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The effect of organic acid and sodium chloride dips on the shelf-life of refrigerated Irish brown crab (*Cancer pagurus*) meat

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Abstract

Crab (*Cancer pagurus*) meat (white and brown) has a short shelf-life. Chemical treatments may inhibit microbial spoilage and extend shelf-life. The effect of 5% organic acids (lactic acid (LA), acetic acid (AA) and citric acid (CA) and 5% sodium chloride (NaCl) on TVC (mesophiles and psychrophiles), Enterobacteriaceae, *Pseudomonas* spp. and lactic acid bacteria (LAB) were investigated during storage (2°C for 12 days). AA was the most effective treatment for white meat, reducing the initial TVCm and TVCp by 1.6 and 1.8 $\log_{10}$ cfu/g, respectively, and extended the shelf life to 8-11.5 days, compared to 5 days for untreated control samples. LA treatment also significantly ($P < 0.05$) reduced the initial TVC, but the shelf life was only increased by 3 days. CA and NaCl treatments had no significant effect ($P > 0.05$). A similar pattern was observed for brown meat samples, although the shelf life was increased by a maximum of 1-3 days. The growth of Enterobacteriaceae, *Pseudomonas* spp. and LAB was significantly ($P < 0.05$) reduced on AA treated samples only. It was concluded that the shelf-life of crab meat may be extended by up to 3 days using lactic acid and more than doubled using acetic acid.

Keywords: *Cancer pagurus*; shelf-life; organic acids; microbial activity; refrigerated storage.

1. Introduction

Irish brown crab, also referred to as ‘edible crab’ (*Cancer Pagurus*) is a commercially important decapod species in Europe. In Ireland approximately 6,000 tonnes are landed annually, worth in excess of €9 million (BIM, 2015). Shelf life is an important consideration, especially as seafood products are highly perishable and the majority of this product is

exported to markets in Europe to be sold unprocessed or as various crab meat products. A longer shelf life would facilitate sales in more geographically distant markets.

In many European countries both the 'white meat' from claws and the 'brown meat' from gonads and hepatopancreas are consumed. The quality of these meat products deteriorates rapidly during processing and subsequent storage, thus limiting their shelf life. In the EU and the USA, seafood products are at the end of their shelf life when the total viable count reaches 5 $\log_{10}$ cfu/g (Robson et al., 2007). Psychrophilic TVC are the most appropriate indicator of shelf life for food products stored at refrigeration temperatures (Nychas et al., 2008), although mesophilic TVC, TEC, *Pseudomonas* spp. and/or LAB counts are used as indicators of microbiological quality (Alonso-Calleja, 2004; Alvarez-Astorga et al., 2002).

Spoilage of crab meat is a complex process. Although chemical and enzymatic reactions trigger the initial decrease in quality (loss of freshness), post-harvest crabs are also contaminated with microorganisms from the harvest site and spoilage is predominantly due to the metabolic activity of bacteria and the production of amines, sulphides, alcohols, aldehydes, ketones and organic acids with associated unpleasant odours, unacceptable appearance and off-flavours (Robson et al., 2007). Thus, activities to extend shelf life should be primarily aimed at retarding or preventing microbial growth. Although there are many ways of preserving seafood, such as drying, salting, smoking, freezing and chemical treatments, many of these can affect the sensory qualities of the food and/or may not be permitted in the EU. At present chilling is the main preservation technology applied and at refrigeration temperatures, crab meat has a shelf life of approximately 5 days when stored under aerobic conditions (Lorentzen et al., 2014). Sodium chloride has been used as a food preservative throughout history and in more recent times the use of lactic, acetic and citric

69 acid has been investigated to prevent bacterial spoilage in a range of foods (Scott et al., 2015,
70 Gonzalez-Fandos and Herrera, 2014). Indeed, citric and lactic acid have been shown to
71 inhibit the growth of spoilage bacteria in freshly shucked oysters (Mahmoud, 2013). Despite
72 these organic acids being cheap, generally regarded as safe (GRAS) and acceptable to
73 consumers, to the best of our knowledge, these antimicrobials have not been applied to crab
74 meat or crab based products.
75
76 The objective of this study was to investigate the effects of lactic acid, acetic acid, citric acid
77 and sodium chloride treatments on the shelf life of both the white and brown meat of edible
78 crab by monitoring TVC (mesophiles and psychrophiles), Enterobacteriaceae, *Pseudomonas*
79 spp. and lactic acid bacteria (LAB).
80

81 **2. Materials and Method**

82 2.1 *Biological and sample preparation*

83 Exactly 20 freshly caught female edible crabs (*Cancer pagurus*) were obtained on three
84 separate occasions from an Irish crab processor. Crabs were stored at 4°C to decrease their
85 metabolism prior to euthanization, which was carried out as recommended by Roth and Øines
86 (2010), whereby both nerve centres were pierced with a steel rod. All crabs were boiled for
87 20 minutes in 5% salt (NaCl) water and air cooled for 1h. White meat (claw and legs) and
88 brown meat (hepatopancreas and gonads) were then picked and separated.

89 *2.2 Immersion solution preparation*

90 All chemical solutions were prepared in sterile distilled water (SDW) and consisted of lactic
91 acid (LA, Sigma Aldrich, Wicklow, Ireland) to 5% (v/v); acetic acid (AA, Sigma Aldrich) to

5% (v/v); citric acid (CA, Sigma Aldrich) to 5% (w/v); and sodium chloride (NaCl, Sigma Aldrich) to 5% (w/v). All dilutions were stored in 1L volumes at 20°C and used within 2 hours.

