

1 **Effect of pig breed and processing stage on the physicochemical**
2 **properties of dry-cured loin**

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15 Abbreviated running title: Quality properties of dry-cured loin using different breeds pig

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24 **Abstract**

25 This study investigated the effect of pig breeds on the quality characteristics of dry-cured
26 loins according to the processing stage. Physicochemical properties of 20 dry-cured processed
27 loins with the different pig breeds (Berkshire vs Landrace × Yorkshire × Duroc (LYD;
28 n=10)) and different processing stages (raw, curing, dry-ripened 15 day and 30 day) were
29 analyzed. The pig breed influenced moisture content and pH with values of 59% and 53%, and
30 6.17 and 5.94, for Berkshire and LYD, respectively, at day 30. Dry-cured loins made with
31 Berkshire showed higher hardness and lower cohesiveness than that of the LYD ($p<0.05$).
32 Redness and yellowness were higher for Berkshire than LYD ($p<0.05$). Lipid oxidation and
33 lightness did not affect by pig breed during processing stages ($p>0.05$). However, sulfhydryl
34 content was significantly higher in Berkshire compared to the LYD after dry-ripened for 15
35 days ($p<0.05$). The concentration of total free amino acids and fatty acids was higher for
36 Berkshire during all processing stages ($p<0.05$). Berkshire may be better quality due to its high
37 moisture content and pH compared to the LYD.

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39 Keywords: Pig breed; Processing stage; Dry-cured loin; Oxidative phenomena;
40 Physicochemical properties

41

42 **1. Introduction**

43 Dry-cured meat product has created from a long stage ago, and there are a wide variety of
44 styles. The characteristics of the final product have been determined by the type of raw meat
45 and chloride salt. Regarding raw material, Cilla et al. (2006) have performed about the effect
46 of Duroc line sire on dry-cured ham. Armenteros et al. (2012) have conducted to replacement
47 of NaCl by other chloride salts. In addition, by monitoring the change in lipid or a protein
48 according to the processing stage, it has been discussed in related to the texture properties or
49 volatile compounds of dry-cured ham (Harkouss et al., 2015; Pérez-Santaescolástica et al.,
50 2018). Dry-cured loin takes third in consumer preferences in Spain and is well accepted (Aliño
51 et al., 2009). The typical manufacturing process is similar to that of the ham, but the time
52 required for each stage is different, and the processing period of dry-cured loin is shorter than
53 the dry-cured ham (50 days vs 18 months) (Abellán et al., 2018).

54 Oxidative reaction in dry-cured meat is an important factor in the determination of quality
55 characteristics of dry-cured meat. During processing, proteins and lipids undergo both
56 oxidation and degradation of myofibrillar proteins or triacylglycerides and phospholipids,
57 respectively, which produce different chemical compounds related to taste (such as free amino
58 acid) or flavor (such as free fatty acid) (Ripollés et al., 2011; Toldra 2006). Also, lipid oxidation
59 can affect the production of numerous volatile compounds due to various decomposition
60 mechanisms, and these compounds have a unique flavor of dry-cured ham (Jin et al., 2010).
61 However, some previous studies have reported that breakdown and oxidation are not
62 particularly relevant in the manufacture of dry-cured ham (Jin et al., 2010; Muriel et al., 2007).
63 Thus, the relationship between breakdown and oxidation in dry-cured meat still need to discuss.

64 Berkshire pigs are a breed of pig originating from the English county of Berkshire that is
65 bred and raised in several parts of the world. The Berkshire pig is not all black but has white,

66 including white socks from the "knee" down and typically a white blaze on its snout (Wikipedia,
67 2019). This breed has remarkable water holding capacity evaluated by cooking loss and drip
68 loss (Lee et al, 2012; Suzuki et al., 2003). In Korea, the most commonly used pork in the
69 commercial market is crossbred pigs, and their characteristics are fast growth, small size, and
70 high meat quantity. The characteristics in pig breeds for the production of crossbred pig
71 followed: Landrace is high daily gain, Yorkshire is thin back fat, and Duroc is high formation
72 of intramuscular fat (Kang et al., 2011). For the reason of production cost based on efficiency,
73 purebred pigs are not suitable in industry. The previous studies have been reported that the
74 crossbred pig has poor meat quality than purebred pigs (Ryu et al., 2008). Ryu et al. (2008)
75 found that most of the samples from Berkshire pigs turned out normal pork (red, firm and non-
76 exudative, RFN). In contrast, approximately 60% of the samples from the Landrace pigs turned
77 out abnormal pork (pale, soft and exudative, PSE; reddish-pink, soft and exudative, RSE; dark,
78 firm and dry, DFD). Also, Subramaniyan et al. (2016) showed that physicochemical
79 characteristics of Berkshire pigs are significantly greater than the LYD, and the authors
80 mentioned that this is a very desirable characteristic for consumers. In summary to the reference,
81 Berkshire has reported higher water holding capacity and palatability than LYD. Thus, it is
82 necessary to compare with LYD how these raw meat characteristics affect the quality of dry-
83 cured loin.

84 For these reasons, the hypothesis of this study is that the quality characteristics of dry-
85 cured loin may be influenced by pig breeding and processing stage. Therefore, the purpose of
86 this study was to determine the effect of pig breed according to the processing stage of dry-
87 cured loin. In addition, oxidative changes on the quality characteristics of dry-cured loin were
88 also investigated.

