Universidad de Extremadura
Departamento de Zootecnia

Evaluación de la calidad y aptitud tecnológica de la carne y productos curados de cerdos ibéricos procedentes de distintos cruces con líneas genéticas de cerdo duroc

Assessment of the quality and technological characteristics of meat and dry-cured products from iberian pigs crossed with different Duroc genetic lines

Rosario Ramírez Bernabé
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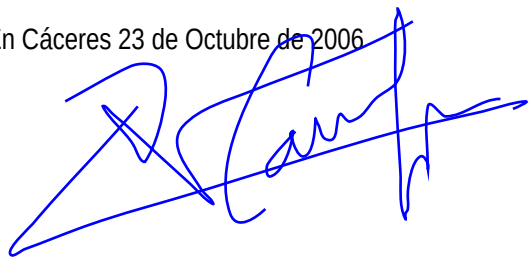
INFORMA

que la Tesis Doctoral titulada "Evaluación De La Calidad Y Aptitud Tecnológica De La Carne Y Productos Curados De Cerdos Ibéricos Procedentes De Distintos Cruces Con Líneas Genéticas De Cerdo Duroc" presentado por la Licenciada Dña María Rosario Ramírez Bernabé, ha sido realizado bajo mi dirección en la Unidad de Tecnología de los Alimentos de la Facultad de Veterinaria. Hallándose concluido y reuniendo a mi entender las condiciones necesarias, autorizo su presentación para su defensa ante el tribunal que ha de juzgarlo.

En Cáceres 23 de Octubre de 2006



Fdo.: Dr. Ramón Cava López

Ahora, cuando se contempla con satisfacción el final del trabajo realizado, creo que es el momento de mostrar mi agradecimiento a muchas personas e instituciones que de forma directa o indirecta han contribuido a que esta Tesis Doctoral haya finalizado.

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A mis padres, a mi hermana.

INDEX

ABBREVIATIONS

ADG. Average daily gain.
BF. *Biceps femoris*.
BFT. Backfat thickness.
BOE. Boletín Oficial del Estado.
CL. Cook loss.
DL. Drip loss.
DMA. Dimethylacetals
DU. Duroc.
FA. Fatty acids.
FAMEs. Fatty acids methyl esters.
FID. Flame ionization detector.
FFA. Free fatty acids.
G6PDH. Glucose 6 phosphate dehydrogenase.
GEN. Genotype.
HFT. Hamfat thickness.
IILP. Iron-induced lipid peroxidation.
IMF. Intramuscular fat.
KOP. Killing out percentage.
LD. *Longissimus dorsi*.
Mb. Myoglobin.
ME. Malic enzyme.
MUFA. Monounsaturated fatty acids.
NL. Neutral lipids.
PL. Polar lipids.
PUFA. Polyunsaturated fatty acids.
SCF. Subcutaneous fat.
SEM. Standard error mean.
SFA. Saturated fatty acids.
TBA. Tiobarbituric acid
TPA. Texture profile analysis.
WBSF. Warner-Bratzler shear force.
WL. Weight loss.

General abstract

GENERAL ABSTRACT

Three different Iberian x Duroc genotype pigs were studied: GEN1: ♂ Iberian x ♀ Duroc1; GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype of pigs Duroc1 (DU1) was selected for the manufacture dry-cured meat products while Duroc2 pigs (DU2) were selected for meat production. Meat quality and storage stability were evaluated in *Longissimus dorsi* and *Biceps femoris* muscles. In addition, dry-cured meat products quality was studied in dry-cured loin and dry-cured ham. No important differences were found between reciprocal crosses, and some of them could by caused by the different slaughter weight. However, the Duroc sire line affected meat and dry-cured meat products quality. Although GEN3 had better production parameters and higher meat pieces yields, meat quality from this genotype in both muscles had characteristics related to PSE condition. Besides, meat from this genotype was most sensitive to changes under storage. Meat products quality from GEN3 was also lower, mainly caused by: the lowest fattening level of the pieces for the manufacture of meat products and the lowest postmortem pH. Therefore, the cross with non-adequate Duroc sire lines could seriously decrease meat and meat products quality from Iberian pigs.

RESUMEN GENERAL

Se estudiaron 3 cruces ibérico x duroc: GEN1 (♂ ibérico x ♀ duroc1); GEN2 (♂ duroc1 x ♀ ibérico); GEN3: (♂ duroc2 x ♀ ibérico). GEN1 y GEN2 son cruces recíprocos, mientras que GEN2 y GEN3 se diferencian en la línea paterna duroc. El genotipo de los cerdos duroc1 correspondió a animales seleccionados para la producción de productos cárnicos, mientras que el genotipo de los cerdos duroc2 correspondió a un genotipo carnicero, con animales seleccionados para la producción de carne. Se evaluó la calidad de la carne fresca y los cambios durante la refrigeración en 2 músculos (*Longissimus dorsi* y *Biceps femoris*). Además se estudió la calidad de los productos curados (lomo y jamón curado). No se encontraron grandes diferencias entre los cruces recíprocos, y las diferencias que se encontraron, pudieron deberse a las diferencias del peso al sacrificio. Sin embargo, la línea paterna utilizada tuvo un gran efecto en la calidad de la carne y los productos cárnicos curados. De este modo, aunque el GEN3 obtuvo mejores parámetros productivos y mayor rendimiento de piezas nobles, la calidad de su carne fresca en los dos músculos analizados fue inferior, ya que ésta mostró características de carne con condición PSE y una mayor susceptibilidad a los cambios durante la refrigeración. La calidad de sus productos curados también fue inferior, principalmente debido 2 causas: el menor engrasamiento de las piezas cárnicas para la elaboración de productos curados y el valor del pH postmortem más bajo en este genotipo. Por tanto, la utilización de genotipos duroc no adecuados podrían comprometer seriamente la calidad de la carne y de los productos curados procedentes del cerdo ibérico.

Introduction

INTRODUCTION

1. - Economical importance of pork production and Iberian production in Spain.

Within the Spanish meat production sector, the importance of pork production (Figure 1) is considerable since in 2005, pork production was the 58% of total production, followed by poultry (24%), beef (13%) and sheep (24%). In addition, pork production has importantly increased from 1989 to 2005, as pork produced has been almost duplicated (1,703,439 vs 3,163,860 Tm) during these years.

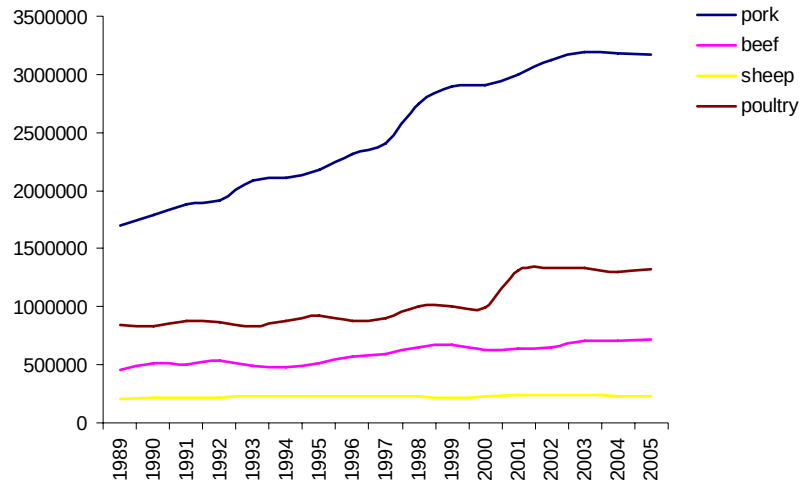

Figure 1.- Meat production (Tm) in Spain in the last years (Source: AICE: asociación de industrias de la carne en España. web: www.aice.es).

A large proportion (38%) of pork produced is destined to the production of meat products. Dry-cured meat products hold a large place on the market, especially in the Mediterranean countries (Chizollini *et al*.,1998). In Spain, the production of dry-cured meat products means the 36% of the total meat products (AICE, 2005). This production, which includes a wide range of products such as dry-cured sausages, loins, shoulder and ham, had slightly increased in the last decade, although recently, it has remained unchanged (Figure 2).

In Spain, among dry-cured meat products, the most valuable are those from Iberian pig. The importance of Iberian pig production is not due to its production levels, but by its turnover volume, as the prices have been continuously rising in the last years. As a result, according to the MAPA (Ministerio de Agricultura Pesca y Alimentación) the number of Iberian pigs in Spain has been duplicated, as in 2003 there were a total of 2,078,365 Iberian pigs, while in 1997 945,887 pigs were slaughtered (Figure 3).

The main reason of this spectacular increase is the growing demand of Iberian dry-cured meat products, as despite of its high price, they are very appreciated by consumers because of their image of traditional products. Beside this, the high consumer acceptability of Iberian dry-cured meat products is mainly sustained on their unique and much appreciated sensory quality. That is consequence of the characteristics of the raw material, which are associated with the characteristics

of the Iberian breed and the traditional rearing system as well as the prolonged traditional processing method, which in the case of dry-cured ham can reach between 1-2 years.

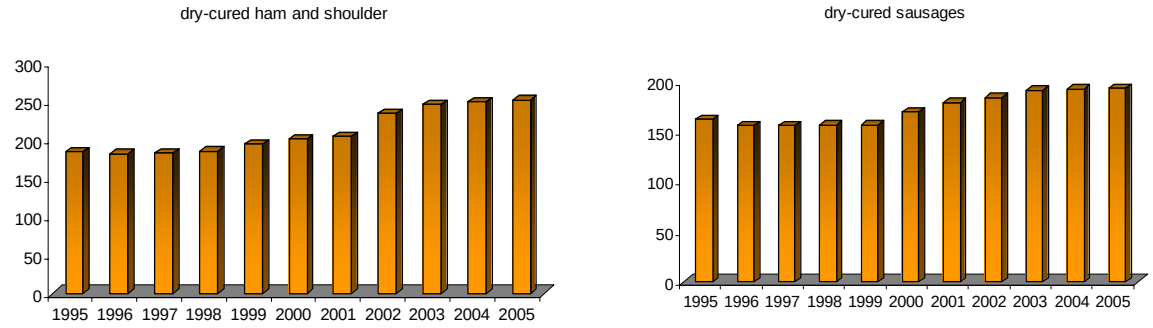

Figure 2.- Dry-cured meat products production (thousands of Tm) in Spain in the last decade (Source: AICE: Asociación de Industrias de la Carne en España. web: www. aice.es)

Moreover, recently, the consumption of Iberian pork has grown (although no data is available). Iberian pork can be considered a high quality alternative to meat from lean pigs commonly consumed in Spain. Different studies (Estévez *et al*., 2003a,b) have compared Iberian and industrial genotypes meat quality and have found better quality traits in Iberian pig such as better visual appearance and shelf-life.

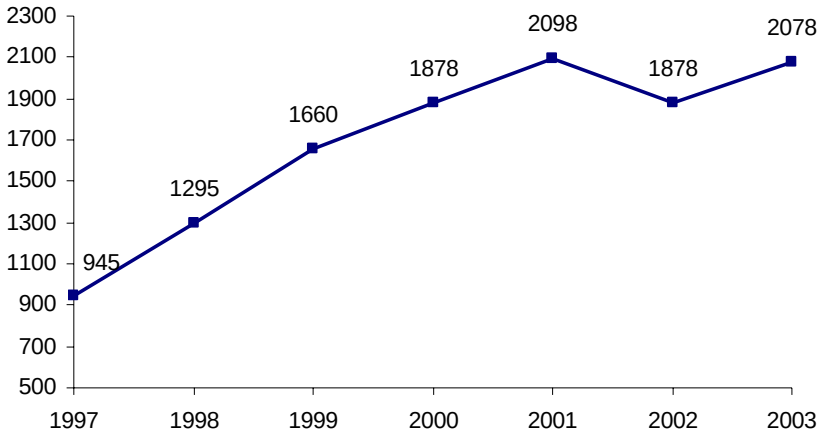

Figure 3.- Thousands of heads of Iberian pig in Spain in the last years (Source www.MAPA.es)

2. – The Iberian pig and its rearing systems.