2.3 Chemical Treatment

Crab meat (white and brown) was prepared as described above, and each meat type was divided into 6 treatment groups. One set of samples was left untreated (untreated control). Each of the other groups were treated by immersion for 30 seconds, in either 500mls of sterile distilled water, lactic acid, acetic acid, citric acid or sodium chloride. Following treatment, samples were immersed in SDW for 30 seconds, and allowed to drain (SDW was changed after each treatment). Each treated meat type sample was divided into 10 gram aliquots in sealable plastic sterile containers (Ramboli 100ml Sterile Specimen Jar). Two samples from each group were immediately subjected to microbiological and physical chemical analysis, as well as after storage at 2°C for 2, 4, 6, 8, 10 and 12 days. All experiments were repeated in duplicate on three separate occasions.

2.4 Microbiological analysis

Of each treated meat type, 10 gram samples were aseptically taken and diluted tenfold with maximum recovery diluent (MRD, Oxoid Ltd., Hampshire,UK) and homogenised for 1min in a stomacher (VWR Starblender LB400). A ten-fold dilution series was then prepared in MRD and plates containing the various agars were inoculated. Total viable mesophilic counts were determined using plate count agar (PCA, Oxoid CM0325) incubated at 30°C for 72 hours. Total viable psychrotrophic counts were determined on PCA plates incubated at 6°C for 10 days. Total enterobacteriaceae counts were carried out using violet red bile glucose agar

(VRBGA, Oxoid CM0485) incubated at 37°C for 24 hours. *Pseudomonas* spp. was determined using Pseudomonas agar base (Oxoid CM0559) with Cephalothin-Sodium Fusidate-Cetrimide (CFC) supplement (Oxoid SR103) incubated at 30°C for 48 hours. LAB were grown on de man Rogosa Sharpe (MRS, Oxoid CM0361) agar at 30°C for 72 hours.

2.5 Physical analysis

The pH was measured at room temperature on undiluted crab meat samples using a surface electrode (Eutech Instruments pH5+ pH meter)

2.5.2 Available water determination

The available water (a_w) was determined at room temperature on undiluted crab meat samples using a water activity meter (Deacagon AquaLab LITE benchtop water activity meter).

2.6 Sensory analysis

In consultation with the Sensory Food Network Ireland, based in Teagasc (Ashtown), the triangle test was selected and used to determine whether consumers could detect a difference between the control samples and those treated with lactic acid, acetic acid, citric acid and/or sodium chloride. Samples of white and brown crab meat were prepared as per the methods outlined in section 2.2 and 2.3. Fifteen taste panellists were then asked to evaluate each of the different treatments. Each panellist was presented with 3 samples (at the same time), 2 alike and 1 different and asked to select (and record) the odd one out based on appearance, odour, taste and texture. Statistical analysis was performed as described by Roessler et al. (1978).

2.7 Statistical analysis

Bacterial counts were converted to $\log_{10}$ cfu/g. Mean generation times (G) for TVC (from time t = 0 to the time where the highest bacterial concentration was recorded) were calculated using the formula: G = t/3.3 logb/B, where t = time interval in h, b = number of bacteria at the end of the time interval, and B = number of bacteria at the beginning of the time interval (Koolman et al, 2014). Lag times and µmax were calculated using the Micro Fit© Software (Version 1.0, Institute of Food Research) and graphs from this software used to calculate stationary, exponential and decline phase information. Micro Fit© is a 32-bit application which is designed to give a graphical representation of microbiological data and fit a growth model to the data to obtain parameters (Sobratee et al., 2009). Statistical comparison of all parameters was performed in GENSTAT by Anova version 14.1 (VSN International Ltd., Hemel, Hempstead, UK) by comparing treatments. Parameters were deemed statistically different at the 5% ($P < 0.05$) level.

3. Results

The pH of the untreated white meat throughout the 12 days storage ranged from pH 6.2 to 7.3 and from pH 5.9 to 6.9 for brown meat (Table 1). Treatment with organic acids reduced the initial pH to as low as pH 4.5 (LA) which subsequently increased up to pH 5.3 to 5.5 by the end of the storage period. The a_w ranged from 0.90 to 0.99, regardless of the meat type or treatment (Table 1).

Growth curves for TVCm and TVCp on white meat subject to the different treatments are shown in Figures 1 and 2 and characterised in terms of initial and maximum bacterial concentration ($\log_{10}$ cfu/g), mean generation time (h), µmax (generations h^{-1}) in Table 2, which also includes the observed shelf life (time to reach 5 $\log_{10}$ cfu/g). Both TVCm and TVCp increased from 2.7 $\log_{10}$ cfu/g (time t = 0) to 7.5 $\log_{10}$ cfu/g in the control samples after 12 days storage at 2°C and a shelf life of 5 days was obtained. SDW did not

significantly ($P > 0.05$) reduce the initial TVCm or TVCp and the mean generation times and µmax were similar resulting in a similar shelf-life (5.5-6d) when compared to the untreated control. Interestingly, while LA significantly ($P < 0.05$) reduced the initial TVCp, TVCm was unaffected. Mean generation times approximately doubled and µmax values halved resulting in an extended shelf life of 7.5-8 days. AA treatment reduced the initial TVCm by 1.6 and TVCp by 1.8 log10 cfu/g. This initial reduction combined with lower growth rates (reduced mean generation times and µmax) resulted in a shelf life of 8 and 11.5 days, respectively. The initial TVCm was unaffected by CA treatment while a 0.9 $\log_{10}$ cfu/g reduction was obtained with NaCl. The corresponding decreases in TVCp for these treatments were 0.8 and 1.2 $\log_{10}$ cfu/g, respectively. The impact of either CA or NaCl treatments on mean generation times was minimal with the exception of CA on TVCp, which increased from 12.8 h (untreated) to 17.9h. Overall, the shelf life of CA and NaCl treated samples when assessed using TVCm and TVCp was 5-6 days which was similar to the untreated controls ($P > 0.05$).