89

90 **2. Materials and methods**

91 ***2.1. Experimental design and dry-cured loin preparation***

92 Animal replication and processing batch effect were randomized for consideration on only
93 experimental effect (breed and processing stage). Animals were reared according to next
94 paragraph, and twenty pigs (Berkshire = 10, LYD = 10 and; n = 10) were evaluated for this
95 study. Also, the dry-cured loin processing was performed 5 times on different day in order not
96 to consider the batch effect.

97 The pigs were raised in the different pen on the same farm and fed the same diet following
98 the commercial production system. The pigs were transferred to a local slaughter house and
99 slaughtered by traditional neck cutting when their live weight reached between 110 kg to 115
100 kg. The carcasses were hung in a chilling room at 0~2°C for 24 h after slaughtering. The pork
101 loins were used deboning and removed the backfat and connective tissue. The pair of pork loins
102 from each pig were removed at 3 cm at both ends and divided in half, and randomly assigned
103 to each processing stage. The trimmed pork loin was rubbed by hand for 10 min with 3.5%
104 purified sodium chloride (99.9%, w/w) and left for 4 days at 4°C and 80 ± 5% room humidity
105 (RH) in salting the bath. After the curing stage, the surface of cured loin was rinsed with tap
106 water and removed moisture with the paper towel. The first drying-ripening stage was
107 performed for 15 days in an artificial environment chamber at 15°C and 80 ± 5% RH, and
108 secondary the drying-ripening stage was carried for 15 days at 12°C and 65 ± 5% RH. After
109 drying-ripening, the dry-cured loins were vacuum-packed and stored at -80°C till further
110 analysis.

111

112 ***2.2. Analytical methods***

113 ***2.2.1. Moisture content and pH***

114 Moisture content was analyzed in duplicate by weight losses after 24 h at $103 \pm 1^\circ\text{C}$
115 (AOAC, 2012). The results were expressed as the percentage of weight. The pH was
116 determined directly with a pH meter (S20 SevenEasyTM, Greifensee, Switzerland). The
117 measurement was taken three times and calibrated with pH 7.00, 4.01, and 9.21.

118

119 **2.2.2. Texture profile analysis (TPA)**

120 TPA was carried out by EZ-SX (Shimadzu corp., Kyoto, Japan) at room temperature. Two
121 cubes (50 mm \times 30 mm \times 25 mm, length \times width \times height) were analyzed, and it was taken at
122 the central portion of each loin. Each section was tested with four replications. The samples
123 were placed with the muscle fiber parallel to the compression plate surface and compressed
124 twice to 50% of their original height with a time interval of 0 s between the two compressions.
125 Force-time curves were recorded with a 500 N load cell applied at a crosshead speed of 100
126 mm/min. The TPA parameters were obtained using the software package and calculated by
127 Bourne (1982).

128

129 **2.2.3. Instrumental color**

130 The instrumental color was measured ten times for each sample by a colorimeter (CR-400,
131 Konica Minolta, Tokyo, Japan). CIE (Commission International del'éclairage) L* (lightness),
132 a* (redness), b* (yellowness), C* (chroma), and h° (hue angle) values were determined with
133 D65 illuminant and 2° standard observer. The instrument was calibrated using a standard white
134 plate (Y = 81.2; x = 0.3191; y = 0.3263). The data were obtained from the average of each
135 measured value.

136

137 **2.2.4. TBARS and sulfhydryl content**

138 The TBARS in dry-cured loin was determined based on the method of Cherian et al. (2007)
139 with some modifications. About 3 g of samples were homogenized with 27 mL of 3.86%
140 perchloric acid. The homogenates were kept at room temperature for 1 h and then centrifuged
141 at $2000 \times g$ for 10 min. The supernatants were filtered through Whatman filter paper No. 1, and
142 the filtrates were mixed with 20 mM TBA solution (1:1, v/v). Then, the mixture was kept in a
143 dark condition for 15 h at room temperature and then read at 531 nm using a spectrophotometer
144 (Cary 60 UV-Vis, Agilent Technologies, CA, USA). The measured value was expressed as mg
145 MDA/kg sample.

146 Sulphydryl was measured following the method of Vossen and De Smet (2015) with some
147 modifications. Two gram samples were homogenized with 25 mL of 1% SDS in 0.10 M Tris
148 buffer. The homogenates were incubated in a water bath for 30 h at 80°C. After cooling at room
149 temperature, the homogenates were centrifuged at $7000 \times g$ for 20 min. For the sulphydryl
150 concentration, 2 mL of 0.1 M Tris buffer (pH 8.0) and 0.5 mL of 10 mM DTNB in 0.1 M Tris
151 buffer were mixed with 0.5 mL of filtered supernatant. For protein concentration, 0.5 mL of
152 filtered supernatant was added to 2.5 mL of 0.1 M Tris buffer. Again, 0.5 mL of 5% SDS in Tris
153 buffer (pH 8.0), 0.5 mL of 10 mM DTNB, and 2.0 mL of 0.1 M Tris buffer were mixed to
154 prepare a blank solution. All mixtures were kept for reaction in the dark room at 4°C for 30 min.
155 The absorbance of sulphydryl concentration in dry-cured loin was measured by
156 spectrophotometer at 412 nm. The sulphydryl concentration was calculated using the Lambert-
157 Beer equation of $\epsilon_{412} = 14,000 \text{ M}^{-1} \text{ cm}^{-1}$ and expressed as nmol sulphydryl/mg protein. The
158 protein concentration in dry-cured loin was measured at 280 nm and calculated based on a
159 bovine serum albumin standard curve.

