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Abstract

This study aimed to determine the effects of breed on meat quality characteristics of porcine Boston butt muscles (*M. subscapularis*) from three different pig breeds: LYD (Landrace×Yorkshire×Duroc), Berkshire, and Ibérico available in Korean market. Ibérico showed significantly higher fat content, yellowness (CIE b*), cooking loss, and lower shear force values than LYD and Berkshire. Moreover, the contents of oleic acid (18:1) and palmitic acid (16:0) were significantly higher in Ibérico breed, but stearic acid (18:0) was higher in LYD. As linoleic acid (18:2) and arachidonic acid (20:4) were higher in Berkshire sows as compared to the other breeds, atherogenicity and thrombogenicity indexes were significantly lower in Berkshire sow. Ibérico had lower the ω-6/ω-3 fatty acids ratio, and higher taurine and free amino acids compared with the others. Ibérico also showed significantly greater lipid oxidation, lower antioxidant capacity, and higher hypoxanthine contents, whereas the Berkshire had higher inosine-5'-monophosphate and lower *K*-index value as compared to the Ibérico. The breed did not impart any significant effect on the size and density of muscle fibers. Thus, quality characteristics of Boston butt varied from breed to breed, and certain consumer preferences for Ibérico can be explained, in part, by the unique quality characteristics imparted by higher contents of intramuscular fat, oleic acid, and free amino acids.

Keywords: breed, *M. subscapularis*, oleic acid, free amino acid, nucleotides

Introduction

Pork is an excellent source of quality proteins, important minerals, vitamins, and fat in the human diet (Li et al., 2013). To be cost-effective, pork quality is an important trait for consumers (Sosnicki et al., 2003). In the present days, South Korean consumers prefer pork with relatively high marbling and redness scores. Interestingly, the Republic of Korea has improved swine breeds in terms of production, and the current focus is now on improving pork quality traits across and within the available breeds (Li et al., 2013). Numerous researches have reported that pork quality traits are affected by the breeds and gender of the animals.

It is reported that muscle pH has a significant effect in different breeds, positively influencing meat quality traits such as meat color (Holmer et al., 2009), tenderness (Savel et al., 2005), and lipid oxidation (Hansen et al., 2004). It is well defined that pork with higher fat content has a significant effect on the ultimate pork quality, and is influenced by various factors (breed, sex, age, feed, species, and environmental conditions), imparting the pH, color, cooking loss, shear force, and sensory attributes (Choi et al., 2014). Higher muscle pH is a desirable trait for any pig breed since it influences certain meat quality parameters. In addition, it is well-documented that gender influences meat tenderness within and across the breeds. Increasing levels of soluble proteins in muscle enhances the binding strength of meat at processing, subsequently affecting the meat quality (Toldrá, 2008).

The fatty acid profile of porcine muscle is specific, independent in its functions, and is affected by several factors associated with the genetic background (breed), gender, age, fatness, body weight, dietary fatty acid composition, energy intake, and de novo synthesis of fatty acids (Wasilewski et al., 2011). Deposition and composition of muscle are highly heritable, and vary among and within breeds (Wood et al., 2004). Atherogenic and thrombogenic index are purportedly lipid quality indicators, depending on the contents of a particular group of fatty acids. They characterize the potential predisposition to atherosclerosis and thrombosis in

humans, and have been used to assess the dietetic values of meat.

Meat freshness is a complex concept that includes physicochemical properties, biochemical attributes including biogenic amine, trimethylamine, and volatile amines (putresine, cadaverine epinephrine, dopamine, histamine), and microbiological spoilage (Gil et al., 2011). Despite the shortcomings that pork imported from other counties should have longer storage and freezing/thawing history resulting in less freshness than domestic pork, Ibérico's preferences are increasing from certain Korean consumers. Nonetheless, a thorough investigation of physicochemical properties and pork freshness of Boston Butt (*M. subscapularis*) muscles of various pork varieties available on the Korean market has not been conducted. Therefore, considering the complexity of pork quality and freshness in the domestic pork market, the present study undertook to investigate the effect of the pig breed on the physicochemical properties of porcine Boston butt (*M. subscapularis*) muscles among the LYD, Berkshire, and Ibérico.

Materials and Methods

Animals facilities and porcine samples

Boston butts (*M. subscapularis*) were randomly selected from the slaughterhouses (Namwon and Suncheon), among the most popular varieties in the Korean pork market. Each five carcasses (grade 1) of LYD (Landrace×Yorkshire×Duroc) barrows, Berkshire sows, or Berkshire barrows weighing about 110 kg were used in the study. Ibérico (bellota) butts were obtained from a direct pork trade company importing from Spain. After collecting available pork samples, the muscles were kept at 4℃ until sampled and analyzed for their physicochemical meat quality traits. Muscle fiber samples for analysis were prepared by cutting each muscle into 1.0×1.0×1.5 cm pieces in a direction parallel to the muscle fiber. The specimens were immediately frozen in isopentane chilled with liquid nitrogen, and stored at -

70℃ until histological analysis.