The growth curves for TVCm and TVCp on brown meat subject to the different treatments are shown in Figures 3 and 4 with the growth parameters summarised in Table 2. There was no significant ($P > 0.05$) difference between the control (untreated) and SDW samples for either TVC or TVCp and their growth parameters were similar resulting in a shelf life of 5-6 days. LA treatment did not affect the initial TVCm while the initial TVCp was reduced by 1.1 $\log_{10}$ cfu/g. Mean generation times and maximum concentrations achieved were also reduced for both TVCm and TVCp and the shelf life was increased by 1 and 3 days, respectively when compared to controls. A similar pattern (reduced initial counts and growth rates) was observed for AA treated samples and the shelf life of 6-8 days was observed. Neither CA nor NaCl treatments resulted in significant ($P > 0.05$) reductions in initial TVCp counts and although growth rates were reduced, the observed shelf lives were 6-7 days.

Levels of TEC, *Pseudomonas* spp. and LAB for white and brown meat are shown in Tables 3 and 4, respectively. For white meat, TEC increased from 'not detected' to 3.7 $\log_{10}$ cfu/g on untreated samples after 12 days at 2°C. TEC increased by approximately 4.5 $\log_{10}$ cfu/g on samples treated by CA and NaCl. In contrast LA and AA limited growth to 2.8 $\log_{10}$ cfu/g ($P > 0.05$). *Pseudomonas* spp. and LAB levels in white meat increased in untreated samples from 0.7 to 8.1 $\log_{10}$ cfu/g and 1.9 to 6.2 $\log_{10}$ cfu/g, respectively, over the course of the study. After storage for 12 days the concentrations of *Pseudomonas* spp. had increased to 8.9, 6.2, 5.6, 6.7 and 8.1 $\log_{10}$ cfu/g and LAB to 5.5, 4.6, 3.1, 3.3 and 4.9 $\log_{10}$ cfu/g on samples treated with SDW, LA, AA, CA and NaCl, respectively. On brown meat the concentrations of TEC, *Pseudomonas* spp. and LAB increased by 3.5, 6.7, 3.3, 3.2, 4.3 and 2.9 $\log_{10}$ cfu/g, 6.3, 7.6, 6.2, 5.4, 7.9 and 7.4 $\log_{10}$ cfu/g, and 3.2, 3.1, 2.4, 1.6, 3.0 and 3.8 $\log_{10}$ cfu/g on untreated, SDW, LA, AA, CA and NaCl treated samples, respectively. The only treatments that showed a statistically significant ($P<0.05$) difference, as compared to the untreated control, were obtained with *Pseudomonas spp.* with AA treatment of white meat at samples times 4, 6, 8 and 12 days and brown meat after 6 and 8 days.

The sensory analysis, using the triangle test, clearly demonstrated that the taste panellists could identify samples treated with 5% (v/v) citric and 5% (v/v) acetic acid, with all 15 correctly identifying the treated samples. In contrast, a significantly ($P < 0.01$) lower detection rate (less than half of the panellists) was obtained with samples treated with 5% (v/v) lactic acid and 5% (w/v) NaCl.

4. Discussion

This study investigated the effects of lactic acid (LA), acetic acid, citric acid and sodium chloride treatments on the shelf life of both the white and brown meat of edible crab by monitoring TVC (mesophiles and psychrophiles), Enterobacteriaceae, *Pseudomonas* spp. and lactic acid bacteria (LAB). The initial TVC on both white and brown crab meat was relatively low (approximately 2.5 $\log_{10}$ cfu/g) suggesting the meat was of good microbiological quality (Li et al., 2017). This is further supported by the low initial TEC. In contrast Gutierrez et al. (2010) report an initial TVC of approximately 5.0 $\log_{10}$ cfu/g for fresh crab meat prepared using similar methods to those applied in this study, while Gates et al. (1995) reported an initial TVC of approximately 4 $\log_{10}$ cfu/g in meat from blue crabs (*Callinectes sapidus*). Environmental conditions, including the quality of the water in the areas where the crabs are captured, and the hygienic handling practices during meat extraction all impact on the microbiological quality of crab meat and may explain differences in the initial microbial counts reported in different crab meat studies.

LA (5% v/v) and AA (5%, v/v) treatments significantly ($P < 0.05$) reduced the TVCp on both white and brown meat. Previous research on the use of LA and AA to decontaminated seafood has demonstrated a significant ($P < 0.05$) decrease in bacterial counts on shrimp (Al Dagal and Bazaraa, 1999; Salem and Amin, 2012), mussels (Terzi and Gucukoglu, 2010) and catfish (Bala and Marshall, 1998). Moreover, LA and AA treated samples had increased mean generation times and longer shelf-lives (defined as the period until 5 $\log_{10}$ cfu/g was achieved) suggesting these organic acids, which have 'generally regarded as safe' (GRAS) status, could be used directly to control microbial spoilage.

In contrast, treatment of white and brown crab meat with CA (5%, w/v) did not significantly ($P > 0.05$) affect the initial TVC and any increase in shelf-life was marginal. The differences observed with the different organic acids was most likely due to the mechanism of action, specifically the requirement that the acid molecule be in the undissociated form to penetrate

the bacterial cell membrane. At pH 4.0, the percentages of LA, AA and CA molecules undissociated are 39.2%, 84.5% and 18.9%, respectively, decreasing to 6.05%, 34.9% and 0.41%, respectively at pH 5.0 (Bell and Kyriakides, 2002). Thus, at the pH of our treated crab samples (pH 4.5 to 4.9), a significant proportion of the LA and AA molecules could enter the bacterial cells, dissociate in the cytoplasm and decrease the intaracellular pH thereby disturbing the transmembrane proton motive force, denaturing acid sensitive proteins and DNA and overall interfering with both metabolic and anabolic processes (Abee and Woulters, 1999; Davidson and Taylor, 2007). In contrast the CA molecules were in the dissociated state and therefore excluded from the bacterial cells and hence the treatment had little or no bacteriocidal or bacteriostatic effect.

