The Effect of Thawing and Storage Temperature on the Microbial Quality of Commercial Frozen Ready Meals and Experimental Reduced Salt Frozen Ready Meals

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Abstract

The effect of thawing at 4ºC or ambient temperature (~20ºC) on the indigenous microflora of commercial regular salt (0.6-1.3% w/w) frozen ready meals was investigated. In a separate trial, the microbial quality of regular salt frozen ready meals was compared with reformulated reduced salt (0.2-0.54%, w/w) counterparts stored at 4ºC, 10ºC or ambient temperature over 8 days. All samples were analysed for psychrophilic, mesophilic, thermophilic and sporeforming bacteria, *Pseudomonas*, *Staphylococcus* and for the presence of *Listeria* species. During storage, psychrophiles, mesophiles, coliforms, *Pseudomonas*, sporeformers and *Listeria* were detected in the commercial regular salt ready meals while mesophiles, thermophiles, coliforms and *Pseudomonas* were detected in the reduced salt counterparts. Levels of mesophilic bacteria ranged from ~3-4 $\log_{10}$ in commercial regular salt meals and ~2-5 $\log_{10}$ in experimental lower salt meals. Overall, a substantial reduction in salt content (50 – 66%) did not appear to adversely impact on the microbial quality of the reduced salt meals.

Keywords: Ready-meals, Salt, Bacteria, Temperature

1. Introduction

Composite consumer foods which do not require significant further processing other than re-heating or completion of a cooking process are designated as ready-to-eat meals by the Food Safety Authority of Ireland (FSAI, 2001). Within the ready meals market, frozen foods comprise the largest sector which has seen a rapid expansion in product range (Department Of Agriculture, 2003). However, in terms of public health issues, excess dietary salt intake has been associated with consumption of foods containing high levels of non-discretionary salt or salt which has added during processing and outside of the control of the consumer. Processed foods such as frozen ready meals may contribute up to 70-80% of an excess dietary salt intake. Excess daily salt intake (~10-12 g) has been strongly linked with an increase in blood pressure ($\geq$ 140mmHg systolic or $\geq$ 90 mmHg diastolic) leading to hypertension a major causative factor in the onset of cardiovascular disease (CVD). In Ireland, CVD is the most common cause of death according to the Irish Heart Foundation (Central Statistics Office, 2002; Durack et al., 2008) . It is now recommended by public health agencies that adults reduce their daily salt intake to a target of 6g (FSAI, 2003; Scientific Advisory Committee on Nutrition, 2003). To assist in reaching this target, various initiatives are underway worldwide involving public health agencies and food manufacturers who have been encouraged to reduce salt in their products (Consensus Action on Salt & Health, 2008; Food Standards Agency, 2008; FSAI, 2003). However, removal of salt from complex food formulations has implications for both sensory and microbiological quality. Salt is routinely used in these product

formulations and acts as both a flavouring agent and a preservative. Salt acts as a preservative by lowering of water activity (a_w) below the minimum values required for bacterial growth (Betts et al., 2007). Following a reduction in a_w, bacterial cells experience osmotic shock and plasmolysis and in order to resume growth, a_w must return to values which allow cells to recover (Davidson, 1997).

Freezing as a means of preservation is used throughout the food industry (O'Leary et al., 2000) and generally frozen ready meals benefit from a long shelf life (McAteer et al. 1995; Nissen et al. 2002; Redmond et al. 2005). Typically, frozen ready meals consist of a complex formulation of ingredients and because of the variation and complexity in these formulations it is likely that varying effects are exerted on bacterial growth and survival during thawing and subsequent storage. Indeed, bacterial species exhibit a variable response to the freezing process while freeze-thawing has been shown not to result in death of all bacteria but merely effect a reduction in population (Doyle & Schoeni, 1984). Surprisingly few studies have been carried out on the microbial quality of frozen ethnic ready meals especially under various thawing and storage conditions. This contrasts with more extensive published studies for other ready meal types including chilled meals, freeze-chilled meals and sous-vide products (McAteer et al., 1995; Nissen et al., 2002; O'Leary et al., 2000; Redmond et al., 2005). Hence, the objectives of this study were to determine the effect of thawing and storage temperature on a range of commercial regular salt frozen ready meals and subsequently to compare the microbial quality of commercial regular salt with experimental reduced salt frozen ready meals stored at various temperatures.

2. Materials and Methods

2.1 Manufacture of commercial regular salt or experimental reduced salt ready meals

The commercial regular salt frozen ready meals, chilli con carne, meat lasagne and chicken curry, were obtained from a leading manufacturer and held at -18ºC until analysis. Experimental reduced salt chilli con carne, meat lasagne and chicken curry meals were manufactured by the commercial supplier using the same proprietary formulations as the commercial regular salt meals but omitting added salt, including low sodium spice blends and in the case of lasagne, the use of reduced salt Cheddar cheese (1.8% w/w, compared with regular salt cheese, 2.7% w/w salt). The experimental reduced salt meals were manufactured on the same processing line as the commercial regular salt products. Typically, the manufacturing process involves cooking of a 600kg batch of Chicken curry or chilli con carne, a 600kg batch of Bolognese mix and a 300kg batch of Béchamel sauce. All products were heated to 90º – 100ºC followed by immediate cooling in a vacuum cooler to 10ºC over 45 mins, a final freezing step involved passage through a spiral freezer for 120 mins to give a final product temperature of -10º to -18º C. In the manufacture of chicken curry and chilli con carne, products are directly hot-filled into bags which are film sealed before freezing. Lasagne manufacture involved filling of plastic trays by personnel at depositing stations where individual layers were added to the tray. Lasagne meals were assembled in the following format: bolognese sauce (77g), pasta (10g), bolognese sauce (77g), pasta (10g), bolognese sauce (77g), pasta (10g), béchamel sauce (109g) and topped with cheddar cheese (5g). The final portion sizes were Chicken Curry 375g, Chilli con carne 350g and Lasagne 375g. The experimental reduced salt meals were manufactured specifically for this study and are not currently available for retail.

