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## Abstract

This study investigated the impacts of gelatin hydrolysate addition on the technological properties and lipid oxidation stability of cooked sausage. Gelatin hydrolysate was prepared from pork and duck skin gelatin, through stepwise hydrolysis using collagenase and pepsin. The cooked sausages were formulated without gelatin (control) or with 1% pork skin gelatin, 1% duck skin gelatin, 1% pork skin gelatin hydrolysate, and 1% duck skin gelatin hydrolysate. The pH, color characteristics, protein solubility, cooking loss, and textural properties were evaluated, and the 2-thiobarbituric acid reactive substances (TBARS) value was measured weekly to determine lipid oxidation stability during 4 wk of refrigerated storage. Enzymatic hydrolysis of gelatin decreased protein content and CIE L\* but increased redness and yellowness ( $p < 0.05$ ). When 1% gelatin or gelatin hydrolysate was incorporated in cooked sausage, however, little to no impacts on pH value, moisture content, protein content, color characteristics, protein solubility, and cooking loss were found ( $p > 0.05$ ). The addition of 1% duck skin gelatin hydrolysate increased the cohesiveness and chewiness of cooked sausages. The inclusion of 1% duck skin gelatin accelerated lipid oxidation of cooked sausages during refrigerated storage ( $p < 0.05$ ), whereas duck skin gelatin hydrolysate caused a lower TBARS value in cooked sausage compared to duck skin gelatin. The results show comparable effects of gelatin and gelatin hydrolysate addition on the technological properties of cooked sausages; however, the oxidative stability of raw materials for gelatin extraction should be evaluated clearly in further studies.

**Keywords:** antioxidant peptide, collagenase, duck skin, enzymatic hydrolysis, pepsin

## 34 **Introduction**

35 In the modern food industry, much effort to meet consumer demand for well-being  
36 trends has been made by replacing artificial food additives with natural ingredients. This  
37 trend has now evolved into the concept of “clean label products” that are formulated with  
38 more natural ingredients and less processed (Aschemann-Witzel et al., 2019). To  
39 manufacture “clean label products”, multifunctional food additives have been considered  
40 extensively to minimize the use of artificial food additives.

41 Gelatin, which is obtained through the thermal hydrolysis of collagen in animal  
42 tissues, has recently been used to produce bioactive hydrolysates or peptides (Lafarga et  
43 al., 2017; Sarbon et al., 2018). Previous studies primarily used mammalian, poultry, and  
44 fish skin as gelatin sources, and several physiological benefits of gelatin hydrolysate,  
45 including antimicrobial, antioxidant, or antihypertensive activities have been found  
46 through *in vitro* and *in vivo* assays (Gómez-Guillén et al., 2011). Moreover, gelatin  
47 hydrolysates with antioxidative and/or antimicrobial activities could be used to extend  
48 the shelf stability of processed foods, resulting from the retardation of oxidative quality  
49 change and the inhibition of harmful microbial growth, respectively (Nikoo et al., 2015;  
50 Zhang et al., 2020).

51 In commercial restructured and/or emulsified meat products, gelatin (generally from  
52 0.5 to 3.0 g/100 g) is used to improve water-holding capacity and textural properties (Lee  
53 and Chin, 2016). Moreover, it has been well documented that the positive functionality  
54 of gelatin in processed meat products results from mainly hydration properties and gel-  
55 forming ability of gelatin (Gómez-Guillén et al., 2011). Beyond the positive functionality,  
56 however, there is no available literature on the impact of gelatin on the oxidative stability  
57 of processed meat products during storage.

58 Commercial gelatin products used in the meat industry is mostly produced from  
59 pork skin and bovine hide (Karim and Bhat, 2009), but gelatin extraction materials have  
60 been diversified now into poultry and fishery sources due to the risk of infectious diseases  
61 and religious reasons (Tümerkan et al., 2019). In particular, poultry by-products such as  
62 feet and skins have been considered as promising materials for gelatin extraction (Noh et  
63 al., 2019; Park et al., 2013). However, although some previous studies have found the  
64 techno-functional advantages of duck skin gelatin in processed meat products (Kim et al.,  
65 2020), the efficiencies of pork and duck skin gelatin on the quality attributes of processed  
66 meat products have not been clearly compared.

67 In this preliminary study, the pork and duck skin gelatin hydrolysates were prepared  
68 through an enzymatic hydrolysis method that produced gelatin hydrolysates with  
69 antioxidant capacity (Lee et al., 2012), and the impacts of gelatin and its hydrolysate on  
70 technological properties and lipid oxidation stability of cooked sausage have been  
71 investigated.

72

## 73 **Materials and methods**

### 74 ***Raw materials***

75 Commercial pork skin gelatin (gel strength: 280 bloom, particle size: 5-15 mesh,  
76 Hangzhou Qunli Gelatin Chemical Co. Ltd., Hangzhou, China) was purchased from a  
77 local market, and frozen duck skin used for gelatin extraction was also provided by a duck  
78 processing company (Farm Duck Co., Jeongeup, Korea). Pork ham and back fat were  
79 purchased from a local market after 48 h postmortem.