160

161 **2.2.5. Fatty acid analysis**

162 The mixture of 5 g of the sample and 1 mg of undecanoic acid as internal standard were
163 homogenized with 25 mL methanol/chloroform solution (methanol:chloroform, 1:2, v/v), and
164 total lipids in the intramuscular were extracted according to the Folch et al. (1957). The solvent
165 in the lipid extract has evaporated at room temperature for 2 h in dark condition. For
166 saponification, the extracted lipids were dissolved with 2 mL methylene chloride, and then 200
167 μL was transferred to a glass vial and boiled with 1 mL of 0.5 N NaOH at 85°C for 10 min.
168 According to Metcalfe et al. (1966), the glass vial was cooled at room temperature and boiled
169 with 1 mL of BF₃-methanol solution at 85°C for 10 minutes for methylation. After methylation,
170 the addition of 3 mL hexane and 8 mL DW were added into the glass vial and vortexed for 10
171 s and centrifuged at 1,000 rpm for 5 min. The 1 mL upper layer was taken for analysis. The gas
172 chromatography running conditions were determined by Lorenzo et al. (2015) with some
173 modifications. The split ratio was 1:10, and 1 μL of the solution was injected. Each peak was
174 identified by comparing their retention times with those of certified standards (Supelco 37
175 component FAME mix). Separation and quantification of the FAMEs was carried out using a
176 gas chromatograph (GC Agilent 6890 N; Agilent Technologies Spain, S.L., Madrid, Spain)
177 equipped with a flame ionization detector and an automatic sample injector HP 7683, and using
178 a Supelco SP-2560 capillary GC column (fused silica, 100 m \times 0.25 mm \times 0.2 μm). The
179 chromatographic conditions were as follows: initial column temperature 120°C, maintaining
180 this temperature for 5 min, programmed to increase at a rate of 5°C/min up to 200°C,
181 maintaining this temperature for 2 min, then at 1°C/min up to 230°C, maintaining this
182 temperature for 3 min. The injector and detector were maintained at 260 and 280°C,
183 respectively. Nitrogen was used as the carrier gas at a constant flow-rate of 1.1 mL/min, with
184 the column head pressure set at 35.56 psi, and the split ratio was 1:20. The results were

185 calculated as compared to the peak area of the internal standard according to the relative
186 quantification method, and expressed as mg fatty acid/g sample.

187

188 **2.2.6. Free amino acid analysis**

189 Free amino acids were analyzed using a method from Franco et al. (2010) with some
190 modifications. Briefly, 10 g of sample was homogenized with 90 mL of DW and centrifuged
191 at $2,000 \times g$ for 10 min. The 10 mL of supernatant was mixed with 10% TCA solution for
192 deproteinization and centrifuged at $10,000 \times g$ for 10 min. The 5 mL of supernatant was mixed
193 with 5 mL of hexane to remove lipid and taken to the lower layer (water layer) filtered through
194 a $0.28 \mu\text{m}$ membrane filter. The filtrate was injected into an amino acid analyzer (Biochrome
195 30 plus, Biochrome, Cambourne, UK).

196

197 **2.3. Statistical analysis**

198 All data were expressed as mean values with a standard error of the means. The statistical
199 model included pig breed and processing stage as fixed factors and animal replication and
200 processing batch as random terms. An analysis of variance (ANOVA) using the ANOVA
201 procedure of the SAS (SAS version 9.4, SAS Institute, Inc., USA) was performed for all
202 variables considered. A Duncan's multiple range test was performed to compare the mean
203 values at a significance level of $p < 0.05$.

204

205 **3. Results and discussion**

206 **3.1. Moisture content and pH values**

207 The results for moisture content and pH are shown in Table 1. The range of moisture
208 content was 59~73% in Berkshire and 52~73% in LYD. The moisture level before day 15 of

209 drying-ripening as 73% was no difference between pig breeds ($p>0.05$). However, the
210 Berkshire was significantly higher than that of the LYD on days 15 of drying-ripening ($p<0.05$).
211 The differences in moisture content between the pig breeds were about 6% even at days 30 of
212 dry aging. Also, it tended to significantly decrease with the processing stage, and LYD showed
213 a higher reduction ($p<0.05$). In pH values, there was no difference in pH values at the raw meat.
214 However, similar to the result of moisture content, there was significantly difference between
215 pig breeds from day 15 of drying-ripening, and Berkshire and LYD were 6.17 and 5.94,
216 respectively on day 30 of drying-ripening ($p<0.05$). Furthermore, the pH was significantly
217 increased in all dry-cured loin as the processing stage progressed ($p<0.05$).