The Iberian pig is considered a rustic breed traditionally free-range reared in the south west of the Iberian Peninsula. The Iberian breed is one of the scarce non-improved breeds which survives to the modern techniques of pig production based on improved genotypes and intensive rearing systems. The origins of the Iberian breed are in ancient pigs named *Sus mediterraneus* or *Sus scrofa meridionalis.* The Iberian pig is perfectly adapted to environment conditions of the Mediterranean evergreen forest in which are free-range reared. The Iberian pig's habitat is the pastureland known

as “Dehesa”, the ecosystem of the “Mediterranean forest”, mainly composed by evergreen oaks (*Quercus ilex*), cork oaks (*Quercus suber*) and gall oaks (*Quercus lusitanica*).

Iberian pig production is unique in Europe and represents a sustainable and friendly system of production, in which this breed is perfectly adapted to the environmental conditions of the south west of the Iberian Peninsula, characterized by cold winters and dry and hot summers, together with periods of abundance and shortage of feed which coincides with the autumn-winter and summer seasons, respectively. Traditionally, the origin of the production system of Iberian pig was to take advantage of the natural resources of the environment, such as acorns and pasture, mainly during the autumn and winter seasons in a productive system named “montanera”. However, natural resources in this rearing system are available only for a limited number of pigs and for a few months (3–4 months) since acorns are only available from late November to March.

“Dehesa’s” sources are a limiting factor for this production as it is not always possible to feed all the pigs by this system. Consequently, it is becoming common the supplementation with concentrate fodders during fattening phase in free-range systems or the use of concentrates and more intensive production systems such as in semi-intensive or in confinement rearing systems (López-Bote, 1998). Nowadays, it is estimated that around the 60% of Iberian pigs are intensively reared (Cruz, 2006).

Although in the last years the number of Iberian pig has been increasing, reaching more than 2 millions of animals (AICE, 2005), this situation was not the same 50 years ago. In the fifties and sixties, the census of Iberian pig was so small that it was nearby to disappear. But in the seventies and especially in the eighties, the demand of dry-cured Iberian meat products increased the same as the income of the Spanish families.

This traditional production system, in which pigs need to be reared for a long time to achieve the high weights necessary for the manufacture of dry-cured meat products and which also need a long time of ripening, has survived due to the high prices of these meat products.

During the fattening phase (free-range reared or semi-intensively reared) Iberian pig will put on a third of its final weight in about 3 months (from 105kg to 145-150kg) mainly in the form of subcutaneous fat and in the muscle (intra and intermuscular fat). Pigs are slaughtered at high live weights (140-160 kg) with around 14 months, because the quality characteristics of the meat products require carcass with high fat content. During this period, subcutaneous adipose tissue and intramuscular lipid content are importantly increased, leading to a raw material very different from industrial genotypes (Coutron-Gambotti *et al*., 1998; López-Bote, 1998).

Dry-cured hams are the most appreciated Iberian product, followed by dry-cured shoulders and dry-cured loins. These products are classified into different commercial types with variable prices according to the fatty acid profile of the subcutaneous fat and the rearing system. The more expensive dry-cured products are those from pigs reared outdoors in the “Dehesas” and fed mainly with acorns and pasture, while the least expensive come from pigs reared indoors with concentrate feeds. In the market, products with different quality can be found depending on these parameters. So, dry-cured meat products are considered as “*de bellota” o “terminado en montanera”*, when the pigs are fattened on acorns and grass; *“de recebo” o “terminado en recebo”* when the end of the

fattening period of the pigs is by acorns and grass as well as with commercial diets and "*de cebo"* when pigs are only fed with commercial diets.

Important differences in the quality of dry-cured hams have been reported depending on the fattening period. In general, dry-cured products from free-range-reared pigs fed on acorn and pasture had better quality than pigs fed on concentrates (i.e. Carrapiso, *et al*., 2002a; Cava *et al* 1997; Petrón *et al*., 2004).

In Iberian pig, the fat content and its fatty acids composition play a determinant role in the nutritive, sensory and technological quality of the meat for the manufacture of dry-cured products. Meat from Iberian pigs free-range reared reflects the fatty acid composition of acorns (with high levels of oleic acid) and grass, being rich in monounsaturated fatty acids, especially oleic acid (C18:1n-9) which is considered one of the main characteristics of Iberian pig's tissues and products (Cava *et al*., 1997). Acorns and pasture are also sources of antioxidant substances (such as alpha and gamma tocopherols) and meat from animals free-range reared contains higher amounts of these compounds than those from pigs reared under other rearing systems (Rey *et al*., 2006; Cava *et al*., 1999). High monounsaturated fatty acids and antioxidant contents provide to the meat from pigs free-range reared a high oxidative stability under storage (Cava *et al*., 2000a; Estévez *et al*., 2003a) or in the manufacture of dry-cured meat products (Cava *et al*., 1997; Ruiz *et al*., 1998, Cava *et al*., 2000b).

3. – The Duroc breed and its relationship with the Iberian breed.

The Iberian pig is one of the non selected pig breeds which has survived without changes from centuries. The lack of selection in terms of productive parameters has conducted to a breed with poor growth performance and production traits in comparison with industrial genotypes, such as low prolificity (7-8 piglets per litter), 1.6-1.7 litter/sow/year and a low growth rate (Daza, 2000). For these reasons, the Iberian pig has been crossed with breeds such as Tamworth, Large Black, Berkshire, etc, to increase its productive parameters. Duroc was the breed which had better results in the crossing with Iberian (Aparicio, 1987). So, nowadays, crossbreeding of Iberian pig is limited to crosses with Duroc breed.

The origin of Duroc breed is in the USA (Briggs, 1969). The Duroc breed was introduced in Europe mainly due to its higher intramuscular fat content compared with other breeds (Barton-Gade, 1987) which has a positive influence on sensory qualities especially in dry-cured meat products (Gandemer, 2002). In addition, Duroc crosses have higher postmortem pH (Tibau *et al*., 1997; García-Macías, *et al*., 1996), higher redness and IMF content (Armero *et al*., 1999) and better juiciness, tenderness and flavour (Candek-Potokar *et al*., 1998) than other breeds.

Iberian pigs have been traditionally crossed with Duroc pigs to improve some production parameters such as the growth rate, the weight at slaughter and the carcass yield (López-Bote, 1998). By this cross, production parameters have been improved such as more piglets per sow, with increases of the prolificity of 2-3 pigs; it also improves the growth rate, the feed efficiency and the lean content, particularly of hams and loins, increasing the weight at weaning and at end of fattening (Dobao *et al*., 1986, Aparicio, 1987).

The cross with Duroc is not considered to produce serious damages in the quality of meat and dry-cured meat products (Tejeda *et al*., 2002, Antequera *et al*., 1994, Andrés *et al*., 2001). However, other authors consider that crossbreeding had a slight effect on dry- cured ham (Carrapiso *et al*., 2003) and dry-cured loin quality (Ventanas *et al*., in press) and others have reported more marked differences in *Biceps femoris* muscles between pure Iberian pigs and Iberian x Duroc genotypes, such as a reduction of IMF and iron levels and the modification of the fatty acid composition (Ventanas *et al*., 2006a).

4. – Policy and regulation of Iberian dry-cured meat products: Quality regulation of Iberian dry-cured meat products (Norma de Calidad para el jamón ibérico, paleta ibérica y caña de lomo ibérico elaborados en España. R.D. 1083/2001 B.O.E. 5th October, 2001).

In the year 2000, the number of Iberian reproductive sows was around 200,000 heads; though only around the 50% were pure Iberian (Buxadé, 2000). Although this percentage seems to be high, it has importantly decreased in the last years since for instance in the seventies most of the sows (85-90%) were crossed (Daza *et al*., 2000).

Because of the increase of the demand of Iberian products and the high frequency of the Iberian x Duroc crosses, a specific law to regulate Iberian market was passed in 2001 (R.D. 1083/2001 B.O.E. 5th October, 2001). One of the main aspects that this law regulates is the pig genotype for the manufacture of Iberian meat products. Besides of pure Iberian pigs (100%), it also permits the crosses with Duroc, but in the crosses it is necessary to use pure Iberian sows, while sire genotype can be Duroc or Iberian x Duroc, in order to preserve the biodiversity of the Iberian breed.

On the other hand, this law has produced important changes in the traditional reproductive scheme in Iberian x Duroc crosses, as Duroc or hybrid sows were preferred instead of Iberian sows since it was generally believed the existence of production advantages. In this respect, a recent study (Morcuende *et al*., in press) has reported an effect of the maternal line on the characteristics of the piglets, especially on production parameters, which is known as “maternal effect”; so pigs from Iberian sows had worse production parameters than those from Duroc sows. However, Iberian x Duroc reciprocal cross does not affect quality traits (Ventanas *et al*., 2006a; Morcuende *et al*., in press).

Nevertheless, Duroc breed can not be considered a homogeneous breed due to its widespread distribution what has made it object of different genetic selections. Important differences have been reported between Duroc lines by Lonergan *et al*., (2001), who found a serious reduction of the meat quality from a selected Duroc line for increased lean growth efficiency. Meat from this line showed higher drip loss and lower early *postmortem* pH values. Moreover, Soriano *et al*., (2005) and Cilla *et al*., (2006) evaluated different Duroc sire lines in crosses with industrial genotypes and found an important influence of the Duroc line on carcass composition, meat and dry-cured ham quality. This means that, when choosing animals for purchase from farms, it will be advisable to take into account the genetic traits selected and not just the animal breed.

5. – Iberian dry-cured meat products manufacture.

The manufacture of Iberian dry-cured loins and hams has an important tradition in Spain. Iberian dry-cured meat products have been traditionally produced by means of natural weather conditions. In traditional process, the pigs are slaughtered in winter and loins, shoulders and legs are salted, dried and matured under natural conditions of temperature and relative humidity: the relative humidity is continuously reduced as long as the temperature is increased. Sodium chloride, nitrites and the dehydratation of the pieces during drying process conduct to the stabilization of the piece without risk of spoilage by reducing water activity.

In the case of Iberian dry-cured ham, the total manufacture process lasts between 20-36 months. The manufacture of Iberian dry-cured ham comprises four stages: 1. Salting “Salado”, 2. Post-salting/resting “Post-salado/Asentamiento”, 3. Drying “Secadero” and 4. Ageing in cellar “Bodega” (Figure 4). In the first, hams are rubbed with salt and nitrites/nitrates and placed in piles with alternate layers of hams and salt at refrigeration temperature (0- 4°C) and high relative humidity (90-95%) for 0.65-2 days per kg (López-Bote, 1998). After salting is completed, ham surface is brushed to remove the rests of salt. Lately, consumer’s demands and health requirements tend to decrease salt content of dry-cured ham; however, this could favour the development of softer textures, which could affect the overall quality of dry-cured Iberian ham (Andrés *et al*., 2004). In the post-salting/resting phase, hams are held at increasing temperature (4-15ºC) and decreasing relative humidity (90-70%) for 80-100days to favour a homogeneous distribution of salt throughout the piece and the loss of water that conduct to the stabilization of the ham. At the end, the piece is stable from a point of view of spoilage. After post-salting, hams are held under natural conditions during the summertime (for around 1-1.5months), temperatures could reach more than 30ºC and the relative humidity is low (less than 50%). High temperature for a long period lead to an intense formation of flavour and taste precursors that will be transformed into flavour and taste compounds in the next phase. Finally, the hams are aged in cellars (“bodegas”) under natural conditions with mild temperatures (14-18ºC) and relative humidity (65-80%) for more than 12 months. This is the most important and distinctive phase in the manufacture of dry-cured Iberian ham. Time and moderate temperature lead to the formation of pleasant odour, taste and flavour compounds derived from fat and protein and the formation of new compounds from the interaction of lipid and protein-derived compounds (Ruiz *et al*., 1999). Microorganisms play a key role in this stage for the generation of flavour precursors in dry-cured meat products (Nuñez *et al*., 1996; Martin *et al*., in press).