2.2 Compositional analysis of all meals

Compositional analysis of products was carried out on day 1 after overnight thawing at ambient temperature for 16 hours. A Jenway (Analytica, Dublin, Ireland) pH meter 3310 was used to measure pH in thawed ready meals. Samples were prepared as follows: 10g of food was homogenized with 90 ml of sterile distilled water, and stomached at high speed for 2 minutes, after which pH was measured directly on the homogenate. Water activity (a_w) in ~5g samples was measured at 23ºC using an Aqualab Series 3 water activity meter (Model TE - Labcell Ltd., Alton, Hants, UK). Moisture content was determined on a 50 g composite food sample prepared by blending using a Moulinex hand blender (Moulinex, Berkshire, UK); a 10 g sub-sample was then weighed into aluminium weighing dishes and placed in an oven at 105ºC for 18-24 hours. After this time period, samples were re-weighed until there was no further drop in weight and differences from initial weights were expressed as percentage dry matter, this figure was subtracted from 100 to give percentage moisture content. Salt content of regular salt ready meals and low salt equivalent meals was measured as follows: 10ml of concentrated nitric acid was added to 0.4-0.5g samples of each meal in acid digester tubes. Samples were digested using a Mars Xpress microwaveable acid digester (CEM Corporation, Matthews, North Carolina, USA). Samples were heated to 200ºC and held at this temperature for 15 minutes. Samples were analyzed using a Varian Atomic Absorption Spectrophotometer (JVA, Dublin, Ireland). Results were converted from ppm and expressed as % NaCl (w/w) for all samples.

2.3 Effect of thawing temperatures on the microbial quality of commercial regular salt frozen ready meals

Commercial regular salt ready meals were removed from storage at -18ºC and thawed up to 24 hours at 4ºC or ambient temperature (~20ºC). Microbiological analysis was carried out in triplicate on samples taken after 4, 8, 16 and 24 hours: 10g of product was weighed into a sterile stomacher bag to which 90 ml of sterile 0.1% peptone water (Oxoid CM0009, Basingstoke, Hants, UK) at 30ºC was added, this ensured that all samples were completely thawed at time of analysis. Samples were then homogenised for 120 seconds in a Seward Stomacher 400 (AGB, Dublin, Ireland) at the high power setting. Serial dilutions from this homogenate were prepared using 0.1% peptone water and inoculated onto media described below. Results are taken from duplicate plates containing between 30 to 300 colonies and expressed as Colony Forming Units (CFU) per gram of product.

2.4 Effect of storage temperature on the microbial quality of commercial regular salt frozen ready meals and experimental low salt frozen ready meals

Frozen commercial regular salt ready meals and experimental reduced salt ready meals were thawed at ambient temperature for 16 hours. After analysis of thawed products on day 1, products were then stored at 4°C, 10°C or ambient temperature (~20°C) over 8 days. Microbiological analysis was carried out on day 3, 5 or 8 at each storage temperature as described below. After 8 days storage at the various temperatures, all meals were microwaved at full power (800 W) for 8 min according to manufacturer's instructions and samples were analysed for total aerobic viable counts as described below.

