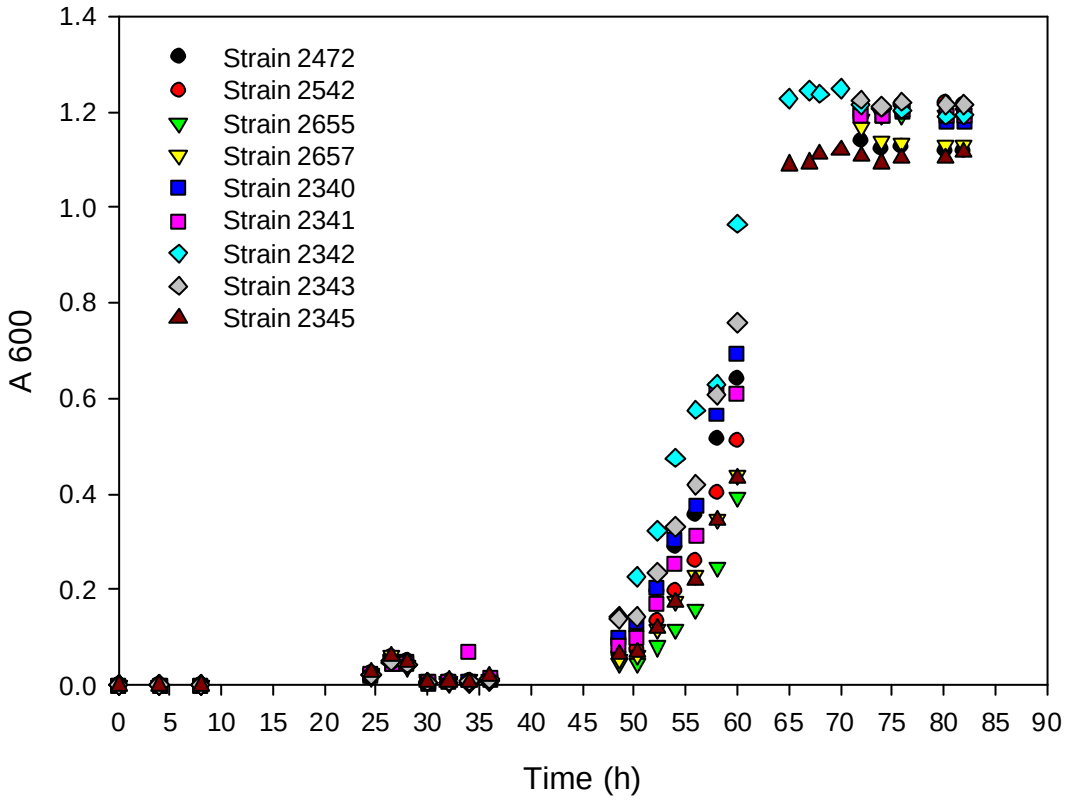

Figure 1. Growth curves of *L. monocytogenes* strains 2472, 2542, 2655, 2657, 2340, 2341, 2342, 2343, 2345. The cells were grown in TSB (pH 6.6) at 15°C in a shaking waterbath (45 strokes/min). Absorbance was measured over time at 600 nm.

High pressure screening of *L. monocytogenes* strains

The nine *L. monocytogenes* strains tested at 600 MPa varied in their sensitivity to high pressure (Figure 2). Strain 2542 was the most resistant, with approximately 1.5-$\log_{10}$ reduction achieved after 60 s of

HPP. Strains 2340, 2342, 2657, 2341 and 2472 were the most sensitive; 60 s of processing at 600 MPa resulted in a 5-$\log_{10}$ or greater reduction in cell numbers.

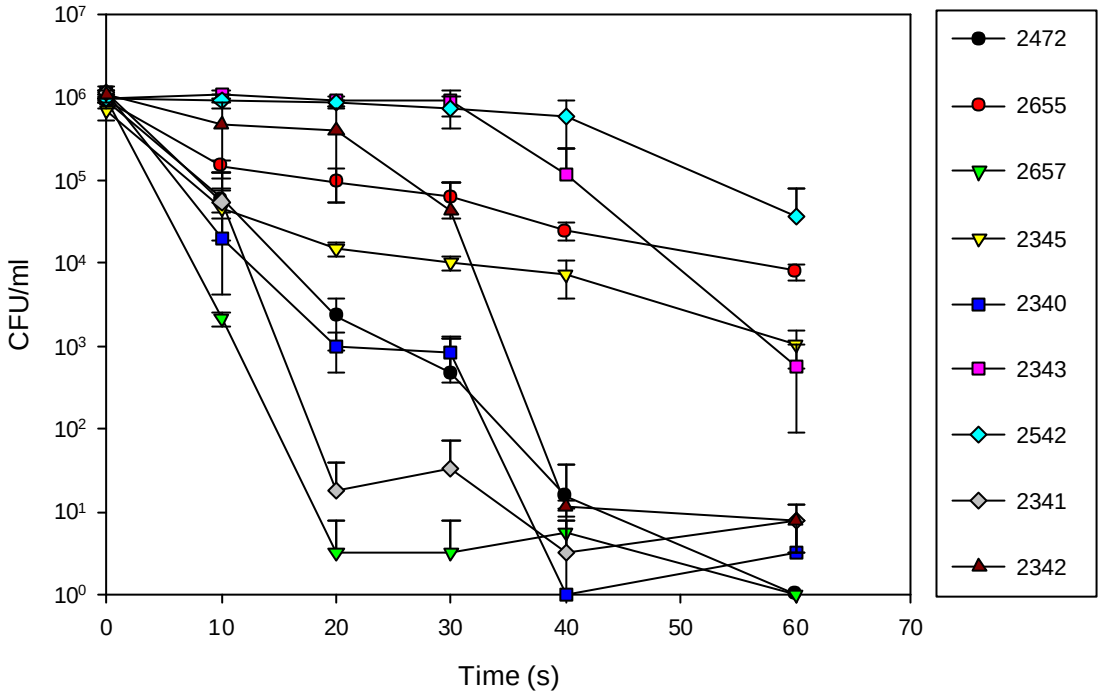

Figure 2 Inactivation curves of *L. monocytogenes* 2472, 2542, 2655, 2657, 2340, 2341, 2342, 2343, 2345. Individual strains were suspended in TSB (pH 6.6), with an initial count of approximately 1 x 10^6 CFU/ml. HPP was 600 MPa for 0, 10, 20, 30, 40 and 60 s. Cells were enumerated on TSYEA, at 37°C for 48 h.

Determination of effect of NaCl on pressure inactivation of *L. monocytogenes* 2542

As *L. monocytogenes* 2542 was the most resistant strain of those examined, it was selected to examine the effect of salt concentration in HPP inactivation. Longer processing times were selected due to the low level of inactivation after 60 s. Inactivation of *L. monocytogenes* 2542 at 600 MPa, 20°C was influenced by the salt level of the medium (Fig. 3). HPP in TSB with no added salt resulted in the fastest inactivation, with > 5-$\log_{10}$ reduction achieved after 90 s at 600 MPa. As the NaCl level was increased, the level of inactivation decreased. For example, when cells were pressure treated at 600 MPa, 20°C for 90s, approximately a 3.5-$\log_{10}$ reduction was achieved. When the NaCl concentration was increased to 3.6%, only a 2.5-$\log_{10}$ reduction was observed after pressure treatment for 90 s at 600 MPa, 20°C.