80

### 81 ***Gelatin extraction from duck skin***

82 Duck skin gelatin was prepared according to a previously described extraction  
83 procedure (Kim et al., 2016; Tümerkan et al., 2019) with minor modifications. The frozen  
84 duck skin was thawed in a 4°C refrigerator for 24 h. The excessive subcutaneous fat was  
85 removed, and the duck skin was washed with tap water several times. The washed duck  
86 skin was ground using a meat grinder (MN-22S, Hankook Fugee Industries Co., Ltd.,  
87 Hwaseong, South Korea) equipped with an 8-mm plate. The ground duck skin was soaked  
88 in 1.5 volumes of 0.1 M NaOH for 16 h to remove non-collagenous proteins and  
89 neutralized with tap water (approximately pH 7.0). Acidic soaking of the duck skin was  
90 performed with 2 volumes of 0.5 M acetic acid for 12 h, and the swelled duck skin was  
91 neutralized again using tap water to pH 6.5–7.0. For hot-water extraction, the duck skin  
92 was blended with distilled water (1:1 ratio, w/w) and heated in a 65°C water bath for 3 h.  
93 After heating, the sample was filtrated through cheesecloth, and the gelatin solution was  
94 set in a 4°C refrigerator for 12 h. The upper fat layer was removed manually, and the  
95 gelatin layer was freeze-dried and pulverized for producing duck skin gelatin powder.

96

### 97 ***Enzymatic hydrolysis of gelatin***

98 Gelatin hydrolysates were prepared through stepwise enzymatic hydrolysis using  
99 collagenase and pepsin, as described by Lee et al. (2012) with minor modifications. Six  
100 grams of pork or duck skin gelatin powder were dissolved in 540 mL of distilled water  
101 and gently homogenized, and a low concentration of gelatin solution was used to prevent  
102 gelation and coagulation. The gelatin solution was heated at 80°C for 10 min to inactivate  
103 any enzymes contained in the gelatin powders and cooled at room temperature for 2 h.  
104 The pH of the gelatin solution was adjusted to pH 7.0 using 1 M NaOH and finally diluted  
105 to 600 mL in a volumetric flask to form 1% gelatin concentration (w/v). The first

106 enzymatic proteolysis of gelatin was performed with collagenase (EC3.4.24.3) at a 1:100  
107 ratio (w/w), and the enzymatic reaction was placed in a 37°C incubator for 12 h with  
108 stirring at 250 rpm. The mixture was heated at 80°C for 10 min and cooled to inactivate  
109 the collagenase. The pH of the mixture was adjusted to pH 2.0 using 6 N HCl for the  
110 second enzymatic hydrolysis, and the mixture was treated with pepsin (EC3.4.23.1) at a  
111 1:50 ratio (w/w). The second step was conducted under the same hydrolysis condition as  
112 above, and the product was heated at 80°C for 10 min and cooled to inactivate the pepsin.  
113 The pH of gelatin hydrolysates was finally adjusted to pH 5.7 using 1 M NaOH and then  
114 centrifuged at 20,000×g for 10 min (4°C). The supernatant was freeze-dried and  
115 pulverized to obtain gelatin hydrolysate powder.

#### 117 ***Manufacture of cooked sausage***

118 The excessive subcutaneous fat and connective tissues on the surface were removed.  
119 The pork ham and back fat were ground using a meat grinder with an 8 mm plate (MN-  
120 22S, Hankook Fugee Industries Co., Ltd., Hwaseong, Korea). The ground pork, ground  
121 back fat, ice, and other ingredients were emulsified using a bowl cutter (Cutter C4W,  
122 Sirman, Marsango, Italy). All treatments were formulated with 60% (w/w) ground pork,  
123 20% (w/w) ground pork back fat, and 20% (w/w) ice. Based on the total sample weight,  
124 1.5% (w/w) NaCl, 0.3% (w/w) sodium tripolyphosphate, and 1% (w/w) gelatin or gelatin  
125 hydrolysates were added as follows: control (without gelatin), 1% (w/w) pork skin gelatin,  
126 1% (w/w) pork skin gelatin hydrolysate, 1% (w/w) duck skin gelatin, and 1% (w/w) duck  
127 skin gelatin hydrolysate, respectively. The emulsified meat batter was stuffed into a  
128 collagen casing (diameter of 25 mm, #240, NIPPI Inc., Tokyo, Japan), cooked in an 80°C  
129 water bath until the core temperature reached 75 °C, and then, cooled in ice-water. The

130 cooked sausages were placed at room temperature for 3 h to evaporate surface moisture.  
131 To determine lipid oxidation stability, the cooked sausages were vacuum-packaged and  
132 stored in a 4°C refrigerator for 4 wk. A total of three independent batches was prepared.