2.5 Microbiological analysis

On each sampling day, 10g portions of each meal were weighed and mixed with 90 ml of sterile 0.1% peptone water in sterile stomacher bags and homogenised for 120 seconds in a Seward Stomacher 400 at the high power setting. Serial dilutions were prepared from this homogenate using 0.1% peptone water and were inoculated in duplicate using standard spread-plate technique onto a range of media sourced from Oxoid. In the case of coliforms, an overlay pour-plate method was used. Total psychrophilic, mesophilic and thermophilic aerobic populations were estimated on Plate Count Agar (PCA - Oxoid CM0325) incubated at 4ºC, 37ºC or 55ºC, respectively. PCA plates at 37ºC and 55ºC were incubated for 48 hours and plates at 4ºC were incubated for 7 days. Total coliform counts were estimated on Violet Red Bile Agar (VRBA) (Oxoid CM0107): 1ml of serial dilutions and 10 ml of molten agar was added into sterile petri dishes and mixed to ensure uniform distribution of sample. After plates had solidified, a layer of ~ 10ml molten VRBA was poured over the set agar. Plates were then incubated at 37ºC for 24 hours. Positive colonies were identified as dark red-purple colonies with a red halo (lactose fermenters) greater than 0.5mm in diameter. Positive colonies were also verified using oxidative and fermentative (O/F) carbohydrate metabolism determination. Representative colonies were inoculated into O/F basal media (AGB, Dublin, Ireland) using a needle which was stabbed through the media to about 1/4 inch of the bottom of 2 tubes. One of the tubes was overlayed with 1-2 ml of mineral oil. All tubes were incubated at 37ºC for 24 hours. Positive results for coliforms produced yellow colour in both tubes after incubation (Becton Dickinson and Company, 2008). Staphylococci were estimated on Baird-Parker Medium (Oxoid CM0275) incubated at 37ºC for 48 hours. Growth of *Pseudomonas* spp. were estimated on Pseudomonas Agar Base (Oxoid CM0559), supplemented with C-F-C supplements (CM0559) and incubated at 25ºC for 48 hours. Total sporeformers and heat resistant bacteria were estimated on Nutrient agar (Oxoid CM0003) from samples of all meals including the separate rice portion which accompanies the chicken curry product. Sporeformer enumeration involved heating of diluted samples at 90ºC for 10 minutes using an Eppendorf Thermomixer comfort (Eppendorf, Hamburg, Germany) heating block and subsequent plating onto Nutrient agar followed by incubation at 30ºC for 24 hours. Novel Enrichment Broth (Oxoid CM1066) and Listeria Selective Agar (Oxoid CM0856) were used for detection of *Listeria* species. The procedure involved taking 25 g of sample and diluting with 225 ml of novel enrichment broth. Samples were then homogenised in a stomacher at the medium speed setting for 30 seconds. These samples were then incubated at 30ºC for 24 ±2 hours. Subsequently, 10 μl of this sample was streaked onto Listeria Selective Agar. Plates were incubated at 37ºC for 24 and/or 48 hours and checked for growth, indicated by the presence of black, shiny, convex colonies. After day 8, all samples stored at various temperatures were microwaved as per manufacturer's instructions (full power in an 800W microwave oven for 8 minutes). After microwave cooking, samples were diluted in sterile 0.1% peptone water, plated onto PCA and incubated at 37ºC for 48 hours. Results are taken from duplicate plates containing between 30 to 300 colonies and expressed as Colony Forming Units (CFU) per gram of product. Where no colonies were present on a spread plate, the estimated count is reported as less than (<) one time the corresponding dilution i.e. no colonies on a 10^{-1} plate are reported as <100 estimated (Swanson et al., 2001).

2.6 Statistics

All graphs and bar charts including means and standard deviations were prepared using GraphPad Prism Version 5.0 (Graph Pad, San Diego, CA, USA). Analysis was carried out using two-way ANOVA comparing bacterial growth in commercial regular salt or in experimental reduced salt frozen ready meals.

3. Results

3.1 Compositional analysis of products

Salt content of commercial regular salt meals ranged from 0.66% to 1.13% and in experimental reduced salt meals ranged from 0.20% to 0.54%. The pH values of all meals were similar at pH, ~ 5.5. Water activity (a_w) of all meals was ~ 0.99, reflecting that of highly perishable foods. Products had moisture contents ranging from ~ 65- 85% (Table 1).

3.2 Effect of thawing at 4℃ or ambient temperature on microbial quality of commercial regular salt ready meals

3.2.1 Aerobic plate count at 4°C or 55°C

Growth was not detected on PCA plates incubated at 4º C or 55ºC for any of commercial regular salt meals when defrosted over 24 hours at 4ºC or at ambient temperature.

3.2.2 Aerobic plate count at 37°C

Meals analysed after 4, 12, 16 and 24 hours had differing aerobic populations. After 4 hours of thawing at both temperatures, the chicken curry product had the lowest population, ~3 $\log_{10}$ CFU/g, followed by chilli con carne, ~4 $\log_{10}$ CFU/g, while the lasagne had the highest population, ~5 $\log_{10}$ CFU/g (Figure 1). For chicken curry ready meals, populations remained constant up to 16 h, when thawed at either 4ºC or ambient temperature, with a slight increase to ~4 - 5 $\log_{10}$ CFU/g noted after 24 h thawing at ambient temperature. Similar trends were noted for chilli con carne, populations increased slightly at 24 h to ~3 $\log_{10}$ CFU/g at ambient temperature thawing. Results for lasagne over 24 h indicated that populations remained constant at either 4ºC or ambient temperature however populations were higher than in the other 2 meals, ~5 $\log_{10}$ CFU/g.

3.2.3 Staphylococci

Staphylococci were detected only in the commercial regular salt chilli con carne meal at ~ 2-3 $\log_{10}$ CFU/g at both thawing temperatures over 24 h period (Figure 2).

3.2.4 *Pseudomonas*

Pseudomonas species were not detected in commercial regular salt lasagne meals thawed at 4ºC or ambient temperature. For chilli con carne and chicken curry, only one sample thawed at ambient temperature at 24 h contained ~3.8 $\log_{10}$ CFU/g or ~3.5 $\log_{10}$ CFU/g colonies, respectively (Data not shown).

3.2.5 *Listeria* species

Of the three commercial regular salt ready meals, only lasagne thawed at 4ºC after 24 h showed positive growth of *Listeria* species and using an API *Listeria* kit (Biomerieux, Basingstoke, Hampshire, UK) these colonies were putatively identified as *L. seeligeri*.

3.2.6 Total coliform count

Coliforms were absent in commercial regular salt meals over 24 hours thawing at either 4ºC or ambient temperature.