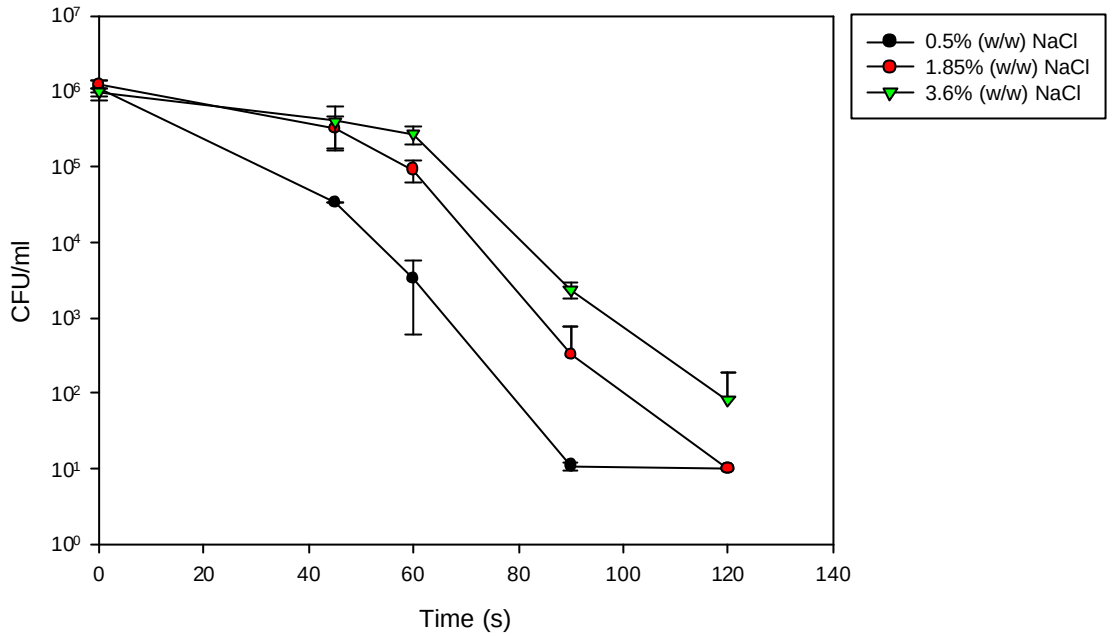

Figure 3. *L. monocytogenes* strain 2542 pressure treated in tryptic soy broth with various NaCl concentrations (pH 6.3, adjusted with 1 M HCl) at 600 MPa, 20°C. The limit of detection was 10 CFU/ml.

Challenge studies

On the initial day of testing the *L. monocytogenes* counts on the inoculated, untreated meat samples were 1.07 x 10^4 CFU/g for pastrami, 1.01 x 10^4 CFU/g for Strassburg, 1.05 x 10^4 CFU/g for export sausage, and 1.00 x 10^4 CFU/g for Cajun beef and the selective enrichment procedure resulted in all samples being positive for *L. monocytogenes*. *L. monocytogenes* was not detected in any of the uninoculated, untreated meat samples by either enumeration or selective enrichment procedures. The pH measurements of the uninoculated untreated meat samples ranged from 6.1 to 6.34 and a_w measurements ranged from 0.956 to 0.967 (Table 3).

Table 3. pH and water activity (a_w) measurements of untreated meat samples. The measurements reported are the average of two samples.

Meat type	**pH**	**a_w**
Low fat pastrami	6.10	0.962
Strassburg	6.34	0.956
Export Sausage	6.13	0.961
Cajun beef	6.32	0.967

Selective enrichment of inoculated samples (approximately 25 g) treated at 600 MPa, 20°C for 180 s showed no positive samples 24 hours after processing (Table 4). After 3 days of storage at 4°C, one of two low-fat pastrami and one of two Strassburg samples tested positive for *L. monocytogenes*. Over the storage period of 13 weeks at 4°C, the Cajun beef samples never had a positive result, the low-fat pastrami had one additionally positive result at week 6 (one of two samples), the export sausage had positive results (one of two samples) at week 4, 10 and 13, while the Strassburg had positive results from day 3 to week 6, with no positive results at week 10 or 13 (Table 4).

Enumeration of *L. monocytogenes* from meat samples pressure treated at 600 MPa, 20°C for 180s, on both nonselective (TSYEA) and selective media (Oxford Agar), showed that all samples tested over the 13-week storage period had levels below the limit of detection (<10 CFU/g) (Tables 5 and 6). Although the Cajun beef did not have a positive result from the selective enrichment procedure throughout the 13-week storage study, on week 13, the Cajun beef sample did have colonies present on both TSYEA and Oxford agar, for one replicate sample (Tables 5 and 6). However, the numbers of colonies on the plate were too great to be confident that the cell morphology was consistent with that typical for *L. monocytogenes*. The Strassburg and the export sausage also had viable counts of *L. monocytogenes* on one replicate sample each on week 13 (Tables 5 and 6), which is consistent with the sporadic positives seen with the enrichment methodology used throughout the storage period. Additionally, the Strassburg sample also had significant background flora present on the TSYEA plate, at levels approximately 2-fold higher than those of the colonies identified as *L. monocytogenes*.

Table 4. Selective enrichment for *L. monocytogenes* in inoculated HPP meat samples (initial levels of approximately 10^4 CFU/g; sample weight approximately 25 g) treated at 600 MPa, 20°C for 180 s and subsequently stored at 4°C. Duplicate samples tested at each pull time. +: sample positive for *L. monocytogenes*, -: sample negative for *L. monocytogenes*

Meat type	Initial	Day 3	Week 2	Week 4	Week 6	Week 10	Week 13
Low-fat pastrami	- / -	+ / -	- / -	- / -	+ / -	- / -	- / -
Strassburg	- / -	+ / -	+ / +	+ / -	+ / +	- / -	- / -
Export sausage	- / -	- / -	- / -	+ / -	- / -	+ / -	+ / -
Cajun beef	- / -	- / -	- / -	- / -	- / -	- / -	- / -

Table 5. Enumeration on tryptic soy agar with yeast extract of *L. monocytogenes* in inoculated HPP meat samples (initial levels of approximately 10^4 CFU/g; sample weight approximately 25 g) treated at 600 MPa, 20°C for 180 s and subsequently stored at 4°C. Duplicate samples tested at each pull time.

Meat type	Initial	Day 3	Week 2	Week 4	Week 6	Week 10	Week 13
Low-fat pastrami	ND[a]	ND	ND	ND	ND	ND	ND
Strassburg	ND	ND	ND	ND	ND	ND	n/a[b]
Export sausage	ND	ND	ND	ND	ND	ND	7.1×10^3 [c]
Cajun beef	ND	ND	ND	ND	ND	ND	TNTC[d]

[a] Not detected in 1 ml of a 10^{-1} dilution (the limit of detection was <10 CFU/g)
[b] Result not available; *L. monocytogenes* colonies were present on one replicate sample only, ND on the second replicate sample, but could not be enumerated as there were mixed colonies on the plate
[c] Result from one replicate only, the other replicate was ND, results were not averaged.
[d] Too numerous to count in at 10^{-2} dilution, result from one replicate sample only, the other replicate was ND

Table 6. Enumeration on Oxford agar (selective medium) of *L. monocytogenes* in inoculated HPP meat samples (initial levels of approximately 10^4 CFU/g; sample weight approximately 25 g) treated at 600 MPa, 20°C for 180 s and subsequently stored at 4°C. Duplicate samples tested at each pull time.