The effect of NaCl (5%) treatment on the initial bacterial counts and subsequent growth rates was also limited. NaCl preserves food by removing water, thereby reducing the aw. However, at 5% (w/v) the aw is reduced to approximately 0.97 (Bell and Kyriakides, 2002), which is not sufficient to retard bacterial growth. Indeed, bacterial will growth until the aw is reduced to below approximately 0.9, which requires NaCl concentrations of at least 9-11% (w/v) (Judge et al., 1989). However, at concentrations above 2-3%, NaCl adversely affects the sensory attributes of food (Sofos, 1986).

For the purpose of this study, the end of shelf life was defined as the point in time when the total bacterial counts reached 5 $\log_{10}$ cfu/g (Robson et al., 2007). The untreated raw crab (*Cancer Pagurus*) meat used in our investigations had a shelf life of 5 days when stored at 2°C. This compares with 10-11 days for whole crabs stored at 4°C (Robson et al., 2007) and 6 days for fresh crab meat, also stored at 4°C (George and Gopakumar, 1988; Gates et al.,

1995) which increased to 15 days when stored at 0°C (Gates et al., 1995). Lorentzen et al., (2016) also reported a shelf life of 10 and 14 days for cooked snow crab (*Chionoecetes opilio*) meat stored at 4°C and 0°C, respectively. Apart from storage temperature, these differences in shelf life are most likely due to differences in initial bacterial contamination levels and variability in spoilage microflora between the different crab species (Robson et al., 2007).

Initial *Pseudomonas* counts were low (0.7 – 1.0 $\log_{10}$ cfu/g). In contrast, Lorentzen et al. (2016) reported an initial level of 2-3.5 $\log_{10}$ cfu/g *Pseudomonas* spp. in raw snow crab (*Chionoecetes opilio*) meat. In our study, these bacteria grew relatively rapidly reaching 7.3 – 8.8 $\log_{10}$ cfu/g after 12 days storage. This observation has also been previously reported in raw crab (*Cancer pagurus*) (Anacleto et al., 2011), cooked crab (Ingham et al., 1990) and in lobster stored at 0°C, 5°C and 20°C (Boziaris et al., 2011). Moreover, *Pseudomonas* spp. have been shown to outgrow and inhibit H_2S producing bacteria, possibly due to their siderophore mediated ability to out-compete other bacteria for iron (Gram and Melchiorsen, 1996). Thus these bacteria are most likely the primary spoilage bacteria in crab meat (Lorentzen et al., 2016). This observation, plus the fact that similar levels were detected in both white and brown meat suggests that *Pseudomonas* spp. counts may be an appropriate spoilage indicator of edible crab (*Cancer Pagurus*) meat, with the product spoiled when the count reaches 4-5 $\log_{10}$ cfu/g. Moreover, the *Pseudomonas* spp. count may be used as an indicator of spoilage with the end of shelf-life obtained when the count reaches 4-5 $\log_{10}$ cfu/g.

Sensory analysis suggested that taste panellists were able to detect CA and AA treated samples but not crab meat treated with LA and NaCl. Although similar data is unavailable for crab meat other relevant studies suggest that treating meat with LA does not adversely affect

the sensory properties probably because LA, unlike CA or AA, does not have a strong taste or odour (Grajales-Lagunes et al., 2012).

5. Conclusion

The data provided in this study provides novel information on the immediate and storage effects of chemical interventions on the natural microflora of white and brown crab meat. It was concluded that treating both white and brown crab meat with 5% (v/v) LA or AA would significantly ($P < 0.05$) reduced the TVC and inhibit the growth of spoilage bacteria thereby increased the shelf-life from 5 days to up to 11.5 days. Furthermore, sensory analysis suggested that LA treatment did not affect the sensory properties of either the white or brown crab meat and this treatment should therefore be considered for application in the crab meat sector subject to consumer acceptability and commercial considerations.

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Table 1. The mean pH and water activity of white (W) and brown (B) crab meat immediately after treatment with lactic acid, acetic acid, citric acid and sodium chloride and during subsequent storage at 2 °C.

Storage time (days)	Untreated		Sterile distilled water		Lactic acid (5%, v/v)		Acetic acid (5%, v/v)		Citric acid (5%, v/v)		Sodium chloride (5%, w/v)	
	W	B	W	B	W	B	W	B	W	B	W	B
Mean pH												
0	7.1	6.4	7.3	6.6	4.5	4.5	4.6	4.6	4.9	4.9	7.1	7.1
2	7.3	6.8	7.5	6.7	5.0	5.0	5.0	5.0	5.3	5.3	6.7	6.7
4	7.1	6.7	7.4	6.6	5.5	5.5	5.2	5.2	5.5	5.5	7	7
6	6.9	6.9	7.4	6.7	5	5.0	5	5	5.1	5.1	6.5	6.5
8	6.9	6.5	7.2	6.4	5.3	5.2	5.2	5.2	5.6	5.6	6.7	6.7
10	6.2	5.9	6.9	5.9	5.4	5.3	5.3	5.3	5.2	5.2	7	7
12	6.8	6.3	6.9	6.1	5.5	5.3	5.3	5.3	5.5	5.5	6.3	6.3
Mean water activity												
0	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99
2	0.96	0.93	0.98	0.99	0.99	0.94	0.99	0.96	0.97	0.97	0.97	0.96
4	0.95	0.93	0.98	0.96	0.97	0.96	0.97	0.94	0.97	0.94	0.99	0.96
6	0.93	0.94	0.97	0.97	0.96	0.96	0.96	0.94	0.98	0.94	0.97	0.95
8	0.90	0.97	0.99	0.96	0.97	0.95	0.96	0.96	0.98	0.96	0.95	0.95
10	0.92	0.98	0.94	0.93	0.99	0.99	0.99	0.90	0.98	0.90	0.97	0.97
12	0.93	0.98	0.96	0.95	0.96	0.93	0.98	0.96	0.96	0.96	0.97	0.98

Table 2. Growth parameters of mesophilic and psychrophilic total viable counts on white and brown crab meat stored at 2°C for 12 days.