3.3 Effect of storage temperature on the microbial quality of commercial regular salt ready meals or experimental reduced salt ready meals

3.3.1 Aerobic plate count at 4°C

Growth was not detected in commercial regular salt lasagne or chilli con carne meals or in any of the experimental reduced salt ready meals at any of the storage temperatures (4ºC 10ºC or ambient temperature). However, the commercial regular salt chicken curry meal contained 4.5 $\log_{10}$ CFU/g on day 8 at 4ºC storage.

3.3.2 Aerobic plate count at 37°C

Initially, aerobic populations after thawing, at day 1, in commercial regular salt ready meals or experimental reduced salt meals were ~3-4 $\log_{10}$ CFU/g and ~2-5 $\log_{10}$ CFU/g, respectively (Figure 3 a, b, c). Of the commercial regular salt meals, chicken curry stored at 4°C developed the lowest population over the 8 day storage ~ 4 $\log_{10}$ CFU/g (Figure 3a). The commercial regular salt chicken curry meal stored at 10°C developed final populations of ~6 $\log_{10}$ CFU/g, an overall increase of 3 $\log_{10}$ CFU/g from day 1. When this product was

stored at ambient temperature, populations increased rapidly, reaching ~ 8 $\log_{10}$ CFU/g by day 3 and remaining at this level to day 8. Populations in experimental reduced salt chicken curry meal stored at 4ºC did not increase over the 8 days storage. During storage at 10ºC, for the experimental reduced salt chicken curry meal there was an increase at day 5 to ~4 $\log_{10}$ CFU/g and the final population reached at day 8 was ~6 $\log_{10}$ CFU/g. However, at ambient storage, populations in the experimental reduced salt ready meal increased to levels comparable to the commercial regular salt meals, ~ 8 $\log_{10}$ CFU/g after 8 days storage. Initial aerobic populations in commercial regular salt chilli con carne were ~4 $\log_{10}$ CFU/g (Figure 3b) and during storage at 4°C increased to ~6 $\log_{10}$ CFU/g at day 8. During storage at 10°C, populations increased to ~7-8 $\log_{10}$ CFU/g at day 5 and remained at this level up to day 8. Commercial regular salt chilli con carne products stored at ambient temperature showed a substantial increase in bacterial populations between day 1 and day 8 with final populations at day reaching ~9 $\log_{10}$ CFU/g. Populations in the experimental reduced salt chilli con carne meals stored at 4ºC reached ~4 $\log_{10}$ CFU/g by day 8 (Figure 3b). When stored at 10ºC the final population in these meals reached ~7 $\log_{10}$ CFU/g. At ambient storage temperature, by day 8 the experimental reduced salt chilli con carne meals contained ~9 $\log_{10}$ CFU/g slightly exceeding populations detected in their commercial regular salt counterparts. In the commercial regular salt lasagne meal, the aerobic bacterial population increased to ~ 5-6 $\log_{10}$ CFU/g after 8 days at 4°C (Figure 3c). Storage at 10ºC of the commercial regular salt lasagne products resulted in an increase in populations to ~ 8 $\log_{10}$ CFU/g by day 8. During storage at ambient temperature, bacterial populations increased to ~8 $\log_{10}$ CFU/g by day 3 and remained at this level to day 8. In the experimental reduced salt lasagne meals, initial populations at day 1 were higher than comparable commercial regular salt meals at ~5 $\log_{10}$ CFU/g (Figure 3c). During storage at 4ºC or 10ºC of the experimental reduced salt lasagne there appeared to be a reduction in populations after day 1 to ~3 $\log_{10}$ CFU/g and at 10ºC storage, after an initial decline the final population was ~5 $\log_{10}$ CFU/g while at 4ºC storage the final population was ~3 $\log_{10}$ CFU/g. During storage at ambient temperature of the experimental reduced salt lasagne, a final population of ~ 9 $\log_{10}$ CFU/g was reached after 8 days, ~1 $\log_{10}$ cycle higher than the commercial regular salt counterpart. Final bacterial populations reached in either commercial regular salt or in experimental reduced salt meals were highest at ambient temperature storage.

Generally, aerobic bacterial populations increased slowly in all thawed commercial regular salt ready meal products stored at 4°C over 8 days. However at 4ºC storage, for the experimental reduced salt meals, an increase in microbial populations was found only in the chilli con carne meal which increased by ~ 1 $\log_{10}$ over 8 days. At 10°C storage, bacterial populations in commercial regular salt chicken curry and chilli con carne meals increased by ~3 $\log_{10}$ CFU/g over 8 days while commercial regular salt lasagne showed a 4 $\log_{10}$ increase in population over this period. Storage of the experimental reduced salt meals at 10ºC resulted in an overall 4 $\log_{10}$ increase in both chicken curry and chilli con carne, while no increase in bacterial populations was found in experimental reduced salt lasagne meals. At ambient temperature storage, for both commercial regular salt or reduced salt products, final populations were 8-9 $\log_{10}$ CFU/g with overall increases between day 1 and day 8 of ~4-6 $\log_{10}$ cycles.

3.3.3 Aerobic plate count at 55°C

Growth was not detected in any of the commercial regular salt meals stored at 4°C, 10°C or ambient temperature on PCA plates incubated at 55ºC. Growth was detected in all experimental reduced salt meals but was present on agar plates as a film and consequently was uncountable; a number of isolates were subsequently identified as *Leuconostoc mesenteroides* using an API 50CH kit (Biomerieux, Basingstoke, Hants, UK).