Meat Type	Initial	Day 3	Week 2	Week 4	Week 6	Week 10	Week 13
Low-fat pastrami	ND[a]	ND	ND	ND	ND	ND	ND
Strassburg	ND	ND	ND	<10	ND	ND	7.6x 10^{3} [c]
Export sausage	ND	ND	ND	ND	ND	ND	6.95x 10^{3} [c]
Cajun beef	ND	ND	ND	ND	ND	ND	TNTC[d]

[a] Not detected in 1 ml of a 10^{-1} dilution (the limit of detection was <10 CFU/g)
[c] Result from one replicate only, the other replicate was ND, results were not averaged.
[d] Too numerous to count on 10^{-2} dilution, result from one replicate sample only, the other replicate was ND

Chemical analyses of smallgoods in shelf life study

The pH and a_w of the four products in the trial was measured for the untreated samples and was measured and followed over the chilled storage of the HPP samples. The pH of the untreated samples was similar, with a range of pH 5.89 to 6.08. HPP did not affect the pH of the products and the pH did not change over 95 days of storage at 4°C (Table 7). Similarly, the a_w of the untreated samples was similar, with a range of a_w 0.955 to 0.973. HPP did not affect the a_w of the products and the a_w did not change over 46 days of storage at 4°C (Table 8).

Table 7. pH (average of two measurements) of the four meat products over storage at 4°C. HPP=high pressure processed at 600 MPa, 20°C for 180 s.

Meat type	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Low-fat Pastrami	5.96	5.98	5.85	5.89	6.05	6.05
Strassburg	6.08	6.08	6.08	6.25	6.18	6.12
Export Sausage	5.89	5.84	5.83	5.94	6.01	5.96
Cajun Beef	6.01	6.10	5.85	6.12	6.12	6.09

Table 8. Water activity (average of two measurements) of the four meat products over storage at 4°C. HPP=high pressure processed at 600 MPa, 20°C for 180 s.

Meat type	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Low-fat Pastrami	0.973	0.973	0.966	0.950	0.967	0.963
Strassburg	0.961	0.955	0.953	0.957	0.958	0.958
Export Sausage	0.957	0.963	0.966	0.961	0.950	0.955
Cajun Beef	0.956	0.959	0.956	0.950	0.954	0.952

Microbiological analyses of smallgoods in shelf life study

Enrichments did not detect *Listeria*, *Salmonella* or coliforms in any of the four untreated samples or the pressure treated samples at any time point during the 95 days of storage at 4°C. The results from the microbiological analyses of the low-fat pastrami, Strassburg, export sausage and Cajun beef are shown in Tables 9, 10, 11 and 12, respectively. The initial microbiology sampling point for this study showed that there were low (<500 CFU/g) levels of *Lactobacillus* spp., anaerobic bacteria and yeast and moulds (mainly yeast) detected in the untreated low-fat pastrami and Cajun beef samples with levels of aerobic bacteria, *Listeria* spp., *Staphylococcus* spp., coliforms spp., and *Brochothrix thermophacta* all being below the detection limit (<10 CFU/g) for all four untreated products.

The levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds in the low-fat pastrami samples were at undetectable levels (<10 CFU/g) 4 days post-HPP processing and remained at undetectable or low levels for 95 days post-HPP (Table 9). The only exception was on day 11, where a low level of aerobic bacteria was detected and sporadic detection of low levels of anaerobic bacteria.

The levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms spp., anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds in the Strassburg samples were at undetectable levels (<10 CFU/g) 4 days post-HPP processing and remained at undetectable levels for 95 days post-HPP (Table 10). The only exception was on day 11, where a low level of aerobic bacteria was detected.

The levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds in the export sausage samples were at undetectable levels (<10 CFU/g) 4 days post-HPP processing and remained at undetectable levels for 95 days post-HPP (Table 11). The two exceptions occurred on day 11, where a low level of yeast and moulds was detected, and on day 46, where a low level of aerobic bacteria was detected.

The levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds in the Cajun beef samples were at undetectable levels (<10 CFU/g) 4 days post-HPP processing and remained at undetectable or low levels for 95 days post-HPP (Table 12). On day 46, low levels of aerobic bacteria, *Lactobacillus* spp., *Staphylococcus* spp., and anaerobic bacteria were detected, while *Listeria* spp., coliforms, *Brochothrix thermophacta*, and yeast and moulds remained at undetectable levels.

Table 9. Results from various plate counts for pressure treated (600 MPa, 180 s) low-fat pastrami stored at 4°C. HPP=high pressure processed

Target Microorganisms (Medium)	CFU/g					
	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Total plate count (SPCA)	<10	< 10	1.25×10^2	< 10	< 10	< 10
Lactobacillus spp. (MRSA)	52	< 10	< 10	< 10	< 10	< 10
Listeria spp. (Listeria selective agar)	<10	< 10	< 10	< 10	< 10	< 10
Staphylococcus spp. (Baird-Parker agar)	<10	< 10	< 10	< 10	< 10	< 10
Coliforms (EMB)	<10	< 10	< 10	< 10	< 10	< 10
Total anaerobic plate count (BHIA)	25	< 10	< 10	10	< 10	40
Brochothrix thermosphacta (STAA)	<10	< 10	< 10	< 10	< 10	< 10
Yeast & Moulds (DRBC)	3.75×10^2	< 10	< 10	< 10	< 10	< 10

Table 10. Results from various plate counts for pressure treated (600 MPa, 180 s) Strassburg stored at 4°C. HPP=high pressure processed

Target Microorganisms (Medium)	CFU/g					
	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Total plate count (SPCA)	<10	< 10	6.55×10^2	< 10	< 10	< 10
Lactobacillus spp. (MRSA)	<10	< 10	< 10	< 10	< 10	< 10
Listeria spp. (Listeria selective agar)	<10	< 10	< 10	< 10	< 10	< 10
Staphylococcus spp. (Baird-Parker agar)	<10	< 10	< 10	< 10	< 10	< 10
Coliforms (EMB)	<10	< 10	< 10	< 10	< 10	< 10
Total anaerobic plate count (BHIA)	<10	< 10	< 10	< 10	< 10	< 10
Brochothrix thermosphacta (STAA)	<10	< 10	< 10	< 10	< 10	< 10
Yeast & Moulds (DRBC)	<10	< 10	< 10	< 10	< 10	< 10

Table 11. Results from various plate counts for pressure treated (600 MPa, 180 s) export sausage stored at 4°C. HPP=high pressure processed

Target Microorganisms (Medium)	CFU/g					
	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Total plate count (SPCA)	<10	< 10	< 10	30	< 10	< 10
Lactobacillus spp. (MRSA)	<10	< 10	< 10	< 10	< 10	< 10
Listeria spp. (Listeria selective agar)	<10	< 10	< 10	< 10	< 10	< 10
Staphylococcus spp. (Baird-Parker agar)	<10	< 10	< 10	< 10	< 10	< 10
Coliforms (EMB)	<10	< 10	< 10	< 10	< 10	< 10
Total anaerobic plate count (BHIA)	<10	< 10	< 10	< 10	< 10	< 10
Brochothrix thermosphacta (STAA)	<10	< 10	< 10	< 10	< 10	< 10
Yeast & Moulds (DRBC)	<10	< 10	90	< 10	< 10	< 10

Table 12. Results from various plate counts for pressure treated (600 MPa, 180 s) Cajun beef stored at 4°C. HPP=high pressure processed

Target Microorganisms (Medium)	CFU/g					
	Untreated	HPP Day 4	HPP Day 11	HPP Day 46	HPP Day 74	HPP Day 95
Total plate count (SPCA)	<10	< 10	< 10	1.18×10^3	< 10	10
Lactobacillus spp. (MRSA)	15	< 10	< 10	8.30×10^2	< 10	15
Listeria spp. (Listeria selective agar)	<10	< 10	< 10	< 10	< 10	< 10
Staphylococcus spp. (Baird-Parker agar)	<10	< 10	< 10	5.50×10^2	< 10	< 10
Coliforms (EMB)	<10	< 10	< 10	< 10	< 10	< 10
Total anaerobic plate count (BHIA)	30	< 10	< 10	8.10×10^2	< 10	< 10
Brochothrix thermosphacta (STAA)	<10	< 10	< 10	< 10	< 10	< 10
Yeast & Moulds (DRBC)	30	< 10	< 10	< 10	< 10	15

Consumer acceptability evaluation of smallgoods in shelf life study

When interpreting the sensory data (appearance, aroma, flavour, texture, aftertaste, and overall liking) in Tables 13-21, refer to the example 9-point hedonic scale shown on page 7. Likewise, when interpreting

the purchase intent data in Tables 13-21, refer to the example 5-point purchase intent scale shown on page 8.