In dry-cured loin, the process is very different. The muscle is rubbed with a seasoning mixture of olive oil, salt and species (garlic, oregano and Spanish paprika) for some days at refrigeration temperature. Then muscles are stuffed into casings and they undergo a drying process during 3-4 months at around 6ºC and 80-85% of relative humidity.

In dry-cured ham, the rise of temperature during several stages of the processing, and the prolonged ripening time in the cellar allow a group of complex chemical reactions (lipolysis and lipid oxidation, protein and amino acids degradation and Maillard reactions) to take place in a greater extent than in other type of dry-cured hams, which contribute to the volatile and non-volatile compounds formation (García *et al*., 1991; López *et al*., 1992).

Fatty acids released during processing of Iberian ham mainly come from phospholipid fraction (Martin *et al*., 1999). The development of lipolytic process enhances lipid oxidation reactions and produces a wide variety of aromatic compounds. Additionally, during the ripening of dry-cured ham, proteins also undergo proteolitic reactions (reviewed by Toldrá *et al*., 1997), which favour the interaction of these compounds with others derived from lipid oxidation by means of Maillard reaction. This is an important source of volatile compounds with great influence in the overall final flavour of Iberian dry-cured ham (Carrapiso *et al*., 2002b). In general, Iberian ham flavour is increased with ripening time (Ruiz *et al*., 1998), however, some authors have reported that an excessive time of ripening decreases the acceptability of dry-cured ham due to the appearance of texture problems (Cilla *et al*., 2005). On the other hand, recently, Ventanas *et al*., (2006b) have reported an increase of protein oxidation associated with lipid oxidation during ripening of dry-cured products, which suggests that oxidation processes not only affect lipids but also proteins.

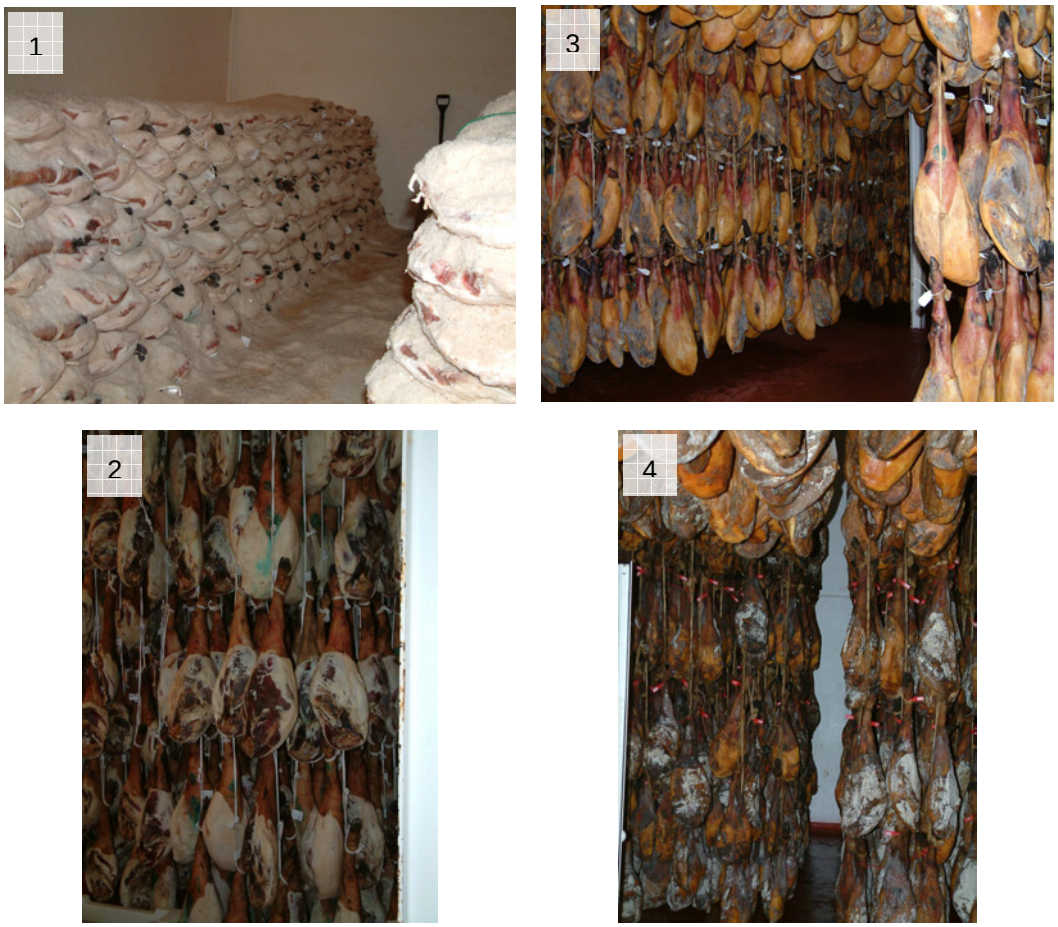

Figure 4.- Stages of the manufacture of Iberian dry-cured ham (1. Salting "*Salado*", 2. Post-salting/Resting "*Post-salado/Reposo*", 3. Dryer "*Secadero*", 4. Ageing in cellar "*Bodega*")

6. – Factors of quality of meat and meat products

The quality of meat and meat products is influenced by intrinsic factors such as breed, genotype, type of muscle, feeding regime, and extrinsic factors such as pre-slaughter handling and slaughter procedure, which influence pH decline and ultimate pH (reviewed by Rosenvold and Andersen, 2003)

6.1. - Breed and genotype

Several studies have been devoted to determine the effect of different pig breeds and crosses on production performance (Blasco *et al*., 1994; Blanchard *et al*., 1999) and meat and meat products quality traits (Guerrero *et al*., 1996).

Two types of genes, polygenes and major genes are distinguished among genes affecting meat quality traits. Polygenes are defined as genes whose individual effect on a trait is small in relation to the total variance of this trait. A gene is considered a major gene when the difference between the mean values of the individual homozygotes for this gene is equal or superior to one phenotypic standard deviation of the trait. Two major genes affecting meat quality traits have been identified in the pig: the Halothane gene and the RN gene (Sellier and Monin, 1994). The abbreviation RN stands for Rendement Napole (Napole yield; Naveau *et al*., 1986).

Halothane gene

The Halothane gene causes malignant hyperthermia, which can be triggered by stress or exposure to the anaesthetic gas halothane (reviewed by Sellier, 1998). The effects of this gene have been closely associated to development of pale, soft and exudative (PSE) meat (Briskey, 1964). It is widely accepted that halothane sensitivity induces an acceleration of the post mortem pH decline, and consequently increases the frequency of PSE meat (Sellier and Monin, 1994) and also affects the colour as this factor is strongly influenced by the post mortem pH decline and ultimate pH (Warris and Brown, 1987; Bendall and Swatland, 1988).

RN gene

The presence of a dominant mutation (denoted RN-) with large effects on meat quality and processing yield in Hampshire pigs is associated with reduced technological yield (Napole yield or in French Rendement Napole, from which the gene has its name) (Naveau, 1986; Lundström *et al*., 1996). This mutation is related to the codification of a muscle-specific isoform of the regulatory gamma subunit of adenosine monophosphate-activated protein kinase (AMPK). AMPK has a key role in regulating energy metabolism in eukaryotic cells and is activated by an increase in the ratio of adenosin monophosphate (AMP) to adenosin triphosphate (ATP). Activated AMPK is expected to inhibit glycogen synthesis and stimulate glycogen degradation. The RN- mutation has only been found in Hampshire pigs or crossbred pigs including Hampshire, and not in any other pig breed (Milan *et al*., 2000), although new alleles has been identified in the pig breeds Hampshire, Large White and Wild Boar (Milan *et al*., 2000). Ciobanu *et al*. (2001) found the new allele in the pig breeds Landrace, Large White, Berkshire, Duroc and Duroc Synthetic and reported that it had effects on glycogen content and on certain meat quality traits, such as ultimate pH and colour.

6.2.- Gender

The influence of sex on pork quality is controversial. Numerous papers have described significant differences between castrated, males and females in parameters closely related with meat quality such as tenderness, juiciness or palatability (Fortin *et al*., 2005; Jeremiah *et al*., 1999; Ellis *et al*.., 1993 and 1996). However others authors did not found differences in meat quality traits as affected by gender (Blanchard *et al*., 1999). Martel *et al*., (1988) and Nold *et al*., (1997) described that meat

from castrated was more tender than meat from entire males and females, probably due to a higher intramuscular fat content. In dry-cured hams, the use of castrated males conducts to improved traits against entire males (Bañón *et al*., 2003). Similarly, Gou *et al*., (1995) reported lower weight loss during drying of dry-cured hams and higher intramuscular fat contents in hams from castrates than those from females.

6.3.- Type of muscle

Differences between oxidative and glycolytic muscles are caused by a different fat content, fatty acid profile of lipid fractions and prooxidant substances content. Glycolytic muscles contain less total lipids, triglycerides and phospholipid than oxidative ones (Alasnier *et al*., 1996). This is caused by the smaller fibre diameters and the higher proportion of mitochondrial membranes of oxidative muscles than in glycolytic ones. Additionally, long-chain PUFA, which are the main substrates for oxidation during meat processing, are more abundant in phospholipids from oxidative muscles than in glycolytic ones (Alasnier and Gandemer, 1998). Fatty acid composition of phospolipids influences oxidative stability of pork, as the higher the proportion of PUFA, the more the susceptibility to oxidation (Cava *et al*., 1999). Moreover, some other prooxidant factors such as heme content in muscle are influenced by muscle fibre type (Aristtoy and Toldrá, 1998; Henckel *et al*., 1997). In this respect, oxidative muscles show higher myoglobin content, so that they have a redder colour (Andrés *et al*., 1999, Cava *et al*., 2003).

As a consequence of the different characteristics of the muscles, oxidative meat is more prone to suffer oxidative and lipolytic deterioration than glycolytic muscles which could reduce as a consequence its shelf-life during refrigerated storage (Morcuende *et al*., 2003, Andrés *et al*., 2001). These effects appear to be more intense in muscles from pigs with a high tendency to fat accumulation and a high content of heme pigments, like Iberian pig muscles, than in lean pigs (Morcuende *et al*., 2003).