3.3.4 Total coliform count

Coliforms were not detected in the commercial regular salt chilli con carne meals at any storage temperature. Coliforms were detected in one of the commercial regular salt chicken curry meals and one of the commercial regular salt lasagne meals at day 3 only when stored at ambient temperature, ~3 $\log_{10}$ CFU/g or ~4 $\log_{10}$ CFU/g, respectively. Coliforms were not detected in any of the experimental reduced salt chicken curry meals at any of the storage temperatures. At 4ºC storage, growth was not detected in the experimental reduced salt chilli con carne meals. At 10ºC storage growth was detected at day 5, at ~3 $\log_{10}$ CFU/g which remained at this level up to 8 days storage. When stored at ambient temperature, growth was not detected until day 5 where ~6 $\log_{10}$ CFU/g were detected and which remained at this level up to day 8. Growth was not detected in the experimental reduced salt lasagne meal at 4ºC or 10ºC storage. However at ambient temperature storage, ~ 2 $\log_{10}$ CFU/g were detected at day 3 or 5 which increased to ~3 $\log_{10}$ CFU/g by day 8.

3.3.5 Staphylococci

Staphylococci were not detected in any of the commercial regular salt or experimental reduced salt ready meals stored at 4°C, 10°C or ambient temperature.

3.3.6 *Pseudomonas*

Pseudomonas spp. were not detected in any of the commercial regular salt meals stored at 4°C. Growth was not detected in commercial regular salt chicken curry when stored at 4 or 10 °C. Storage of commercial regular salt chicken curry meals at ambient temperature resulted in growth of *Pseudomonas* spp. to ~4 $\log_{10}$ CFU/g at day 3 with final populations of ~7.5 $\log_{10}$ CFU/g at day 8 (Figure 4a). In the case of commercial regular salt chilli con carne, *Pseudomonas* spp. were detected at ambient temperature storage at day 3, ~4 $\log_{10}$ CFU/g, and these populations reached > 6 $\log_{10}$ CFU/g at day 8 (Figure 4b). Growth of *Pseudomonas* species was detected in commercial regular salt lasagne stored at 10°C at day 5 with a final population of ~ 4 $\log_{10}$ CFU/g at day 8. Growth of *Pseudomonas* species was detected at ambient temperature storage of the commercial regular salt lasagne meal. This population increased from ~4 $\log_{10}$ CFU/g at day 5 to a final population of ~6 $\log_{10}$ CFU/g at day 8. Overall, populations of *Pseudomonas* species detected in commercial regular salt meals stored at ambient temperature decreased in the order: chicken curry > chilli con carne > lasagne. In the case of the experimental reduced salt chicken curry, growth of *Pseudomonas* spp was not detected at 4°C over the 8 day period. At 10°C growth was only detected at day 8 at ~3 $\log_{10}$ CFU/g. At ambient temperature storage, growth was detected at day 3 at 5 $\log_{10}$ CFU/g, decreasing at day 5 to < 4 $\log_{10}$ CFU/g with a final population at day 8 of ~ 4 $\log_{10}$ CFU/g. Growth was detected in experimental reduced salt chilli con carne at all storage temperatures (Figure 4b). At 4°C storage, populations detected were 5 $\log_{10}$ CFU/g at day 8. At 10°C storage, growth was detected at day 3 at 3 $\log_{10}$ CFU/g, and increased thereafter to ~6 $\log_{10}$ CFU/g at day 8. At ambient storage ~7 $\log_{10}$ CFU/g were detected at day 3 but appeared to decrease to a final population of ~ 6 $\log_{10}$ CFU/g at day 8. Growth was not detected in the experimental reduced salt lasagne meal at any of the storage temperatures

3.3.7 Sporeformers and heat resistant bacteria

Spore forming or heat resistant bacteria were not detected in the experimental reduced salt meals or in the commercial regular salt chilli con carne at any storage temperature. Colonies were not detected in commercial regular salt chicken curry meal at day 1 at any of the storage temperatures. However, on day 3 at ambient temperature storage, a number of colonies were present in the commercial regular salt chicken curry, which, on further investigation were determined as Gram negative, catalase-negative cocci and not *Bacillus* species. In rice samples stored at 10°C, a small number of colonies were detected at day 5 of storage and were subsequently determined to be Gram positive bacilli with a slow catalase reaction and were putatively identified as *Lactobacillus* species using API 50CHL (Biomerieux). A number of colonies, <30 per plate, were detected on day 3 in the commercial regular salt lasagne meal stored at 10°C, further examination of these colonies including gram staining and identification using API 50CHL (Biomerieux) determined them to be Gram positive cocci, not *Bacillus* spp.

3.3.8 *Listeria* species

Growth was detected only in commercial regular salt chicken curry meals after 8 days storage at 4°C and using an API Listeria kit, colonies were putatively identified as *Listeria grayii*. *Listeria* spp. were not detected in any of the experimental reduced salt meals.

3.3.9 Total bacterial count after microwaving

Growth was not detected in either of the commercial regular salt or experimental reduced salt meals following microwaving.