The median scores provided in brackets in Tables 13-21 are shown in addition to the mean values as the measurement scales are categorical in nature, allowing consumers to rate samples using discrete values. Therefore, the median score corresponds to a discrete point on the categorical scale. For example, a median score of 7.00 on the 9-point hedonic scale relates to 'like moderately', while a median score of 4.00 on the 5-point purchase intent scale relates to 'probably would buy'.

Day 7

Consumer hedonic evaluation of the control and HPP meat samples on day 7 of storage is shown in Table 13.

Table 13. Mean consumer acceptability scores for cold meat samples on day 7 of storage

Sample	Appearance	Aroma	Flavour	Texture	Aftertaste	Overall Liking	Purchase intent
Strassburg control	5.50 (5.50) *1.754*	5.95 (6.00) *1.300*	6.38 (7.00) *1.409*	6.13 (6.00) *1.399*	6.03 (6.00) *1.459*	6.03 (6.00) *1.349*	3.08 (3.00) *1.163*
Strassburg HPP	5.80 (6.00) *1.924*	5.75 (6.00) *1.676*	6.73 (7.00) *1.198*	6.40 (6.00) *1.336*	6.58 (7.00) *1.152*	6.63 (7.00) *1.254*	3.58 (4.00) *1.238*
Export sausage control	4.53 (4.00) *1.585*	4.50 (4.00) *1.664*	5.35 (6.00) *2.058*	5.08 (5.00) *1.685*	5.13 (5.00) *1.924*	5.05 (5.00) *1.839*	2.45 (2.00) *1.260*
Export sausage HPP	5.15 (5.00) *1.875*	4.68 (5.00) *1.774*	5.20 (5.50) *2.345*	5.13 (5.00) *1.951*	5.15 (6.00) *2.237*	5.13 (6.00) *2.162*	2.63 (3.00) *1.372*
Pastrami control	6.35 (7.00) 1.777	6.63 (7.00) *1.234*	5.85(6.00) *1.748*	5.95 (6.00) *1.694*	5.63 (6.00) *1.779*	5.98 (6.00) *1.672*	3.03 (3.00) *1.310*
Pastrami HPP	6.45 (7.00) *1.663*	6.15 (6.00) *1.562*	6.25 (7.00) *1.765*	5.93 (6.00) *1.940*	6.08 (6.00) *1.655*	6.30 (7.00) *1.772*	3.48 (4.00) *1.320*
Cajun beef control	4.55 (4.00) *1.894*	4.18 (4.00) *1.599*	4.70 (5.00) *1.870*	5.18 (6.00) *1.781*	4.53 (4.50) *1.797*	4.35 (4.00) *1.718*	1.98 (2.00) *1.143*
Cajun beef HPP	4.93 (5.00) *1.913*	4.25 (4.00) *1.676*	4.70 (5.00) *1.990*	5.40 (6.00) *1.692*	4.90 (4.50) *1.945*	4.80 (4.50) *1.977*	2.28 (2.00) *1.320*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control and corresponding HPP meat sample after 7 days of storage. **No significant differences were established at the significance level p = 0.05:**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control and corresponding HPP meat sample after 7 days of storage. **No differences in purchase intent ratings were identified at the significance level p = 0.05**.

Day 14

Consumer hedonic evaluation of the control (day 7) and HPP meat samples on day 14 of storage is shown in Table 14.

Table 14. Mean consumer acceptability scores for the control (day 7) and HPP cold meat samples on day 14 of storage

Sample	Appearance	Aroma	Flavour	Texture	Aftertaste	Overall Liking	Purchase intent
Strassburg control	5.50 (5.50) *1.754*	5.95 (6.00) *1.300*	6.38 (7.00) *1.409*	6.13 (6.00) *1.399*	6.03 (6.00) *1.459*	6.03 (6.00) *1.349*	3.08 (3.00) *1.163*
Strassburg HPP	5.79 (6.00) *1.533*	6.24 (6.00) *1.046*	6.74 (7.00) *1.163*	6.18 (6.00) *1.336*	6.44 (6.00) *1.133*	6.47 (7.00) *1.134*	3.50 (4.00) *0.961*
Export sausage control	4.53 (4.00) *1.585*	4.50 (4.00) *1.664*	5.35 (6.00) *2.058*	5.08 (5.00) *1.685*	5.13 (5.00) *1.924*	5.05 (5.00) *1.839*	2.45 (2.00) *1.260*
Export sausage HPP	4.21 (4.00) *1.719*	4.71 (5.00) *1.750*	5.29 (5.00) *2.008*	5.03 (5.00) *1.992*	5.15 (5.50) *1.987*	4.71 (5.00) *2.195*	2.44 (2.00) *1.397*
Pastrami control	6.35 (7.00) 1.777	6.63 (7.00) *1.234*	5.85(6.00) *1.748*	5.95 (6.00) *1.694*	5.63 (6.00) *1.779*	5.98 (6.00) *1.672*	3.03 (3.00) *1.310*
Pastrami HPP	6.74 (7.00) *1.377*	6.29 (7.00) *1.679*	6.24 (7.00) *1.759*	5.94 (7.00) *1.953*	6.18 (6.00) *1.714*	6.21 (7.00) *1.771*	3.26 (3.00) *1.263*
Cajun beef control	4.55 (4.00) *1.894*	4.18 (4.00) *1.599*	4.70 (5.00) *1.870*	5.18 (6.00) *1.781*	4.53 (4.50) *1.797*	4.35 (4.00) *1.718*	1.98 (2.00) *1.143*
Cajun beef HPP	5.26 (6.00) *1.990*	4.41 (4.50) *1.708*	5.26 (6.00) *2.020*	5.50 (6.00) *1.674*	4.91 (5.00) *1.798*	5.03 (5.50) *1.784*	2.44 (2.00) *1.186*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control and corresponding HPP meat sample after 14 days of storage. **No significant differences were established at the significance level p = 0.05:**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control and corresponding HPP meat sample after 14 days of storage. **No differences in purchase intent ratings were identified at the significance level p = 0.05**.

Day 49

The export sausage meat sample was excluded for consumer hedonic evaluation on day 49 due to discolouration (Figure 4). Such discolouration (red/pink to grey) was possibly due to residual oxygen retained around the export Sausage sample during vacuum packaging (day 1). Inclusion of this product in the consumer study at day 49 would have introduced bias to the study and compromised hedonic test validity.

Figure 4. Discolouration present in the export sausage meat sample in comparison to the remaining cold meat samples 49 days post HPP

The samples are arranged from left to right as follows: low-fat pastrami, Strassburg, export sausage and Cajun beef.

