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

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21 Abstract

22 Crab (*Cancer pagurus*) meat (white and brown) has a short shelf-life. Chemical treatments
23 may inhibit microbial spoilage and extend shelf-life. The effect of 5% organic acids (lactic
24 acid (LA), acetic acid (AA) and citric acid (CA) and 5% sodium chloride (NaCl) on TVC
25 (mesophiles and psychophiles), Enterobacteriaceae, *Pseudomonas* spp. and lactic acid
26 bacteria (LAB) were investigated during storage (2°C for 12 days). AA was the most
27 effective treatment for white meat, reducing the initial TVC_m and TVC_p by 1.6 and 1.8 log₁₀
28 cfu/g, respectively, and extended the shelf life to 8-11.5 days, compared to 5 days for
29 untreated control samples. LA treatment also significantly ($P < 0.05$) reduced the initial TVC,
30 but the shelf life was only increased by 3 days. CA and NaCl treatments had no significant
31 effect ($P > 0.05$). A similar pattern was observed for brown meat samples, although the shelf
32 life was increased by a maximum of 1-3 days. The growth of Enterobacteriaceae,
33 *Pseudomonas* spp. and LAB was significantly ($P < 0.05$) reduced on AA treated samples
34 only. It was concluded that the shelf-life of crab meat may be extended by up to 3 days using
35 lactic acid and more than doubled using acetic acid.

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

38

39 1. Introduction

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

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

46

47 In many European countries both the 'white meat' from claws and the 'brown meat' from
48 gonads and hepatopancreas are consumed. The quality of these meat products deteriorates
49 rapidly during processing and subsequent storage, thus limiting their shelf life. In the EU and
50 the USA, seafood products are at the end of their shelf life when the total viable count
51 reaches $5 \log_{10}$ cfu/g (Robson et al., 2007). Psychrophilic TVC are the most appropriate
52 indicator of shelf life for food products stored at refrigeration temperatures (Nychas et al.,
53 2008), although mesophilic TVC, TEC, *Pseudomonas* spp. and/or LAB counts are used as
54 indicators of microbiological quality (Alonso-Calleja, 2004; Alvarez-Astorga et al., 2002).

55

56 Spoilage of crab meat is a complex process. Although chemical and enzymatic reactions
57 trigger the initial decrease in quality (loss of freshness), post-harvest crabs are also
58 contaminated with microorganisms from the harvest site and spoilage is predominantly due to
59 the metabolic activity of bacteria and the production of amines, sulphides, alcohols,
60 aldehydes, ketones and organic acids with associated unpleasant odours, unacceptable
61 appearance and off-flavours (Robson et al., 2007). Thus, activities to extend shelf life should
62 be primarily aimed at retarding or preventing microbial growth. Although there are many
63 ways of preserving seafood, such as drying, salting, smoking, freezing and chemical
64 treatments, many of these can affect the sensory qualities of the food and/or may not be
65 permitted in the EU. At present chilling is the main preservation technology applied and at
66 refrigeration temperatures, crab meat has a shelf life of approximately 5 days when stored
67 under aerobic conditions (Lorentzen et al., 2014). Sodium chloride has been used as a food
68 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

92 5% (v/v); citric acid (CA, Sigma Aldrich) to 5% (w/v); and sodium chloride (NaCl, Sigma
93 Aldrich) to 5% (w/v). All dilutions were stored in 1L volumes at 20°C and used within 2
94 hours.

