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# Characterization of *Longissimus thoracis*, *Semitendinosus* and *Masseter* muscles and relationships with technological quality in pigs. 2. Composition of muscles

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ABSTRACT

The composition of three porcine muscles (*Longissimus thoracis*: LT, *Semitendinosus*: ST, *Masseter*: MS) was characterized and its link with muscle quality was evaluated. The LT muscle had a higher content of tyrosine, tryptophan, and carbohydrates and a lower content of vitamin E and haem iron than the MS muscle, while the ST had similar composition to MS but a lower content of haem iron. Large differences between muscles were observed in relative amounts of most of the major fatty acids. The LT muscle had higher saturated fatty acids (SFA) and $n-6$:$n-3$ fatty acid ratio, and lower polyunsaturated fatty acids (PUFA), PUFA:SFA ratio, unsaturation index and average fatty acid chain length than the ST and MS muscles. Muscle pH, redness and chroma were positively correlated with vitamin E and unsaturated lipids and negatively correlated with tyrosine, tryptophan, carbohydrates and saturated lipids, whereas muscle lightness and expressible juice showed similar correlations but an opposite sign with these variables.



## 1. Introduction

Meat and meat products contribute substantially to the human diet as a source of proteins with a high biologic value, essential fatty acids, vitamins (B group) and micronutrients (Zn, Se, Fe) (Higgs, 2000). World pork production occupied the first place in 2009 among other types of meat, representing 40% followed by poultry with 30% and beef with 23% (FAO, 2011). Iron deficiency in humans is one of the most common nutritional problems in both the developed and developing countries (Monsen, 1999), especially in neonates, fertile women and vegetarians. Meat consumption not only supplies haem iron which has higher bioavailability than vegetable non-haem iron (Hunt & Roughead, 2000), but also enhances non-haem iron absorption from other diet components (Mulvihill & Morrissey, 1998). However, pork meat is often controversial because it is considered that it contributes to an excess of fat, saturated fatty acids and cholesterol (Hernandez, Navarro, & Toldrá, 1998). Among the compositional traits related to meat quality, the quantity and nature of the muscle lipids are known to be very important (Fernandez, Mourot, Mounier, & Ecolan, 1995). It is well known that the predominant characteristic of a given muscle is determined by its relative composition in the different types of myofibres, and the lipid content and composition vary among the different pork muscles. However, there are still conflicting results with regard to the effect of muscle type on the content and composition of lipids. Generally, oxidative muscles contain more lipids and the lipids are less saturated than glycolytic ones (Cava, Estévez, Ruiz, & Morcuende, 2003; Hernandez et al., 1998; Leseigneur-Meynier & Gandemer, 1991). In contrast, some authors found no association between total lipid content (Leseigneur-Meynier & Gandemer, 1991) or composition (Kang et al., 2011) and the type of muscular metabolism. In addition, Purchas, Morel, Janz, and Wilkinson (2009) indicated that there are some aspects of muscle composition for pork that have not received much attention because their possible importance from a nutritional perspective has only recently been appreciated, including some of the fatty acids present at low concentrations (Enser, 2001), and some muscle components other than fatty acids that have potential bioactive properties (Purchas, Rutherfurd, Pearce, Vather, & Wilkinson, 2004).

As discussed by Realini et al. (2013), muscle models are needed to evaluate the effect of different processing parameters on the technological and nutritional qualities of meat. The European project 'Design and development of REAlistic food Models with well-characterized micro- and macro-structure and composition' (*DREAM*, 2013, 2009-222654-2; Mackie et al., 2012) is developing food models for use as standards in food research and industry. More specifically, meat models are needed to understand reaction routes promoted by heating such as protein modifications (denaturation, oxidation, aggregation) which may have an impact on the nutritional value of meat (Gatellier et al., 2009). The present study was focused on the selection of well characterized meat categories which could be used as homogeneous 'standard' meat samples. Results concerning some structural characteristics of three porcine muscles (*Longissimus thoracis*, *Semitendinosus*, and *Masseter*) selected as meat categories and its relationship with technological quality were presented in a companion article (Realini et al., 2013). The objective of this study was to characterize the

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nutritional composition of these muscles and to evaluate its link with technological quality (pH, water holding capacity, instrumental colour) of meat.

## 2. Materials and methods

### 2.1. Animals and muscle samples

Heads, loins and hams were obtained from carcasses of ten entire male [Pietrain × (Duroc × Landrace)] pigs reared under the same conditions on the same farm (carcass weight 89 ± 7 kg; lean percent 56 ± 3) at 24 h post-mortem from a commercial abattoir and transported to the IRTA-Monells centre in Spain. *Longissimus thoracis* (LT), between the 10th and 14th thoracic vertebra, and *Semitendinosus* (ST) muscles from the right-side of the carcass, and *Masseter* (MS) muscle from both right and left-sides of the carcass were excised at 48 h post-mortem from the loin, ham and head, respectively. LT and ST muscles were transversally cut from the cranial and the proximal end, respectively, into slices of different thicknesses: 4 cm for pH, instrumental colour, composition (moisture, fat, protein, collagen) and fatty acids, 1.5 cm for expressible juice, 2 cm for content determination of the different types of iron, 1 cm for aromatic amino acids, 1 cm for vitamin E content and 1 cm for the determination of total carbohydrates. MS samples were cut with the same thickness as the LT and ST muscles for pH, instrumental colour, composition and fatty acid determinations from the left-side of the carcass, while samples for expressible juice, vitamin E, aromatic amino acids, iron and total carbohydrate analyses were removed from the right-side of the carcass.