Treatment	Initial concentration (log_{10} cfu/g)		Mean generation time (h)[5]		µmax (generations h^{-1})		Maximum concentration observed (log_{10} cfu/g)		Shelf life[6] (days)	
	TVCm	TVCp	TVCm	TVCp	TVCm	TVCp	TVCm	TVCp	TVCm	TVCp
	White meat									
Untreated	2.7	2.7	10.7	12.8	0.10	0.08	7.5	7.5	5	5
SDW	2.6	2.0	10.1	14.3	0.10	0.06	8.7	8.0	5.5	6
LA (5%, v/v)	2.1	1.0	20.2	15.1	0.04	0.05	6.5	5.3	7.5	8
AA (5%, v/v)	1.1	0.9	12.3	17.9	0.06	0.15	8.2	5.7	8	11.5
CA (5%, v/v)	2.9	1.9	12.1	17.9	0.30	0.42	7.9	6.2	6	5
NaCl (5%, w/v)	1.8	1.5	10.8	12.9	0.07	0.06	7.2	7.4	6	6
	Brown meat									
Untreated	2.3	2.4	11.5	13.9	0.07	0.05	7.7	8.7	5	5
SDW[1]	2.3	1.7	13.3	14.8	0.08	0.07	6.7	7.6	6	6
LA[2]	2.1	1.3	12.8	16.7	0.07	0.04	6.7	6.6	6	8
AA[3]	1.1	1.0	13.1	16.7	0.06	0.04	7.8	6.2	8	6
CA[4]	1.9	2.2	13.3	20.0	0.12	0.10	7.4	5.8	6	6
NaCl	2.8	1.8	11.2	15.4	0.31	0.08	7.8	7.4	6.5	7

[1] SDW = sterile distilled water

[2]LA = lactic acid

[3]AA = acetic acid

[4]CA = citric acid

[5] Calculated using the formula G = t/3.3 logb/B, where t = time interval in h to when the late lag phase was reached, b=number of bacteria at the end of the time interval, and B = number of bacteria at the beginning of the time interval (Koolman et al, 2014).

[6]Shelf-life is defined as the time required for the TVC to reach 5 $\log_{10}$ cfu/g

Table 3. Spoilage (TEC, *Pseudomonas* spp. and lactic acid bacteria) bacterial counts (log_{10} cfu/g) on white crab meat immediately after treatment with lactic acid, acetic acid, citric acid and sodium chloride and during subsequent storage at 2 °C.

Storage time (days)	Untreated		Sterile distilled water		Lactic acid (5%, v/v)		Acetic acid (5%, v/v)		Citric acid (5%, v/v)		Sodium chloride (5%, w/v)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE
	Total Enterobacteriaceae count (TEC)											
0	ND		0.8	0.43	0.1	0.11	0.5	0.2	ND		ND	
2	0.4	0.22	1.18	0.61	0.4	0.18	ND		ND		0.4	0.28
4	0.8	0.38	1.9	0.65	1.3	0.36	0.2	0.16	ND		0.7	0.45
6	1.6	0.57	3.49	0.75	1.3	0.41	0.4	0.34	1.4	0.49	1.9	0.50
8	4.7	1.03	4.4	0.59	1.1	0.44	0.8	0.52	2.2	0.48	4.5	0.42
10	3.6	.23	4.7	0.61	2.3	0.35	2.0	0.42	3.0	0.36	3.6	0.18
12	3.6	.25	6.7	0.52	2.8	0.12	2.8	0.27	4.7	0.401	4.6	0.49
	Pseudomonas spp.											
0	0.7	0.19	1.7	0.55	ND		ND		ND		0.3	0.46
2	2.8	0.52	2.1	0.47	1.5	0.31	0.5	0.20	0.6	0.352	1.7	0.25
4	3.8	0.34	3.2	0.71	1.6	0.24	0.5	0.21	0.9	0.133	2.3	0.27
6	5.6	0.27	4.6	0.41	2.6	0.20	0.8	0.52	2.7	0.308	4.4	0.37
8	5.9	0.49	5.8	0.32	4.0	0.50	2.4	0.73	4.9	0.390	6.2	0.14
10	6.9	0.74	7.6	0.59	4.9	0.24	3.9	0.45	5.5	0.166	7.2	0.63
12	8.1	0.72	8.9	0.18	6.2	0.27	5.6	1.00	6.7	0.110	8.1	0.33
	Lactic acid bacteria											
0	1.9	0.14	2.2	0.10	0.9	0.57	1.4	0.55	1.5	0.37	1.4	0.44
2	2.8	0.23	3.2	0.25	2.6	0.47	2.8	0.24	2.8	0.16	2.6	0.54

4	3.3	0.26	2.9	0.21	2.6	0.15	2.9	0.13	2.8	0.18	2.9	0.58
6	3.4	0.42	3.3	0.79	2.6	0.17	2.7	0.22	2.6	0.12	2.5	0.46
8	4.3	0.40	3.9	0.16	2.8	0.37	3.4	0.43	3.5	0.35	3.9	0.67
10	5.1	0.40	4.9	1.11	3.5	0.27	3.0	0.12	3.4	0.12	4.1	0.65
12	6.2	0.20	5.5	0.80	4.6	0.81	3.1	1.1	3.3	0.23	4.9	0.38

ND = not detected

SE = standard error

Table 4. Spoilage (TEC, *Pseudomonas* spp. and lactic acid bacteria) bacterial counts (log_{10} cfu/g) on brown crab meat immediately after treatment with lactic acid, acetic acid, citric acid and sodium chloride and during subsequent storage at 2 °C.