95 *2.3 Chemical Treatment*

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

106

107 *2.4 Microbiological analysis*

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

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

119

120 2.5 Physical analysis

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

123 2.5.2 Available water determination

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

126 2.6 Sensory analysis

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

135

136 2.7 Statistical analysis

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

149 **3. Results**

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

155
156 Growth curves for TVC_m and TVC_p on white meat subject to the different treatments are
157 shown in Figures 1 and 2 and characterised in terms of initial and maximum bacterial
158 concentration (\log_{10} cfu/g), mean generation time (h), μ_{max} (generations h^{-1}) in Table 2,
159 which also includes the observed shelf life (time to reach 5 \log_{10} cfu/g). Both TVC_m and
160 TVC_p increased from 2.7 \log_{10} cfu/g (time $t = 0$) to 7.5 \log_{10} cfu/g in the control samples
161 after 12 days storage at 2°C and a shelf life of 5 days was obtained. SDW did not

162 significantly ($P > 0.05$) reduce the initial TVC_m or TVC_p and the mean generation times and
163 μ_{max} were similar resulting in a similar shelf-life (5.5-6d) when compared to the untreated
164 control. Interestingly, while LA significantly ($P < 0.05$) reduced the initial TVC_p, TVC_m was
165 unaffected. Mean generation times approximately doubled and μ_{max} values halved resulting
166 in an extended shelf life of 7.5-8 days. AA treatment reduced the initial TVC_m by 1.6 and
167 TVC_p by 1.8 log₁₀ cfu/g. This initial reduction combined with lower growth rates (reduced
168 mean generation times and μ_{max}) resulted in a shelf life of 8 and 11.5 days, respectively. The
169 initial TVC_m was unaffected by CA treatment while a 0.9 log₁₀ cfu/g reduction was obtained
170 with NaCl. The corresponding decreases in TVC_p for these treatments were 0.8 and 1.2 log₁₀
171 cfu/g, respectively. The impact of either CA or NaCl treatments on mean generation times
172 was minimal with the exception of CA on TVC_p, which increased from 12.8 h (untreated) to
173 17.9h. Overall, the shelf life of CA and NaCl treated samples when assessed using TVC_m
174 and TVC_p was 5-6 days which was similar to the untreated controls ($P > 0.05$).

175
176 The growth curves for TVC_m and TVC_p on brown meat subject to the different treatments
177 are shown in Figures 3 and 4 with the growth parameters summarised in Table 2. There was
178 no significant ($P > 0.05$) difference between the control (untreated) and SDW samples for
179 either TVC or TVC_p and their growth parameters were similar resulting in a shelf life of 5-6
180 days. LA treatment did not affect the initial TVC_m while the initial TVC_p was reduced by 1.1
181 log₁₀ cfu/g. Mean generation times and maximum concentrations achieved were also reduced
182 for both TVC_m and TVC_p and the shelf life was increased by 1 and 3 days, respectively
183 when compared to controls. A similar pattern (reduced initial counts and growth rates) was
184 observed for AA treated samples and the shelf life of 6-8 days was observed. Neither CA nor
185 NaCl treatments resulted in significant ($P > 0.05$) reductions in initial TVC_p counts and
186 although growth rates were reduced, the observed shelf lives were 6-7 days.

187
188 Levels of TEC, *Pseudomonas* spp. and LAB for white and brown meat are shown in Tables 3
189 and 4, respectively. For white meat, TEC increased from 'not detected' to 3.7 log₁₀ cfu/g on
190 untreated samples after 12 days at 2°C. TEC increased by approximately 4.5 log₁₀ cfu/g on
191 samples treated by CA and NaCl. In contrast LA and AA limited growth to 2.8 log₁₀ cfu/g (P
192 > 0.05). *Pseudomonas* spp. and LAB levels in white meat increased in untreated samples
193 from 0.7 to 8.1 log₁₀ cfu/g and 1.9 to 6.2 log₁₀ cfu/g, respectively, over the course of the
194 study. After storage for 12 days the concentrations of *Pseudomonas* spp. had increased to 8.9,
195 6.2, 5.6, 6.7 and 8.1 log₁₀ cfu/g and LAB to 5.5, 4.6, 3.1, 3.3 and 4.9 log₁₀ cfu/g on samples
196 treated with SDW, LA, AA, CA and NaCl, respectively. On brown meat the concentrations
197 of TEC, *Pseudomonas* spp. and LAB increased by 3.5, 6.7, 3.3, 3.2, 4.3 and 2.9 log₁₀ cfu/g,
198 6.3, 7.6, 6.2, 5.4, 7.9 and 7.4 log₁₀ cfu/g, and 3.2, 3.1, 2.4, 1.6, 3.0 and 3.8 log₁₀ cfu/g on
199 untreated, SDW, LA, AA, CA and NaCl treated samples, respectively. The only treatments
200 that showed a statistically significant (P<0.05) difference, as compared to the untreated
201 control, were obtained with *Pseudomonas* spp. with AA treatment of white meat at samples
202 times 4, 6, 8 and 12 days and brown meat after 6 and 8 days.