4. Discussion

To date, little published information exists on the effects of thawing temperature and storage time and temperature on the microbial quality of frozen commercial ethnic ready meals. Additionally, to our knowledge, a comparison of the effects of salt reduction on the microbiological quality of frozen ready meals during storage has not been undertaken. Despite manufacturers' instructions or public health safety information, consumers may thaw out frozen foods for inappropriate times and temperatures prior to cooking. Similarly, subsequent storage by the consumer of thawed ready meals may occur for prolonged periods at temperatures likely to allow microbial growth to exceed recommended levels. Consumer surveys have consistently revealed a lack of knowledge of correct refrigeration temperatures which may be due to the absence of thermometers in domestic refrigerators and this may lead to temperature abuse by the consumer (Worsfold & Griffith, 1997). Hence the temperatures selected in this study were designed to simulate thawing/storage either in a domestic refrigerator

operating at 4º to 10ºC or in a consumer temperature abuse scenario where the thawed meals were held at ambient temperature. All commercial regular salt or experimental reduced salt ready meals contained an aerobic population at day 1, (~2 - 5 $\log_{10}$ CFU/g) within FSAI recommendations (aerobic plate count of 4 - < 5 and ≥ 5 $\log_{10}$ CFU/g) (FSAI, 2001) with subsequent outgrowth on extended storage at various temperatures.

Data from the thawing trial provides evidence of an indigenous microbial flora present in the products after processing and which were detectable after 4 h thawing of all commercial regular salt products. Generally, the duration of frozen storage and food composition affects bacterial survival. Survival of bacteria following freezing is also dependant on the species and strain, the growth phase of the cells and the physiological condition of the cells prior to freezing (Dykes & Moorhead, 2001; Uljas & Ingham, 1999). Available water, aw, in the food matrix also affects growth, respiration and enzyme synthesis (Blondeaux et al., 1999) and the freezing process of food products reduces the available water content for the microorganisms present (Leistner & Gorris, 1995). This may be due to the fact that freezing of foods leads to formation of ice crystals which affects the mechanical structure of the food and also moisture migration (Durack et al., 2011)

The microbial load detected in the three regular salt ready meals during the 24 h thawing trial indicates a tolerance and an ability of bacterial strains to survive following a relatively severe industrial manufacturing regime. However, in this study it is not clear at what stage during the industrial process bacterial survival occurs.

Regarding the effects of storage temperature on microbial quality, all thawed commercial regular salt ready meals stored at 4ºC developed substantial bacterial populations over 8 days. Of the regular salt meals, bacterial numbers in lasagne and chilli con carne meals reached unacceptable levels at 8 days storage at 4ºC. Elevation of storage temperature to 10ºC resulted in higher developed bacterial populations which by day 3 in lasagne and chilli con carne meals exceeded recommended levels (FSAI, 2001). Continued elevations of storage temperature resulted in rapid increases in bacterial numbers in all meals by day 3, and were well in excess of recommended levels. Microbial counts of the experimental reduced salt meals differed from the commercial regular salt meals. In the case of aerobic plate counts (APC), initial microbial levels at 4ºC storage for both experimental reduced salt chicken curry and chilli con carne populations were much lower than in the commercial regular salt meals, 1 or 3 $\log_{10}$ cycle, respectively. However, final populations after 8 days at 4ºC storage in all three experimental reduced salt meals were significantly lower (P <0.001) than those in commercial regular salt meals. The populations of these experimental reduced salt meals at 4ºC storage were within FSAI microbiological guidelines (APC of 4 - < 5 and ≥ 5 $\log_{10}$ CFU/g) (FSAI, 2001). Experimental reduced salt meals and commercial regular meals salt meals stored at ambient temperature and 10ºC storage contained similar final bacterial populations. Evidence of the ability of bacteria to survive freeze-chilling was reported by O' Leary et al. (2000). These workers found that salmon blast frozen to -35ºC for 2.5h, stored at -25ºC for 7 days, thawed overnight at 4ºC and subsequently stored at 4ºC for 5 days had higher total viable counts in comparison with freshly prepared products (cooked and analysed on the same day). These authors proposed that the thawed product may have had a more open structure due to freeze damage, with more free liquid containing cell nutrients making it more susceptible to microbial growth during subsequent chilling.

During the current study, the removal of a substantial level of salt (~50%) from the commercial regular salt products did not appear to adversely impact on microbial safety, as populations in the reduced salt experimental meals were similar to commercial regular salt meals. The use of salt as a method for food preservation has been well established (Desmond, 2006; Guinee & Fox, 2004). Boziaris et al., (2007) found that salt had a slightly inhibitory affect on bacteria especially at initial exposure but after adaptation, salt may actually stimulate bacterial growth. In contrast to the noted salt tolerance of *Listeria* spp, Conner et al., (1986) found that cabbage juice containing 1% salt caused an initial decrease in populations of *Listeria*. In ready meals the presence of *Enterobacteriaceae* may indicate inadequate heat processing or post pasteurization contamination. Indeed, while certain coliform species i.e. *E.coli*, *Yersinia enterocolitica* may experience some damage during freezing they are also capable of withstanding freezing and subsequent thawing cycles (Gurtler & Beuchat, 2005; Koujitani et al., 2006; Raccach et al., 2002; Warseck et al., 1973). In the present study, except for experimental low salt chilli con carne stored at ambient temperature, coliform populations were within FSAI recommendations for *Enterobacteriaceae* of ≤4 $\log_{10}$ CFU/g (FSAI, 2001).

