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Abstract

The aim of this study was to compare the effect of seawater to that of conventional salt (NaCl) on the technological properties of chicken emulsion sausages in a model system. Chicken sausages were prepared with seawater at three levels (10%, 15%, and 20%) in iced water (10%, 5%, and 0%, respectively) or with iced water (20%) and salt (1.2%). There was no difference in pH values and fat loss from emulsion stability between the two treatments. In general, with an increase in the amount of seawater, the water holding capacity (cooking yield and water loss), protein solubility (total and myofibrillar protein), and viscosity were increased. The addition of 20% seawater induced greater ($p < 0.05$) water holding capacity, protein solubility, and viscosity compared to the control sample treated with salt, which was accompanied by an increase in the level of myosin heavy chain (MHC) protein of samples with 10% and 20% seawater. Furthermore, addition of at least 15% seawater increased all of the main textural properties except for cohesiveness along with the moisture of sausage, whereas the fat and protein contents were decreased. Based on these results, the addition of $\geq 15\%$ seawater to chicken breast sausage can induce equivalent or enhanced technological properties to those induced with salt, including water holding capacity, protein solubility, viscosity, and textural properties.

Key words: seawater, chicken sausage, protein solubility, technological property

Introduction

Salt is a major ingredient in processed meat products owing to its functional capacities, including improving the emulsion stability, water holding capacity, textural properties, flavor, and shelf-life (Bowen et al., 1974; Seman et al., 1980; Watts, 1954). In particular, the protein extracted by salt in raw meat plays an important role in forming a stable gel matrix comprising insoluble fat and other components, resulting in a final product of high quality (Choe et al., 2017).

As an alternative source of salt, seawater has been utilized for the processing of various foods, including meat, for the curing of *Baechu* (Chinese cabbage), and in the formation of soybean curd (tofu), and has been shown to improve the quality characteristics (Ko et al, 2013; Ryu et al., 2014; Uttaro, 2011). Seawater contains sodium chloride (NaCl; about 80%), sulfates, and various minerals, including zinc, magnesium, calcium, and arsenic (Moon et al., 2004). The pH of seawater is largely dependent on the salt component and is generally around 8.3, which can improve the water holding capacity of meat products (Kim et al., 2016). As the number of negative ions increases as pH high, empty space creates water molecules to settle. However, to our knowledge, there has been no previous attempt at using seawater to improve the technological properties of emulsified meat products. Therefore, the objective of this study was to compare the effects of seawater and salt addition at different addition levels on the technological properties of a chicken sausage model system.

Materials and Methods

Preparation of Seawater and Sausage Samples

The seawater was provided by Yeorumul (Incheon, Korea), comprising 20% NaCl, 0.007% zinc, 3.3% sodium, 1.8% potassium, and 0.0004% arsenic. The chicken breast meat was obtained from a local butcher (Jungwoo-food, Pocheon, Korea). All of the subcutaneous and intramuscular fat, and visible connective tissues were removed from the chicken meat muscle. The meat was cut and ground using a grinder (PA-82, Mainca, Barcelona, Spain) equipped with 3 mm plates. The ground chicken meat (60%) was mixed with pork backfat (20%), iced water (20%), salt (1.2%), sugar (1%), and mixed spice (0.6%) in a bowl-cutter (K-30, Talsa, Valencia, Spain).

The samples were treated with seawater at three levels (10%, 15%, and 20%, respectively) in iced water (10%, 5%, and 0%, respectively) or with iced water and salt as the conventional

61 method. The control is manufactured by adding 1.2% of NaCl and 20% of Iced water, and the
62 treatments were treated with seawater at three levels (10%, 15%, and 20%, respectively) in iced
63 water (10%, 5%, and 0%, respectively). The meat emulsion samples were divided into two parts.
64 One portion was used for measurement of pH values, protein solubility, cooking yield, emulsion
65 stability, and viscosity, and the other port
66 ion was stuffed into hog casings (Hog casing, Woosing Foodtec, Seoul, Korea) using a stuffer
67 (EM-12, Mainca, Barcelona, Spain) and heated using a chamber (10.10ESI/SK, Alto Shaham,
68 Menomonee Falls, USA) at 85°C until the internal temperature of the samples reached 75°C. The
69 cooked sausage samples were subsequently cooled in iced water for 20 min, and then stored at
70 5°C until analysis (proximate composition, pH, and textural properties).