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

208

209 4. Discussion

210 This study investigated the effects of lactic acid (LA), acetic acid, citric acid and sodium
211 chloride treatments on the shelf life of both the white and brown meat of edible crab by
212 monitoring TVC (mesophiles and psychrophiles), Enterobacteriaceae, *Pseudomonas* spp. and
213 lactic acid bacteria (LAB). The initial TVC on both white and brown crab meat was relatively
214 low (approximately $2.5 \log_{10}$ cfu/g) suggesting the meat was of good microbiological quality
215 (Li et al., 2017). This is further supported by the low initial TEC. In contrast Gutierrez et al.
216 (2010) report an initial TVC of approximately $5.0 \log_{10}$ cfu/g for fresh crab meat prepared
217 using similar methods to those applied in this study, while Gates et al. (1995) reported an
218 initial TVC of approximately $4 \log_{10}$ cfu/g in meat from blue crabs (*Callinectes sapidus*).
219 Environmental conditions, including the quality of the water in the areas where the crabs are
220 captured, and the hygienic handling practices during meat extraction all impact on the
221 microbiological quality of crab meat and may explain differences in the initial microbial
222 counts reported in different crab meat studies.

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

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

235 the bacterial cell membrane. At pH 4.0, the percentages of LA, AA and CA molecules
236 undissociated are 39.2%, 84.5% and 18.9%, respectively, decreasing to 6.05%, 34.9% and
237 0.41%, respectively at pH 5.0 (Bell and Kyriakides, 2002). Thus, at the pH of our treated crab
238 samples (pH 4.5 to 4.9), a significant proportion of the LA and AA molecules could enter the
239 bacterial cells, dissociate in the cytoplasm and decrease the intracellular pH thereby
240 disturbing the transmembrane proton motive force, denaturing acid sensitive proteins and
241 DNA and overall interfering with both metabolic and anabolic processes (Abee and Wouters,
242 1999; Davidson and Taylor, 2007). In contrast the CA molecules were in the dissociated state
243 and therefore excluded from the bacterial cells and hence the treatment had little or no
244 bacteriocidal or bacteriostatic effect.

245

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

253

254 For the purpose of this study, the end of shelf life was defined as the point in time when the
255 total bacterial counts reached $5 \log_{10}$ cfu/g (Robson et al., 2007). The untreated raw crab
256 (*Cancer Pagurus*) meat used in our investigations had a shelf life of 5 days when stored at
257 2°C. This compares with 10-11 days for whole crabs stored at 4°C (Robson et al., 2007) and
258 6 days for fresh crab meat, also stored at 4°C (George and Gopakumar, 1988; Gates et al.,

259 1995) which increased to 15 days when stored at 0°C (Gates et al., 1995). Lorentzen et al.,
260 (2016) also reported a shelf life of 10 and 14 days for cooked snow crab (*Chionoecetes*
261 *opilio*) meat stored at 4°C and 0°C, respectively. Apart from storage temperature, these
262 differences in shelf life are most likely due to differences in initial bacterial contamination
263 levels and variability in spoilage microflora between the different crab species (Robson et al.,
264 2007).