133

## 134 ***Physicochemical analysis***

### 135 *1. Chemical composition*

136 The pH of the gelatin and hydrolysate powder (1% solution, w/v) was measured three  
137 times using an electronic pH-meter (Orion Star™ A211 pH Benchtop Meter, Thermo  
138 Scientific, USA). For meat batter and cooked sausage, the homogenate, which was  
139 prepared with 3 g of sample and 27 mL of distilled water, was used for pH analysis.

140 The moisture content of the cooked sausage was determined using the oven air-  
141 drying method (AOAC, 2007). The protein content of gelatin powder and cooked sausage  
142 was measured using a nitrogen protein analyzer (Rapid N Cube, Elementar,  
143 Langenselbold, Germany) and calculated using nitrogen-protein conversion factors of  
144 5.55 and 6.25, respectively (Mariotti, 2008).

145

### 146 *2. Color characteristics*

147 The color characteristics of gelatin powder and cooked sausage were measured using  
148 a colorimeter (CR-400, Konica Minolta Sensing, Inc., Osaka, Japan) with an 8 mm  
149 aperture and 2° observer. The setting for the illuminant was a D<sub>65</sub> source. Calibration of  
150 the instrument was conducted with a calibration tile (CIE L\*: +93.01, CIE a\*: -0.25, CIE  
151 b\*: +3.50), according to the manufacturer's manual. The CIE L\*, a\*, and b\* values were  
152 taken five times on the cross section of each sample (internal color).

153



154        *3. Protein solubility*

155        The protein solubility of the meat batter was measured using the method described  
156 by Warner et al. (1997). Two grams of sample were homogenized with a buffer solution  
157 (1.1 M potassium iodide in 0.1 M potassium phosphate buffer, pH 7.2) using a  
158 homogenizer (HG-15A, Daihan Sci., Seoul, Korea) at 12,000 rpm for 2 min. The  
159 homogenate was stored in a 2°C refrigerator overnight and centrifuged at 1,500 × g for  
160 20 min (4°C). The supernatant was filtered through a filter paper (Whatman no. 1), and  
161 the protein concentration of the filtrate was quantified using the Biuret method (Gornall  
162 et al., 1949). Protein solubility was expressed in mg protein soluble fraction per gram of  
163 emulsified meat batter (mg/g).

164  
165        *4. Cooking loss*

166        The cooking loss of cooked sausage was determined by the percent weight difference  
167 between the raw and cooked samples (Kim et al., 2015).

168  
169        *5. Texture profile analysis*

170        Texture profile analysis of cooked sausage was conducted using a texture analyzer  
171 (CT3, Brookfield Engineering Laboratories, INC. Middleboro, MA). The sausages were  
172 equilibrated to room temperature (22°C) for 3 h, and four samples (2.5 cm height) were  
173 prepared from the middle portion of each sample. A twice compression cycle test (70%  
174 compression of the original sample height) was performed with a cylinder probe  
175 (diameter in 4 cm). Sample deformation curves were obtained with a 50-kg maximum  
176 load cell, and the analysis condition was as follows: pre-test speed 1.0 mm/s, post-test

177 speed 5.0 mm/s, and head speed 2.0 mm/s. The values for hardness (kg), springiness  
178 (ratio), cohesiveness, gumminess (kg), and chewiness (kg) were presented (Bourne, 1978).

179

## 180 *6. Lipid oxidation*

181 Lipid oxidation of the cooked sausage was determined weekly using the 2-  
182 thiobarbituric acid reactive substance (TBARS) method of Buege and Aust (1978). The  
183 results were expressed as malondialdehyde mg/kg sample per kg of sample (mg MDA/kg  
184 sample).

185

## 186 *Statistical analysis*

187 The experimental design was a completely randomized block design with three  
188 independent batches. An analysis of variance (ANOVA) was performed on the measured  
189 variables using the one-way ANOVA procedure of the SPSS program (SPSS Inc.,  
190 Chicago, IL, USA). Duncan's multiple range test was used to determine significant  
191 differences between means ( $p < 0.05$ ). For lipid oxidation, a two-way ANOVA was  
192 conducted, in which treatment and storage effect as the main effects, and their interaction  
193 were found.

194

## 195 **Results and discussion**

### 196 *Physicochemical properties of gelatins and gelatin hydrolysates*

197 The protein content, pH, and color characteristics of pork and duck skin gelatin and  
198 their hydrolysates are shown in Table 1. The protein content of pork skin gelatin was  
199 93.54 g/100 g, whereas duck skin gelatin showed a considerably lower protein content  
200 (78.71 g/100 g) ( $p < 0.05$ ). This result was likely that the commercial pork skin gelatin

201 product was industrially purified through filtration and deionization processes. After  
202 stepwise enzymatic hydrolysis using collagenase and pepsin, the hydrolysates of pork and  
203 duck skin gelatins showed similar protein contents of 76.77 and 72.17 g/100 g,  
204 respectively ( $p>0.05$ ). Previously, Kim et al. (2013) reported that the protein content of  
205 pork skin gelatin hydrolysate prepared through 0.3% flavourzyme treatment was 511.53  
206 mg/g dry weight.