71

72 **Proximate Composition**

73 The proximate composition of each sample was analyzed as described by Choe and Kim (2019)
74 following standard AOAC (2012) methods.

75

76 **pH**

77 Each sample (4 g) was homogenized with 16 mL distilled water using a homogenizer (HMZ-
78 20DN, Pooglim Tech, Seongnam, Korea). The pH value of the homogenate was then measured
79 with a pH meter (Model S220, Mettler-Toledo International Inc., Greifensee, Switzerland).

80

81 **Cooking Yield**

82 Cooking yield was determined as the percent weight of each meat sample after cooking. The
83 samples were heated in a water bath at 85°C for 35 min until reaching an internal temperature of
84 72°C and then cooled in ice water. Cooking yield was determined using the following formula:

$$\text{Cooking yield (\%)} = \frac{\text{Weight after cooking (g)}}{\text{Weight before cooking (g)}} \times 100$$

85

86 **Emulsion Stability**

87 The manufactured emulsifiers were sealed a glass tube with the wire cut to 4×4 cm. After heating

88 the glass tube in the chamber (10.10ESI/SK, Alto Shaam, Menomonee Falls, WI, USA) for 35
89 min at 85°C, it was cooled at 10°C for 1 h. After cooling down completely, the amounts of water
90 and oil were measured, and the loss value was used to determine the emulsion stability according
91 to the following formula:

$$\text{Loss value (\%)} = \frac{\text{Water (or fat) exudation layer (mL)}}{\text{Weight before cooking (g)}} \times 100$$

93

94 **Protein Solubility**

95 Protein solubility (total, myofibrillar, and sarcoplasmic proteins) of the meat emulsion was
96 determined as described by Choe et al. (2017). In brief, for assessment of the total protein content,
97 2 g of the raw sample was added to 20 mL of 1.1 M potassium iodide and 0.1 M potassium
98 phosphate (pH 7.4). The sarcoplasmic protein was quantified by adding 2 g of the raw sample to
99 0.025 M potassium phosphate (pH 7.4). For both methods, the samples were homogenized for 2
100 min and incubated overnight at 2°C. The samples were then centrifuged at 6,000 rpm for 15 min
101 at 4°C and filtered using Whatman No. 1 filter paper to prepare for quantification. The absorbance
102 of the sample was measured at 540 nm to obtain the optical density (OD) value using the Spectra-
103 photometer (SpectraMax iD3, Molecular devices., Sanjose, USA), and the protein content in each
104 sample was determined by according to the following formula:

105 Total protein (mg/g)=OD value×upper diluent multiples×buffer dilution factor×a value (protein
106 concentrate)

107

108 **Apparent Viscosity**

109 The flow behavior and time dependence of the batter for each sample were investigated at 20±1°C
110 using a rotational viscometer (MerlinVR, Rheosys, Princeton, USA). The meat emulsion was
111 allowed to equilibrate for 5 min at room temperature (25±1°C), and the time dependency of the
112 meat emulsion viscosity was determined by measuring the apparent viscosity under a constant
113 shear rate of 20 rpm for 60 s.

114

115 **Texture Profile Analysis (TPA)**

116 To determine the texture profile of the cooked sausage samples, the samples were cut into cubes
117 of 2.5×2.5×2.0 cm (width×length×height) and placed in the Texture Analyzer system (TA 1,
118 Lloyd Co., Largo, FL, USA) equipped with a 25 mm cylinder probe. The analysis conditions
119 were set to a pre-test speed of 2.0 mm/s, post-test speed of 5.0 mm/s, head speed of 2.0 mm/s,
120 distance of 8.0 mm, and force of 0.4 N. The measured hardness (N), springiness, and
121 cohesiveness were recorded, and the gumminess (N) and chewiness (N) were calculated using
122 these values.