265 Initial *Pseudomonas* counts were low (0.7 – 1.0 log₁₀ cfu/g). In contrast, Lorentzen et al.
266 (2016) reported an initial level of 2-3.5 log₁₀ cfu/g *Pseudomonas* spp. in raw snow crab
267 (*Chionoecetes opilio*) meat. In our study, these bacteria grew relatively rapidly reaching 7.3 –
268 8.8 log₁₀ cfu/g after 12 days storage. This observation has also been previously reported in
269 raw crab (*Cancer pagurus*) (Anacleto et al., 2011), cooked crab (Ingham et al., 1990) and in
270 lobster stored at 0°C, 5°C and 20°C (Boziaris et al., 2011). Moreover, *Pseudomonas* spp.
271 have been shown to outgrow and inhibit H₂S producing bacteria, possibly due to their
272 siderophore mediated ability to out-compete other bacteria for iron (Gram and Melchiorsen,
273 1996). Thus these bacteria are most likely the primary spoilage bacteria in crab meat
274 (Lorentzen et al., 2016). This observation, plus the fact that similar levels were detected in
275 both white and brown meat suggests that *Pseudomonas* spp. counts may be an appropriate
276 spoilage indicator of edible crab (*Cancer Pagurus*) meat, with the product spoiled when the
277 count reaches 4-5 log₁₀ cfu/g. Moreover, the *Pseudomonas* spp. count may be used as an
278 indicator of spoilage with the end of shelf-life obtained when the count reaches 4-5 log₁₀
279 cfu/g.

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

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

285

286 **5. Conclusion**

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

295

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301

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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		μ_{max} (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

¹SDW = sterile distilled water

²LA = lactic acid

³AA = acetic acid

⁴CA = citric acid

⁵Calculated using the formula $G = t/3.3 \log b/B$, 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).

⁶Shelf-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
<i>Pseudomonas</i> 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
	<i>Pseudomonas</i> 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