207 The pH value (4.50) of pork skin gelatin was significantly lower than that of duck  
208 skin gelatin (6.17); however, their hydrolysates showed similar pH values ( $p>0.05$ ) since  
209 the final pH of gelatin hydrolysates were equally adjusted at the end of enzymatic  
210 hydrolysis process. According to a previous study, the final pH of gelatin is mainly  
211 affected by the acid/alkali treatment and neutralization in the gelatin extraction process  
212 (Kim et al., 2012), and the general pH after neutralization is about 5.0–7.0 (Noh et  
213 al., 2019). Thus, in this study, the pH difference between pork and duck skin gelatin could  
214 be associated with different manufacturing processes.

215 In terms of color characteristics, duck skin gelatin showed higher CIE L\* but lower  
216 CIE b\* (yellowness) than pork skin gelatin ( $p<0.05$ ); there was no significant difference  
217 in redness between the two different gelatin sources. Stepwise enzymatic hydrolysis  
218 decreased lightness of both gelatin sources but increased redness ( $p<0.05$ ). After  
219 enzymatic hydrolysis, the yellowness of duck skin gelatin significantly increased, but no  
220 change in yellowness was observed between pork skin gelatin and its hydrolysate.  
221 Between pork and duck skin gelatin hydrolysates, similar lightness and redness were  
222 observed, but duck skin gelatin hydrolysate showed a slightly higher yellowness than  
223 pork skin gelatin hydrolysate ( $p<0.05$ ).

224 Previously, Chuaychan et al. (2016) suggested that an increase in temperature during  
225 the drying process could increase the yellowness of gelatin hydrolysate powder, resulting  
226 from a Maillard reaction between reducing sugars and amino acids. In this study, since  
227 the duck skin gelatin hydrolysate powder was prepared through a lyophilization process,  
228 the increased yellowness was probably due to the heating process (80°C) for the  
229 inactivation of proteases during enzymatic proteolysis. In general, commercial gelatin  
230 generally presents a white color; however, the color characteristics of gelatin are not  
231 related to functional properties such as gel-forming ability, gel strength, and emulsifying  
232 capacity (Ockerman and Hansen, 1988). Therefore, the increased yellowness of gelatin  
233 hydrolysates may have little to no impact on the functional properties of gelatin  
234 hydrolysates.

235

236 ***Chemical composition and color of cooked sausages with pork skin gelatin, duck skin***  
237 ***gelatin, and their hydrolysates***

238 The chemical composition and color characteristics of cooked sausages prepared  
239 with gelatin or gelatin hydrolysates are shown in Table 2. The pH values of meat batter  
240 and cooked sausages were unaffected by the incorporation of gelatin or gelatin  
241 hydrolysate ( $p=0.174$ ). Although there was significant difference in pH between pork and  
242 duck skin gelatin and gelatin hydrolysates (Table 1), it seemed that the inclusion level  
243 might be too small to change the pH of meat batter and cooked sausages. The addition of  
244 gelatin and gelatin hydrolysates had no significant effect on the moisture and protein  
245 content of the cooked sausage. The moisture content of the control and all treatments  
246 ranged from 61.19 to 62.41 g/100 g, and the protein content was from 16.36 to 19.89  
247 g/100 g. As a similar result, it has been reported that the addition of 1% gelatin slightly

248 increased the protein content of cooked sausage, whereas the moisture content was  
249 relatively decreased (Lee and Chin, 2016).

250 There was no change in the lightness and yellowness of the cooked sausages when  
251 1% gelatin or gelatin hydrolysate were added ( $p>0.05$ ). While the addition of gelatin and  
252 gelatin hydrolysate decreased the redness of the cooked sausage ( $p<0.05$ ); however, the  
253 difference between the control and treatment was numerically too small. As a similar  
254 result, Lee and Chin (2016) reported that the addition of 0.5%, 1.0%, and 1.5% pork skin  
255 gelatin had no impacts on lightness, redness, and yellowness of pork sausages. Our results  
256 indicate that the color changes of gelatin hydrolysate due to enzymatic hydrolysis may  
257 have no impacts on color characteristics of cooked sausages and suggest that this  
258 phenomenon could be affected by the inclusion level of gelatin and gelatin hydrolysate.

259

260 ***Protein solubility and cooking loss of cooked sausages with pork skin gelatin, duck skin***  
261 ***gelatin, and their hydrolysates***

262 The addition of 1% gelatin and gelatin hydrolysate caused an increasing trend in  
263 protein solubility of the meat batters ( $p=0.080$ ; Table 3). This was probably related to the  
264 increased protein content with adding gelatin or gelatin hydrolysates. In terms of cooking  
265 loss, a decreasing trend was found due to the addition of 1% gelatin and gelatin  
266 hydrolysates ( $p=0.081$ ). Previously, Lee and Chin (2016) reported that the addition of 1%  
267 pork skin gelatin had little effect on the water loss of cooked sausage. Recently, Noh et  
268 al. (2019) reported that the positive impacts of gelatin on the water-holding capacity of  
269 processed meat products could be associated with the functional properties rather than the  
270 interaction between myofibrillar proteins and gelatin added. Thus, the inclusion level of

271 1% gelatin or gelatin hydrolysate might be insufficient to form a gel matrix to entrap  
272 moisture in the meat batter.