123

124 **SDS-PAGE**

125 Relevant protein levels were assessed with SDS-PAGE analysis using the method of Laemmli
126 (1993) using a 12% separating gel and 4% stacking gel. The supernatants of the samples were
127 mixed with 3 mM phosphate buffer and 5X sample buffer. Fifteen microliters of each sample was
128 added to each well of the gel and processed for 1 h and 20 min. The gel was removed and fixed
129 in a fixing solution, incubated overnight using a rocker, and stained with Coomassie brilliant blue
130 for 20 min with gentle agitation. The dye was removed with a destaining solution for 1 h, and the
131 gel was stored in a storage solution and scanned.

132

133 **Statistical Analysis**

134 All data were analyzed using one-way ANOVA in SAS (version 9.3), and the results are
135 expressed as mean values with standard deviations. Significant differences ($p < 0.05$) among the
136 mean values were determined using Duncan's multiple range test. For pH values, the differences
137 between raw and cooked samples between groups were tested with the independent samples t-
138 test. Correlation coefficients between variables (proximate composition, pH cooking yield,
139 emulsion stability, protein solubility, and TPA) were tested using the Pearson correlation
140 coefficient.

141

142

Results and Discussion

Proximate Composition and pH

The addition of seawater and the amount of seawater added affected the proximate composition of the emulsified sausage (Table 1). The moisture and fat contents were not significantly affected by the level or addition of seawater, but the crude protein and ash contents differed significantly according to the presence and the level of seawater addition. The seawater-treated groups showed significantly higher crude protein contents compared to those of the salt-treated control group. In addition, the ash content was significantly the highest in the group processed with 20% seawater. This could reflect the natural salt and ash content in the seawater.

The presence and level of seawater addition did not influence the pH in the samples either before or after cooking, but the cooking treatment significantly increased the pH ($p < 0.05$ or < 0.001) in all treatment groups (Table 1). This increase in pH from cooking is due to the non-reactive alkaline amino acids generated by the heat-induced denaturation of the proteins in the emulsion, thereby releasing large amounts of cations (Forrest et al., 1975).

Cooking Yield and Emulsion Stability

The presence and level of seawater addition clearly affected the cooking yield of the sausage (Table 2). The group treated with 10% seawater showed a lower cooking yield compared to that of the other seawater groups and the salt-treated control group ($p < 0.05$), whereas there was no significant difference in cooking yield between the control group and the 15% and 20% seawater groups. Therefore, the addition of 15% or more seawater resulted in similar retention ability as the addition of salt. Froning and Sackett (1984) reported that increasing the concentration of NaCl added to turkey breast muscle increased the protein solubility, which in turn increased the cooking yield, consistent with the present findings. In this study, as shown in Table 3, the protein solubility (total and myofibrillar protein) was increased ($p < 0.05$) as increasing the addition level of seawater and the group with over 15% seawater induced higher ($p < 0.05$) protein solubility compared to group with salt. However, the significant difference in protein solubility did not affect cooking yield ($p > 0.05$). It could be speculated that salt and seawater have the different

172 degree of myofibrillar protein swelling capacity affecting WHC of meat emulsion, depending on
173 content of chloride (Gordon and Barbut, 1992).

174 With respect to the emulsion stability, the addition of seawater affected the water loss of the
175 sausage, but it did not affect fat loss (Table 2). The group treated with 10% seawater showed the
176 highest water loss, whereas the group treated with 20% seawater showed the lowest water loss,
177 and both effects were statistically significant. This suggests that seawater has similar salt-soluble
178 protein extraction capacity, emulsion formation capacity, and protein-binding capacity to those
179 of salt (Kim et al., 2010). Kwak et al. (2010) reported that the salt-soluble protein derived from
180 anchovies could effectively separate fat and water during the production of emulsified sausage
181 to produce a stable emulsion, which would influence the salt-soluble protein measurement as
182 discussed further below in the next section. Surh et al. (2006) also reported that samples with
183 high emulsion stability showed minimal separation of water and fat, which allowed for the
184 production of a high-quality meat product.