Storage time (days)	Untreated		Sterile distilled water		Lactic acid (5%, v/v)		Acetic acid (5%, v/v)		Citric acid (5%, v/v)		Sodium chloride (5%, w/v)	
	mean	SE	mean	SE	mean	SE	mean	SE	mean	SE	Mean	SE
	Total Enterobacteriaceae count (TEC)											
0	0.3	0.24	0.2	0.16	0.3	0.33	ND		ND		0.9	0.54
2	0.5	0.36	0.3	0.27	0.6	0.39	0.8	0.08	0.7	0.45	0.5	0.28
4	0.8	0.35	1.5	0.49	1.4	0.45	0.9	0.31	0.8	0.36	1.2	0.41
6	1.5	0.56	1.7	0.73	1.1	0.54	0.8	0.22	2.7	0.55	2.0	0.24
8	4.9	0.76	4.4	0.57	1.2	0.74	1.2	0.30	4.2	0.51	3.5	0.47
10	3.2	0.42	4.6	0.05	2.1	0.31	1.9	0.14	3.7	0.43	3.3	0.44
12	3.8	0.22	6.9	0.14	3.6	0.1	3.2	0.18	4.3	0.71	3.8	0.08
Pseudomonas spp.												
0	1.0	0.39	0.9	0.27	ND		ND		ND		ND	
2	2.2	0.6	1.2	0.58	ND		ND		ND		0.9	0.22
4	3.1	0.21	3.4	0.13	1.22	0.18	0.7	0.37	0.6	0.16	1.4	0.45
6	5.1	0.43	3.6	0.40	3.47	0.32	1.4	0.73	3.5	0.18	3.1	0.36
8	6.6	0.28	5.8	0.93	5.97	0.43	2.7	0.11	5.0	0.39	5.4	0.33
10	6.7	0.73	6.3	0.34	5.56	0.20	4.9	0.11	5.9	0.43	6.5	0.33
12	7.3	0.19	7.5	0.41	6.17	0.43	5.4	0.15	7.9	0.18	7.4	0.54
Lactic acid bacteria												
0	2.2	0.28	1.9	0.56	1.6	0.56	1.4	0.50	1.4	0.54	1.5	0.99

2	2.3	0.59	2.1	0.61	3.1	0.27	3.1	0.19	3.1	0.26	2.9	0.12
4	3.3	0.17	3.1	0.18	3.1	0.27	2.7	0.25	2.8	0.12	2.7	0.14
6	3.4	0.44	3.8	0.43	3.2	0.36	2.7	0.38	3.3	0.16	2.7	0.51
8	4.5	0.35	4.5	0.42	3.5	0.25	3.6	0.12	3.5	0.29	3.2	0.40
10	4.	0.24	4.3	0.11	2.8	0.93	3.3	0.53	3.0	0.34	4.1	0.98
12	5.4	0.53	5.0	0.20	4.0	0.198	3.0	0.24	4.4	0.26	5.3	0.51