218 Seong et al. (2014) reported that there was no significant difference in moisture content
219 between dry-cured ham of Berkshire and LYD and dry-cured ham of Berkshire showed a
220 numerically higher moisture content of about 3% compared to those of LYD. However, these
221 authors reported that dry-cured ham of Berkshire was about 10% lower in total weight loss than
222 those of LYD. In addition, Berkshire reported lower drip loss compared to those of LYD,
223 Yorkshire, Landrace and Duroc in raw meat (Lee et al., 2012; Ryu et al., 2008). Based on this,
224 one hypothesis may be that due to greater water holding capacity of Berkshire, it may have a
225 higher moisture content than LYD on the day 30 of drying-ripening. Moisture and pH are key
226 factors in the manufacture of dry-cured meat product and have a great influence on the overall
227 quality characteristics. In particular, it was found that these factors had a great influence on
228 proteolysis and lipolysis through previous studies, and Rico et al. (1993) reported that the
229 proteolytic enzymes cathepsin B and L were slightly affected by the decrease in salt and water
230 activity, and showed the most optimal activity at pH 5.7. Also, Petrova et al. (2015) reported
231 that triacylglycerol and phospholipids were hydrolyzed by lipolytic enzymes during the
232 processing, and they were finally decomposed into free fatty acids, and the optimum pH range

233 was different depending on the type of lipolytic enzyme.

234

235 **3.2. Color values**

236 The instrumental color parameters for dry-cured loin are shown in Table 2. Lightness was
237 not significantly difference between pig breeds at the initial stage ($p>0.05$), but Berkshire had
238 significantly higher lightness on day 30 of drying-ripening from the curing stage ($p<0.05$). The
239 lightness of two pig breeds significantly decreased depending on the processing stage ($p<0.05$).
240 At this stage, lightness decreased more for LYD compared to Berkshire. Redness was
241 significantly difference between pig breeds from the beginning to day 30 of drying-ripening,
242 excluding the curing stage ($p<0.05$). In the raw meat stage, redness of Berkshire and LYD was
243 5.81 and 5.20, respectively, the values gradually increased until the day 30 of dry ripening to
244 8.63 and 6.24, respectively. The dry-cured loin of Berkshire was about 2 higher than that of
245 LYD ($p<0.05$). Contrary to lightness, it significantly increased with the processing stage
246 ($p<0.05$), and Berkshire showed a greater lightness than LYD. The values of yellowness and
247 chroma were significantly higher in Berkshire than in LYD at each stage, and significantly
248 increased according to processing stage in all pig breeds ($p<0.05$). The hue angle was
249 significantly higher for Berkshire from the beginning to the curing stage ($p<0.05$), but there
250 were no significant differences between the pig breeds on day 30 of drying-ripening ($p>0.05$).
251 Likewise, the lightness decreased significantly with the processing stage ($p<0.05$). Overall,
252 Berkshire showed higher values for color parameters on day 30 of drying-ripening than LYD.
253 In addition, there was a tendency to increase as processing progressed except for lightness.

254 A color has a great influence on basic product purchasing decisions alike technical
255 characteristics and textural properties (Faustman and Cassens, 1990). Ryu et al. (2008)
256 compared the meat quality of various pig breeds, and Berkshire reported higher redness and

257 lower lightness than those of the LYD, Landrace, and Yorkshire, and these results were similar
258 to our results. Lightness is correlated with water holding capacity, which was revealed through
259 previous studies (Huff-Lonergan et al., 2002). In addition, the authors reported that drip loss
260 and cooking loss are representative methods of measuring water holding capacity of meat and
261 also reported a positive correlation with lightness and mentioned that this is closely related to
262 pH. Therefore, a higher water holding capacity of Berkshire could be likely to result in
263 relatively little leakage of water on the surface, resulting in reduced reflection during
264 measurement and low lightness. In addition, a higher water holding capacity of Berkshire
265 would have an effect on the amount of water evaporated over the processing stage. As a result,
266 the higher moisture content of Berkshire would have a higher lightness. Redness of Berkshire
267 was higher than those of LYD from the beginning, and this was maintained until day 30 of
268 drying-ripening. This result may be due to differences in raw meat. In addition, the cured meat
269 color forms NO-MetMb through a chemical reaction between myoglobin in meat and
270 nitrate/nitrite, which is reduced to nitrosylmyoglobin in an anaerobic state, and at this stage, it
271 has a red color (Suman and Joseph, 2013). Furthermore, increased nitrosylmyoglobin due to
272 nitrate/nitrite reduction through continuous dehydration and microbial activity until the product
273 reaches its final stage may be the cause (Arnau et al., 2007; Salazar et al., 2015). However,
274 since the salt used in this study consisted of purified NaCl with a purity of 99% or more, the
275 effect of raw meat would have been greater than the effect of nitrate or nitrite. Ramírez et al.
276 (2007) reported similar to our yellowness results, and the authors reported that the yellowness
277 of a dry-cured loin made from a crossbreed of Iberian and Duroc was 5.1 to 6.3. On the other
278 hand, Pateiro et al. (2014) reported that dry-cured loins made with Celta decreased with the
279 processing stage, and the authors mentioned that there was a positive correlation with moisture
280 loss. Also, yellowness is related to the fat content in meat, and Li et al. (2013) reported that

281 yellowness and fat content had a positive correlation.