6.4.- Feeding

Body fat comes from a combination of fats produced endogenously and those from the diet. Therefore, the body fat composition reflects the composition of the dietary fat to some extent, especially in monogastric animals. In pigs, dietary fatty acids are absorbed unchanged from the intestine and incorporated into tissue lipids. Fatty acids absorbed from the diet, especially polyunsaturated ones, specifically inhibit endogenous synthesis of fatty acids, inflating the effect of dietary fat composition on body fat composition (reviewed by Pettigrew and Esnaola, 2001). Therefore, fatty acid composition of feeding fat is a great influencing factor for fat composition in pigs (i.e. Wood, 1984; Warnants *et al*., 1999; Cava *et al*., 1997, Pettigrew and Esnaola, 2001, Rosenvold and Andersen 2003). According to Fontanillas *et al*., (1998) and Romans *et al*., (1995), dietary polyunsaturated fatty acids (PUFA) are readily incorporated into pig fat, whereas dietary monounsaturated (MUFA) and saturated fatty acids (SFA) have less influence on fat composition. Similarly, Warnants *et al*., (1996) found a linear relationship between the feed PUFA content and the PUFA content of intramuscular fat (IMF) and backfat.

In the case of Iberian pig, feeding affects importantly the quality of meat and meat products. Meat from pigs reared outdoors, in “Montanera”, has high amounts of monounsaturated fatty acids (MUFA) in the total lipids; triglyceride and phospholipid fractions since acorns are rich in them (Cava *et al*., 1997). In addition, pork from Iberian pigs has lower content of PUFA than those fed with commercial fodders (Estévez and Cava, 2003b). Mixed diets from pigs reared in intensive are commonly composed by cheap raw materials with abundant PUFAs, which could cause undesirable technological and sensory consequences on meat quality (Morrissey *et al*., 1998).

Beside this, the feeding with natural pastures and acorns causes an increase of natural antioxidants concentration (tocopherols) in meat (Cava *et al*., 2000a). The higher lipid oxidative stability of meat as a result of the incorporation of antioxidant from grass has been described in previous papers (Cava *et al*., 2000a; Nilzén *et al*., 2001; Estévez and Cava, 2003a). Other authors have described the protecting role of natural antioxidants, mainly tocopherols in refrigerated meat and in dry-cured meat products (Alasnier *et al*., 2000a; Cava *et al*., 1999).

Logically, fattening di*et al*so affects the quantity and composition of the lipid of dry-cured hams. Total intramuscular lipids and triacylglycerols content were higher in hams from pigs reared in Montanera than those from pigs fed with commercial fodders (Petrón *et al*., 2004). In addition, these hams contained more oleic acid and less stearic and palmitic acids (Petrón *et al*., 2004).

6.5.- Intramuscular fat content

It is generally recognized that differences in IMF content explain an important part of the genetic variation in eating quality of porcine meat. In this respect, non selected breeds have higher IMF content than selected genotypes as these pigs have been generally selected to improve lean growth.

The decrease of IMF content has been so important in the last years, especially in industrial genotypes, that it affected the quality of meat, decreasing the juiciness. Nowadays, one of the strategies to improve IMF content is the genetic selection. Heritability for IMF content indicates substantial genetic variation in this trait but it differs considerably among studies from 0.26 to as high as 0.86 with an average of 0.5 (reviewed by Gerbens, 2004). Some authors have found significant correlations between backfat thickness and IMF content (Hovenier *et al*., 1993). However, in general, carcass fatness and marbling are not perfectly correlated, but ordinarily, nutritional interventions that alter one, cause the other to move in the same direction also. Nevertheless, this is not a problem that affects Iberian pig, as due to its adipogenic character and the heavy slaughter weights, they have very high levels of intramuscular fat, which is a factor of prime importance for the production of dry-cured meat products (Gandermer, 2002), although by the inclusion of selected genotypes, it could be altered.

Generally, the increase of IMF content in meat is mainly due to an increase in triglycerides content as demonstrate different studies in pigs (Cameron and Enser, 1991; Essén-Gustavsson *et al*., 1994) and rabbits (Gondret *et al*., 1998). The synthesis of adipose tissue triglycerides, the major constituents of depot fat, either proceeds from fatty acids synthesised “de novo” (especially from dietary carbohydrates) in that tissue (O'Hea and Leveille, 1969) or from fatty acids obtained from

circulating triglycerides as a result of adipose tissue lipoprotein lipase activity (Steffen *et al*., 1978). However, the liver has little importance in fatty acid synthesis in pig (O'Hea and Leveille, 1969).

Several authors have evaluated lipogenic enzyme activity. The main enzymes assessed are glucose 6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) due to their importance in the production of NADPH (Figure 5). Lipogenic enzyme activities are markedly influenced by the animal breed and to a lesser extent by the diet (Morales *et al*., 2002). Some studies have demonstrated the influence of pig genotype on lipogenesis, as indicated the comparison of the lipogenic activity between Iberian and Landrace breeds (Morales *et al*., 2002) and between Meishan and Large White breeds (Mourot and Kouba, 1998).

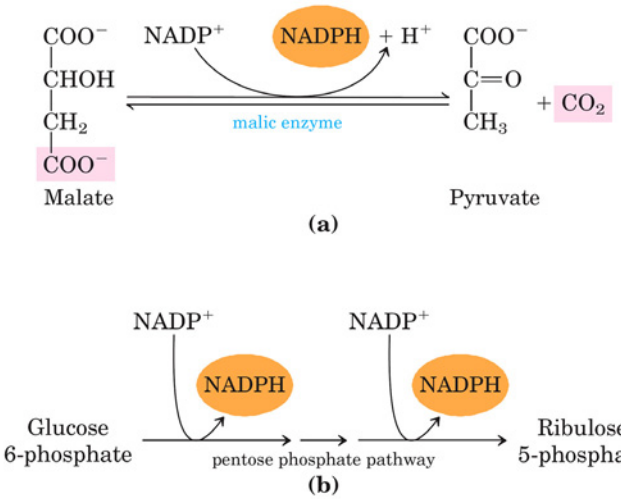

Figure 5.- Production of NADPH by malic enzyme activity (a) and by the pentose phosphate pathway (b). (Source: Lehninger Principles of Biochemistry, Fourth Edition (Hardcover, 2004) Albert L. Lehninger, David L. Nelson, Michael M. Cox).

Tissues that carry out extensive fatty acid synthesis (liver, adipose, lactating mammary gland) or very active synthesis of cholesterol and steroid hormones (liver, adrenal gland, gonads) require NADPH.

In hepatocytes and adipocytes, cytosolic NADPH is largely generated by the pentose phosphate pathway (by G6PDH) and by ME. In these tissues, the oxidation of glucose 6-phosphate by the pentose phosphate pathway is of particular importance (Figure 6). The first reaction of the pentose phosphate pathway is the oxidation of glucose 6-phosphate to form 6-phosphoglucono-δ-lactone by the action of the enzyme G6PDH. This pathway produces NADPH as well as ribose 5-phosphate, which serves as precursor for nucleotides, coenzymes, and nucleic acids. In cells that are not using ribose 5-phosphate for biosynthesis, they recycles six molecules of the pentose into five molecules of the hexose glucose 6-phosphate, allowing continued production of NADPH and converting glucose 6-phosphate (in six cycles) to CO_2.

Acetyl-CoA used in fatty acid synthesis is formed in mitochondria from pyruvate oxidation and from the catabolism of the carbon skeletons of amino acids, but the pathway for fatty acid synthesis occurs in the cytoplasm and for that reason this molecule has to be transported out of the mitochondria. The mitochondrial inner membrane is impermeable to acetyl-CoA, so an indirect shuttle transfers acetyl group equivalents across the inner membrane (Figure 10). Intramitochondrial

acetyl-CoA first reacts with oxaloacetate to form citrate, which then passes through the inner membrane on the citrate transporter. In the cytosol, acetyl-CoA is regenerated in an ATPdependent reaction. Oxaloacetate cannot return to the mitochondrial matrix directly, so cytosolic malate dehydrogenase reduces the oxaloacetate to malate, which returns to the mitochondrial matrix. Alternatively, the malate produced in the cytosol is used to generate cytosolic NADPH through the activity of malic enzyme. These molecules of NADPH produced will be used in fatty acids synthesis.

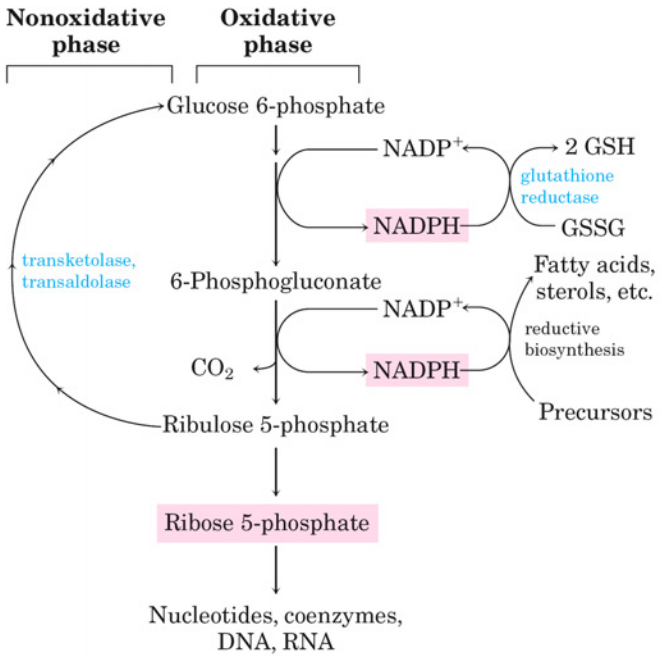

Figure 6.- General scheme of the pentose phosphate pathway. (Source: Lehninger Principles of Biochemistry, Fourth Edition (Hardcover, 2004) Albert L. Lehninger, David L. Nelson, Michael M. Cox).

The first step of the fatty acids synthesis is the formation of malonyl-CoA from acetyl-CoA which are used by the fatty acid synthase for the synthesis of fatty acids. All the reactions of fatty acid synthesis are carried out by the multiple enzymatic activities of fatty acid synthase. The primary fatty acid synthesized by fatty acid synthase is palmitate. Palmitate is then released from the enzyme and can then undergo separate elongation and/or unsaturation to produce other fatty acid molecules.

Several studies revealed that IMF content is one of the most important traits that influence eating quality traits such as tenderness, juiciness, flavor and taste (reviewed by Verbeke *et al*., 1999). In terms of technological quality, fat distribution and its fatty acid composition regulate water migration during drying of dry-cured meat (Arnau, 1998). An inadequate intramuscular fat content conducts to a fast dehydratation of the dry-cured products, leading to an inappropriate development of quality attributes. Furthermore, the level and composition of IMF play an important role in textural aspect such as in hardness, juiciness and brightness as well as in the production of flavour compounds (Ruiz *et al*., 2000, 2002; Cava *et al*., 1999, Carrapiso *et al*., 2002b). In addition, the juiciness perceived during chewing is one of the main factors for the acceptability of dry-cured Iberian ham (Ruiz *et al*., 2002). The juiciness of dry-cured meat products is determined by two factors: at the beginning, it is influenced by the water content, however, as dry-cured products have low moisture,

juiciness is mainly affected by the intramuscular fat content (Ruiz *et al*., 2002, Cava, *et al*., 2000b). Ruiz-Carrascal (2000) reported that high IMF content has a positive influence on some texture and appearance traits of ham, such as oiliness, brightness, juiciness and marbling; however, it was negatively related to dryness, fibrousness and hardness. Cilla *et al*. (2005) found that some of the factors that were positively related to the acceptability of dry-cured ham were IMF content and subcutaneous fat level while lipid oxidation, adhesiveness, pastiness, saltiness and rancidity were negatively correlated with their acceptability.