273

274 ***Textural properties of cooked sausages with pork skin gelatin, duck skin gelatin, and***  
275 ***their hydrolysates***

276 The addition of 1% gelatin and gelatin hydrolysate did not affect hardness,  
277 springiness, and gumminess of cooked sausage ( $p>0.05$ ; Table 3). However, the addition  
278 of duck skin gelatin hydrolysate significantly increased the cohesiveness of cooked  
279 sausage. As a result, higher chewiness was observed for gelatin or gelatin hydrolysate  
280 treatments compared to the control ( $p<0.05$ ). Previously, the impacts of gelatin on textural  
281 properties of cooked sausages have been inconsistent. Lee and Chin (2016) reported that  
282 the hardness, cohesiveness, gumminess, and chewiness of emulsion sausages decreased  
283 as the gelatin level increased. In addition, they reported that gelatin addition did not affect  
284 the springiness of emulsion sausage, since only a weak interaction between muscle  
285 protein and gelatin could occur (Lee and Chin, 2016). On the other hands, Jridi et al.  
286 (2015) indicated that fish gelatin addition (0–1.5%) increased the hardness of turkey  
287 sausage but decreased cohesiveness. However, our results show that the addition of duck  
288 skin gelatin hydrolysate could increase the cohesiveness of cooked sausage. This is  
289 probably because the peptide groups in gelatin hydrolysate, which are available for the  
290 protein-protein interaction with muscle protein, could be exposed additionally. Moreover,  
291 since there are differences in amino acid composition and molecular size of gelatin, such  
292 positive effects of gelatin hydrolysate may be also different depending on gelatin  
293 extraction source.

294

### 295 *Lipid oxidation of cooked sausages with gelatin hydrolysates*

296 The effect of gelatin and gelatin hydrolysate addition on the lipid oxidation of cooked  
297 sausage during 4 wk of refrigerated storage is shown in Fig. 1. At the initial storage time  
298 (0 wk), the incorporation of duck skin gelatin or hydrolysate significantly increased the  
299 TBARS value of cooked sausages. Throughout the refrigerated storage period, the lipid  
300 oxidation of cooked sausages prepared with duck skin gelatin or hydrolysate accelerated  
301 consistently ( $p < 0.05$ ). As a result, the TBARS value of cooked sausage prepared with  
302 duck skin gelatin at 2 wk reached 1 mg MDA/kg sample, which is recognized as a limit  
303 of sensorial acceptance. For duck skin gelatin hydrolysate, the phenomenon of rapid lipid  
304 oxidation was alleviated slightly (0.88 mg MDA/kg at 4 wk). No changes in the TBARS  
305 value of the pork and duck skin gelatin hydrolysate treatments were observed during 4  
306 wk of the refrigerated storage period ( $p > 0.05$ ). The pork skin gelatin hydrolysate  
307 treatment presented similar TBARS value to the control during overall storage period  
308 ( $p > 0.05$ ). According to Ch'ng et al. (2014), when 0.5% of commercial gelatin, cold water  
309 fish skin gelatin, and bovine gelatin was added to chicken sausages, rapid lipid oxidation  
310 occurred during 3 wk of refrigerated storage. Thus, fatty acids and/or other pro-oxidants  
311 contained in raw skin materials for gelatin extraction could be incorporated into gelatin  
312 powder, which might be associated with the accelerated lipid oxidation of meat products  
313 formulated with gelatin.

314

### 315 **Conclusion**

316 In conclusion, this study shows comparable effects of gelatin and gelatin hydrolysate  
317 addition on the technological properties of cooked sausages. In particular, the addition of  
318 1% gelatin hydrolysate could increase the cohesiveness and chewiness of cooked

319 sausages. However, duck skin gelatin considerably accelerated the lipid oxidation of  
320 cooked sausages during 4 wk of the refrigerated storage period, although duck skin gelatin  
321 hydrolysate prepared through the stepwise enzymatic hydrolysis using collagenase and  
322 pepsin could alleviate the accelerated lipid oxidation. In further studies, an extraction  
323 process that can minimize the incorporation of lipid and/or pro-oxidant compounds  
324 should be considered for developing a multifunctional gelatin hydrolysate that provides  
325 antioxidant capacity as well as technological benefits.