Proximate composition, pH, cooking loss, meat color, and shear force

Moisture contents of *M. subscapularis* muscle excised from the three different pig breeds were determined by drying the samples (3 g) at 102℃ (AOAC, 2000). The crude protein content was measured by the method suggested by the (AOAC, 2000). Lipids were extracted from 5 g of muscle with chloroform/methanol (2:1), according to the (Folch and Lees, 1951). pH values of *M. subscapularis* were measured using a pH meter (Seven Excellence™, METTLER TOLEDO, Switzerland). The lightness (CIE L*), redness (CIE a*), and yellowness (CIE b*) of Boston butt samples from the different pigs were measured using a colorimeter (CR-410, Minota Co. Ltd., Japan). All values of color were taken in triplicate for each sample. Shear force values were measured using a Warner-Bratzler shear attachment on a texture analyzer (TA-XT2, Stable Micro System Ltd., Surrey, U. K.).

Fatty acid composition and nutritional quality indices

The fatty acids composition of porcine *M. subscapularis* muscle was estimated by the method of (O'fallon et al., 2007), with a minor modification. The assay was performed using a Gas Chromatograph-Flame Ionization Detector (Agilent, 7890 series, USA) under the following conditions: injector split mode with split ratio of 25:1, temperature 250℃. High purity air, high purity H_2, and high purity He were used as carrier gases. The flow rate was maintained at 40 mL/min for H_2 and 400 mL/min for air. An HP-88 column (60 m ×250 μm ×0.2 mm) was used for the analysis. Fatty acid composition is expressed as a percentage. The nutritional quality indexes of lipid for porcine *M. subscapularis* muscle were analyzed from the fatty acid composition data obtained for each group of pigs, according to the equations proposed by (Santos-Silva et al., 2002), as shown below:

105 Atherogenicity index **(AI)** = (C12:0 + 4 × C14:0 + C16:0)/[MUFA + ∑ (ω-6) + ∑ (ω-3)]

106 Thrombogenicity index **(TI)** = (C14:0 + C16:0 + C18:0)/[0.5 × ∑ MUFA + 0.5 × ∑ (ω-6) + 3

107 × ∑ (ω-3) + ∑ (ω-3)/∑ (ω-6)]

108

109 **Analysis of free amino acids (FAA)**

110 The soluble amino acids composition of porcine *M. subscapularis* muscle was determined

111 by using a slightly modified method described by (Hughes et al., 2002). HPLC analyses of free

112 amino acids were obtained using an S433 auto analyzer, cation separation column (LCAK07/li;

113 4.6 × 150 mm), buffer change (A: pH 2.90; B: pH 4.20; C: pH 8.00), lithium citrate buffer

114 solution having a buffer flow rate 0.45 mL/min, ninhydrin flow rate 0.25 mL/min, and column

115 temperature 37℃.

116

117 **Lipid oxidation and antioxidant capacity**

118 Lipid oxidation rate of the Boston butt porcine muscle (*M. subscapularis*) was assessed

119 according to the procedure described by (Ahn et al., 1998), with a slight modification in the

120 thiobarbituric acid-reactive substances assay (TBARS). Anti-oxidant capacity of *M.*

121 *subscapularis* porcine muscle from the three different pig breeds was determined by applying

122 the free radical scavenging assay, according to a method described by (Blois, 1958), and is

123 expressed as the DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity (%). The

124 porcine samples were inspected on d 1 and 7 of refrigeration at 4℃.

125

126 **Muscle fiber size and fiber density**

127 Muscle fiber characteristics were determined by the method of (Choi et al., 2012), with slight

128 modifications. Frozen muscles were cut into 10 μm thick transverse sections using a

129 cryomicrotome (CM1860, Leica Biosystems Inc., USA) at -20℃. Each sample was mounted

on 76×26×1 mm adhesive microscope slides (HistoBond®, Paul Marienfeld GmbH & Co. KG, Lauda-Königshofen, Germany), coated with a drop of aqueous mounting medium (S3023, Dako, Carpinteria, CA, USA), and covered with a 22×22 mm coverslip (100 Deckglaser, Menzel-Glaser). All samples were viewed and photographed using a fluorescent microscope (BX51, Olympus, Tokyo, Japan) equipped with a DP72 digital camera (Olympus). Using a Photoshop CC (Adobe, California, USA), cross-sectional area (CSA; μm^2) and muscle fiber density (fiber number/mm^2) was determined from approximately 900 fibers per section.