185

186 **Protein Solubility**

187 Protein solubility is a key factor in sausage production for producing a high-quality product.
188 During the production of emulsified sausage, improvement in the protein solubility allows for
189 formation of a stable emulsion, which in turn enhances product characteristics such as moisture
190 retention power, yield, and texture (Aminlari et al., 2009; Marco et al., 2010). According to
191 Briskey and Sayre (1964), enhancing the content of salt-soluble proteins increases the binding
192 power during the production of meat products to ultimately improve the retention power and
193 texture. The group treated with 10% seawater showed similar total protein and salt-soluble
194 proteins to those of the control group (Table 3). However, groups treated with 15% or 20%
195 seawater showed higher total protein and salt-soluble proteins. This difference in total protein
196 solubility may be related to the difference in the salt-soluble proteins between treatment groups
197 (Table 3; $R^2=0.72$).

198 Xiong et al. (2010) found that the addition of increased concentrations of NaCl also increased
199 the content of water-soluble proteins. Kim et al. (2003) reported that the addition of NaCl in DFD
200 meat increased the total protein solubility, which improved the quality of the meat product.
201 Therefore, addition of more than 15% seawater could improve the quality of the sausage.

202 **Viscosity and TPA**

203 The changes in apparent viscosity according to the presence and the level of seawater addition
204 are shown in Fig. 1. The groups treated with 15% and 20% seawater showed significantly higher
205 viscosity of 102.91 Pa·s and 128.34 Pa·s, respectively, at 60 s compared to that of the control
206 group and the 10% seawater group (96.71 Pa·s and 101.33 Pa·s, respectively). In general, the
207 control group showed the lowest viscosity values, and an increasing trend with increased
208 seawater addition was observed. This suggests that the increased salt from seawater acted on the
209 myofilaments of the meat protein to extract salt-soluble proteins, which further stabilized the
210 water, protein, and fat (Park and Kim, 2016).

211 Excluding cohesiveness, the other physical properties (hardness, springiness, gumminess, and
212 chewiness) of the control group were significantly lower than those of the seawater treatment
213 groups (Table 4). Hardness, gumminess, and chewiness values were the lowest in the control
214 group and the highest in the 20% seawater group. Samples treated with 10% and 15% seawater
215 showed the lowest levels of cohesiveness ($p<0.05$), and samples treated with 20% seawater had
216 the highest cohesiveness values ($p<0.05$).

217 Moreover, the solubility of total, water-soluble, and salt-soluble proteins was closely correlated
218 to the physical characteristics of the sausage. The solubility of total protein and salt-soluble
219 protein was significantly correlated with hardness ($R^2=0.83$ and $R^2=0.56$, respectively),
220 springiness ($R^2=0.99$ and $R^2=0.25$), cohesiveness ($R^2=0.30$ and $R^2=0.84$), gumminess ($R^2=0.77$
221 and $R^2=0.77$), and chewiness ($R^2=0.94$ and $R^2=0.62$), which increased with addition of seawater,
222 leading to formation of a stable emulsion. Fat loss and hardness were negatively correlated,
223 which suggests that an increased fat content leads to reduced hardness (Table 5).