326

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### 332 **Reference**

- 333 1. AOAC. 2007. Official methods of analysis of AOAC International. 18th ed.  
334 AOAC International, Gaithersburg, MD, USA.
- 335 2. Aschemann-Witzel J, Varela P, Peschel AO. 2019. Consumers' categorization of  
336 food ingredients: Do consumers perceive them as 'clean label' producers expect?  
337 An exploration with projective mapping. *Food Qual Prefer* 71:117-128.
- 338 3. Bourne MC. 1978. Texture profile analysis. *Food Technol* 32:62-66.
- 339 4. Buege JA, Aust SD. 1978. Microsomal lipid peroxidation. *Method Enzymol* 52:  
340 302-310.
- 341 5. Ch'ng SE, Ng MD, Pindi W, Kang OL, Abdullah A, Babji AS. 2014. Chicken  
342 sausages formulated with gelatin from different sources: A comparison of sensory



- 343 acceptability and storage stability. *World Appl Sci J* 31:2062-2067.
- 344 6. Chuaychan S, Benjakul S, Sae-leaw T. 2016. Gelatin hydrolysate powder from  
345 the scales of spotted golden goatfish: Effect of drying conditions and juice  
346 fortification. *Dry Technol* 35:1195-1203.
- 347 7. Gómez-Guillén MC, Giménez B, López-Caballero ME, Montero MP. 2011.  
348 Functional and bioactive properties of collagen and gelatin from alternative  
349 sources: A review. *Food Hydrocolloid* 25:1813-1827.
- 350 8. Gornall AG, Bardawill CJ, David MM. 1949. Determination of serum proteins  
351 by means of the biuret reaction. *J Biol Chem* 177:751-766.
- 352 9. Jridi M, Abdelhedi O, Souissi N, Kammoun M, Nasri M, Ayadi MA. 2015.  
353 Improvement of the physicochemical, textural and sensory properties of meat  
354 sausage by edible cuttlefish gelatin addition. *Food Biosci* 12:67-72.
- 355 10. Karim AA, Bhat R. 2009. Fish gelatin: properties, challenges, and prospects as  
356 an alternative to mammalian gelatins. *Food Hydrocolloid* 23:563-576.
- 357 11. Kim DW, Park K, Ha G, Jung JR, Chang O, Ham JS, Jeong SG, Park BY, Song  
358 J, Jang A. 2013. Anti-oxidative and neuroprotective activities of pig skin gelatin  
359 hydrolysates. *Food Sci Anim Resour* 33:258-267.
- 360 12. Kim HW, Lee YJ, Kim YHB. 2015. Efficacy of pectin and insoluble fiber  
361 extracted from soy hulls as a functional non-meat ingredient. *LWT-Food Sci*  
362 *Technol* 64:1071-1077.
- 363 13. Kim HW, Song DH, Choi YS, Kim HY, Hwang KE, Park JH, Kim YJ, Choi JH,  
364 Kim CJ. 2012. Effects of soaking pH and extracting temperature on the  
365 physicochemical properties of chicken skin gelatin. *Food Sci Anim Resour*  
366 32:316-322.

- 367 14. Kim HW, Yeo IJ, Hwang KE, Song DH, Kim YJ, Ham YK, Jeong TJ, Choi YS,  
368 Kim CJ. 2016. Isolation and characterization of pepsin-soluble collagens from  
369 bones, skins, and tendons in duck feet. *Food Sci Anim Resour* 36:665-670.
- 370 15. Kim SM, Kim TK, Ku SK, Kim MJ, Jung S, Yong HI, Choi YS. 2020. Quality  
371 characteristics of semi-dried restructured jerky: combined effects of duck skin  
372 gelatin and carrageenan. *J Anim Sci Technol* 62:553-564.
- 373 16. Lafarga T, Álvarez C, Hayes M. 2017. Bioactive peptides derived from bovine  
374 and porcine co-products: a review. *J Food Biochem* 41:e12418.
- 375 17. Lee CH, Chin KB. 2016. Effects of pork gelatin levels on the physicochemical  
376 and textural properties of model sausages at different fat levels. *LWT-Food Sci  
377 Technol* 74:325-330.
- 378 18. Lee SJ, Kim KH, Kim YS, Kim EK, Hwang JW, Lim BO, Moon SH, Jeon BT,  
379 Jeon YJ, Ahn CB, Park PJ. 2012. Biological activity from the gelatin hydrolysates  
380 of duck skin by-products. *Process Biochem* 47:1150-1154.
- 381 19. Mariotti F, Tomé D, Mirand PP. 2008. Converting nitrogen into protein-Beyond  
382 6.25 and Jones' factors. *Crit Rev Food Sci Nutr* 48:177-184.
- 383 20. Nikoo M, Benjakul S, Xu X. 2015. Antioxidant and cryoprotective effects of  
384 Amur sturgeon skin gelatin hydrolysate in unwashed fish mince. *Food Chem*  
385 181:295-303.
- 386 21. Noh SW, Song DH, Ham YK, Kim TK, Choi YS, Kim HW. 2019. Interaction of  
387 porcine myofibrillar proteins and various gelatins: Impacts on gel properties.  
388 *Food Sci Anim Resour* 39:229-239.
- 389 22. Ockerman HW, Hansen CL. 1988. *Glue and gelatin animal by-product  
390 processing*. Ellis Horwood Ser Food Sci Technol, New York, NY, USA. pp 132–