Nucleotides contents

Nucleotide contents were determined by the method of (Lee et al., 2017), with slight modifications. Briefly, the *M. subscapularis* muscle (5 g) was homogenized with 20 mL of 0.6 M perchloric acid. The homogenate was centrifuged at 2,265 ×g for 15 min (Continent 512R, Hanil Co., Ltd., Incheon, Korea) and filtered through a filter paper (Whatman No. 4, Whatman PLC., Brentford, UK). The filtrate was titrated to pH 5.5 using 0.6 N and 6 N KOH. After titration, the samples were transferred to a volumetric flask and the resultant solution was filtered through a membrane filter (0.2 μm) into a glass vial. Nucleotides were quantified using high performance liquid chromatography (HPLC; Ultimate 3000, Thermo Fisher Scientific Inc., Waltham, MA, USA) with a Synergi™ Hydro-RP 80 Å column (250×4.6 mm, 4 μm particles; Phenomenex Inc., Seoul, Korea). The 20 mM phosphate buffer (pH 5.5) was eluted at a flow rate 1.0 mL/min, detection wavelength 254 nm, and column temperature 30°C. The meat freshness indicator, known as the *K*-index, was determined using the ATP degradation nucleotides formula suggested by (Gil et al., 2011):

$$K\text{-index} = \frac{[\text{Ino}] + [\text{Hx}]}{[\text{IMP}] + [\text{Ino}] + [\text{Hx}]} \times 100$$

Statistical analysis

Data obtained were analyzed by multiple assay techniques, applying the Student-Newman-Keuls for significance test ($p<0.05$) using the general linear model of the SAS program (SAS, 2003). Significant differences were determined by applying the one-way ANOVA. Each treatment was performed in five replication (carcasses), and results are presented as the standard error of the means (SEM).

Results and Discussion

Proximate composition, color, pH, cooking loss, and shear force values

Proximate composition and meat quality characteristics (color, pH, cooking loss, and shear force value) of porcine *M. subscapularis* muscle were examined (Table 1). Results show that moisture contents ranged from 62.11 to 74.16%, with Ibérico pigs harboring significantly lower content than other breeds ($p<0.05$). However, previous studies have reported lower moisture contents ranging from 61.86 to 63.74% in pigs, which is similar to a study by (Lim et al., 2014). It was demonstrated that the Ibérico pigs have a significantly higher lipid content as compared to other groups. The variation of fat content among groups is probably due to the breed effect (Stanišić et al., 2013). Moreover, the higher moisture loss in Ibérico pigs could be attributed to longer storage time under freezing conditions. The crude protein contents in Ibérico pigs were significantly higher than LYD pigs. Besides the ash content, no significant differences were found among the breeds. Our results indicate that except the yellowness value (CIE b*) of Ibérico pigs, the breed does not affect the overall meat color. The enhanced yellowness (CIE b*) for Ibérico pigs is associated with higher fat oxidation and pigment lability, as compared to

other groups (Fernandez-Lopez et al., 2004). It is also noteworthy that Ibérico pigs significantly lost a higher amount of water during cooking than other breeds. It has previously been reported that lower cooking loss is associated with heavier pigs as compared to lighter animals (Magowan et al., 2011), and is also related to cooking processes, such as temperature and time at the heating phase (Madzimure et al., 2017). Considering the shear force value, *M. subscapularis* porcine muscle from Ibérico pigs show remarkably lower shear force value (kg.f) which is associated with more tender meat, as compared to other pig breeds (Table 1). Tenderer meat is related to proteolysis of muscles, specifically myofibrillar and cytoskeletal protein degradation including titin, desmin, nebulin, and troponin-T (Jeleníková et al., 2008). Meat tenderness is also affected by the origin of the animal as well as their age, breed, gender, and environmental conditions, and period of meat ageing (Ouali, 1990).

Fatty acid analysis

The fatty acid composition of muscles is an important factor in determining the nutritional quality of meat or adipose tissue subjected with special attention in human health**.** In our study, we determined the free fatty acid composition of porcine *M. subscapularis* muscle from different pig breeds (LYD, Berkshire, and Ibérico) (Table 2). Our results reveal that the SFA content for Berkshire sow is significantly lower than other breeds ($p<0.05$). Conversely, Berkshire sow harbored a higher content of UFA than the LYD, Berkshire barrow, and Ibérico pigs. The predominant fatty acids, (18:1) and (16:0), were significantly higher in Ibérico *M. subscapularis* porcine muscle. Moreover, significantly higher levels of (18:2) and (20:4) were obtained in Berkshire sow, as compared to the other tested breeds. The PUFA/SFA (P/S) ratio of meat contributes a favorable balance between the ω-6/ω-3 PUFA; the recommended P/S value is greater than 0.4, although meat from some natural sources has a value of around 0.1 (Wood et al., 2004). The P/S values obtained from the three different pig breeds were 0.38, 0.58,