Consumer hedonic evaluation of the control (day 7) and HPP meat samples on day 49 of storage is shown in Table 15.

Table 15. Mean consumer acceptability scores for the control (day 7) and HPP cold meat samples on day 49 of storage

Sample	Appearance	Aroma	Flavour	Texture	Aftertaste	Overall Liking	Purchase intent
Strassburg Control	5.50 (5.50) *1.754*	5.95 (6.00) *1.300*	6.38 (7.00) *1.409*	6.13 (6.00) *1.459*	6.03 (6.00) *1.459*	6.03 (6.00) *1.349*	3.08 (3.00) *1.163*
Strassburg HPP	5.94 (6.50) *1.585*	6.39 (6.00) *1.202*	6.75 (7.00) *1.251*	6.42 (7.00) *1.461*	6.50 (7.00) *1.254*	6.47 (7.00) *1.444*	3.47 (4.00) *1.158*
Pastrami Control	6.35 (7.00) *1.777*	6.63 (7.00) *1.234*	5.85 (6.00) *1.784*	5.95 (6.00) *1.694*	5.63 (6.00) *1.779*	5.89 (6.00) *1.672*	3.03 (3.00) *1.310*
Pastrami HPP	6.86 (7.00) *1.246*	6.58 (7.00) *1.422*	6.19 (7.00) *1.600*	6.17 (6.00) *1.342*	6.44 (7.00) *1.382*	6.44 (6.50) *1.362*	3.42 (3.50) *1.204*
Cajun beef Control	4.55 (4.00) *1.894*	4.18 (4.00) *1.599*	4.70 (5.00) *1.870*	5.18 (6.00) *1.781*	4.53 (4.50) *1.797*	4.35 (4.00) *1.718*	1.98 (2.00) *1.143*
Cajun beef HPP	4.67 (4.00) *2.084*	4.64 (5.00) *1.944*	5.56 (6.00) *2.104*	5.94 (6.00) *1.706*	5.50 (6.00) *1.964*	5.25 (6.00) *2.156*	2.58 (2.50) *1.273*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests elicited significant differences in the hedonic scores given by consumers to the control and corresponding low-fat pastrami and Cajun beef HPP meat samples following 49 days of storage. **The following differences were significant (p = 0.05) and only significant results are mentioned:**

Aftertaste:

The aftertaste of the bw-fat pastrami sample on day 49 post HPP was more preferred compared to the aftertaste of the control low-fat pastrami sample.

The aftertaste of the Cajun beef sample on day 49 post HPP was more preferred compared to the aftertaste of the control Cajun beef sample

Overall liking:

The Cajun beef sample on day 49 post HPP was more preferred overall compared to the control Cajun beef meat sample.

Day 76

The discoloration on the exposed side of the HPP export sausage meat sample remained apparent after 76 days of refrigerated storage (Figure 5). Furthermore, the Cajun beef sample also exhibited some discoloration after 76 days of refrigerated storage (Figure 5). Such discolouration (red/pink to grey) was possibly due to residual oxygen, which was retained during vacuum packaging (day 1) and ultimately surrounded each sample during storage.

Figure 5. Discolouration present in the export sausage and to a lesser extent the Cajun beef meat samples in comparison to the remaining cold meat samples 76 days post HPP. The samples are arranged from left to right as follows: Strassburg, low-fat pastrami, export sausage and Cajun beef.

Microbiological testing revealed that all meat samples were safe to consume following 76 days of refrigerated storage. To facilitate inclusion of the HPP export sausage meat samples in the consumer trial, suitable cuts of the product were removed as outlined in Figure 6.

Figure 6. Preparation of suitable cuts of the export sausage for consumer evaluation

Consumer hedonic ratings of the control (day 7) and HPP meat samples on day 76 of storage are shown in Table 16.

Table 16. Mean consumer acceptability scores for the control (day 7) and HPP cold meat samples on day 76 of storage.

Sample	Appearance	Aroma	Flavour	Texture	Aftertaste	Overall Liking	Purchase intent
Strassburg control	5.50 (5.50) *1.754*	5.95 (6.00) *1.300*	6.38 (7.00) *1.409*	6.13 (6.00) *1.399*	6.03 (6.00) *1.459*	6.03 (6.00) *1.349*	3.08 (3.00) *1.163*
Strassburg HPP	5.61 (6.00) *1.619*	6.00 (6.00) *1.500*	6.12 (6.00) *1.763*	6.00 (7.00) *1.750*	5.91 (6.00) *1.665*	5.91 (6.00) *1.809*	3.00 (3.00) *1.323*
Export sausage control	4.53 (4.00) *1.585*	4.50 (4.00) *1.664*	5.35 (6.00) *2.058*	5.08 (5.00) *1.685*	5.13 (5.00) *1.924*	5.05 (5.00) *1.839*	2.45 (2.00) *1.260*
Export sausage HPP	4.27 (5.00) *1.825*	4.70 (5.00) *1.686*	4.94 (5.00) *2.277*	5.27 (6.00) *2.309*	5.30 (5.00) *2.023*	4.67 (5.00) *2.341*	2.52 (3.00) *1.395*
Pastrami control	6.35 (7.00) 1.777	6.63 (7.00) *1.234*	5.85(6.00) *1.748*	5.95 (6.00) *1.694*	5.63 (6.00) *1.779*	5.98 (6.00) *1.672*	3.03 (3.00) *1.310*
Pastrami HPP	6.64 (7.00) *1.432*	6.27 (6.00) *1.353*	6.70 (7.00) *1.237*	6.27 (7.00) *1.376*	6.09 (6.00) *1.487*	6.52 (7.00) *1.482*	3.48 (3.00) *1.064*
Cajun beef control	4.55 (4.00) *1.894*	4.18 (4.00) *1.599*	4.70 (5.00) *1.870*	5.18 (6.00) *1.781*	4.53 (4.50) *1.797*	4.35 (4.00) *1.718*	1.98 (2.00) *1.143*
Cajun beef HPP	5.27 (6.00) *2.004*	4.94 (5.00) *1.836*	5.85 (7.00) *2.210*	5.55 (5.00) *1.954*	5.91 (7.00) *1.942*	5.33 (6.00) *2.231*	2.85 (3.00) *1.523*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests elicited significant differences in the hedonic scores given by consumers to the control and corresponding low-fat pastrami and Cajun beef HPP meat samples following 76 days of storage. The following differences were significant ($p = 0.05$) and only significant results are mentioned:

Flavour:

The flavour of the low-fat pastrami sample on day 76 post HPP was more preferred compared to the flavour of the control low-fat pastrami sample.

The flavour of the Cajun beef sample on day 76 post HPP was more preferred compared to the flavour of the control Cajun beef sample.

Aftertaste:

The aftertaste of the Cajun beef sample on day 76 post HPP was more preferred compared to the aftertaste of the control Cajun beef sample.

Overall liking:

The Cajun beef sample on day 76 post HPP was more preferred overall compared to the control Cajon beef meat sample.

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control and corresponding HPP meat sample after 76 days of storage. **The following difference was identified at the significance level p = 0.05**.

Cajun beef:

Based solely on sensory character, consumers were more willing to purchase Cajun beef on day 76 post HPP compared to the control Cajun beef sample.

Day 97

Figure 7 depicts the discoloration in the export sausage samples following 96 days of storage. As revealed, only the side of the Cajun beef sample in contact with the packaging material (top slice) experienced discoloration. In a similar manner to sample preparation on day 76, suitable cuts of export sausage were removed for consumer evaluation on day 97.