- 391 157.
- 392 23. Park JH, Choe JH, Kim HW, Hwang KE, Song DH, Yeo EJ, Kim HY, Choi YS,  
393 Lee SH, Kim CJ. 2013. Effects of various extraction methods on quality  
394 characteristics of duck feet gelatin. *Food Sci Anim Resour* 33:162-169.
- 395 24. Sarbon NM, Badii F, Howell NK. 2018. Purification and characterization of  
396 antioxidative peptides derived from chicken skin gelatin hydrolysate. *Food*  
397 *Hydrocolloid* 85:311-320.
- 398 25. Tümerkan ETA, Cansu Ü, Boran G, Mac Regenstein J, Özoğul F. 2019.  
399 Physiochemical and functional properties of gelatin obtained from tuna, frog and  
400 chicken skins. *Food Chem* 287:273-279.
- 401 26. Warner RD, Kauffman RG, Greaser ML. 1997. Muscle protein changes post  
402 mortem in relation to pork quality traits. *Meat Sci* 45:339-352.
- 403 27. Zhang L, Shan Y, Hong H, Luo Y, Hong X, Ye W. 2020. Prevention of protein  
404 and lipid oxidation in freeze-thawed bighead carp (*Hypophthalmichthys nobilis*)  
405 fillets using silver carp (*Hypophthalmichthys molitrix*) fin hydrolysates. *LWT-*  
406 *Food Sci Technol* 123:109050.
- 407

## Table Lists

408

409

410 **Table 1. Physicochemical properties of pork and duck skin gelatins and their**  
411 **hydrolysates<sup>1)</sup>**

412

413 **Table 2. Chemical composition and color characteristics of cooked sausages**  
414 **formulated with 1% pig and duck skin gelatins and their hydrolysates<sup>1)</sup>**

415

416 **Table 3. Protein solubility, cooking loss, and textural properties of cooked sausages**  
417 **formulated with 1% pig and duck skin gelatins and their hydrolysates<sup>1)</sup>**

418

419 **Table 1. Physicochemical properties of pork and duck skin gelatins and their**  
 420 **hydrolysates<sup>1)</sup>**

Trait	Pork skin		Duck skin		SEM <sup>1)</sup>	p value
	Gelatin	Gelatin hydrolysate <sup>1)</sup>	Gelatin	Gelatin hydrolysate		
Protein content (g/100 g)	93.54a	76.77b	78.71b	72.17b	2.374	<0.001
pH value	4.50c	5.71b	6.17a	5.71b	0.188	<0.001
<i>Color characteristics</i>						
CIE L* (lightness)	89.91b	84.60c	95.69a	79.16c	1.866	<0.001
CIE a* (redness)	-0.43b	2.02a	-0.99b	2.02a	0.513	0.002
CIE b* (yellowness)	13.99b	14.08b	5.99c	16.52a	1.231	<0.001

421 <sup>1)</sup>Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

422 <sup>2)</sup>SEM: standard error of the means.

423 a-c Means sharing the same letters within a row are not significantly different (p>0.05).

424

425 **Table 2. Chemical composition and color characteristics of cooked sausages**  
 426 **formulated with 1% pig and duck skin gelatins and their hydrolysates<sup>1)</sup>**

Trait	Control <sup>2)</sup>	Pork skin		Duck skin		SEM <sup>3)</sup>	P value
		Gelatin	Gelatin hydrolysate	Gelatin	Gelatin hydrolysate		
pH (meat batter)	5.91	5.85	5.96	5.95	5.91	0.016	0.312
pH (cooked sausage)	6.36	6.24	6.24	6.31	6.31	0.017	0.174
Moisture content (g/100 g)	62.41	61.49	61.19	61.87	62.22	0.222	0.429
Protein content (g/100 g)	16.36	18.12	19.89	18.76	19.23	0.821	0.896
<i>Color characteristics</i>							
CIE L* (lightness)	77.10	78.22	77.80	77.80	77.54	0.156	0.269
CIE a* (redness)	3.55a	3.54b	3.23b	3.14b	3.26b	0.058	0.029
CIE b* (yellowness)	11.16	11.19	11.25	11.50	11.44	0.073	0.541

427 <sup>1)</sup>Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

428 <sup>2)</sup>Control was prepared without gelatin or gelatin hydrolysate, and other treatments were formulated with  
 429 1% gelatin or gelatin hydrolysate.

430 <sup>3)</sup>SEM: standard error of the means.

431 a, b Means sharing the same letters within a row are not significantly different (p>0.05).