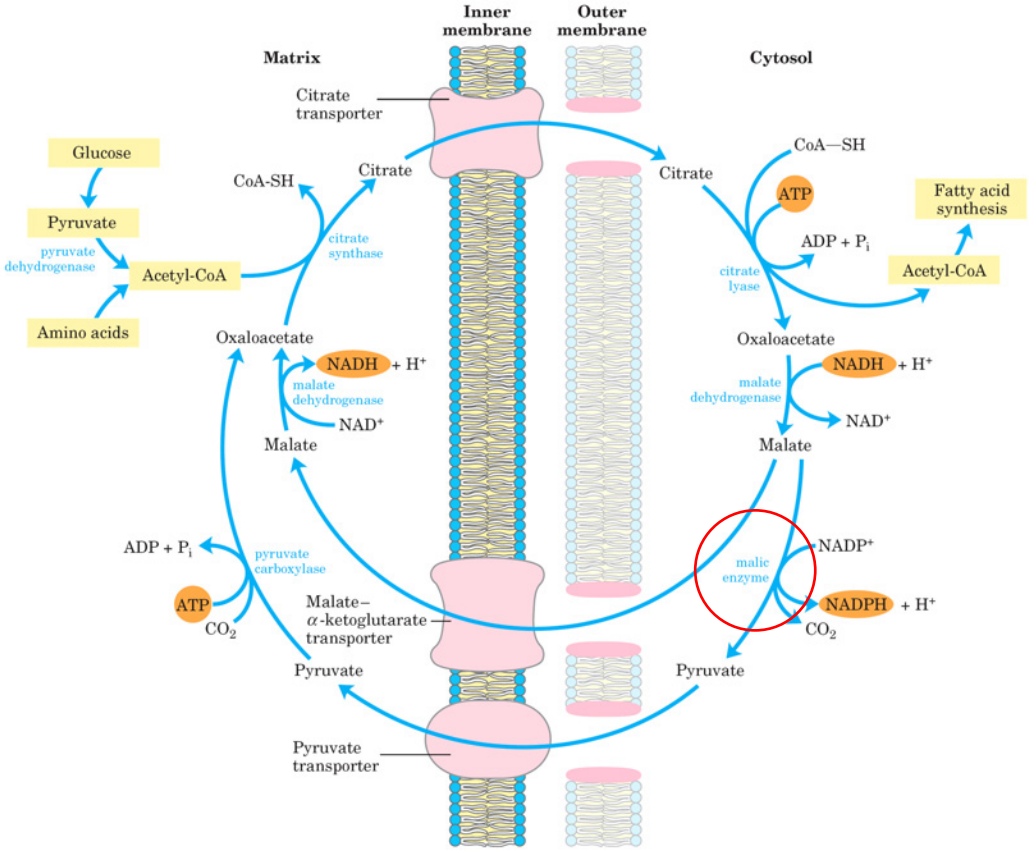

Figure 7.- Function of malic enzyme in the mitochondrial membrane for the fatty acid synthesis (Source: Lehninger Principles of Biochemistry, Fourth Edition (Hardcover, 2004) Albert L. Lehninger, David L. Nelson, Michael M. Cox).

In addition, intramuscular fat content has been reported to be genetically correlated with various other meat quality traits such as drip loss, water-holding capacity, cooking loss, reflectance, tenderness, juiciness, overall acceptability, marbling, flavour and firmness of the meat (Gerbens, 2004), which affect positively the overall quality of meat and especially of dry-cured meat products.

It is well known that the typical ham aroma is related to intramuscular lipid composition and to the extent of lipolysis and oxidation of lipids during processing (Martín, 1999; Cava *et al*., 1999; Carrapiso *et al*., 2002b). During the first steps of processing (salting, drying and first part of ripening), volatiles mainly arise from lipid oxidation, while those formed during the second part of ripening stage are formed from both lipid and amino acid degradation (Ruiz *et al*., 1999; Carrapiso *et al*., 2002b).

The overall acceptance of meat products depends to a large extent on their flavour, which is mainly determined by taste and odour compounds. Different researches have deal with the odour active

volatile compounds responsible of the characteristic aroma of the Iberian dry-cured ham (Carrapiso *et al*., 2002ab). In comparison with other types of dry-cured hams, Iberian ham has significantly higher aldehyde content (Sabio *et al*., 1998). Moreover, Iberian ham is richer in volatile compounds than other types such as Bayonne ham, Corsican ham, Serrano ham, Parma ham and light Italian country ham (Sabio *et al*., 1998). Most of the volatiles of Iberian dry-cured ham are formed by lipid oxidation. High intramuscular fat content favours aroma compounds retention in meat because triacylglycerols are a good solvent for these compounds which mainly arise from lipid oxidation (García *et al*., 1991; López *et al*., 1992). The main volatiles generated through oxidation process are aldehydes, which have large impact on the overall aroma of dry-cured meat products because of their typical aroma and their low odour threshold (Shahidi *et al*., 1986).

6.6.- pH decline and ultimate pH

Meat quality is closely linked to the genotype and those changes related with pH decline and ultimate pH values. Thus, the existence of the halothane and the RN- gene ("Rendement Napole") is associated with PSE meat.

In pigs subjected to a short-stress situation, the relationship between the genetic susceptibility to stress and meat quality has been demonstrated to be the main cause of pale, soft and exudative meat (PSE) (Cassens *et al*., 1975). Stressing environmental conditions such as the density of animals and inadequate transports, favour the development of abnormal meat quality. In addition, behavioural, physiological and metabolic responses to aversive situations depend on genetic background and prior experience of the animals (Terlow, 2005), so Duroc pigs are relatively insensitive to slaughter conditions in comparison with other breeds such as Large White (Terlow, 2005).

All these factors affect the development of *rigor mortis* and pH decline. After slaughter, along the ATP available to break down the actin and myosin bonds is decreasing, muscles cannot relax and become inextensible (Aberle *et al*., 2001), which is one of the characteristics of *rigor mortis*. Moreover, after bleeding, anaerobic muscular metabolism continues in the absence of oxygen and nutrients to produce lactic acid. So, the lactate formation increases while the pH is progressively declining. Normally, the pH in the muscle decreases from 7.0 to around 5.3–5.8 (Smulders *et al*., 1992).

One of the aspects related to *postmortem* pH is water holding capacity due to the fact that the decrease of pH causes protein denaturation (Offer, 1991; Offer and Knight, 1988) and ultimate pH causes myofibrillar repulsion (Elliot, 1968) as at pH values around 5.0, the electrical net charge of repulsion of the myofibrils and myofibrillar volume is minimal, which as a result decreases water holding capacity (Figure 8).

The relation between *postmortem* changes and water holding capacity has been extensively studied (reviewed by Huff-Lonergan and Lonergan, 2005). Much of the water inside living muscle cells is located within the myofibril. In fact, it is estimated that around the 85% of the water in a muscle cell is held in the myofibrils (Huff-Lonergan and Lonergan, 2005) by capillary forces arising from the filaments within the myofibril. When pH of porcine muscle is reduced from physiological values to

5.2–5.5 (near the isoelectric point of myosin), the distance between the thick filaments declines an average of 2.5 nm (Diesbourg *et al*., 1988) and as a consequence, water is driven towards the extracellular spaces (Wismer-Pedersen, 1987). Consequently, it is likely that the gradual mobilization of water from the intramyofibrillar spaces to the extramyofibrillar spaces may be the key in providing a source of drip (Huff-Lonergan and Lonergan, 2005).

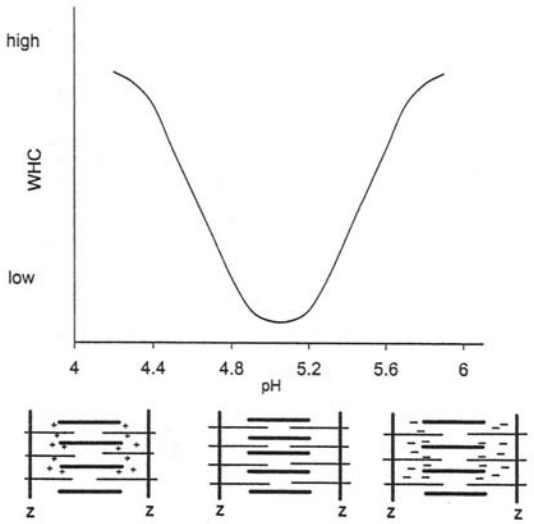

Figure 8.- Relationship between water holding capacity (WHC) and pH and the effect on myofilaments structure.

A fast decline of pH causes the denaturation of muscle proteins, due to the association of low pH and relatively high muscle temperature; producing low water holding capacity and losses of colour because of changes in myoglobin structure (Lawrie, 1998) as well as a decrease of firmness, which is known as pale, soft and exudative meat (PSE-meat; Briskey, 1964). Therefore, degree of pre-slaughter stress and temperature early post mortem is crucial in relation to colour development and colour stability (Rosenvold and Andersen, 2003) as the oxidation/peroxidation induced by the presence of myoglobin is highly pH dependent (Baron and Andersen, 2002).

The resolution of r*igor mortis* could affect other quality parameters such as the oxidative stability. In this respect, Juncher *et al*., (2001) reported that one of the most important parameters that affects oxidative stability of meat were pH24h, since they found that lightness, lipid oxidation, drip loss were significantly higher in pork with lower *postmortem* pH.

In dry-cured ham industry, *post-mortem* pH is the one of the main parameter used to classify the raw material for the manufacture of dry-cured meat products. García Rey *et al*., (2004) classified raw material for the production of dry-cured ham as normal-pH and low-pH hams taking as cut-off point pH of 5.55. They found significant correlations between pH values and the appearance of defective textures in dry-cured ham such as pastiness and crusting as well as with the colour in the final product. Furthermore, Ruiz-Ramírez, *et al*. (2005) reported that hams with low pH had higher hardness, cohesiveness and springiness than those from hams with higher pH. On the other hand, DFD hams were softer, pastier, more crumbly and more adhesive than normal ones (Guerrero *et al*., 1999).

Postmortem pH is important for the colour of cured meat products, since both formation of nitrosylmyoglobin during curing and the formation of nitrosyl heme pigments from oxidized pigments during storage is acid catalyzed (Andersen and Skibsted, 1992). Moreover, a low initial pH in muscles favours lipid oxidation in dry-cured hams (Buscailhon *et al*., 1994) and therefore, the development of rancidity. Chizzolini *et al*., (1996) evaluated the relationship between raw material and dry-cured ham quality. They found that pH was the most determinant factor measured in fresh for meat products quality, as they found a close relation between this parameter and sensory and instrumental colour values. However, this relationship was not strong enough as maturing appears to have a positive effect on the colour of pale meat. They also associated lean firmness of matured hams with the colour and pH of the fresh meat.

The incidence of exudative meats in Iberian pigs is practically non-existent, whereas the cross of Iberian pigs with selected Duroc lines could introduce undesirable characteristics, which could affect the quality of fresh meat and dry-cured meat products.

7. – Deterioration of meat and meat products during refrigerated storage.

7.1.- Color changes.

The colour of fresh pork is influenced by intrinsic factors such as genotype, gender, type of muscle, dietary supplementation with antioxidants (vitamin E), and extrinsic factors such as pre-slaughter handling and slaughter procedure, which influence pH decline and ultimate pH, and beside this, storage time and conditions (temperature, atmosphere, light, etc) (Faustman and Cassens, 1990; Renerre, 1990; Sellier and Monin, 1994; Rosenvold and Andersen, 2003).