0.41, and 0.20 for LYD, Berkshire sow, Berkshire barrow, and Ibérico pigs, respectively. The ratio of ω-6/ω-3 unsaturated fatty acids of *M. subscapularis* porcine muscle was determined to be 14.32, 17.34, 14.45, and 7.71 for LYD, Berkshire sow, Berkshire barrow, and Ibérico pigs, respectively. The significantly lower ratio of ω-6/ω-3 fatty acid noted for Ibérico *M. subscapularis* porcine muscle attributes as a desirable characteristic in terms of reducing chronic diseases, especially blood clot formations leading to heart attack, in humans (Wood et al., 2004). The recommended ω-6/ω-3 ratio is less than 4.00; however, some meats are higher than the recommended level which, however, can be manipulated by maintaining the P/S ratio in meat. The differences observed in the study were associated to breed effect and different genetic factors. In Berkshire barrow, the SFA level was higher than the Berkshire sow ($p<0.05$), which was in agreement with results reported by (Kasprzyk et al., 2015). However, PUFA levels in the Berkshire sow were significantly higher than other breeds ($p<0.05$), mainly due to the high content of linoleic acid (Table 2). We infer that the higher ω-6/ω-3 ratio is due to the excessive saturated fat and lower levels or deficiency of ω-3 fatty acids in the diet, as well as the influence of breed attributes (Enser et al., 2000).

Lipid quality indexes

The nutritional quality indexes of lipid for *M. subscapularis* porcine muscle excised from three different pig breeds were evaluated (Table 2). Ulbricht and Southgate, (1991) reported that the atherogenic or hyperlipidemic SFAs are (C12:0), (C14:0), and (C16:0) acids, while (C14:0), (C16:0), and (C18:0) acids are thrombogenic SFAs. The long chain unsaturated fatty acids, especially ω-6 (linoleic) and ω-3 (linolenic) fatty acids, are thought to be anti-atherogenic and anti-thrombogenic, indicating that diets should contain lower index values to reduce human cardiovascular diseases (Cebulska et al., 2018). Lower AI value represents a lower proportion of saturated to unsaturated fatty acids, which subsequently reduces the endothelial strength of

blood vessels owing to collapsed lipids and plaque formation (Cebulska et al., 2018). Conversely, lower TI values determined from the proportion of other fatty acids, indicates a lower risk of disturbance to blood coagulation and clotting. In the current study, AI and TI index values obtained were 0.52, 0.46, 0.51, 0.52, and 1.24, 1.01, 1.14, 1.15, respectively, for LYD, Berkshire sow, Berkshire barrow, and Ibérico pigs, respectively. These results indicate that Berkshire sow has significantly lower AI and TI values, as compared with other tested breeds ($p<0.05$); this could be attributed to the higher content of unsaturated fatty acids.

Free amino acids

The free amino acids composition of porcine *M. subscapularis* muscle of the three different pig breeds was analyzed (Table 3). It has previously been classified that lysine, leucine, isoleucine, valine, phenylalanine, histidine, methionine, and threonine are the essential amino acids, whereas aspartic acid, serine, arginine, glutamic acid, tyrosine, glycine, and alanine are non-essential amino acids. Previous studies report that except lysine and histidine, all essential amino acid levels were significantly higher in Ibérico pigs as compared to other breeds ($p<0.05$). Taurine, the functional compound related to ATP production in muscles, was significantly higher in the Ibérico pigs. However, the glutamic amino acid which imparts the umami taste in meat, was significantly higher in LYD pigs as compared to Ibérico pigs (Wood et al., 2004). In the present study, amino acid levels were higher in Ibérico pigs than other breeds, and enriched with almost all essential amino acids, an important factor for eating quality and also imparting numerous health benefits for meat consumers (Subramaniyan et al., 2016). The increment of amino acids in Ibérico pigs can be attributed to breed effect, which depends on the amino peptidase and hydrolytic activity toward increased group with the proteolysis of muscle by enzyme known as calpain (Nishimura et al., 1988; Feidt et al., 1996). Moreover, Ibérico pigs harbor a higher amount of the total tasty and bitter amino acids, as compared with LYD,

253 Berkshire sow, and Berkshire barrow pigs ($p<0.05$).

254

255 **Oxidative stability**

256 We examined the oxidative stability (lipid oxidation) of *M. subscapularis* muscle excised
257 from LYD, Berkshire sow, Berkshire barrow, and Ibérico pigs (Table 4). Lipid oxidation value
258 was significantly higher for Ibérico pigs at both d 1 and 7 of storage, compared to other breeds,
259 as determined by the TBARS value, which revealed a significantly increasing trend with
260 increasing d of storage. Lipid oxidation is one of the primary causes for loss in meat quality,
261 and generates the volatile compounds responsible for rancidity of fresh meat (Gray et al., 1996).
262 Lipid oxidation results in deterioration of some meat quality traits like flavor, texture, and color,
263 and also decreases the shelf life, along with the production of some toxic compounds (Mohamed
264 et al., 2008). In addition to lipid oxidation, it is postulated that muscles with higher fat content
265 in between or across the muscles, show a greater tendency to be oxidized via a continuous free-
266 radical chain reaction (Ruban, 2009). The oxidation rate of post-mortem muscle depends on the
267 antioxidant capacity existing in the muscle of the animal, which can be retarded by the action
268 of endogenous antioxidant enzymes, and differ among different species, different breeds, and
269 or even among animals of a single species (Ren et al., 2013).