224

225 **SDS-PAGE**

226 Myosin and actin are important proteins that contribute to the main functional properties of
227 myofibrillar protein (Chan et al., 2011). Furthermore, myosin heavy chain (MHC) and
228 tropomyosin in a protein-based gel or emulsion are associated with the gel-forming capacity of
229 comminuted meat products (Kamath et al., 1992; Benjakul et al., 2004). The addition of seawater
230 and the seawater level induced different protein patterns (Fig. 2). The band of MHC appeared in
231 the 15% and 20% seawater-treated samples, whereas no such band appeared in the control and

232 10% seawater samples. This difference can explain the improvement of viscosity, protein
233 solubility (total and myofibrillar protein), and some of the textural properties with seawater
234 addition. C-protein, which is affected by actinidin and a component of myosin filaments
235 (Aminlari et al., 2009; Asghar et al., 1985), was much more strongly expressed in the 15% and
236 20% seawater samples compared to the control and 10% seawater samples. There was no
237 difference in the extent of actin expression among the treatments. In general, tropomyosin, which
238 is related with protein solubility (Grossi et al., 2016), was not affected by the addition of seawater.
239 However, for the upper band of tropomyosin, the control and 10% seawater groups showed a
240 slightly stronger band density compared with those in the 15% and 20% seawater groups.

241

242 **Conclusions**

243 The results of this study suggest that seawater can be used as a source of salt in emulsion-type
244 meat products when added at $\geq 15\%$ without an adverse effect. Moreover, the addition of 20%
245 seawater can enhance the technological properties of chicken emulsion sausages, including the
246 total and myofibrillar protein solubility, viscosity, and textural properties compared to the
247 conventional use of salt. Furthermore, seawater with chicken meat would probably result in better
248 than those that use salt.