Figure 7. HPP export sausage sample following 96 days of storage. The exposed side of the sample is shown on the right.

Consumer hedonic ratings of the control (day 07) and HPP meat samples on day 97 of storage are shown in Table 17.

Table 17. Mean consumer acceptability scores for the control (day 7) and HPP cold meat samples on day 97 of storage

Sample	Appearance	Aroma	Flavour	Texture	Aftertaste	Overall Liking	Purchase intent
Strassburg control	5.50 (5.50) *1.754*	5.95 (6.00) *1.300*	6.38 (7.00) *1.409*	6.13 (6.00) *1.399*	6.03 (6.00) *1.459*	6.03 (6.00) *1.349*	3.08 (3.00) *1.163*
Strassburg HPP	5.58 (6.00) *1.723*	6.28 (7.00) *1.396*	6.45 (7.00) *1.535*	5.85 (6.50) *1.703*	6.10 (6.50) *1.614*	6.15 (7.00) *1.545*	3.28 (3.00) *1.109*
Export sausage control	4.53 (4.00) *1.585*	4.50 (4.00) *1.664*	5.35 (6.00) *2.058*	5.08 (5.00) *1.685*	5.13 (5.00) *1.924*	5.05 (5.00) *1.839*	2.45 (2.00) *1.260*
Export sausage HPP	5.03 (6.00) *2.057*	5.28 (5.00) *1.840*	5.50 (6.00) *2.276*	5.50 (6.00) *2.124*	5.28 (6.00) *2.195*	5.20 (6.00) *2.244*	2.60 (2.50) *1.411*
Pastrami control	6.35 (7.00) 1.777	6.63 (7.00) *1.234*	5.85(6.00) *1.748*	5.95 (6.00) *1.694*	5.63 (6.00) *1.779*	5.98 (6.00) *1.672*	3.03 (3.00) *1.310*
Pastrami HPP	6.28 (6.50) *1.797*	6.43 (7.00) *1.599*	6.48 (7.00) *1.601*	5.95 (6.00) *1.739*	6.08 (7.00) *1.730*	6.20 (7.00) *1.604*	3.18 (3.00) *1.217*
Cajun beef control	4.55 (4.00) *1.894*	4.18 (4.00) *1.599*	4.70 (5.00) *1.870*	5.18 (6.00) *1.781*	4.53 (4.50) *1.797*	4.35 (4.00) *1.718*	1.98 (2.00) *1.143*
Cajun beef HPP	4.58 (4.00) *1.810*	4.38 (5.00) *1.877*	5.13 (6.00) *2.289*	5.50 (6.00) *2.112*	5.00 (5.00) *2.184*	4.97 (5.00) *2.166*	2.50 (2.50) *1.396*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control and corresponding HPP meat sample after 97 days of storage. **No significant differences were established at the significance level p = 0.05:**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control and corresponding HPP meat sample after 97 days of storage. **No differences in purchase intent ratings were identified at the significance level p = 0.05**.

Hedonic measurement from 7 to 98 days
Strassburg control and HPP cold meat sample

The results of consumer evaluations of the control Strassburg cold meat sample and the HPP Strassburg cold meat sample over the 97-day storage period are shown in Table 18.

Table 18. Mean consumer acceptability scores for the control Strassburg and HPP Strassburg cold meat samples over storage time.

	Control	Day 7	Day 14	Day 49	Day 76	Day 97
Appearance	5.50 (5.50) *1.754*	5.80 (6.00) *1.924*	5.79 (6.00) *1.533*	5.94 (6.50) *1.585*	5.61 (6.00) *1.619*	5.58 (6.00) *1.723*
Aroma	5.95 (6.00) *1.300*	5.75 (6.00) *1.676*	6.24 (6.00) *1.046*	6.39 (6.00) *1.202*	6.00 (6.00) *1.500*	6.28 (7.00) *1.396*
Flavour	6.38 (7.00) *1.409*	6.73 (7.00) *1.198*	6.74 (7.00) *1.163*	6.75 (7.00) *1.251*	6.12 (6.00) *1.763*	6.45 (7.00) *1.535*
Texture	6.13 (6.00) *1.399*	6.40 (6.00) *1.336*	6.18 (6.00) *1.336*	6.42 (7.00) *1.461*	6.00 (7.00) *1.750*	5.85 (6.50) *1.703*
Aftertaste	6.03 (6.00) *1.459*	6.58 (7.00) *1.152*	6.44 (6.00) *1.133*	6.50 (7.00) *1.254*	5.91 (6.00) *1.665*	6.10 (6.50) *1.614*
Overall liking	6.03 (6.00) *1.349*	6.63 (7.00) *1.254*	6.47 (7.00) *1.134*	6.47 (7.00) *1.444*	5.91 (6.00) *1.809*	6.15 (7.00) *1.545*
Purchase intent	3.08 (3.00) *1.163*	3.58 (4.00) *1.238*	3.50 (4.00) *0.961*	3.47 (4.00) *1.158*	3.00 (3.00) *1.323*	3.28 (3.00) *1.109*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control Strassburg and HPP Strassburg meat samples over the 97-day storage period. **No significant differences were established at the significance level p = 0.05.**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control Strassburg and HPP Strassburg meat samples over the 97-day storage period. **No differences in purchase intent ratings were identified at the significance level p = 0.05**.

Export Sausage control and HPP cold meat sample

The results of consumer evaluations of the control export sausage cold meat sample and the HPP export sausage cold meat sample over the 97-day storage period are shown in Table 19.

Table 19. Mean consumer acceptability scores for the control export sausage and HPP export sausage cold meat samples over storage time.

	Control	Day 7	Day 14	Day 49	Day 76	Day 97
Appearance	4.53 (4.00) *1.585*	5.15 (5.00) *1.875*	4.21 (4.00) *1.719*		4.27 (5.00) *1.825*	5.03 (6.00) *2.057*
Aroma	4.50 (4.00) *1.664*	4.68 (5.00) *1.774*	4.71 (5.00) *1.750*		4.70 (5.00) *1.686*	5.28 (5.00) *1.840*
Flavour	5.35 (6.00) *2.058*	5.20 (5.50) *2.345*	5.29 (5.00) *2.008*		4.94 (5.00) *2.277*	5.50 (6.00) *2.276*
Texture	5.08 (5.00) *1.689*	5.13 (5.00) *1.951*	5.03 (5.00) *1.992*		5.27 (6.00) *2.309*	5.50 (6.00) *2.124*
Aftertaste	5.13 (5.00) *1.924*	5.15 (6.00) *2.237*	5.15 (5.50) *1.987*		5.30 (5.00) *2.023*	5.28 (6.00) *2.195*
Overall liking	5.05 (5.00) *1.839*	5.13 (6.00) *2.162*	4.71 (5.00) *2.195*		4.67 (5.00) *2.341*	5.20 (6.00) *2.244*
Purchase intent	2.45 (2.00) *1.260*	2.63 (3.00) *1.372*	2.44 (2.00) *1.397*		2.52 (3.00) *1.395*	2.60 (2.50) *1.411*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control export sausage and the HPP export sausage samples over the 97-day storage period. **No significant differences were established at the significance level p = 0.05.**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the export sausage HPP samples over the 97-day storage period. **No difference in purchase intent ratings was identified at the significance level p = 0.05**.

Low-fat pastrami control and HPP cold meat sample

The results of consumer evaluations of the control low-fat pastrami cold meat sample and the HPP low-fat pastrami cold meat sample over the 97-day storage period are shown in Table 20.