270

271 **Antioxidant capacity**

272 Antioxidants are compounds which combat free radicals in the system by intervening in any
273 of the three steps of lipid oxidation, viz., initiation, propagation, and termination (Cui et al.,
274 2004). The total antioxidant capacity of the *M. subscapularis* porcine muscles collected from
275 three different pig breeds was measured by performing the free radical scavenging assay (Table
276 4), and was found to be affected by pig breeds. Ibérico pigs had lower antioxidant capacity
277 compared to LYD, Berkshire sow, and Berkshire barrow at d 1 and 7 of storage ($p<0.05$).

Except the Ibérico pig meat, all other tested groups presented no significant differences between the two storage conditions. Free radical inhibition percentage ranged from 43.06 to 52.73% and 38.95 to 52.56% for d 1 and 7, respectively. In Ibérico pigs, the free radical scavenging activity showed a decreasing trend with increasing number of d. Higher MDA compounds in Ibérico pigs revealed a higher content of lipids that deteriorate the quality of meat, thereby indicating lower antioxidant capacity. Apart from the MDA compounds, the antioxidant activities are also affected by endogenous, non-enzymatic antioxidants, breed, diet, and muscle types (Králová, 2015).

Muscle fiber size and fiber density

Examination of the muscle fiber size and fiber density of porcine *M. subscapularis* from LYD, Berkshire sow, Berkshire barrow, and Ibérico pigs showed no significant variation among the breeds (Table 5). Furthermore, different genders within breeds also had no significant impact on the muscle fiber size and fiber density. Moreover, the muscle fiber number is determined before birth genetically, and only the length and cross sectional area of the muscle fibers increases with age (Wigmore and Stickland, 1983). In addition, it is postulated that the total number, density, and size of different muscle fiber area as well as their composition are important histochemical attributes which impact the fresh meat or cooked meat during conversion from muscle to meat (Joo and Kim, 2011). Considering the muscle fiber density and fiber size, it has been determined that muscle fiber size positively correlates with the carcass weight, backfat thickness and loin eye area, whereas fiber density has a negative correlation in pork quality traits (Ryu et al., 2004). The density, compactness, and space of muscle fiber have been shown in Fig. 1. In our study, breed of the pigs imparted no effect on muscle fiber size and density. More research on muscle fiber types and composition is required to clarify the effect of breed on meat quality traits.

303

304 **Nucleotides contents**

305 The nucleotide contents of porcine *M. subscapularis* muscle from LYD, Berkshire sow,
306 Berkshire barrow, and Ibérico pigs were determined and are presented in Table 6. Ibérico pigs
307 harbored significantly higher amounts of AMP and hypoxanthine than Berkshire sow and LYD
308 pigs ($p<0.05$). AMP content differed depending on the animal age, sex, quality grade, and
309 different cuts, possibly due to higher nucleotide contents. Regardless of the sex, Berkshire pigs
310 had significantly higher IMP compared to Ibérico pigs ($p<0.05$). Nucleotides, AMP, GMP, and
311 IMP are related to the umami and savory taste, while inosine and hypoxanthine impart the bitter
312 taste (Dashdorj et al., 2015). Moreover, IMPs are important metabolites in meat flavor due to
313 the synergistic effect with glutamic acid via the maillard reaction (Lee et al., 2017). The
314 decomposition of ATP in different muscles is considered the most useful and reliable approach
315 to evaluate the correct meat freshness. Owing to ATP decomposition, the analysis is based on
316 the concept that after exemplification of pork, the ATP in meat decomposes in the following
317 sequence: ATP-ADP-AMP-IMP-Ino-Hx (Hernández-Cázares et al., 2010). During these
318 consequential changes, the smell and taste of meat also changes at different intervals. A similar
319 autolytic process takes place in all animal, with variation among the different species. The
320 concentration of ATP alone cannot be implied to measure the freshness index of meat, since it
321 disappears within approximately 24 hours after post-mortem (Karube et al., 1984). In addition,
322 a similar disappearing phenomenon is also observed for ADP and AMP. However, ATP
323 degradation products generated have been suggested as indicators of meat freshness, and hence
324 the concept of *K*-index was developed and introduced by (Gil et al., 2011). Low *K*-index value
325 is considered to indicate fresher meats than higher values. We therefore determined the *K*-
326 index value of porcine *M. subscapularis* muscle from LYD, Berkshire sow, Berkshire barrow,
327 and Ibérico pigs (Table 6). The *K*-index value of LYD, Berkshire sow, Berkshire barrow, and

Ibérico pigs were found to be 79.92, 70.55, 66.80, and 94.21 respectively. Table 6 shows that LYD and Berkshire pigs presented with significantly lower *K*-index value than Ibérico pigs ($p<0.05$). Thus, the significantly lower *K*-index and hypoxanthine content of Berkshire *M. subscapularis* muscle indicates meat quality close to freshness and superior to Ibérico pigs (Hernández-Cázares et al., 2010; Nishimura et al., 1988).