249

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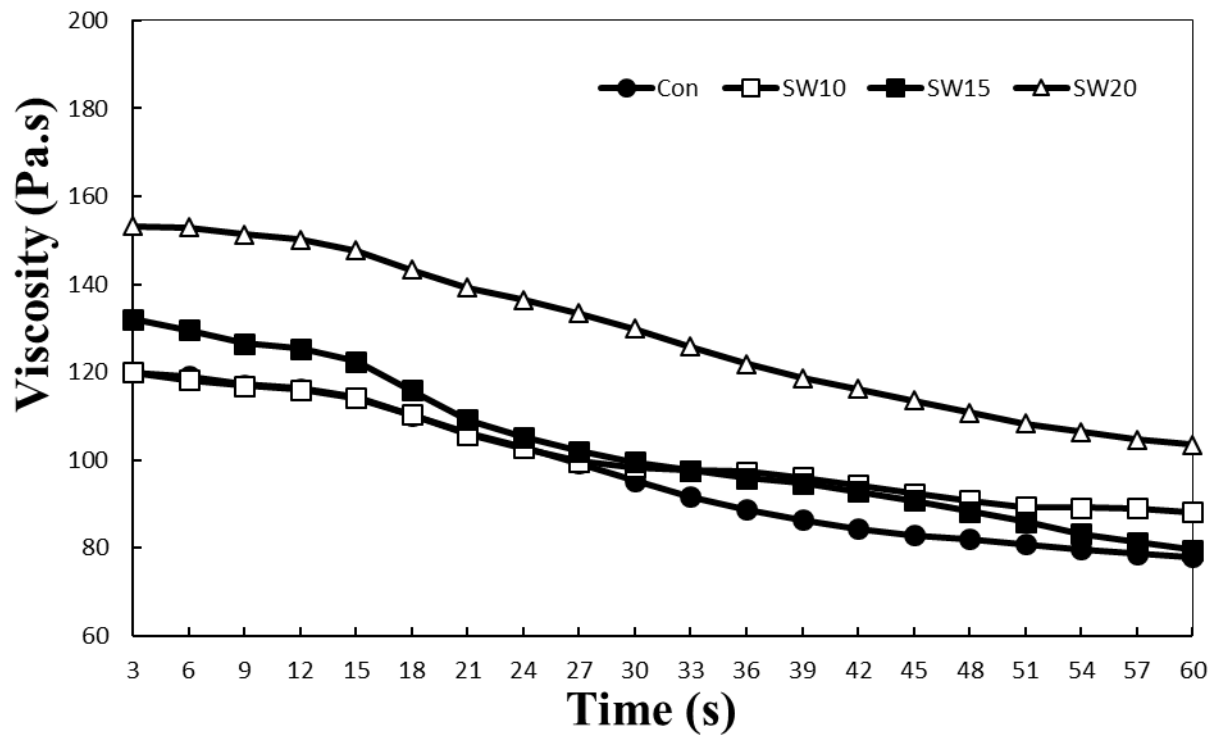
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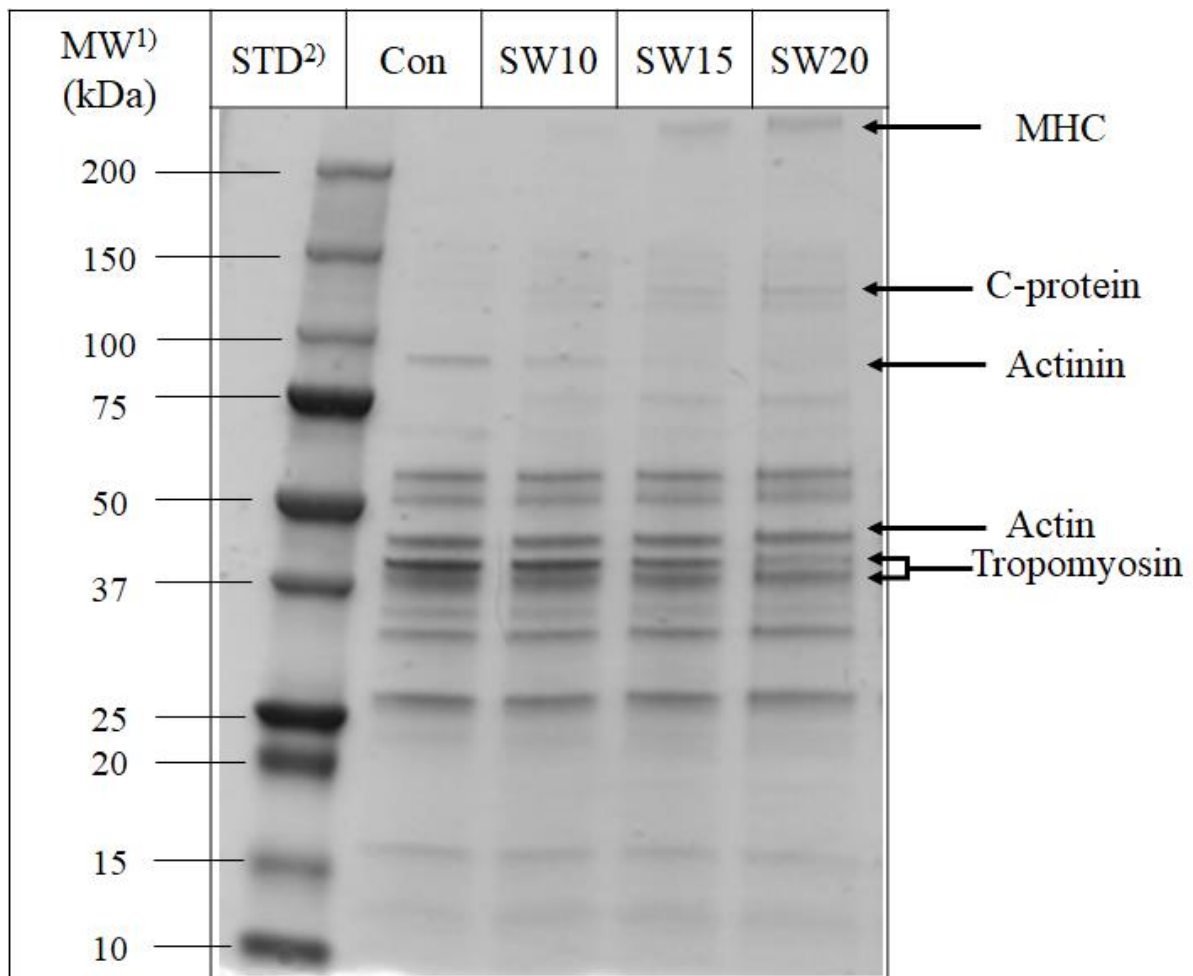
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323