Psychrophilic bacteria were detected in the commercial regular salt chicken curry meal stored at 4ºC at day 8 only. Psychrophiles or psychrotrophs generally have the ability to grow in temperature environments below ~ 15ºC to 20ºC (Cousin et al., 2001). Conversely, *Pseudomonas* spp. was detected in all commercial regular salt meals stored at ambient temperature but growth was not detected in any of these meals stored at 4ºC. In the experimental low salt meals *Pseudomonas* was only detected in the experimental low salt chilli con carne at 4ºC. The spoilage problem posed by growth of *Pseudomonas* spp. especially in meat products at refrigeration

temperatures has been highlighted a number of workers (Liu et al., 2006; Rajmohan, 2002). Another bacterial species well known for its ability to survive and grow at refrigeration temperatures (3-7ºC) is *Listeria monocytogenes* (Carlin et al., 1995; Carlin & Nguyen-The, 1994; Francis & Beirne, 1997; Rosso et al., 1996). Leistner (2000) found that *Listeria innocua* had a higher survival in margarine when stored at 7ºC compared with an ambient temperature of 25ºC. In this study, *Listeria* species were detected during thawing of regular salt lasagne at 4ºC or in the regular salt chicken curry meal stored at 4ºC on day 8. Indeed the commercial regular salt chicken curry meal was the only meal where general growth of psychrotrophs was evident. Although *L. grayii* is regarded as non-pathogenic it may indicate deterioration in hygiene standards or inadequate process conditions, leading to an increased risk of contamination by other pathogenic *Listeria* species (Greenwood et al., 2005). Lekroengsin et al. (2007) demonstrated that cooking processes, i.e. roasting, steaming and frying, are sufficient to destroy any *Listeria* species present in raw materials. Hence, the presence of *Listeria* spp. in the thawed ethnic ready meals may indicate some post-heat processing contamination.

In the manufacture of chicken curry and chilli con carne, products are directly hot-filled into bags which are film sealed and thereafter enter a spiral freezer. This process minimises human contact with the food after heating and therefore a lower risk of possible contamination by *Staphylococcus* species is present. However, lasagne manufacture involves minimal contact with personnel at depositing stations where individual layers are added to the tray. Hence, this particular process is potentially more susceptible to entry of S*taphylococcus* species. Staphylococci were detected in the commercial regular salt chilli meal only during thawing trials. It is quite possible for packs to be contaminated by Staphylococci post processing through human contact or processing vessels or equipment. In the storage trials, Staphylococci were absent in all of the ready meals indicating a good standard of general personal hygiene of workers in contact with the products and adequate cleaning of utensils and work surfaces (Lancette & Bennett, 2001).

The main ingredient of ready meals potentially susceptible to contamination by sporeforming bacteria is the cooked rice portion, which on extended storage may allow germination and outgrowth. Bacterial spores e.g. *Bacillus cereus*, are capable of withstanding refrigeration temperatures and can germinate and grow in foods stored under inappropriate conditions (Collado et al., 2003). However, in this study only a small number of possible sporeforming or heat resistant bacteria were detected in commercial regular salt lasagne, chicken curry and in the separate rice portion which accompanies the chicken curry meal and were not *Bacillus* spp.

Growth of bacterial species on plates incubated at 55ºC was detected in all experimental low salt meals but not in the commercial regular salt meals. Some isolates were putatively identified as *Leuconostoc mesenteroides*, a species involved in vegetable fermentation (Eom et al., 2007), cheesemaking (Clementi & Rossi, 1984), but which are also commonly associated with spoilage of cooked meat products (García-Gimeno et al., 2005).

In agreement with Aziz et al. (2002), Canumir et al. (2002) and Pucciarelli and Benassi (2005), the effectiveness of microwaving as a means of bacterial inactivation was demonstrated even in the heavily contaminated products after 8 days storage. However, this heat treatment does not inactivate pre-formed toxins or preclude the survival of pathogens or spoilage microorganisms in the viable-but-non-culturable state in these products. Indeed, Woo et al. (2000) noted that while microwaving of suspensions of *E. coli* or *B. subtilis* resulted in a significant reduction in cell viability, cell density, as measured by optical density at 600nm, did not decrease. These authors suggested that after exposure to microwave radiation, cells despite becoming non-viable, were not autolysed**.**

All of the ready meals in this study have slightly acidic pH values with relatively high moisture levels. The moisture levels in the experimental reduced salt chilli con carne and chicken curry were similar to their commercial regular salt counterparts, however, moisture levels in low salt lasagne were ~20% lower. The addition of salt to foods containing meat can increase water holding capacity. Conversely, removal of salt from meat-containing products such as lasagne may release water which is subsequently evaporated during heat processing at 100ºC (Aktas et al., 2003; Cheng & Sun, 2008). Regarding water activity, the majority of microorganisms grow at a_w values >0.90 (FSAI, 2005) and both commercial and experimental meals had a_w values in excess of this, making them suitable for bacteria growth.

In order for a food to bear a claim that it is low in sodium/salt, it must contain no more than 0.12g of sodium, (equivalent to 0.3g of salt, per 100g or per 100ml). To bear a claim of Very Low Sodium/Salt the product must contain no more than 0.04g of sodium (equivalent to 0.1g salt, per 100g or per 100ml) and in order to make a claim of Sodium-Free or Salt-Free the product must contain no more than 0.005g of sodium (equivalent to 0.0125g salt per 100g) according to EU Directive 80/777/EEC. Likewise in order for a product to claim that sodium/salt has been reduced there must be a 25% reduction difference compared with a similar product under the EU Directive 90/496/EEC (FSAI, 2007). In this study, the commercial regular salt meals contained ~1.75g –

2.0g salt per serving, providing up to 30% of recommended 6g daily salt intake. In the experimental meals salt was reduced substantially (50-66%) enabling experimental reduced salt chicken curry meal to be declared a low salt product while the experimental reduced salt chilli con carne or lasagne meals could bear a reduced salt claim.