Conclusion

The current investigation demonstrates that breed has a significant impact on pork quality due to the genetic makeup, nutrient composition, and muscle rheological properties. Meat quality traits such as fat content, yellowness, and cooking loss were significantly higher, and shear force value was lower in Ibérico pork, as compared to others. In addition, meat from Ibérico pigs had significantly lower ω-6/ω-3 ratios than LYD or Berkshire, while the content of free amino acids, taurine, and oleic was significantly higher. As a result, compared to other pig breeds, Ibérico pigs have the desired characteristic meat quality attributes for consumers who want highly marbled meat. However, Berkshire pigs had fresher values (lower *K*-index) as compared to other breeds. Therefore, a systematic evaluation of the breeding effects among meat quality parameters of Ibérico pigs can be used for further studies to improve high marbled pork.

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Tables and Figures

Table 1. Proximate composition and meat quality characteristics of porcine *M. subscapularis* muscle from LYD, Berkshire, and Ibérico pigs

Items	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
Moisture (%)	71.33[b]	74.16[a]	73.62[a]	62.11[c]	0.44
Crude protein (%)	19.38[b]	20.84[ab]	20.17[ab]	22.33[a]	0.87
Fat (%)	5.98[b]	4.91[b]	5.62[b]	13.72[a]	0.34
Crude ash (%)	1.06	1.08	1.06	1.04	0.03
CIE L*[2)]	43.25	45.83	47.03	51.42	2.41
CIE a*[3)]	16.98	18.62	17.41	20.52	1.12
CIE b*[4)]	7.48[b]	9.59[b]	8.70[b]	13.48[a]	0.98
pH	5.98	6.02	6.17	6.31	0.11
Cooking loss (%)	10.10[b]	11.96[b]	15.10[b]	27.00[a]	2.63
Shear force (kg.f)	4.82[a]	4.17[a]	3.12[b]	1.78[c]	0.37

[a-c] Values with different superscripts letters within the same row differ significantly (p<0.05).

[1)] SEM: standard error of the means (n=12).

[2)] CIE L*: lightness.

[3)] CIE a*: redness.

[4)] CIE b*: yellowness.

468 **Table 2.** Fatty acid composition (%) and lipid quality indexes of porcine *M. subscapularis*

469 muscle from LYD, Berkshire, and Ibérico pigs

Items	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
10:0	0.11[a]	0.10[ab]	0.12[a]	0.09[b]	0.00
12:0	0.14[a]	0.15[a]	0.14[a]	0.08[b]	0.01
14:0	1.52	1.52	1.55	1.46	0.07
16:0	23.81[b]	22.22[b]	23.47[b]	25.53[a]	0.35
16:1	2.28[b]	2.94[a]	2.97[a]	3.54[a]	0.12
18:0	13.38[a]	9.87[c]	11.53[b]	10.07[c]	0.26
18:1	40.08[b]	38.47[b]	40.68[b]	48.81[a]	0.75
18:2	12.07[b]	15.81[a]	12.39[b]	5.74[c]	0.61
18:3	0.75	0.73	0.75	0.78	0.02
20:2	0.42[a]	0.52[a]	0.44[a]	0.23[b]	0.03
20:3	0.23[b]	0.34[a]	0.23[b]	0.09[c]	0.02
20:4	1.49[b]	2.27[a]	1.35[b]	0.65[c]	0.15
24:1	0.33[b]	0.46[a]	0.34[b]	0.12[c]	0.02
∑SFA[2)]	38.96[a]	33.86[b]	36.80[a]	37.23[a]	0.56
∑UFA[3)]	57.65[c]	61.55[a]	59.14[bc]	59.95[b]	0.45
∑MUFA[4)]	42.70[b]	41.87[b]	43.98[b]	52.46[a]	0.80
∑PUFA[5)]	14.96[b]	19.68[a]	15.15[b]	7.49[c]	0.74
UFA/SFA	1.48[b]	1.82[a]	1.61[b]	1.61[b]	0.04
PUFA/SFA	0.38[b]	0.58[a]	0.41[b]	0.20[c]	0.02
∑ω-6	13.98[b]	18.61[a]	14.18[b]	6.62[c]	0.73
∑ω-3	0.98[ab]	1.07[a]	0.98[ab]	0.87[b]	0.03
ω-6/ω-3	14.32[b]	17.34[a]	14.45[b]	7.71[c]	0.60
AI[6)]	0.52[a]	0.46[b]	0.51[a]	0.52[a]	0.01
TI[7)]	1.24[a]	1.01[b]	1.14[a]	1.15[a]	0.02

470 [a-c] Values with different superscripts letters within the same row differ significantly (p<0.05).