324
 325 **Fig. 1. Change in the apparent viscosity on chicken-breast sausage formulated with various**
 326 **levels of seawater. Con, sausage with 1.2% NaCl; SW10, sausage with 10% seawater; SW15,**
 327 **sausage with 15% seawater; SW20, sausage with 20% seawater.**



328
 329 **Fig. 2. SDS-PAGE gel and comparative band (pixel) intensity from chicken-breast sausage**
 330 **samples formulated with various levels of seawater.** Con, sausage with 1.2% NaCl; SW10,
 331 sausage with 10% seawater; SW15, sausage with 15% seawater; SW20, sausage with 20%
 332 seawater. ¹⁾MW, molecular weight; ²⁾ STD, standard marker.

333

334 **Table 1. Proximate composition and pH of chicken-breast sausage formulated with various**
 335 **levels of seawater or salt**

Traits	NaCl (Con)	SW10	SW15	SW20	
Moisture	58.75 ± 0.71	54.14 ± 1.16	56.63 ± 1.08	57.92 ± 1.46	
Protein	19.29 ± 1.59 ^b	22.49 ± 0.23 ^a	22.92 ± 1.00 ^a	23.37 ± 1.20 ^a	
Fat	21.25 ± 0.18	23.69 ± 0.66	20.63 ± 1.81	19.31 ± 1.21	
Ash	2.22 ± 0.18 ^b	2.34 ± 0.15 ^b	2.96 ± 0.32 ^{ab}	3.40 ± 0.27 ^a	
pH	Uncooked	5.52 ± 0.49 ^{**}	5.58 ± 0.03 ^{***}	5.64 ± 0.02 ^{***}	5.63 ± 0.01 ^{***}
	Cooked	6.04 ± 0.01 ^{**}	5.73 ± 0.05 ^{***}	5.78 ± 0.01 ^{***}	5.78 ± 0.01 ^{***}

336 All values are mean ± SD.

337 Con, sausage with 1.2% NaCl and 20% iced water; SW10, sausage with 10% seawater and 10% iced water; SW15, sausage with
 338 15% seawater and 5% iced water; SW20, sausages with 20% seawater.

339 ^{a-b} Means in the same row with different letters are significantly different (p<0.05).

340 ^{**} p<0.01, ^{***} p<0.001.

341

342 **Table 2. Cooking yield and emulsion stability of chicken-breast sausage formulated with**
 343 **various levels of seawater or salt**

Traits		NaCl (Con)	SW10	SW15	SW20
Cooking yield		79.02 ± 0.03 ^a	74.46 ± 0.77 ^b	79.90 ± 1.00 ^a	81.36 ± 0.48 ^a
Emulsion stability	Water loss	24.05 ± 0.15 ^{ab}	26.51 ± 0.78 ^a	25.65 ± 1.07 ^{ab}	23.62 ± 0.30 ^b
	Fat loss	1.46 ± 0.03	1.37 ± 0.01	1.10 ± 0.03	0.38 ± 0.05

344 All values are mean ± SD.

345 Con, sausage with 1.2% NaCl and 20% iced water; SW10, sausage with 10% seawater and 10% iced water; SW15, sausage with
 346 15% seawater and 5% iced water; SW20, sausages with 20% seawater.

347 ^{a-c} Means in the same row with different letters are significantly different (p<0.05).