ND = not detected

SE = standard error

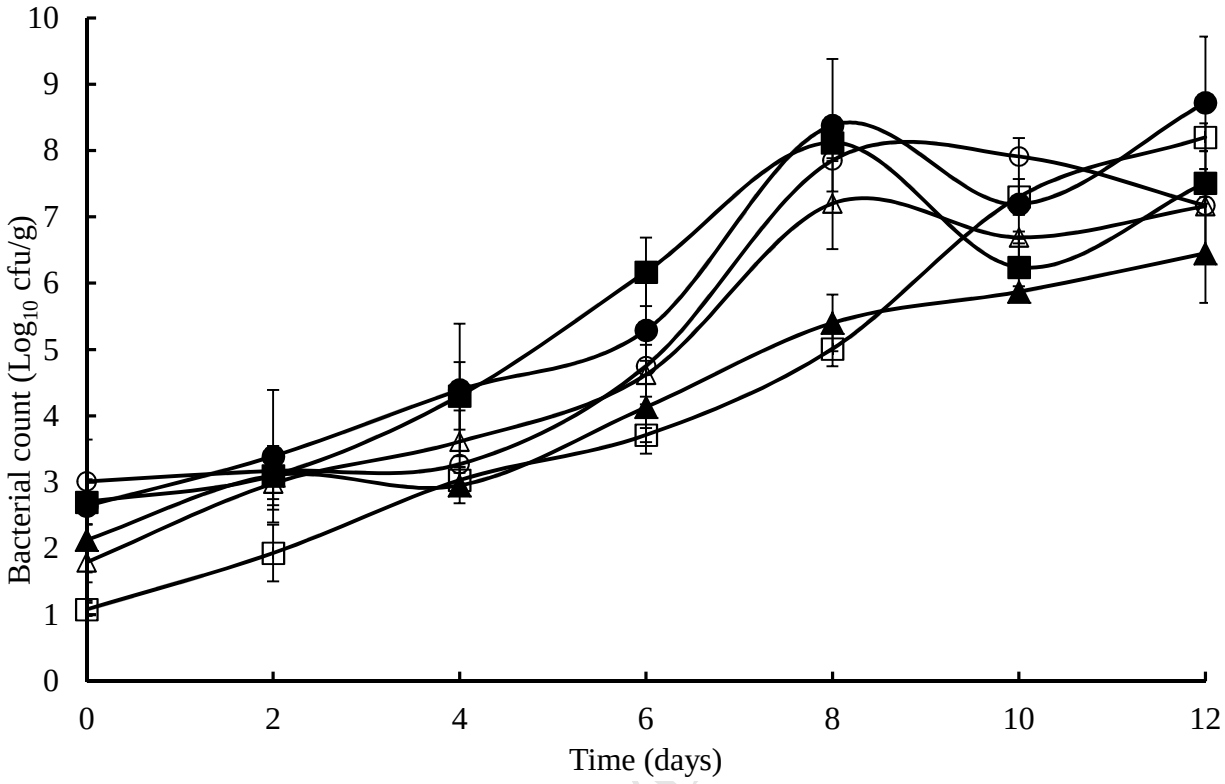

Figure 1. The total viable count (mesophilic) in white meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5% v/v lactic acid (▲), 5% v/v acetic acid (□), 5%, v/v citric acid (O) and 5%, w/v sodium chloride (Δ).

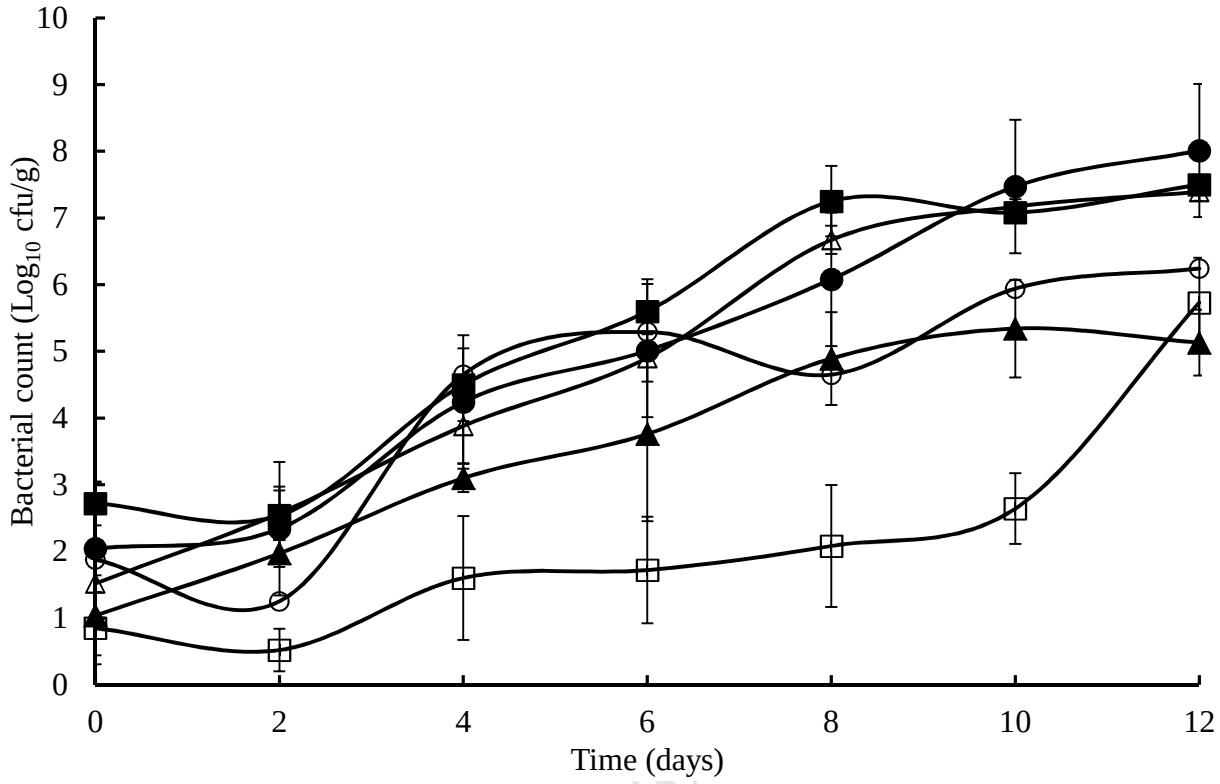

Figure 2. The total viable count (psychrophilic) in white meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (O) and 5%, w/v sodium chloride (Δ).