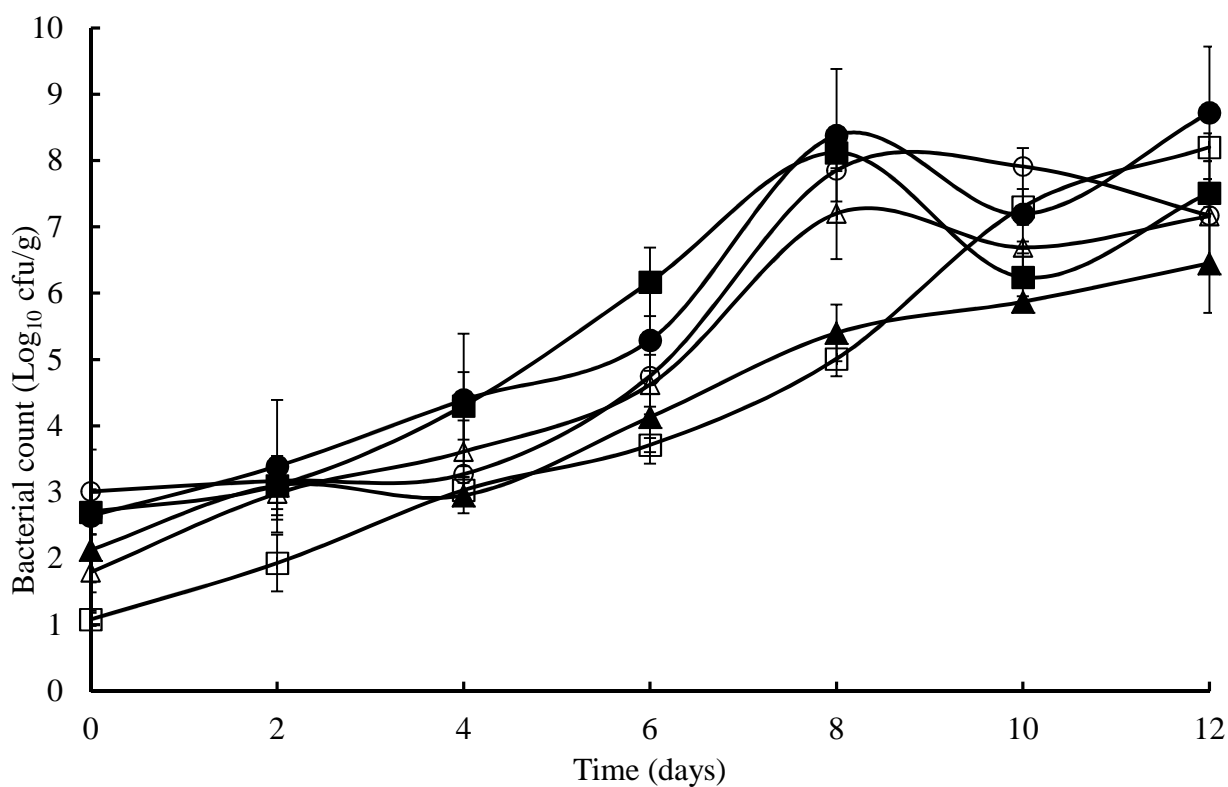


Figure 1. The total viable count (mesophilic) in white meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5% v/v lactic acid (▲), 5% v/v acetic acid (□), 5% v/v citric acid (○) and 5% w/v sodium chloride (△).