Table 20. Mean consumer acceptability scores for the control low-fat pastrami and HPP low-fat pastrami cold meat samples over storage time.

	Control	Day 7	Day 14	Day 49	Day 76	Day 97
Appearance	6.35 (7.00) *1.777*	6.45 (7.00) *1.663*	6.74 (7.00) *1.377*	6.86 (7.00) *1.246*	6.64 (7.00) *1.432*	6.28 (6.50) *1.797*
Aroma	6.63 (7.00) *1.234*	6.15 (6.00) *1.562*	6.29 (7.00) *1.679*	6.58 (7.00) *1.422*	6.27 (6.00) *1.353*	6.43 (7.00) *1.599*
Flavour	5.85 (6.00) *1.748*	6.25 (7.00) *1.765*	6.24 (7.00) *1.759*	6.19 (7.00) *1.600*	6.70 (7.00) *1.237*	6.48 (7.00) *1.601*
Texture	5.95 (6.00) *1.694*	5.93 (6.00) *1.940*	5.94 (7.00) *1.953*	6.17 (6.00) *1.342*	6.27 (7.00) *1.376*	5.95 (6.00) *1.739*
Aftertaste	5.63 (6.00) *1.779*	6.08 (6.00) *1.655*	6.18 (6.00) *1.714*	6.44 (7.00) *1.382*	6.09 (6.00) *1.487*	6.08 (7.00) *1.730*
Overall liking	5.98 (6.00) *1.672*	6.30 (7.00) *1.772*	6.21 (7.00) *1.771*	6.44 (6.50) *1.362*	5.52 (7.00) *1.482*	6.20 (7.00) *1.604*
Purchase intent	3.03 (3.00) *1.310*	3.48 (4.00) *1.320*	3.26 (3.00) *1.263*	3.42 (3.50) *1.204*	3.48 (3.00) *1.064*	3.18 (3.00) *1.217*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control low-fat pastrami and the HPP low-fat pastrami meat samples over the 97-day storage period. **No significant differences were established at the significance level p = 0.05:**

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control low-fat pastrami and the HPP low-fat pastrami meat samples over the 97-day storage period. **No differences in purchase intent ratings were identified at the significance level p=0.05**.

Cajun Beef control and HPP cold meat sample

The results of consumer evaluations of the control Cajun beef cold meat sample and the Cajun beef cold meat sample over the 97-day storage period are shown in Table 21.

Table 21. Mean consumer acceptability scores for the control and HPP Cajun beef cold meat samples over storage time.

	Control	Day 7	Day 14	Day 49	Day 76	Day 97
Appearance	4.55 (4.00) *1.894*	4.93 (5.00) *1.913*	5.26 (6.00) *1.990*	4.67 (4.00) *2.084*	5.27 (6.00) *2.004*	4.58 (4.00) *1.810*
Aroma	4.18 (4.00) *1.599*	4.25 (4.00) *1.676*	4.41 (4.50) *1.708*	4.64 (5.00) *1.944*	4.94 (5.00) *1.836*	4.38 (4.00) *1.877*
Flavour	4.70 (5.00) *1.870*	4.70 (5.00) *1.990*	5.26 (6.00) *2.020*	5.56 (6.00) *2.104*	5.85 (7.00) *2.210*	5.13 (6.00) *2.289*
Texture	5.18 (6.00) *1.781*	5.40 (6.00) *1.692*	5.50 (6.00) *1.674*	5.94 (6.00) *1.706*	5.55 (5.00) *1.954*	5.50 (6.00) *2.112*
Aftertaste	4.53 (4.50) *1.797*	4.90 (4.50) *1.945*	4.91 (5.00) *1.798*	5.50 (6.00) *1.964*	5.91 (7.00) *1.942*	5.00 (5.00) *2.184*
Overall liking	4.35 (4.00) *1.718*	4.80 (4.50) *1.977*	5.03 (5.50) *1.784*	5.25 (6.00) *2.156*	5.33 (6.00) *2.231*	4.97 (5.00) *2.166*
Purchase intent	1.98 (2.00) *1.143*	2.28 (2.00) *1.320*	2.44 (2.00) *1.186*	2.58 (2.50) *1.273*	2.85 (3.00) *1.523*	2.50 (2.50) *1.396*

Note: Median scores are shown in brackets; Standard deviations are shown in italics.

One-way ANOVA and Duncan's multiple comparison tests were used to elicit significant differences in the hedonic scores given by consumers to the control Cajun beef and the HPP Cajun beef meat samples over the 97-day storage period. **The following difference was significant (p = 0.05):**

Aftertaste:

The aftertaste of the Cajun beef sample on day 76 post HPP was more preferred compared to the aftertaste of the control Cajun beef sample and the Cajun beef meat sample following 7 (control and HPP meat samples) and 14 (HPP meat sample) days of storage.

The Chi-square statistic was used to determine significant differences in purchase intent ratings given by consumers to the control Cajun beef and the HPP Cajun beef meat samples over the 97-day storage period. **No differences in purchase intent ratings were identified at the significance level p = 0.05**.

Discussion

The nine *L. monocytogenes* strains examined in this study varied in their resistance to HPP, with between a 1.5-$\log_{10}$ to a >5-$\log_{10}$ reduction occurring after 60 s at 600 MPa, 20°C, depending on the strain. The five most resistant strains were 2472, 2542, 2345, 2343 and 2655, and therefore these strains were used as a cocktail for the inoculated challenge tests. As the products used in this study had various NaCl concentrations (1.85 – 3.6%), the effect of NaCl concentration on HPP inactivation was examined. *L. monocytogenes* 2542 was the most resistant of the nine strains examined and therefore it was selected for these inactivation kinetic studies. The HPP inactivation kinetics were different when cells were treated in TSB with varying salt concentrations. As the level of salt was increased from 0.5% to 3.6% (highset NaCl concentration in the 4 meat products used in the challenge studies) the level of inactivation decreased. These results indicate that NaCl may afford protection to *L. monocytogenes* during high pressure processing.

The results from inactivation kinetics studies, particularly those with strain 2542 when HPP in TSB with 3.6% NaCl, indicated that processing times at 600 MPa, 20 °C would need to be greater than 120s to achieve a 4-$\log_{10}$ CFU/ml reduction of *L. monocytogenes*. Additionally, preliminary inoculated challenge tests (data not shown) indicated that processing inoculated meat samples (initial inoculum levels of 10^4 CFU/ml) for 180 s at 600 MPa, 20°C would allow for no detectable levels of *L. monocytogenes*

immediately after processing, using enrichment techniques. For theses reasons, HPP at 600 MPa, 20°C for 180 s was the process chosen for both the inoculated challenge studies and the shelf life studies. The inoculated challenge studies showed that levels of *L. monocytogenes* would remain below detectable levels over at least a 10-week storage period at 4°C for some products, but other products did have sporadic positive results from selective enrichment procedures, and that some packages had countable levels of *L. monocytogenes* at 13 weeks post HPP. These results indicate the possibility that there was recovery and growth of *L. monocytogenes* cells over a 13-week storage at 4°C. This finding would need to be confirmed with further studies. Additionally, the results indicate that a slightly longer processing time or higher pressure may be necessary to assure nondetectable levels if the initial level of *L. monocytogenes* on product is assumed to be 10^4 CFU/g. However, a recently published survey of *L. monocytogenes* in ready-to-eat foods indicated that levels of *L. monocytogenes* in luncheon meats are typically lower that 10^4 CFU/g (Gombas, et al., 2003). Ready-to-eat luncheon meats were one of eight categories of foods examined over a 23-month period. Of the 9,199 pre-sliced luncheon meat [ham (pork or poultry); bologna (pork, beef, turkey or a mixture of these); poultry (turkey or chicken; smoked or not smoked)] samples examined, only 82 (0.89%) tested positive for *L. monocytogenes*. The majority of samples had levels of < 10^2 CFU/g, with only one sample having initial levels of 10^3-10^4 CFU/g. This indicated that the majority of retail ready-to-eat meats would have levels much lower than the level chosen for these challenge studies, indicating that HPP of 600 MPA for 180 s could be used successfully as final in-package pasteurisation step in the commercial production of smallgoods. Further evaluation of the effects of various components in the meat products, such as fat level, spices, acids, on the effectiveness of HPP, would aid in the development of commercial in-package pasteurisation processes which would ensure the safety and extend the shelf life of refrigerated, smallgoods products.