282

283 **3.3. Texture profile analysis**

284 The textural characteristics were analyzed according to the pig breed and processing stage
285 (Table 3). In hardness, the significant difference between pig breeds was in raw meat stage and
286 day 30 of drying-ripening ($p < 0.05$), and Berkshire was higher than LYD. There was a
287 significant difference depending on the processing stage ($p < 0.05$). The hardness value of
288 Berkshire decreased from 7.17 N in the raw meat stage to 2.69 N on the day 15 of drying-
289 ripening and increased to 5.06 N on the day 30 of drying-ripening again. The LYD was similar
290 result to that of Berkshire. The cohesiveness was not significantly different depending on the
291 pig breed ($p > 0.05$), but there was a significant difference depending on the processing stage
292 ($p < 0.05$). Also, it gradually increased as the processing stage progressed, and it tended to be
293 maintained from day 15 of dry-ripening. The springiness showed a significant difference based
294 on the processing stage ($p < 0.05$), and on the contrary, the cohesiveness showed a tendency to
295 decrease with the processing stage. The significant difference between the pig breeds was only
296 in the cured meat, and it was seen that Berkshire was higher than LYD ($p < 0.05$). There was no
297 significant difference in chewiness between pig breeds ($p > 0.05$), but there was a significant
298 difference in processing stage ($p < 0.05$). It decreased from the raw meat stage until day 15 of
299 drying-ripening, but on the contrary, it increased again on day 30 of drying-ripening. In
300 summary, there was no difference according to the pig breed, but there was a change in textural
301 characteristics according to the processing stage. The textural component of dry cured meat is
302 final result of the physical part expressed by various factors (oxidation, lipolysis, proteolysis,
303 etc.) in quality characteristics, and it could be affected by consumer preference (Ruiz-Ramírez
304 et al, 2005).

305 Based on this study results, day 30 drying-ripening of Berkshire has relatively low protein
306 deterioration and its moisture content is high compared to those of LYD. Thus, it would have
307 been little empty space inside of muscle by loss of moisture. For this reason, dry-cured loin of
308 Berkshire could be a higher hardness than those of LYD on day 30 drying-ripening. The result
309 of texture properties was very similar to the changes over the processing period of *m.*
310 *semimembranosus* and *m. biceps femoris* as reported by Harkouss et al. (2015). The discussion
311 of these authors is not sufficient to understand our results, but the trend in the results of the
312 hardness was the same. This trend could be presumed by salting, water evaporation, and
313 contraction. The salting could be led to internally deterioration of the loin, which can lead to
314 loosen tissue. Also, during the initial drying-ripening, the pore will be formed in the place
315 where moisture has evaporated, and the hardness will continue to decrease. However, as the
316 drying-ripening continues, the loin is contracted, and the pores disappear, and the hardness will
317 increase again. Also, Ruiz-Ramírez et al. (2005) found that moisture and water activity had a
318 positive correlation with cohesiveness, and hardness and chewiness had a negative correlation.
319 In view of this, the change in our textural properties is also closely related to moisture, and as
320 the moisture evaporates over the processing stage, the pork loin partially contracts. Thus, the
321 use of dry matter such as proteins could be increased during analysis resulting in higher
322 hardness. In addition, high hardness requires a lot of strength in TPA analysis, and since this
323 requires high strength in structural destruction, cohesiveness can be perceived to be rather low
324 (Ramírez and Cava, 2005; Serra et al., 2005).

325

326 **3.4. Lipid oxidation and sulfhydryl group**

327 The result for lipid oxidation and sulfhydryl group are presented in Fig. 1 and Fig. 2,
328 respectively. There was no significant difference in TBARS depending on the pig breed, but it

329 gradually increased as the processing stage progressed ($p<0.05$). On the other hand, sulfhydryl
330 groups were significantly higher in Berkshire from the beginning to day 30 of drying-ripening,
331 and it decreased continuously from the beginning based on the processing stage ($p<0.05$). From
332 our results, the moisture content of Berkshire was higher than those of LYD, which would be
333 closely related to the reduction of sulfhydryl. Traore et al. (2012) reported a relationship
334 between protein oxidation and drip loss for longissimus muscle, and the authors were found
335 that there was a very high correlation between drip loss and protein oxidation. In addition, the
336 high moisture content is thought to have a positive effect on the product yield and textural
337 characteristics. TBARS is an important indicator for lipid oxidation, and which increase in lipid
338 oxidation during processing is directly linked to the formation of free fatty acids and volatile
339 compounds that affect the flavor (Jin et al., 2010). TBARS found in this study are in accordance
340 with Ventanas et al. (2005). There was no statistical difference, but numerically, Berkshire had
341 a high TBARS value, and which would have affected our fatty acid results (Table 4).