Meat colour influences consumer's choice and acceptance as meat with a grey-brown colour is generally associated with non-fresh products (Krammer, 1994). Meat colour depends on the pigments concentration, mainly myoglobin and its chemical state as well as on the overall physical state of meat such as pH, myofibril proteins state, denaturation degree (Ledward, 1992, Renerre, 1990). Myoglobin and haemoglobin, together with mitochondrial citocrome c, are responsible for the characteristic red colour of meat (Govindarajan, 1973; Giddings, 1977); although myoglobin is the main muscle heme pigment (Figure 9). Myoglobin is a globular protein formed by an apo protein (globin) and an iron-containing heme group, Fe-protoporphyrin, which is the chromophore of myoglobin (Stryer, 1981). The apo protein fraction consists of 8 α-helices linked by short non-helical sections which are around the heme group. The iron atom bind to the four nitrogens in the centre of the protoporphyrin ring can form two additional bonds, one on either side of the heme plane, the fifth and sixth coordinating positions. The fifth coordinating position is bound to histidine on the globin, and the sixth is free for binding to different small ligands such as O_2, H_2O, OH^-, NO and CO (Hamm, 1975; Stryer, 1981).

The colour of myoglobin (Figure 10) is determined by the redox state of the iron atom, ferrous (Fe^{2+}) and ferric (Fe^{3+}), and by the type of ligand bound (Govindarajan, 1973; Hamm, 1975). Oxygen can only be bound to myoglobin in the ferrous redox state, whereas H_2O is bound in the ferric redox state at physiological pH and below (Govindarajan, 1973). The colour cycle of fresh meat is reversible and dynamic (Figure 10) with constant interconversion of mainly three species: deoxymyoglobin (Mb),

oxymyoglobin (MbO_2) and metmyoglobin (MetMb) (Govindarajan, 1973). Myoglobin, provides a purple colour to meat and can be transformed into oxymyoglobin (MbFe(II) O) when it is linked to oxygenum, with an attractive bright red colour. The surface colour changes from purple to bright red, due to oxygenation of Mb to MbO_2, when fresh meat is exposed to oxygen, this reaction is known as blooming (Govindarajan, 1973; Giddings, 1977). Finally, metmyoglobin (MbFe(III)) is formed when Fe^{2+} of the myoglobin is oxidized to Fe^{3+} and has brown colour very undesirable for consumers as it is associated with non-fresh products.

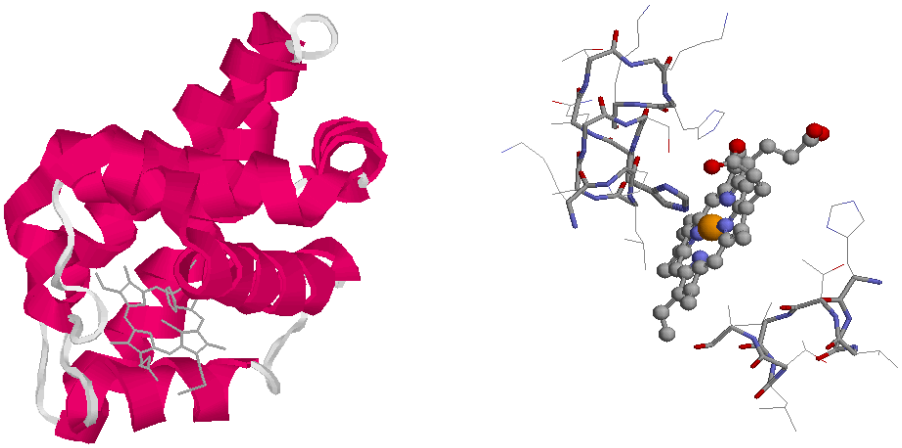

Figure 9.- Myoglobin structure (left) and heme group (right)

On the other hand, colour formation and stability are very important quality traits of dry-cured meat products. During manufacture, nitrates and nitrites added to meat to form the characteristic pigment of cured-meat: nitrosylmyoglobin. Nitrosylmyoglobin is a myoglobin derivative, where the ligand nitric oxide (NO), formed from nitrite in acid media, is coordinated to central Fe^{2+} in heme group. The same reaction is produced with the haemoglobin, the blood pigment, which can also be present in low concentrations in meat. Oxidative discoloration of cured meats converts the nitrosylmyoglobin, MbFe(II)NO, to nitrate and metmyoglobin, MbFe(III), which is not only detrimental for appearance, but also for the oxidative stability of unsaturated lipids. The stability of the total pigments and nitroso myoglobin formation depends on microbiological, enzymatic and chemical processes, which are in turn influenced by factors such as pH, redox potential, curing salt concentration, temperature and moisture content (Chasco *et al.,* 1996).

The relationship between lipid oxidation and oxymyoglobin oxidation has been the subject of numerous research papers over the years as the oxidation of myoglobin to metmyoglobin during the storage results in meat discolouration (reviewed by Baron and Andersen, 2002). At high unsaturated fatty acid/heme protein ratios both oxymyoglobin and metmyoglobin are denatured resulting in exposure of the heme group to the environment, which raises a hematin-induced peroxidation mechanism. Such processes might contribute to the poor stability of meat and meat products with high fat content (Baron and Andersen, 2002)

On the other hand, meat stored under low temperatures has a limited shelf-life due to biochemical changes developed after slaughter such as lipolysis and lipid oxidation of muscle lipids (Gray *et al*., 1996; Kanner, 1994; Morrissey *et al*., 1998).

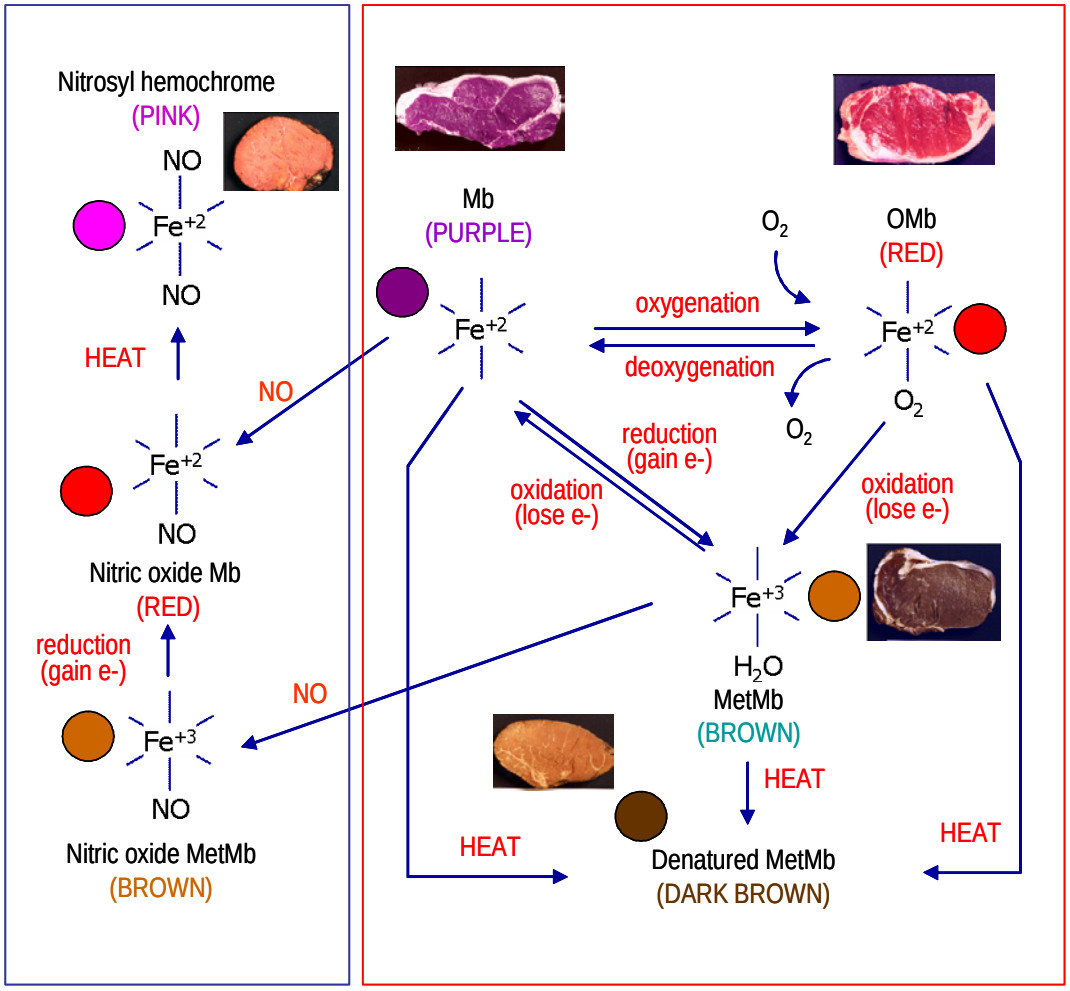

Figure 10,- Chemical species of myoglobin.

7.2.- Lipid oxidation.

Regardless of microbial spoilage, lipid oxidation is the main factor reducing the quality and shelf-life of meat and meat products (Morrissey *et al*., 1998). The main consequences of lipid oxidation are i. the generation of toxic compounds such as MDA and hydroperoxides, ii. the losses in the nutritional value due to vitamins and essential fatty acids oxidation and iii. the development of rancid flavours and undesirable colours, among others (Gray *et al*., 1996).

The overall mechanism of lipid oxidation is generally a free radicals chain reaction including three steps: i. initiation, ii. propagation and iii. termination (Frankel, 1982, 1985). The first step of lipid oxidation involves the abstraction of a hydrogen atom from a methylene carbon in an unsaturated fatty acid (RH) to form fatty acyl ($R^{\cdot}$) and peroxyl radicals ($RO_2^{\cdot}$). The generation of the primary radicals is facilitated by the presence of oxidation initiators such as transition metals (iron and copper), oxidants, various homolysis –prone substances or enzymes (Kanner *et al*., 1994; Gray *et al*., 1996). Once a free radical is generated, the chain reaction of oxidation is initiated, new free

radicals are formed and the process is easily propagated to other fatty acids which are consequently oxidised forming new peroxyl radicals and lipid hydroperoxides (ROOH) (Morrissey *et al*., 1998). Both primary lipid oxidation products undergo carbon-carbon cleavage to form small molecular weight stable breakdown products (secondary lipid oxidation products) including aldehydes, ketones, alcohols, hydrocarbons, esters, furans and lactones (Frankel, 1985; Morrissey *et al*., 1998).

Intensity of lipid oxidative reactions depend on several factors such as the amount and characteristics of the muscle lipids (i.e. lipid content, fatty acid composition, distribution of unsaturated lipids in lipid fractions), the presence of prooxidant factors (i.e. iron, copper, sodium chloride) and antioxidant factors (i.e. tocopherols, antioxidant enzymes, nitrite) and the balance between prooxidants and antioxidants factors (Morrissey *et al*., 1998).

Oxidative stability of muscle foods is largely determined by the amount of muscle lipids (Morrissey *et al*., 1998) being positively correlated fat content and lipid oxidation. Additionally, the fatty acid composition of muscle lipids affects the sensitivity of meat to oxidative reactions. Thus, the susceptibility of fatty acids to oxidation increases with the number of double bonds and therefore, polyunsaturated fatty acids are more prone to suffer oxidation reactions than monounsaturated and saturated fatty acids (Nawar, 1996). In this sense, membrane phospholipids, due to their rich polyunsatured fatty acid composition and the proximity to prooxidant systems are the place for the initial development of oxidised flavours in raw meat during storage.

The role of iron and muscle heme pigments as lipid oxidation promoters is well established (Kanner, 1994). Heme and non-heme iron in the forms oxidized or reduced have the ability to promote the catalysis of oxidation of unsaturated fatty acids (Halliwell and Gutteridge, 1986) by different mechanisms, including direct and indirect initiation and indirect initiation-propagation (Schaich, 1992).