432

433 **Table 3. Protein solubility, cooking loss, and textural properties of cooked sausages**  
 434 **formulated with 1% pig and duck skin gelatins and hydrolysates<sup>1)</sup>**

Trait	Control <sup>2)</sup>	Pork skin		Duck skin		SEM <sup>3)</sup>	P value
		Gelatin	Gelatin hydrolysate	Gelatin	Gelatin hydrolysate		
Protein solubility (mg/g)	74.77	93.68	80.81	90.52	83.90	2.681	0.080
Cooking loss (%)	2.37	1.82	1.63	1.99	1.90	0.089	0.081
<i>Textural properties</i>							
Hardness (kg)	7.61	8.82	8.21	9.15	8.12	0.303	0.596
Springiness (ratio)	0.68	0.68	0.73	0.79	0.77	0.009	0.051
Cohesiveness (unitless)	0.16bc	0.16c	0.21ab	0.21ab	0.22a	0.019	0.045
Gumminess (kg)	1.20	1.37	1.68	1.90	1.90	0.090	0.195
Chewiness (kg)	0.14c	0.17bc	0.26a	0.21ab	0.24ab	0.015	0.021

435 <sup>1)</sup>Gelatin hydrolysates were prepared through a stepwise enzymatic hydrolysis using collagenase and pepsin.

436 <sup>2)</sup>Control was prepared without gelatin or gelatin hydrolysate, and other treatments were formulated with  
 437 1% gelatin or gelatin hydrolysate.

438 <sup>3)</sup>SEM: standard error of the means.

439 a-c Means sharing the same letters within a row are not significantly different (p>0.05).

440

## Figure Legends

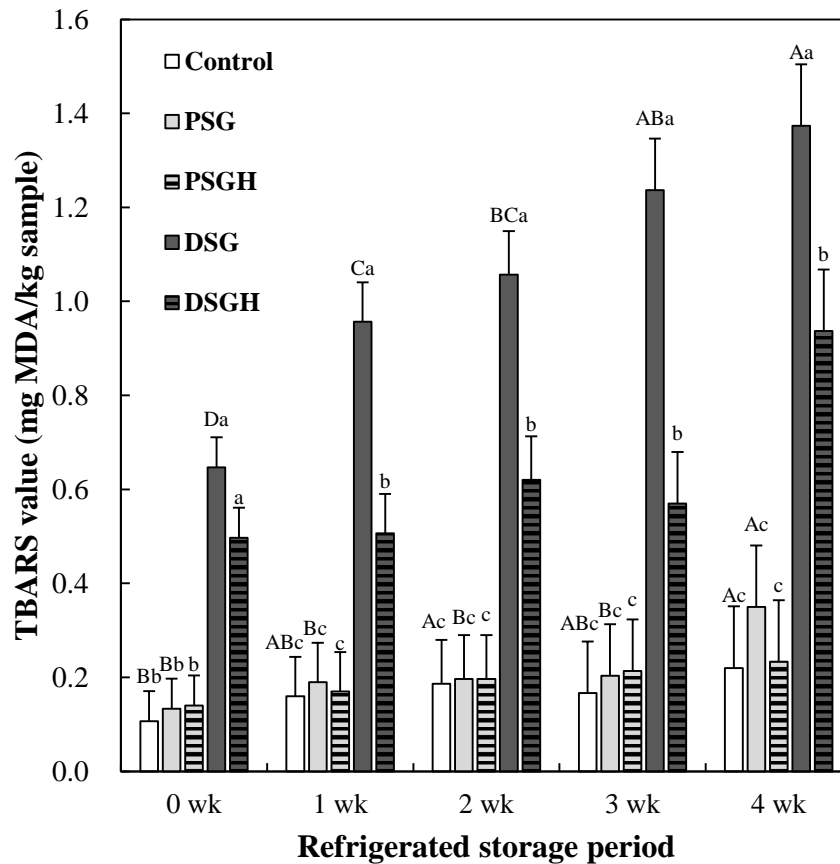
441

442 **Fig. 1. Changes in 2-thiobarbituric acid reactive substances (TBARS) of cooked**  
443 **sausages formulated with 1% pork and duck skin gelatins and their**  
444 **hydrolysates.** Gelatin hydrolysates were prepared through a stepwise enzymatic  
445 procedure using collagen and pepsin. Control, without gelatin or gelatin  
446 hydrolysate, PSG, 1% pork skin gelatin; PSGH, 1% pork skin gelatin hydrolysate;  
447 DSG, 1% duck skin gelatin; DSGH, 1% duck skin gelatin hydrolysate. Error bars  
448 represent standard error of the means. A-D Means with the same letter within each  
449 treatment are not significantly different ( $p>0.05$ ). a-c Means with the same letter  
450 within each storage period are not significantly different ( $p>0.05$ ).

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452

453 **Fig. 1. Changes in 2-thiobarbituric acid reactive substances (TBARS) of cooked**  
 454 **sausages formulated with 1% pork and duck skin gelatins and their**  
 455 **hydrolysates.** Gelatin hydrolysates were prepared through a stepwise enzymatic  
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 459 represent standard error of the means. A-D Means with the same letter within each  
 460 treatment are not significantly different ( $p > 0.05$ ). a-c Means with the same letter  
 461 within each storage period are not significantly different ( $p > 0.05$ ).

462