The microbiological analyses of smallgoods products utilized in the shelf life study (Strassburg, low-fat pastrami, export sausage and Cajun beef) showed that HPP (600 MPa, 180 s) was effective in keeping levels of aerobic bacteria, *Lactobacillus* spp., *Listeria* spp., *Staphylococcus* spp., coliforms, anaerobic bacteria, *Brochothrix thermophacta*, and yeast and moulds to below the detectable limits (<10 CFU/g), or at low levels throughout the 95 days of storage at 4°C. Additionally, at no time during the shelf life trail did any samples test positive (via enrichment methods) for *L. monocytogenes*, coliforms or *Salmonella spp.* These results indicate that HPP can successfully be used as an in-package pasteurisation method to significantly extend the refrigerated shelf life of ready-to-eat meat products with regards to microbial safety and stability.

The chemical analyses of the four products in this study, namely low-fat pastrami, Strassburg, export sausage and Cajun beef, showed that HPP (600 MPa, 20° for 180 s) had no affect on the pH or water activity of the products and the pH and water activity did not change over time.

Comparison of consumer hedonic ratings for the control (non-processed) and corresponding HPP meat samples on 7, 14 and 97 days refrigerated storage revealed no differences in consumer appreciation of the meat samples. After 49 days refrigerated storage, the aftertaste of the HPP low-fat pastrami and Cajun beef samples were more preferred than the corresponding control sample (hedonic measurement generated at 7 day storage). Furthermore, consumer ratings for overall liking of the HPP Cajun beef sample was higher at 49 days refrigerated storage compared to the control sample, which was evaluated after 7 days refrigerated storage. After 76 days refrigerated storage, the flavour of the HPP low-fat pastrami and Cajun beef samples was more preferred than that of the corresponding control meat sample. Furthermore, consumer ratings of aftertaste, overall liking and purchase intent for the HPP Cajun beef sample was higher at 76 days refrigerated storage compared to the corresponding control meat sample. While it is unlikely that HPP contributed to the apparent enhancement of sensory character during storage (based on existing scientific evidence), conclusive elucidation of such an effect would require comparative studies using sensory profiling in conjunction with separative volatile measurements. Notwithstanding this, the results demonstrate how HPP maintained the sensory quality of Strassburg, low-fat pastrami and Cajun beef samples for an extended (96 days) refrigerated storage period. While the HPP export sausage meat sample was not evaluated by consumers on day 49 refrigerated storage, consumer hedonic ratings on day 76 and 97 revealed that the sensory quality of this meat sample was maintained by HPP.

Comparison of consumer hedonic ratings for the control (day 7) and corresponding HPP meat samples over the evaluated storage period (97 days) revealed no deterioration in the sensory quality of the Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples. Moreover, the aftertaste of

the HPP Cajun beef meat sample was more preferred at day 76 refrigerated storage compared to days 7 (control and HPP meat samples) and 14 (HPP meat sample). In the open-ended responses consumers were afforded the opportunity to make explicit comments, sensory or otherwise, about each sample. Comparison of open-ended responses over the evaluated storage period revealed that the majority comments were hedonic in nature, when consumers commented on their like or dislike of the specific test samples. On day 76 and 97 some comment was made about the apparent discoloration around the edges of the Cajun beef meat samples. Such discoloration, which was also present in the export sausage meat samples after 49 days refrigerated storage, was likely to have been caused by vacuum packing rather than HPP treatments.

Comparison of consumer hedonic ratings at five time points over an evaluated storage period, provided the statistical power necessary to prove that HPP does not adversely affect the sensory quality of Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples. Moreover, this study revealed that HPP is an effective means of maintaining the sensory quality of Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples for an extended refrigerated storage period (97 days). It is noteworthy however, that 'sensory quality' refers specifically to 'eating quality', an integrated sensory assessment provided by the untrained consumer. To conclusively determine if HPP has an effect on the 'sensory properties' of Strassburg, export sausage, low-fat pastrami, and Cajun beef meat samples, objective assessment of specific sensory attributes using a panel of trained assessor (i.e. sensory profiling) must be performed.

HPP is not a complex process, however the components that comprise the system are specialised to cope with the pressures required for food processing. As a result of the specialised nature of the equipment, HPP is not a low cost technology from the point of view of either the capital or running costs. On the basis of an average 12 minute cycle time, running a two-shift operation 50 weeks per year, or 20,000 cycles, the cost per cycle, including maintenance and labour is approximately A$25.01 or A$0.05 per packaged unit[1]. For the Australian small processor or start up operation, contract processing is available from Australian High Pressure Processor (AHPP), an Adelaide based company set up specifically to provide HPP services. AHPP have quoted a rate of A$100 per cycle and a cost of A$0.20 per package based on a 100mm x 130mm x 25mm, 300 g package. This rate includes handling. AHPP have plans to set up similar processing facilities in other capital cities in the future, making the technology easily available to all food producers[1].

Many of the benefits of HPP of foods are indirect. The process does not improve production rates or organoleptic quality of cooked, ready-to-eat meats, however it will extend shelf life, with benefits in restocking frequencies, production size runs, stock levels and pallet utilisation improvements in the warehouses of major retail chains[1]. Extended shelf life has particular benefits in the servicing of overseas markets and may allow sea freight to be used rather than airfreight, with substantial cost savings. HPP may also give the product a premium edge in the market place, using the Avure Technologies, Inc. patented "Fresher Under Pressure" logo. HPP will eliminate the risk of contaminated product entering the consumer stream and guarantee microbiological integrity. HPP may also allow the reduction in Quality Assurance staff. The HPP treatment becomes a verifiable traceable HACCP step in the smallgoods manufacturing process, post-cooking, portioning and packaging, which may be lacking in the producer's current process[1].

The results from this study show that HPP (as an in-package nonthermal pasteurisation method) can be a powerful intervention strategy for controlling *L. monocytogenes* in ready-to-eat refrigerated smallgoods, as part of a good overall HACCP program. Although the inoculated challenge testing showed sporadic positives for the presence of *L. monocytogenes*, a greater than 4-$\log_{10}$ CFU/g reduction was achieved, as indicated by in enumeration methods over at least 10 weeks storage at 4°C and enrichment techniques performed immediately post-processing. The shelf life studies, which included both microbiological analyses and consumer acceptance testing, indicated that the refrigerated shelf life of commercially available ready-to-eat sliced meat products could be greatly extended from their current shelf life (approximately 45-50 days) to at least 98 days while maintaining the eating quality and microbiological safety and quality.

[1] Eyes, L. (2003) A Financial Evaluation of High Pressure Processing of Smallgoods. MLA Project PRMS.033A, available from Meat & Livestock Australia Limited.

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