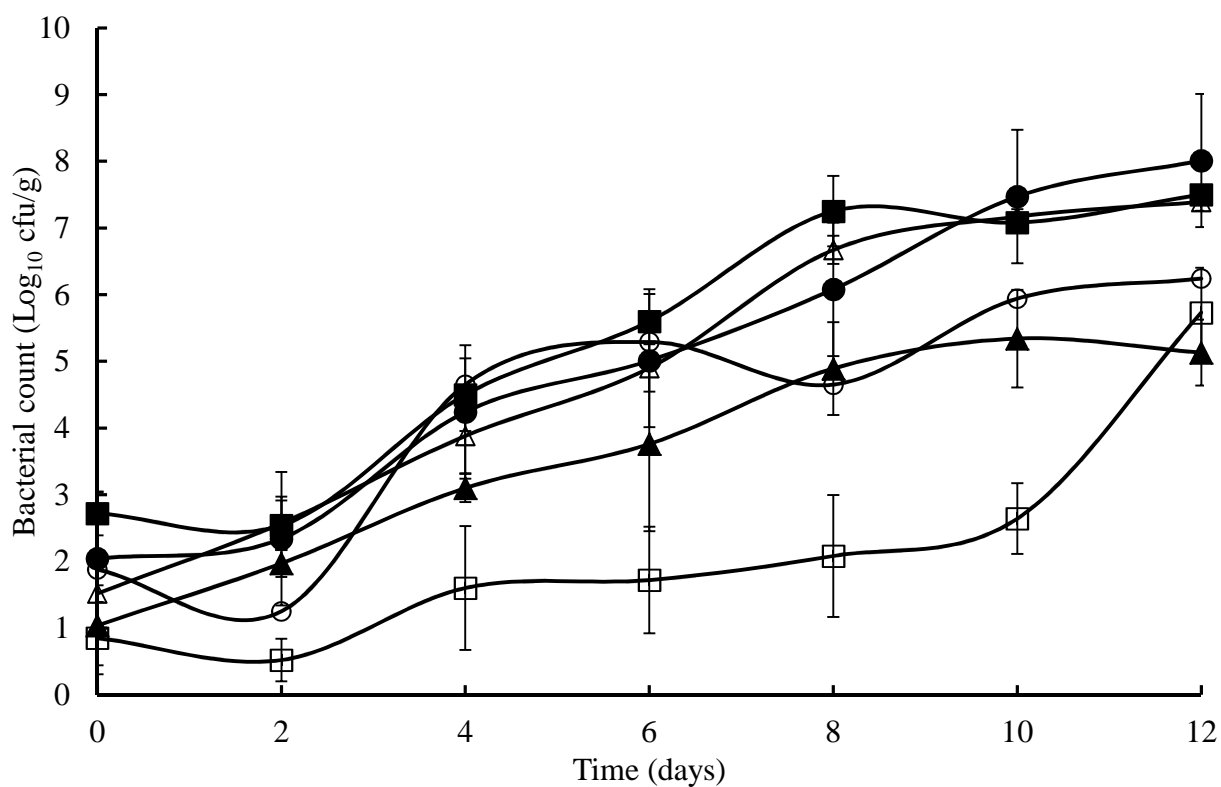


Figure 2. The total viable count (psychrophilic) in white meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (○) and 5%, w/v sodium chloride (△).

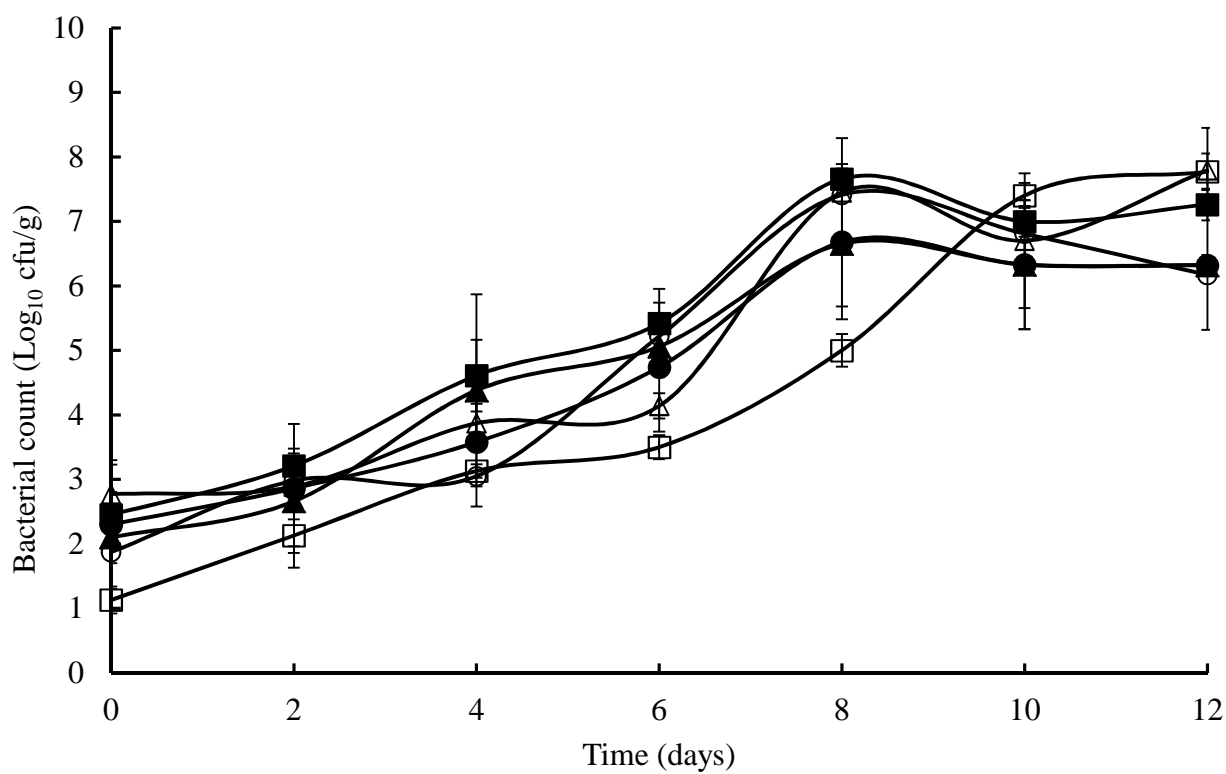


Figure 3. The total viable count (mesophilic) in brown meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (○) and 5%, w/v sodium chloride (Δ).