Muscle antioxidants enhance the oxidative stability of tissues reducing the development of oxidative reactions during storage and processing of meat and meat products and minimizing the adverse effects on quality (Morrissey *et al*.., 1998). Thus, tocopherols inhibit oxidative decomposition of polyunsaturated fatty acids as well as oxymyoglobin oxidation (Faustman *et al*., 1989; Yin *et al*., 1993).

Undesirable flavours appearance is related to oxidation development as lipid oxidation products favour the development of off-flavours, particularly during storage. However, lipid oxidation also has positive implications especially in dry-cured meat products, as some of the volatile compounds which show pleasant flavour notes in Iberian dry-cured meat products arise from the oxidation of unsaturated fatty acids (Carrapiso *et al*., 2002b).

7.3.- Lipolytic changes

Lipolysis is considered a promoter of lipid oxidation because fatty acids released from neutral and polar lipids are highly susceptible to oxidation (Moerck and Ball, 1974; Nawar, 1996; Alasnier *et al*., 2000b). Adipose tissue and mucles lipolytic enzymes, such as lipases, sterases, phospholipases and lysophospholipases, are involved in the hydrolytic processes of muscle lipids during refrigeration and maturation of meat and meat products (Flores *et al*., 1996; Hernández *et al*., 1998). Lipolysis

releases free fatty acids from both triacylglycerols and phospholipids (Alasnier *et al*., 2000b), and thus, favour lipid oxidation because free fatty acids are very sensitive to lipid oxidation (Nawar, 1996). Moreover, most of those free fatty acids are long chain polyunsaturated fatty acids (Alasnier *et al*., 2000b), which enhance oxidative reactions.

7.4.- Protein oxidation

In meat and meat products, both protein and lipid oxidation are processes closely related (Mercier *et al.,* 1998, 2004; Ventanas *et al*, 2006b), being protein oxidation affected by similar oxidation promoters capable to oxidize unsaturated fatty acids (muscle heme pigments, transition metals, oxidative enzymes…). Protein oxidation undergoes similar processes to those described for lipid oxidation, involving initiation, propagation and termination stages (Schaich, 1980). Oxidative processes of proteins involve the oxidation of aminoacids, especially aminoacids with reactive side chains (sulphydryl, thioether, amino, imidazole or indole ring) by free-radicals and non-radicals reactive oxygen substances. The oxidation of proteins leads to the loss of sulphydryl groups and the formation of carbonyl compounds (Xiong, 2000) which have been used to measure the extent of protein oxidation (Levine *et al*., 1990; Mercier *et al*., 1998; Ventanas *et al*, 2006a). In meat, protein oxidation processes occur after slaughter in myofibrillar proteins (Martinaud *et al*., 1997). The consequences of protein oxidation are not clear but they could be linked with a decrease in muscle protein functionality contributing to decrease water holding capacity and changes in textural characteristics (Xiong, 2000; Huff-Lonergan and Lonergan, 2005) and modifications in the nutritional value (Meucci *et al*., 1991; Liu and Xiong, 1997).

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Approach, objectives and experimental design

2. APPROACH, OBJECTIVES AND EXPERIMENTAL DESIGN.

Iberian dry-cured meat products are highly appreciated by consumers because of their high sensory quality. This is a result of three main factors the rearing system, the manufacture process and the characteristics of the Iberian pig breed.

In the last years, the high consumer's demand of the Iberian meat products has conducted to a fast growing of this market which is still increasing. In order to provide raw materials to the industry, the traditional rearing system has changed, and nowadays, semi-intensive systems and crossbreeding with Duroc breed are common in the Iberian pig productive sector.

The high growth of Iberian sector has needed a specific law, which was passed in 2001 (BOE, 2001) and that regulates the manufacture of dry-cured products with the label of "*Iberian*". This law regulates the rearing and feeding of the animals (free-range reared or fed on concentrates, age and slaughter weight …), the conditions of maturation of the products (relative humidity and temperature conditions, duration of each maturation phase and the length of the process…) and specially the breed of the pigs (pure Iberian pigs or Duroc x Iberian crossed pigs). Concerning this last point, it is required the use of pure Iberian pigs (labelled as "Ibérico puro", "pure Iberian") or Duroc x Iberian crossed pigs (labelled as "Ibérico", "Iberian") in which the females must be a pure Iberian pig sow, in order to preserve the purity of Iberian breed and their biodiversity.

The crosses with Duroc breed are so popular because they improve productive parameters of Iberian pigs, such as the increase in 1-2 piglets/sow. Besides, they also improve the growth rate, and the weight at weaning and at the end of fattening (Dobao *et al.*., 1986, Aparicio, 1987) and without an important decrease in the quality of meat and dry-cured meat products (Tejeda *et al.*., 2002, Antequera *et al.*., 1994, Andrés *et al.*., 2001). However, there is some controversy about the effect of crossbreeding with Duroc. Some authors (Carrapiso *et al.*., 2003) have reported that crossbreeding had a slight effect on dry- cured ham quality. In the contrary, other authors (Ventanas *et al.*., 2006, Ventanas *et al.*., in press) have reported a marked decrease of meat and dry-cured loin quality in Iberian x Duroc genotypes respect to those from pure Iberian pigs.

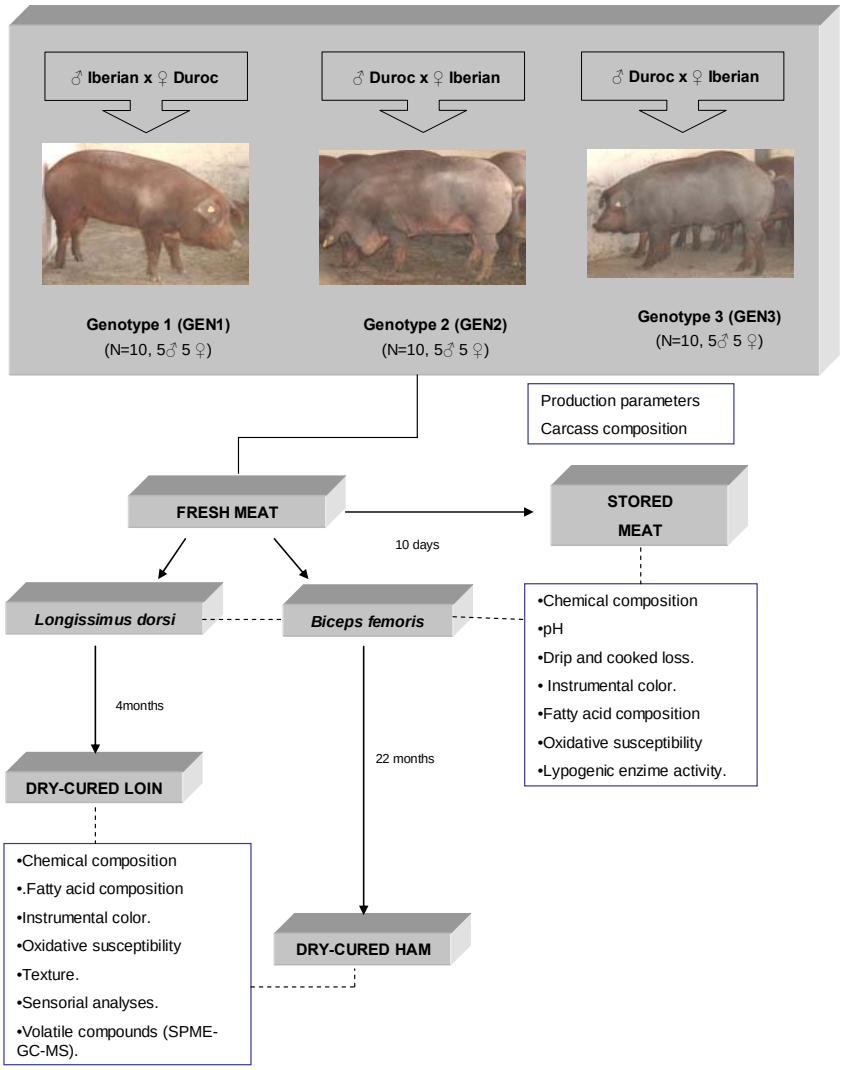
However, the Duroc genotype used in these crosses also should be taken into account, as Duroc cannot be considered a homogeneous breed due to its widespread distribution. Therefore, the characterization of the different lines of Duroc for Iberian production seems to be of prime importance. There are previous studies which evidence some differences between Duroc lines (Soriano *et al.*., 2005, Cilla *et al.*., 2006) in terms of carcass composition and fresh meat and dry-cured ham quality.

In addition, Lonergan *et al.*., (2001) compared different selected Duroc lines and found that the Duroc line selected to increase lean growth efficiency decreased meat quality. In addition, there are some previous studies about the effect of the reciprocal cross (Morcuende *et al.*., in press; Ventanas *et al.*., 2006) but there are no previous research about the influence of the Duroc line in the crosses with Iberian and their consequences the productive parameters and the quality of meat and dry-cured meat products.

Therefore, the main general objectives of this thesis are:

- To evaluate the effect of the Duroc line on productive characteristics as well as on the quality of meat and dry-cured loin and ham.
- To evaluate the influence of the Iberian x Duroc reciprocal cross on productive characteristics as well as on the quality of meat and dry-cured loin and ham.
- To evaluate the main parameters of the raw material that affect dry-cured meat products quality and to establish its possible effect on dry-cured meat products.

For the achievement of these objectives, the experimental design scheme showed in the Figure 1 was set out. 3 Iberian x Duroc genotypes (GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian) were studied. Iberian sows and sires and Duroc line 1 were provided by CENSYRA (*Centro de Selección y Reproducción Animal, Junta de Extremadura*) and Duroc line 2 sires were provided by an international pig breeding stock company. Genotypes GEN1 and GEN2 were reciprocal crosses; GEN3 was similar to GEN2 but with a different Duroc sire line. Right loins (m. *Longissimus dorsi*) and legs were used for fresh and refrigerated studies while left ones were cured into dry-cured loins and hams.



In order to facilitate the presentation and discussion of findings, this thesis has been divided in 8 chapters:

- **Chapter 1.** "CARCASS COMPOSITION AND MEAT QUALITY OF 3 DIFFERENT IBERIAN X DUROC GENOTYPE PIGS"
- **Chapter 2.** "FATTY ACID COMPOSITION AND ADIPOGENIC ENZYME ACTIVITY OF MUSCLE AND ADIPOSE TISSUE AS AFFECTED BY IBERIAN x DUROC PIG GENOTYPE"
- **Chapter 3.** "THE CROSSBREEDING OF DIFFERENT DUROC LINES WITH IBERIAN PIG AFFECTS COLOR AND OXIDATIVE STABILITY OF MEAT DURING STORAGE"
- **Chapter 4.** "CHANGES IN FATTY ACID COMPOSITION OF 2 MUSCLES FROM 3 IBERIAN x DUROC GENOTYPES AFTER REFRIGERATED STORAGE"
- **Chapter 5.** "EFFECT OF IBERIAN x DUROC GENOTYPES ON DRY-CURED LOIN QUALITY"
- **Chapter 6.** "COMPOSITION, INSTRUMENTAL AND SENSORY ANALYSIS OF DRY-CURED HAMS AS AFFECTED BY THE IBERIAN x DUROC GENOTYPE"
- **Chapter 7.** "VOLATILE PROFILE OF DRY-CURED MEAT PRODUCTS FROM 3 DIFFERENT IBERIAN x DUROC GENOTYPES"
- **Chapter 8.** "EFFECT OF PHYSICO-CHEMICAL CHARACTERISTICS OF RAW MUSCLES FROM THREE IBERIAN x DUROC GENOTYPES ON DRY-CURED MEAT PRODUCTS QUALITY**"**

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Materials and methods

1.- MATERIALS

1.1.- Samples

1.1.a.- Animals. 3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian (Image 1). The genotype Duroc1 (DU1) was selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fat. The genotype Duroc2 (DU2) was selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were provided by the CENSYRA (*Centro de Selección y Reproducción Animal, Junta de Extremadura*) which is a reproductive centre which developed the trial to obtain the different genotypes. In the project were used 21 sows and 6 barrows (2 from each genotype). Iberian and one Duroc line were provided by CENSYRA (*Centro de Selección y Reproducción Animal, Junta de Extremadura*). The other Duroc sire line was provided by an international pig breeding stock company. 101 piglets were born, among which were randomly selected 10 pigs from each genotype. Pigs were all castrated as is traditionally done. Males were castrated and females ovariectomized. Surgical procedure performed under local anesthesia (2% de lidocaine hydrochloride) previous sedation with azaperone (Stresnil) 1h before intervention. At 3 months age, pigs were moved to the farm of the Faculty of Veterinary Science (University of Extremadura) in Cáceres and raised all together in a semi-intensive system. They were fed ad libitum with diets shown in Table 1 and provided by the company PICAB.