In order to respond to calls for salt reduction in their products and to achieve ambitious salt reduction targets set by worldwide public health agencies the food industry urgently requires scientific data on the consequent effects of substantial salt reduction on food safety. This report attempts to provide such information, overall, it would appear that salt reduction of the order of 50% does not adversely impact on the microbiological quality of a range of ready meals. However, this study also highlights the short shelf life of thawed frozen ready meals even after the relatively severe manufacturing processes imposed on them. It is also clear from the data, of the requirement for adequate refrigerated storage conditions and the need for strict adherence to manufacturer's instructions regarding microwaving by the consumer.

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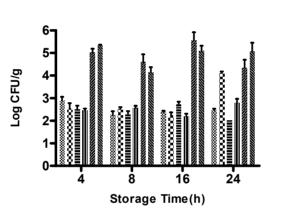
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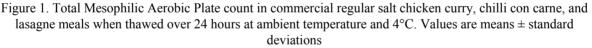
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Table 1. Salt content, pH, a_w and moisture content of commercial regular salt and experimental low salt chilli con carne, chicken curry and lasagne. Values are means ±standard deviations

	Salt Content (%)		pH		Moisture		Water Activity	
	Regular	**Low salt**	**Regular**	**Low salt**	**Regular**	**Low salt**	**Regular**	**Low salt**
Chilli Con Carne	0.66 ± 0.06	0.20 ± 0.01	5.46 0.03	5.30 0.02	79.62 1.28	76.22 ±1.27	0.992 0.00	0.996 ± 0.00
Chicken Curry	1.13 ± 0.15	0.47 ± 0.02	5.84 0.02	5.49 0.03	74.54 0.30	78.01 ±0.42	0.990 0.00	0.996 ± 0.00
Meat Lasagne	1.01 ± 0.14	0.54 ± 0.02	5.58 0.09	5.37 0.09	84.60 0.15	66.09 ±0.49	0.984 0.00	0.977 ± 0.00

Figure 1. Total Mesophilic Aerobic Plate count in commercial regular salt chicken curry, chilli con carne, and lasagne meals when thawed over 24 hours at ambient temperature and 4°C. Values are means ± standard deviations
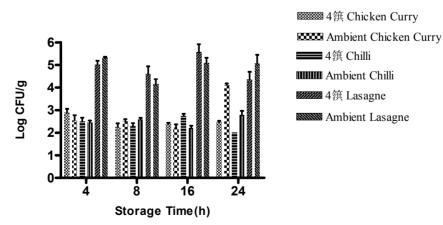

Figure 2. Total Staphylococci count in commercial regular salt chilli con carne meals when thawed over 24 hours at ambient temperature and 4°C storage. Values are means ± standard deviations
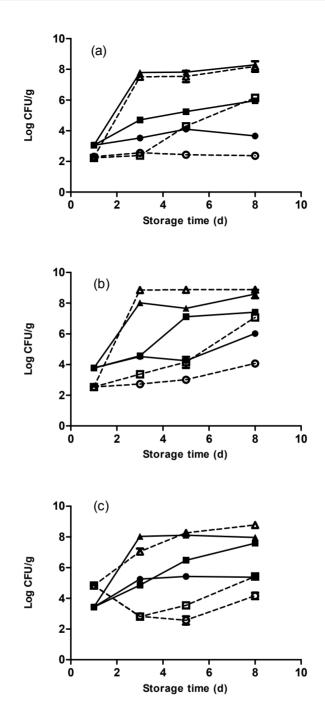
Figure 3. Total Mesophilic Aerobic Plate count in commercial regular salt (___) and experimental low salt (-----) (a) Chicken Curry (b) Chilli Con Carne and (c) Lasagne meals stored over 8 days at various temperatures; 4°C storage (● regular and ○low salt), 10°C (■ regular and □ low salt) or ambient temperature(▲ regular and Δ low salt). Values are means ± standard deviations
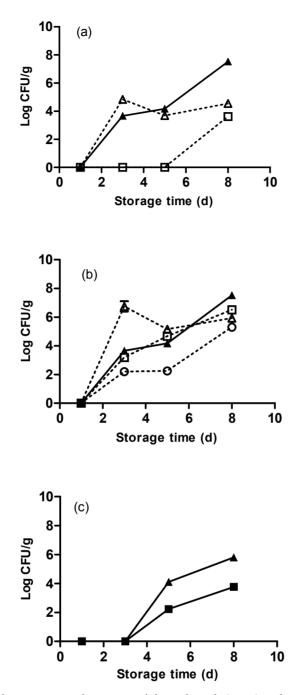
Figure 4. Total *Pseudomonas* count in commercial regular salt (____) and experimental low salt (----) (a) Chicken Curry (b) Chilli Con Carne and (c) Lasagne meals stored over 8 days at various temperatures; 4°C storage (● regular and ○low salt), 10°C (■ regular and □ low salt) or ambient temperature(▲ regular and Δ low salt). Values are means ± standard deviations