471 [1)] SEM: standard error of the means (n=12).

472 [2)] SFA: saturated fatty acid.

473 [3)] UFA: unsaturated fatty acid.

474 [4)] MUFA: monounsaturated fatty acid.

475 [5)] PUFA: polyunsaturated fatty acid.

476 [6)] AI: atherogenicity index.

477 [7)] TI: thrombogenicity index.

478 **Table 3.** Free amino acids (mg/100g) of porcine *m. subscapularis* muscle from LYD, Berkshire,

479 and Ibérico pigs

Free AA[2)]	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
Taurine	608.65^{b}	640.60^{b}	597.73^{b}	758.73^{a}	32.29
Aspartic acid	193.99	167.27	178.35	164.58	16.93
Threonine	66.26^{b}	68.21^{b}	61.06^{b}	155.58^{a}	3.14
Serine	75.72^{b}	100.25^{b}	98.37^{b}	239.59^{a}	6.09
Asparagine	107.84^{b}	105.80^{b}	95.34^{b}	142.85^{a}	8.59
Glutamic acid	663.40^{a}	465.70^{ab}	384.93^{ab}	251.83^{b}	87.49
Glycine	297.30^{b}	286.15^{b}	295.57^{b}	414.04^{a}	15.14
Alanine	513.90^{b}	447.36^{b}	483.17^{b}	950.96^{a}	28.03
Valine	52.87^{b}	54.74^{b}	55.11^{b}	145.18^{a}	7.91
Methionine	19.98^{b}	18.46^{b}	21.78^{b}	57.08^{a}	3.07
lsoleucine	26.92^{b}	30.52^{b}	29.79^{b}	100.59^{a}	3.47
Leucine	67.00^{c}	86.96^{b}	82.14^{b}	228.34^{a}	4.55
Tyrosin	34.61^{b}	38.04^{b}	41.50^{b}	51.22^{a}	2.71
Phenyalanine	37.96^{b}	37.57^{b}	35.47^{b}	104.00^{a}	2.13
Tryptophan	398.81^{ab}	466.76^{a}	394.62^{ab}	337.08^{b}	23.81
Carnorsine	9.33	215.39	101.9	155.91	84.22
Lysine	46.96	136.79	73.66	106.62	28.17
Ammonia	117.19	161.46	93.82	141.8	34.1
Arginine	72.54	70.6	16.39	65.39	34.56
Histidine	15.36	3.53	1.67	15.4	6.63
∑Tasty AA	2507.76^{b}	2349.93^{b}	2207.08^{b}	3029.21^{a}	136.25
∑Bitter AA	277.27^{b}	298.85^{b}	240.69^{b}	700.59^{a}	34.43
Tasty/bitter AA	9.88	8.16	9.26	4.33	1.35

480 $^{a\text{-}c}$ Values with different superscripts letters within the same row differ significantly (p<0.05).

481 [1)] SEM: standard error of the means (n=12).

482 [2)] AA: amino acids.

Table 4. Lipid oxidation expressed as TBARS value (mg MDA/kg) and antioxidant activity expressed as DPPH radical scavenging activity (%) of porcine *M. subscapularis* muscle from LYD, Berkshire, and Ibérico pigs at different storage period

Items	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
TBARS[3)] (mg MDA/kg)					
1 d	0.20^{by}	0.16^{by}	0.15^{by}	0.35^{ay}	0.02
7 d	0.54^{dx}	0.56^{cx}	0.59^{bx}	0.75^{ax}	0.00
SEM[2)]	0.00	0.02	0.01	0.00	
DPPH[4)] radical scavenging activity (%)					
1 d	50.89^{a}	52.73^{a}	49.27^{a}	43.06^{bx}	1.37
7 d	50.51^{a}	50.51^{a}	52.56^{a}	38.95^{by}	1.27
SEM[2)]	0.81	1.00	1.39	0.59	

$^{a-d}$Values with different superscripts letters within the same row differ significantly (p<0.05).

$^{x-y}$Values with different letters within the same column differ significantly (p<0.05).

[1, 2)] SEM: standard error of the means (n=12).

[3)]TBARS: thiobarbituric acid-reactive substances.

[4)]DPPH: (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity.

501 **Table 5.** Muscle fiber size (μm^2) and fiber density (fiber number/mm^2) of porcine *M.*
502 *subscapularis* muscle from LYD, Berkshire, and Ibérico pigs

Items	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
Muscle fiber density	170.11	183.39	197.42	180.02	9.37
Muscle fiber size (CSA[2)])	5949.81	5558.32	5215.65	5638.26	296.38

503 [1)] SEM: standard error of the means (n=12).