343 ***3.5. Free amino acids and fatty acid compositions***

344 The contents of free amino acids and fatty acids are shown in Table 4. There was no
345 difference in the free amino acid content at the initial stage ($p>0.05$), however, the free amino
346 acids content for Berkshire was significantly higher from day 15 of drying-ripening to day 30
347 of drying-ripening ($p<0.05$). In addition, both of pig breeds were significantly increased until
348 day 30 of drying-ripening ($p<0.05$). In the initial fatty acid content, there was no significant
349 difference in PUFA between pig breeds ($p>0.05$). On the other hand, Berkshire was
350 significantly higher in SFA, UFA, and MUFA ($p<0.05$). In addition, all fatty acid parameters
351 significantly increased based on processing stage ($p<0.05$). Free amino acids and fatty acids
352 are the final breakdown products of proteins and lipids and are the most important factors for

353 taste and flavor in meat products (Martin et al., 1999; Ramalingam et al., 2019). In this regard,
354 various attempts are still being made to measure and study their compositional forms in order
355 to understand the relationships with sensory characteristics in detail. As a result, Berkshire
356 showed a higher content of free amino acids and fatty acids than those of LYD, and this trend
357 was maintained until the end of the processing. Abellán et al. (2018) reported similar results
358 for the free amino acid content of dry-cured loin using refrigerated or frozen raw meat prepared
359 for 50 days, and also, Martin et al. (2008) showed that the fatty acid content of dry cured loin
360 prepared from pigs fed conjugated linoleic acid was similar to our results. The content of free
361 fatty acids increases with the production stage because the reduction of neutral and polar lipids
362 leads to the release of free fatty acids (Martin et al., 2008). The cause of this phenomenon may
363 be enzyme activity and fat breakdown by oxidation. Muriel et al. (2007) investigated the
364 changes in neutral and polar lipids in raw meat and dry-cured loin and reported that they
365 decreased by 81% and 32%, respectively, compared to raw meat. In addition, the authors
366 suggested that if oxidation is the cause of the decrease in neutral or polar lipids, SFA would be
367 more prominent in PUFA than MUFA. It has been reported by many studies that the content of
368 free amino acids increases as the dry ripening period increases in the manufacture of dry cured
369 meat products (Abellán et al., 2018; Armenteros et al., 2012). These are cathepsins, an enzyme
370 degrading in meat, dipeptidylpeptidases, and aminopeptidases. Toldrá et al., (1993)
371 investigated the enzyme activity in dry-cured ham in relation to cathepsins and stated that
372 cathepsins B, H, and L were active until the end of the product processing, but that the activity
373 of cathepsin D was only a few months in the initial period. In our experiment, protein and lipid
374 oxidation of Berkshire were lower than those of LYD (Fig. 1). Therefore, it is not appropriate
375 to interpret that Berkshire had a higher free amino acids and fatty acids due to oxidation. Rather,
376 as mentioned above, the assumption that the enzyme showed higher activity than LYD due to

377 a higher moisture and pH would be more appropriate. In addition, there were no studies on
378 enzyme activity in Berkshire and LYD, but studies mentioning that the genetic line of pigs may
379 be strongly related to enzyme activity (Monin et al., 2003; Cava et al., 2004).

380

381 **Conclusion**

382 The present study compared the physicochemical properties of dry-cured loin
383 manufactured with Berkshire and LYD. Depending on the manufacturing stage, dry-cured loin
384 of Berkshire had higher moisture and pH compared to those of LYD. Because of that, there
385 were higher content of fatty acid and amino acid than final product of LYD. Moreover,
386 Berkshire showed a high lightness and redness, and it could be advantageous in consumer
387 preference. Therefore, a study on the sensory characteristics of dry-cured loin manufactured
388 with Berkshire should be carried out in the further study.

389

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394

395 **Conflict of Interest**

396 The authors declare no potential conflicts of interest.

397

398 **Ethics Approval**

399 This article does not require IRB/IACUC approval because there are no human and animal

400 participants.

401

402 **Author Contribution**

403 Conceptualization: Yang HS, Seo JK. Data curation: Seo JK, Ko JH. Formal analysis: Seo
404 JK, Ko JH, Park JY, Eom JU. Methodology: Seo JK, Yang HS. Software: Seo JK, Park JY.
405 Validation: Seo JK, Eom JU. Writing -original draft: Seo JK. Writing -review & editing: Seo
406 JK, Ko JH, Park JY, Eom JU, Yang HS.

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517

518 **Figure legends**

519

520 **Fig. 1.** The TBARS of dry-cured loin in different pig breeds and processing stage. Capital letter
521 (^{A-C}) indicate significantly ($p < 0.05$) differences between processing stage.

522

523 **Fig. 2.** The sulfhydryl of dry-cured loin in different pig breeds and processing stage. Capital
524 letter (^{A-C}) indicate significantly ($p < 0.05$) differences between processing stage. Small letter (^{a-}
525 ^b) indicate significantly ($p < 0.05$) differences between pig breeds.

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527 **Table 1**

528 Effect of pig breed and processing stage on moisture content and pH of dry-cured loin.

Parameters	Pig breeds	Stage				SEM
		RM	CM	DR15	DR30	
Moisture (%)	Berkshire	73.11 ^A	73.22 ^A	65.28 ^{Ba}	59.60 ^{Ca}	0.49
	LYD	73.49 ^A	72.05 ^A	62.83 ^{Bb}	53.29 ^{Cb}	0.54
	SEM	0.45	0.44	0.49	0.68	
pH	Berkshire	5.75 ^B	5.87 ^B	5.99 ^{Ba}	6.17 ^{Aa}	0.09
	LYD	5.65 ^B	5.73 ^B	5.84 ^{ABb}	5.94 ^{Ab}	0.07
	SEM	0.08	0.10	0.07	0.07	

529 LYD: Landrace × Yorkshire × Duroc.

530 SEM: Standard error of the means.

531 ^{A-C}Means with upper letter in same animal significantly differ.