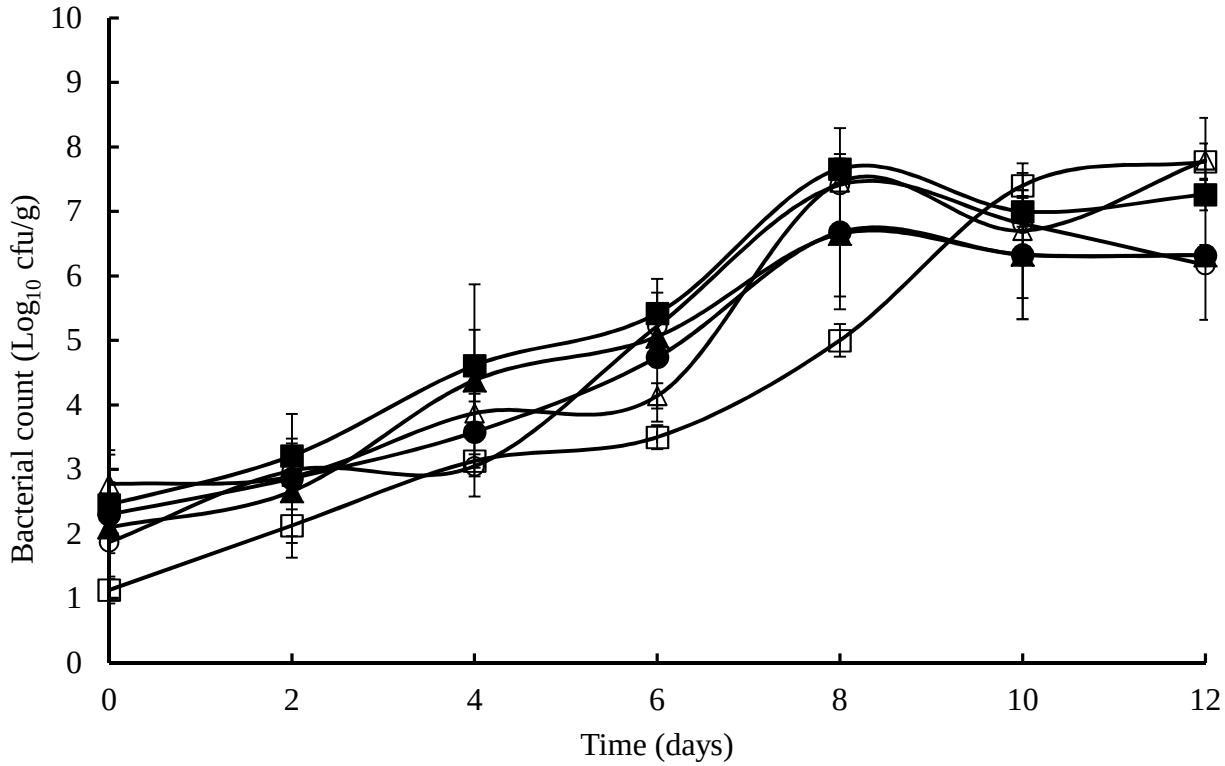

Figure 3. The total viable count (mesophilic) in brown meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (O) and 5%, w/v sodium chloride (Δ).

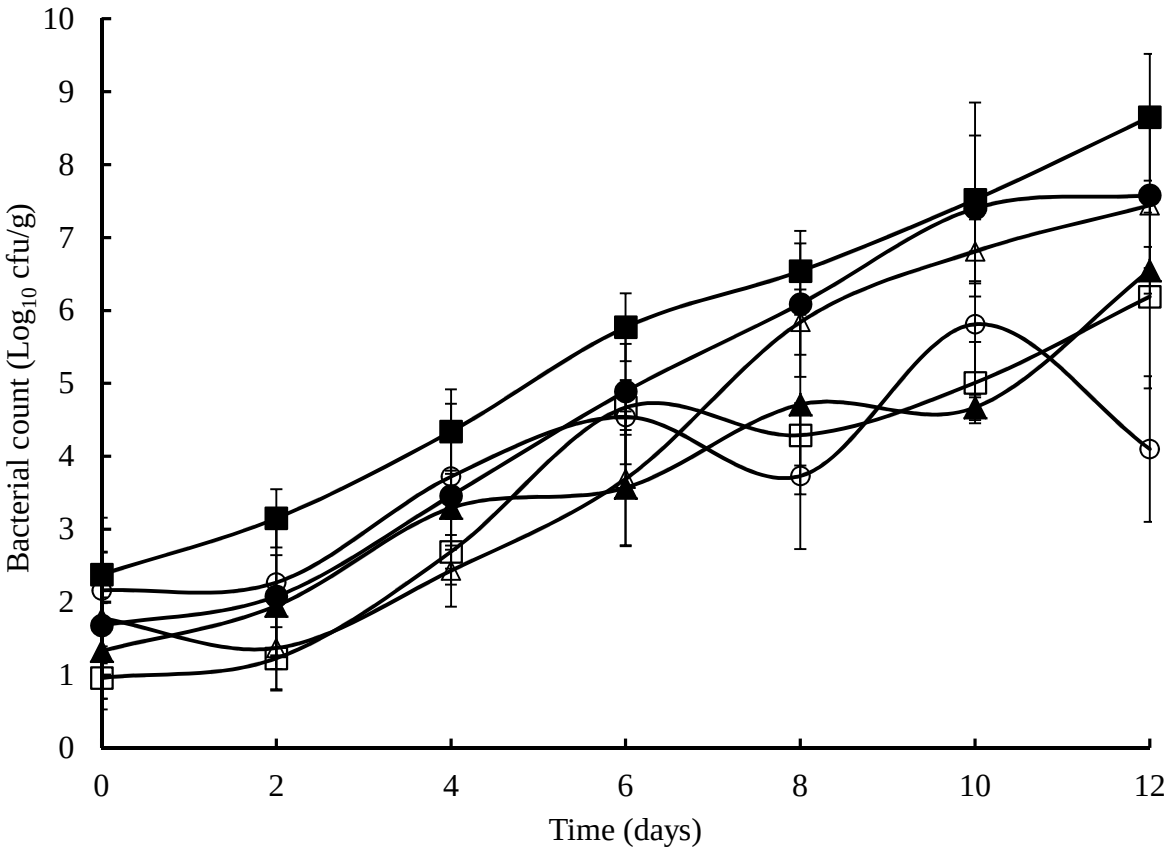

Figure 4. The total viable count (psychrophilic) in brown meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (O) and 5%, w/v sodium chloride (Δ).

The effect of organic acids and sodium chloride on the shelf-life of Irish brown crab (*Cancer pagurus*) meat

Highlights

- Lactic acid (5%, v/v) increased the shelf-life by 3 days.
- Acetic acid (5%, v/v) more than doubled the shelf-life.
- Citric acid (5%, v/v) and sodium chloride (5%, w/v) treatments did not affect shelf-life.