504 [2)] CSA: cross-sectional area.

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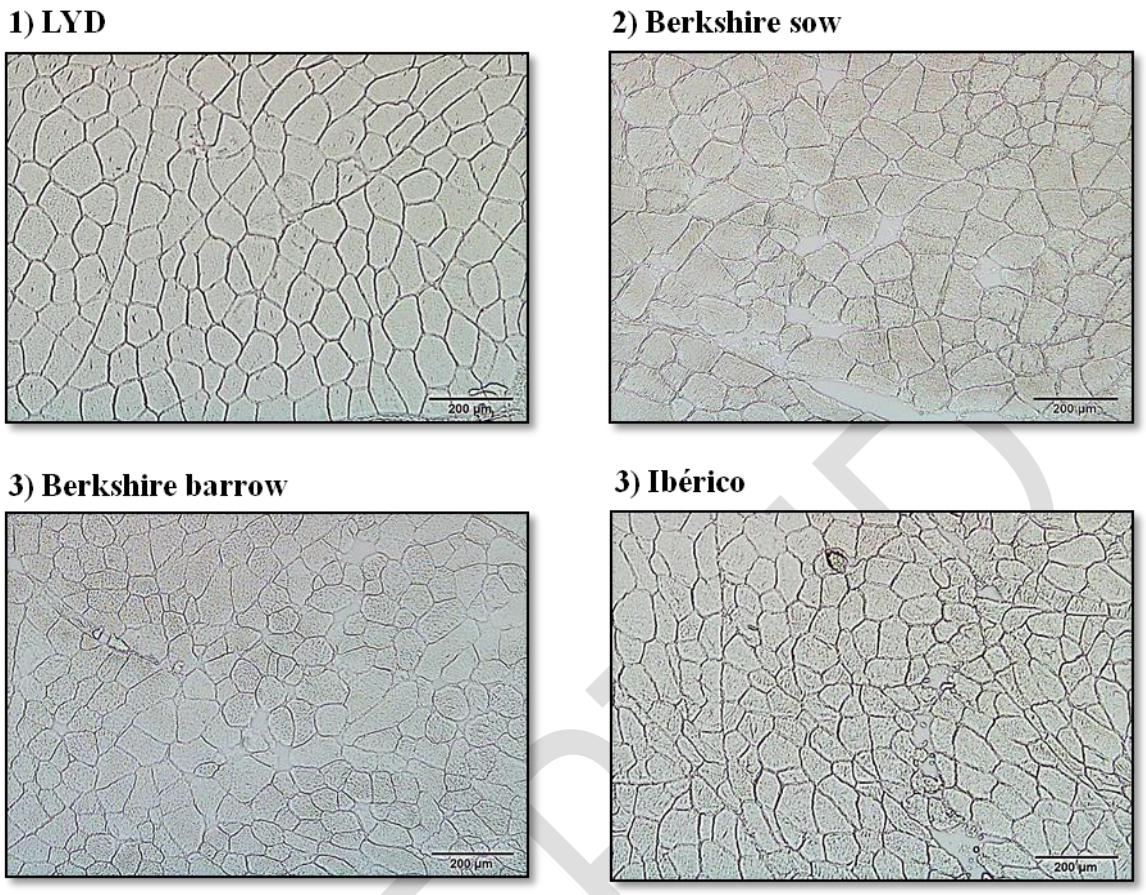

Fig. 1. Optical microscopic structure of porcine *M. subscapularis* muscle from LYD, Berkshire, and Ibérico pigs

Table 6. Nucleotide contents (mg/100 g) and *K*-index (%) of porcine *M. subscapularis* muscle from LYD, Berkshire, and Ibérico pigs

Items	LYD	Berkshire sow	Berkshire barrow	Ibérico	SEM[1)]
AMP[2)]	4.64[b]	4.28[b]	5.16[ab]	5.72[a]	0.23
GMP[3)]	2.21	2.41	2.26	1.43	0.29
IMP[4)]	68.35[a]	103.82[a]	104.45[a]	28.47[b]	10.51
Inosine	43.90	49.07	48.31	35.78	5.87
Hypoxanthine	88.73[b]	71.95[b]	57.84[b]	160.76[a]	9.44
K-index[5)]	79.92[b]	70.55[b]	66.80[b]	94.21[a]	3.37

[a-b] Values with different superscripts letters within the same row differ significantly ($p<0.05$).

[1)] SEM: standard error of the means (n=12).

[2)] AMP: adenosine-5'-monophosphate.

[3)] GMP: goanosine monophosphate.

[4)] IMP: inosine-5'-monophosphate.

[5)] *K*-index = [Inosine]+[Hypoxanthine]/[IMP]+[Inosine]+[Hypoxanthine] x 100.