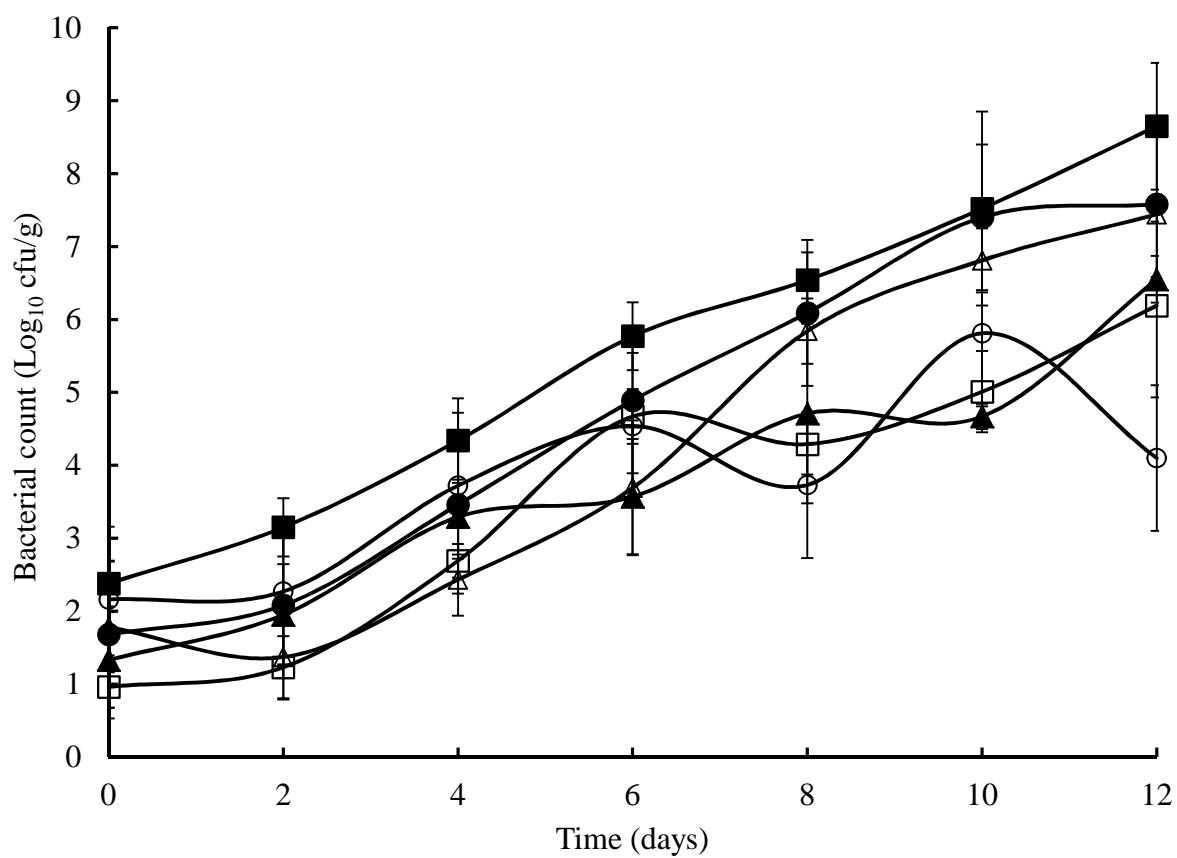


Figure 4. The total viable count (psychrophilic) in brown meat samples stored at 2°C with the following treatments; untreated (■), SDW (●), 5%, v/v lactic acid (▲), 5%, v/v acetic acid (□), 5%, v/v citric acid (○) and 5%, w/v sodium chloride (△).

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.