532 ^{a-b}Means with small letter in same stage significantly differ.

533 Stage: RM, raw meat; CM, cured meat; DR15, day 15 of drying-ripening; DR30, day 30 of drying-
534 ripening.

535

536 **Table 2**

537 Effect of pig breed and processing stage on color of dry-cured loin.

Parameters	Pig breeds	Stage				SEM
		RM	CM	DR15	DR30	
Lightness	Berkshire	51.20 ^A	47.36 ^{Ba}	45.50 ^{Ba}	44.88 ^{Ba}	0.66
	LYD	52.43 ^A	45.04 ^{Bb}	43.03 ^{Bb}	40.30 ^{Cb}	0.27
	SEM	0.70	0.19	0.17	0.81	
Redness	Berkshire	5.81 ^{Ca}	5.69 ^C	6.99 ^{Ba}	8.38 ^{Aa}	0.70
	LYD	5.20 ^{Cb}	5.44 ^C	6.03 ^{Bb}	6.24 ^{Ab}	0.35
	SEM	0.30	0.31	0.19	0.31	
Yellowness	Berkshire	5.14 ^{Ba}	4.86 ^{Ba}	5.23 ^{Ba}	6.59 ^{Aa}	0.51
	LYD	4.21 ^{Bb}	4.15 ^{Bb}	4.72 ^{Ab}	5.82 ^{Ab}	0.28
	SEM	0.40	0.31	0.34	0.51	
Chroma	Berkshire	7.76 ^{Ba}	8.00 ^{Ba}	8.61 ^{Aa}	9.61 ^{Aa}	0.52
	LYD	6.72 ^{Bb}	6.88 ^{Bb}	7.88 ^{Bb}	8.39 ^{Ab}	0.73
	SEM	0.45	0.34	1.24	0.47	
Hue angle	Berkshire	41.55 ^{Ba}	38.17 ^{Ba}	37.49 ^B	43.22 ^A	1.52
	LYD	38.19 ^{Bb}	36.92 ^{Bb}	37.32 ^B	43.91 ^A	0.94
	SEM	1.06	1.51	0.92	1.43	

538 LYD: Landrace × Yorkshire × Duroc.

539 SEM: Standard error of the means

540 ^{A-C}Means with upper letter in same animal significantly differ.

541 ^{a-b}Means with small letter in same stage significantly differ.

542 Stage: RM, raw meat; CM, cured meat; DR15, day 15 of drying-ripening; DR30, day 30 of drying-
543 ripening.

544

545 **Table 3**

546 Effect of pig breed and processing stage on texture properties of dry-cured loin.

Parameters	Pig breeds	Stage				SEM
		RM	CM	DR15	DR30	
Hardness (N)	Berkshire	7.17 ^{Aa}	4.33 ^B	2.69 ^C	5.06 ^{ABa}	0.66
	LYD	5.66 ^{Ab}	4.18 ^{AB}	2.29 ^B	4.47 ^{Ab}	0.60
	SEM	1.09	0.54	0.26	0.61	
Cohesiveness	Berkshire	0.38 ^C	0.47 ^B	0.55 ^A	0.51 ^{ABb}	0.01
	LYD	0.39 ^B	0.48 ^{AB}	0.56 ^A	0.54 ^{Aa}	0.03
	SEM	0.04	0.01	0.01	0.01	
Springiness (mm)	Berkshire	0.92 ^A	0.91 ^{Aa}	0.84 ^B	0.82 ^B	0.02
	LYD	0.95 ^A	0.81 ^{Bb}	0.81 ^B	0.84 ^B	0.02
	SEM	0.01	0.03	0.02	0.01	
Chewiness (Nm)	Berkshire	2.45 ^A	1.85 ^{AB}	1.24 ^B	2.11 ^A	0.26
	LYD	2.81 ^A	1.62 ^{BC}	1.03 ^C	1.99 ^{AB}	0.25
	SEM	0.41	0.21	0.11	0.29	

547 LYD: Landrace × Yorkshire × Duroc.

548 SEM: Standard error of the means.

549 ^{A-C}Means with upper letter in same animal significantly differ.550 ^{a-b}Means with small letter in same stage significantly differ.551 Stage: RM, raw meat; CM, cured meat; DR15, day 15 of drying-ripening; DR30, day 30 of drying-
552 ripening.