348

349 **Table 3. Protein solubility of chicken-breast sausage formulated with various levels of**
 350 **seawater or salt**

Traits	NaCl (Con)	SW10	SW15	SW20
Total protein solubility	1451.38 ± 0.06 ^b	1816.35 ± 0.08 ^{ab}	1947.34 ± 0.03 ^a	2005.18 ± 0.18 ^a
Sarcoplasmic protein solubility	279.13 ± 1.11	237.73 ± 0.79	243.36 ± 0.67	231.1 ± 0.40
Myofibrillar protein solubility	1172.24 ± 0.23 ^b	1578.61 ± 0.22 ^b	1703.97 ± 0.38 ^a	1774.08 ± 0.27 ^a

351 All values are mean ± SD

352 Con, sausage with 1.2% NaCl and 20% iced water; SW10, sausage with 10% seawater and 10% iced water; SW15, sausage with
 353 15% seawater and 5% iced water; SW20, sausages with 20% seawater

354 ^{a-b} Means in the same row with different letters are significantly different (p<0.05).

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356

357 **Table 4. Texture properties of chicken-breast sausage formulated with various levels of**
 358 **seawater or salt**

Traits	NaCl (Con)	10	15	20
Hardness (N)	12.65 ± 0.18 ^c	25.10 ± 0.12 ^b	22.65 ± 0.12 ^b	30.10 ± 0.15 ^a
Springiness	0.86 ± 0.03 ^b	0.90 ± 0.03 ^{ab}	0.83 ± 0.03 ^a	0.86 ± 0.03 ^{ab}
Cohesiveness	0.32 ± 0.02 ^{ab}	0.34 ± 0.03 ^c	0.30 ± 0.04 ^c	0.40 ± 0.03 ^a
Gumminess (N)	4.99 ± 0.02 ^c	8.65 ± 0.11 ^b	6.87 ± 0.02 ^b	12.12 ± 0.03 ^a
Chewiness (N)	3.40 ± 0.05 ^c	7.81 ± 0.07 ^b	6.22 ± 0.01 ^b	10.03 ± 0.02 ^a

359 All values are mean ± SD.

360 Con, sausage with 1.2% NaCl and 20% iced water; SW10, sausage with 10% seawater and 10% iced water; SW15, sausage with
 361 15% seawater and 5% iced water; SW20, sausages with 20% seawater.

362 ^{a-c} Means in the same row with different letters are significantly different (p<0.05).

Table 5. Pearson correlation between the various technological parameters of chicken emulsion sausages in a model system

	Water	Protein	Fat	Ash	pH	Cooking yield	Water loss	Fat loss	TP	SP	MP	Hardness	Springiness	Gumminess	Chewiness	Cohesiveness
Water		0.95**	0.12	0.30	0.97**	0.91**	0.97**	0.23	0.35	0.21	0.96**	0.37	0.47	0.38	0.21	0.93**
Protein			0.64	0.12	0.99** *	0.17	0.94**	0.58	0.28	0.26	0.20	0.85	0.39	0.44	0.33	0.25
Fat				0.85	0.71	0.44	0.36	0.68	0.19	0.19	0.23	0.94**	0.95**	0.37	0.39	0.28
Ash					0.82	0.26	0.62	0.35	0.79	0.69	0.19	0.76	0.91**	0.90**	0.35	0.36
pH						0.74	0.26	0.31	0.25	0.43	0.68	0.94**	0.05	0.36	0.40	0.34
Cooking yield							0.74	0.07	0.30	0.17	0.25	0.95**	0.85	1.00** *	0.26	0.98**
Water loss								0.80	0.23	0.21	0.14	0.78	0.76	0.92**	0.88	0.22
Fat loss									0.74	0.16	0.21	0.29	0.94**	0.88	0.84	0.71
TP										0.20	0.56	0.83	0.99	0.77	0.94*	0.30
SP											0.90**	0.20	0.74	0.68	0.79	0.91**
MP												0.56	0.25	0.84	0.77	0.62
Hardness													0.94	0.09	0.35	0.38
Springiness														0.90	0.99*	0.38
Gumminess															0.75	0.15

Chewiness

0.86

Cohesiveness

* p<0.05; ** p<0.01; *** p<0.001.

¹⁾ TP, Total protein; ²⁾ SP, Sarcoplasmic protein; ³⁾ MP, Myofibrillar protein