### 2.2. Meat quality traits

Muscle pH, instrumental colour and expressible juice were determined as described by Realini et al. (2013). The ST muscle is not homogeneous in colour showing two major coloured zones. Thus, pH, expressible juice and instrumental colour parameters were determined in two areas of the ST, the dark and deep and the light and superficial parts of the muscle. Samples for the determination of meat composition were analyzed as described by Realini et al. (2013). Meat samples for the determination of fatty acids, vitamin E, aromatic amino acids, and iron content were vacuum packaged and stored at −20 °C until analyses.

### 2.3. Chemical analysis

Lipids were extracted using the chloroform–methanol procedure of Folch, Lees, & Stanley (1957). After evaporation of the extract, fatty acids were converted to fatty acid methyl esters (FAME) following the method ISO 5509-1978 (E) by using 14% $BF_3$ in methanol, and analyzed by gas chromatography (Hewlett–Packard 5890 Series II GC, Avondale, PA, USA) in duplicate using tripentadecanoin (T4257, Sigma-Aldrich, Madrid, Spain) as internal standard. Individual fatty acids were identified by retention time with reference to standards (FA methyl ester mixture 189-19; Sigma-Aldrich, Madrid, Spain). Unsaturation index (UI) and average chain length (ACL) were calculated according to Cava et al. (2003) and the nutritional ratio was calculated as reported by Estevez, Morcuende, and Cava (2006) as indicated in Table 2.

For the determination of tocopherols, muscle samples were extracted with a solution of n-hexane/2-propanol (3:2, v/v) by sonication. The obtained solutions were centrifuged and the supernatant was evaporated to dryness in a stream of nitrogen. The residue was redissolved in 1 ml of n-hexane/ethyl acetate (80:20, v/v) and 20 μl aliquot of the filtered extract was injected into the HPLC system. Samples and standards (5 and 10 ng/ml of α-tocopherol and δ-tocopherol in mobile phase) were analyzed by a normal phase in an Agilent Technologies HP 1100 system (Agilent, Palo Alto, CA, USA) equipped with a fluorescence detector (FLD model 1046A, Hewlett Packard, Palo Alto, CA, USA). Chromatographic separation was performed on a Luna 3 μm, $C_{18}$ (150 mm × 3 mm i.d.) column (Phenomenex, Torrance, USA). The mobile phase consisted of n-hexane/ethyl acetate (80:20, v/v) at a flow rate of 0.55 ml/min. Detection was carried out by fluorescence measurements (excitation wavelength = 290 nm, emission wavelength = 330 nm). Total carbohydrates were determined following the method of AOAC 958.06 (1980), using a spectrophotometer Shimadzu UV-1603 (Shimadzu Europa GmbH, Duisburg, Germany) at 630 nm, and results were expressed as the percentage of total glucose.

The aromatic amino acid content was determined for phenylalanine, tyrosine and tryptophan using derivative spectrophotometry following the procedure described by Gatellier et al. (2009) and all measurements were performed in triplicate.

Non-haem iron was measured spectrophotometrically by the Ferrozine method according to Carter (1971), and haem iron according to Hornsey (1956) as described by Realini et al. (2013).

### 2.4. Statistical analysis

Analyses of variance were performed using the GLM procedure of SAS (SAS Inst. Inc., Cary, NC) with animal and muscle as fixed effects in the model and means separated by the Tukey's Studentized range test. Correlation Principal Component Analysis (PCA) was carried out using the JMP software (SAS Inst. Inc., Cary, NC) in order to evaluate the relationships between the technological quality and the chemical characteristics of the three pork muscles. Correlations among variables were computed by the CORR procedure of the SAS system. Muscle pH, expressible juice and instrumental colour data for the ST muscle were averaged across the light and dark coloured areas of the muscle for correlation analysis.

## 3. Results and discussion

### 3.1. Composition of muscles

The samples used in this study were characterized using microscopy and meat quality characteristics and proximate composition as reported by Realini et al. (2013). Meat composition of aromatic amino acids, vitamin E, total carbohydrates, and iron is shown in Table 1 for LT, ST and MS pork muscles. Gatellier et al. (2009) indicated that the determination of aromatic amino acids is of great relevance to assess the nutritional value of meat since tryptophan and phenylalanine are essential amino acids for humans, and tyrosine can become essential in the diet of individuals living with phenylketonuria. In addition, cooking of meat favours protein oxidation and aromatic amino acids have been identified as particularly sensitive to the oxidative processes (Davies, 1987; Davies, Delsignore, & Lin, 1987). In turn, oxidations in meat induced by heating may limit amino acid bioavailability and are a leading cause of the decrease of nutritional value of meat (Gatellier et al., 2009). In bovine meat, Gatellier et al. (2009) have observed an important decrease of aromatic amino acids with increasing cooking times and temperatures and the indole ring of tryptophan was more stable to the heating treatments than the phenyl ring of phenylalanine and especially of tyrosine. These results are probably transposable to pork during heating. The oxidative degradation of aromatic amino acids depends on the pro- and anti-oxidant status of muscles which vary with the type of fibre. Therefore, the presence of natural antioxidants such as vitamin E, may be expected to have a role in the amino acid bioavailability from meat. No significant differences were detected among muscles in the content of phenylalanine, but the tyrosine percentage was higher in LT than ST and MS, and the tryptophan percentage was higher in LT than MS. Purchas et al. (2009) evaluated the chemical composition of pork muscles and found higher contents of tyrosine