553

554 **Table 4**

555 Effect of pig breed and processing stage on free amino acid and fatty acid of dry- cured loin.

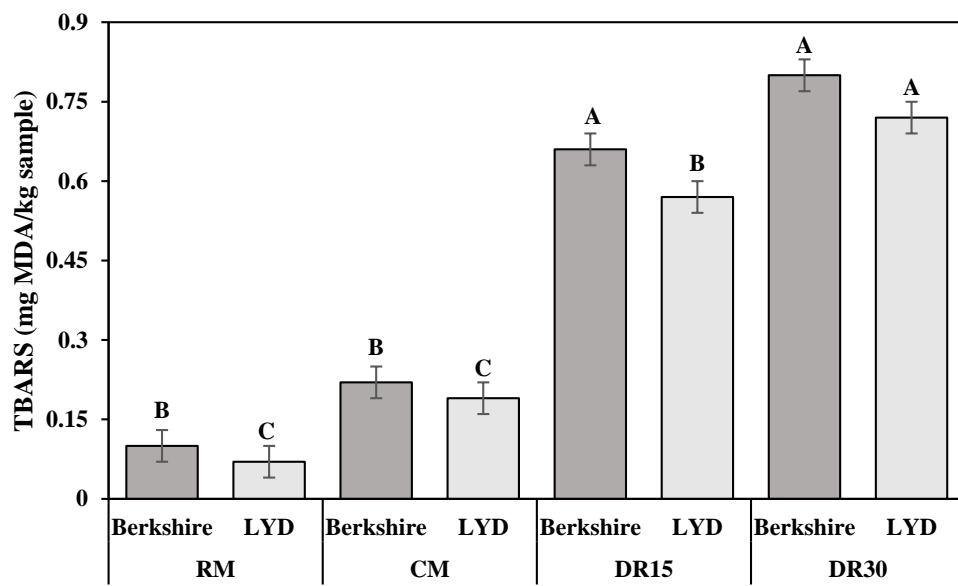
Parameters	Pig breeds	Stage				SEM
		RM	CM	DR15	DR30	
TFAA (mg/100g)	Berkshire	1956.98 ^C	2165.75 ^C	2465.154 ^{Ba}	2690.32 ^{Aa}	35.25
	LYD	1798.34 ^B	1965.56 ^B	2095.36 ^{ABb}	2137.40 ^{Ab}	32.88
	SEM	20.36	30.54	15.32	28.65	
SFA (mg/g)	Berkshire	9.51 ^{Ca}	9.54 ^{Ca}	13.06 ^B	17.09 ^{Aa}	0.77
	LYD	7.75 ^{Bb}	7.60 ^{Bb}	12.93 ^{AB}	15.92 ^{Ab}	0.59
	SEM	0.74	0.43	0.69	0.76	
UFA (mg/g)	Berkshire	15.89 ^{Ca}	15.86 ^{Ca}	22.84 ^{Ba}	27.73 ^{Aa}	0.42
	LYD	10.38 ^{Cb}	11.18 ^{Cb}	17.31 ^{Bb}	25.28 ^{Ab}	0.77
	SEM	0.39	0.59	0.06	0.35	
MUFA (mg/g)	Berkshire	12.19 ^{Ca}	11.57 ^{Ca}	16.94 ^{Ba}	21.92 ^{Aa}	0.74
	LYD	8.28 ^{Cb}	8.27 ^{Cb}	13.53 ^{Bb}	19.61 ^{Ab}	0.45
	SEM	0.08	0.32	0.55	0.45	
PUFA (mg/g)	Berkshire	3.70 ^B	4.30 ^{Ba}	5.90 ^{Aa}	5.81 ^{Aa}	0.79
	LYD	2.09 ^B	2.91 ^{Bb}	3.78 ^{Bb}	4.67 ^{Ab}	0.47
	SEM	0.36	0.35	0.73	0.29	

556 LYD: Landrace × Yorkshire × Duroc.

557 SEM: Standard error of the means.

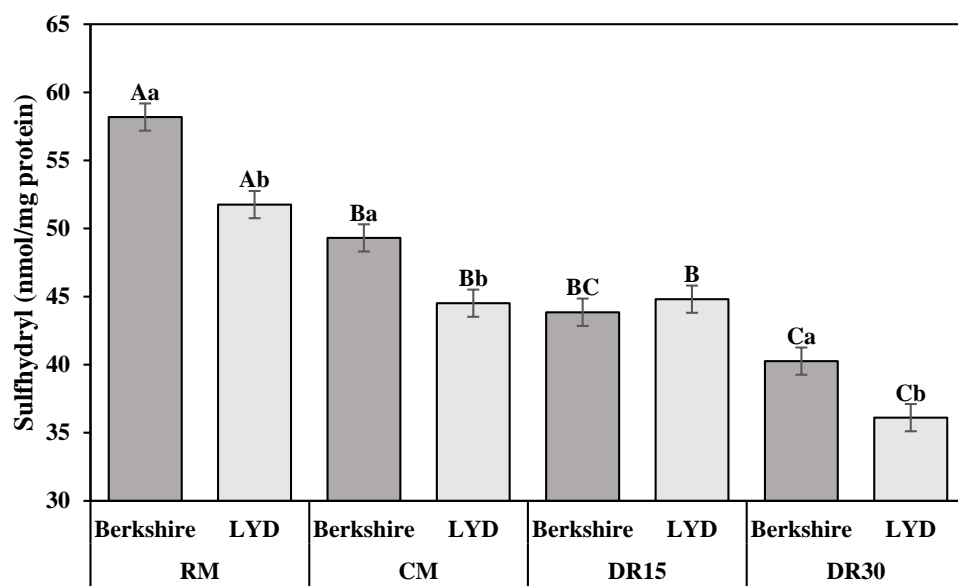
558 ^{A-C}Means with upper letter in same animal significantly differ.559 ^{a-b}Means with small letter in same stage significantly differ.560 Stage: RM, raw meat; CM, cured meat; DR15, day 15 of drying-ripening; DR30, day 30 of drying-
561 ripening.562 TFAA, total free amino acid; SFA, saturated fatty acid; UFA, unsaturated fatty acid, MUFA,
563 monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

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