Table 1. Proximate composition (%) and fatty acid composition (% total fatty acids) of the pig diets.

	MIXED DIET I from 60 to 100kg l.w.	MIXED DIET II from 100 to 165kg l.w.
***Proximate composition** (%)*		
Crude protein	16.0	13.5
Crude fat	3.3	5.0
Crude fiber	4.8	3.7
Ash	6.9	6.2
Lysine	0.9	0.5
Metabolizable energy (Kcal./Kg.)	3071.5	3183.7
***Fatty acid composition** (% total fatty acids)*		
C14:0	0.1	0.1
C16:0	14.6	21.0
C18:0	4.4	5.6
C18:1n-9	23.3	31.3
C18:2n-6	34.7	35.1
C18:3n-6	2.0	2.3

Pigs were randomly slaughtered after 316 days of rearing with 150-165Kg live weight in the slaughter plant VIDOSA (Higuera La Real, Badajoz). After slaughter, loins and legs, subcutaneous fat samples were removed from the carcasses. Subcutaneous fat samples were taken at 5^{th}-6^{th} rib. Right loins (m. *Longissimus dorsi*) and legs were used for fresh and refrigerated studies while left ones were cured into dry-cured loins and hams. M. *Biceps femoris* were dissected from raw legs and dry-cured hams.

1.1.b.- Refrigerated storage trial. After slaughter, *Longissimus dorsi* (LD) and *Biceps femoris* (BF) were removed from carcass and sliced (1.5 cm thick) using a slicing machine. They were placed on Styrofoam meat trays, over-wrapped with PVC oxygen permeable films and stored under fluorescent

light at +4ºC for 10 days. To avoid microbial growth, a solution of chloramphenicol (4mg/ml)/ciclohexamide (1mg/ml) was sprayed on the surface of the chops. After instrumental colour measurements, samples were vacuum packaged and stored at -80ºC until analyses.

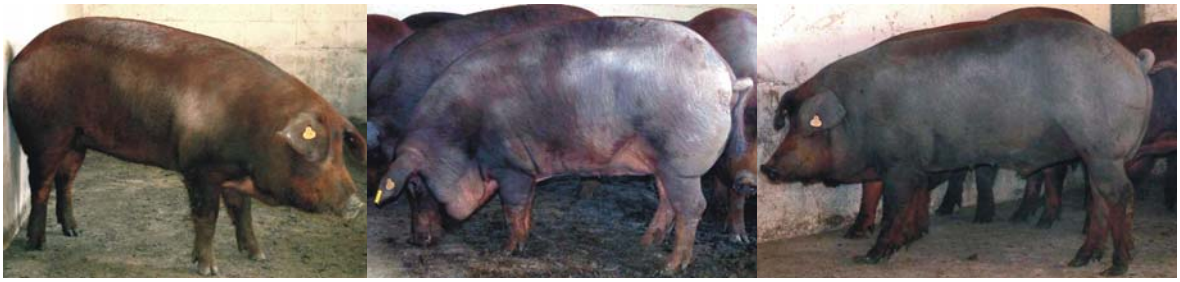

Image 1. Pigs from genotypes studied. (From left to right, GEN1, GEN2 and GEN3).

1.1.c.- Dry-curing process. Dry-cured loins and hams (Image 2 and 3, respectively) were processed in the processing plant of JAIBESA (Fregenal de la Sierra, Badajoz). Left loins *(m. Longissimus dorsi*) were removed from the carcasses (10loins/genotype) and processed into dry-cured loins. The average weight of fresh muscle was 2.5-3.2Kg and pH values <6.0. Loins were seasoned by rubbing a mixture of salt, nitrite, olive oil and spices such as Spanish paprika (*Capsicum annuum, L.*), oregano (*Origanum vulgare L.*) and garlic (*Altium sativum, L.*). Loins were kept at 4°C for 4 days to allow the seasoning mixture to penetrate. Then, loins were stuffed into collagen casings and held at 4°C at a relative humidity of ~80% for 30 days. Finally, loins were ripened at ~12°C and at ~70% relative humidity for 90 days. Loins were processed for a total dry-curing time of 4 months.

Image 2. Dry-cured loin slices from the 3 genotypes studied.

For the manufacture of dry-cured hams, the left leg from each animal was processed (10ham/genotype). After salting, hams were kept at 0–3ºC and 80–90% relative humidity for ~6 months. Then, the hams were ripened at 10–25ºC and 60–80% relative humidity for ~18 months. Hams were processed for a total dry-curing time of 24 months. After the ripening process, the *Biceps femoris* muscles were removed from the hams for the analysis.

2.- METHODS

2.1.-Growth performance and carcass composition. The weights of hams, shoulders and loins were taken 5 hours post-mortem. The backfat thickness (BFT) and ham fat thickness (HFT) were measured in the 5th rib and in the m. *Gluteo-biceps* in the carcass and ham, respectively. Samples of

subcutaneous fat (taken from backfat at 5^{th}-6^{th} rib) and *Biceps femoris* (BF) and *Longissimus dorsi* (LD) muscles were dissected from the carcasses and stored at -80ºC until analyses.

2.2.- pH. pH values at 45 min (pH_{45}) and 24h (pH_U) *post-mortem* in the BF and LD muscles were measured with a puncture pHmeter Crisol mod. 507.

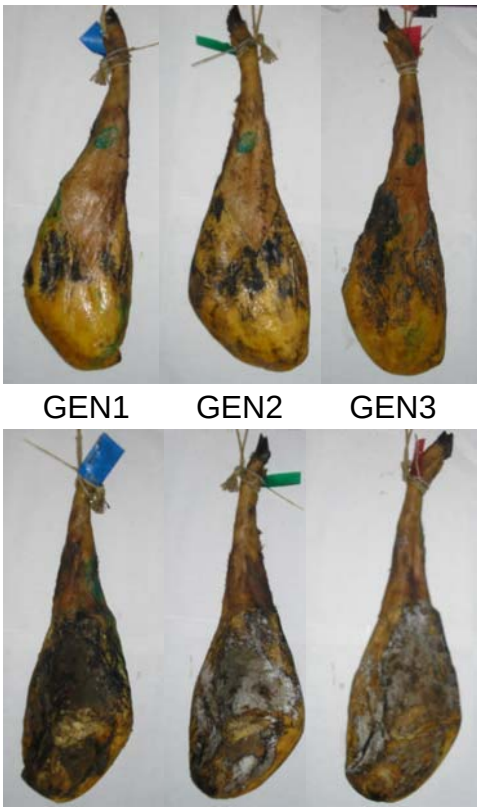

Image 3. Dry-cured hams (lateral –up- and medial –down- views) from the 3 genotypes studied.

2.3.- Drip and cook loss. Drip loss was measured following the method of Honikel (1998). The samples were weighed and suspended in two plastic bags, the inner bag perforated, and the exudates were collected in the outer bag. Meat was stored for 10 days at refrigerated storage (4ºC). Drip loss (%) was calculated by difference in weight between day 0 and day 10 of storage. For cook loss (%), each chop was placed in a plastic bag and cooked by immersion at 80ºC for 60min. The difference of weight before and after cooking was used to calculate cook loss percentage.

2.4.- Lipid extraction. Lipids were extracted from 5g of meat according to the method described by Bligh and Dyer (1959). The meat was homogenized in 15mL chloroform/methanol (1.2) with 0.005% BHT. Then the mix has centrifuged at 2000 r.p.m. at 4ºC for 5min. Then, the supernatant was filtered and 5ml of distilled water were added, the mix was shaken and centrifuged in the same conditions as before. The aqueous phase was removed with a pasteur pipette and the lower organic phase was filtered through sodium sulphate anhydrous into a round flask, previously weighed. The total lipids were dissolved in 10 mL of hexane and kept frozen at -80ºC. The solvent was evaporated in a rotary evaporator (Rotavapor, Giralt Heidolph VV2000) and the remaining solvents were removed under a nitrogen stream. Extracted fat content was calculated by difference of weight.

2.5.- Protein content. Protein content was determined by the Kjieldahl method (AOAC, 2000). 1g of minced sample was digested with 20ml of concentrate sulphuric acid, with potassium sulphate anhydro, cuprum and selenium as catalysers. After that, it was distilled after adding sodium hydroxide (30%). Distillate was collected in 100mL of boric acid (2%). The ammoniac taken was titrated with clorhidric acid (0.1N). The protein content was calculated with the following formula:

$$\%protein=\%N*6.25$$

2.6.- Moisture content. Moisture was determined by drying the samples (~5 g) at 102ºC (AOAC, 2000). 2g of minced meat was mixed with sea sand in a capsule previously dried and weighed (capsule + sand). 5mL of ethanol were mixed with the sand and meat. Then they were dried in an electric stove at 102ºC until constant weight. The moisture content was calculated by difference of weight.

2.7.- Haem pigments. Heme pigments were assessed following the method of Hornsey (1956). 10g of minced meat were placed in a 100mL Erlenmeyer flask and mixed with 40mL of acetone, 2mL of mili-Q water and 1mL of 12M HCl. The samples, immersed in an ice bath, were homogenized at 2000 rpm for 2 min on ice and incubated at 4ºC for 12h in darkness. After incubation, the homogenate was filtered and the absorbance at 640nm of the supernatant was measured in a spectrophotometer at 640nm. The hematine content (in ppm) was calculated by multiplying the absorbance by 680.

2.8.- Instrumental colour. Colour measurements were made following the recommendations on colour determination of the American Meat Science Association (Hunt *et al.*., 1991). The following colour coordinates were determined: lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue). Colour parameters were assessed using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a calibration plate (Y:93.6, x: 0.3154, y: 0.3320 for D65). In addition, hue angle, which describes the hue or colour was calculated (H^o=arctg b*/ a* · 360/2π) as well as the saturation index or chroma (C*) ($C= (a^{*2}+b^{*2})^{0.5}$), which describes the brightness or vividness of colour. The measurements were repeated at 5 randomly selected places on each slice and averaged.

2.9.- Intramuscular lipids fractionation. Intramuscular lipids were fractionated into NL, FFA and PL using propylene NH_2 – aminopropyl minicolumns following the method described by Monin *et al.*., (2003). Previously to fractionation, columns were activated with hexane. ~20mg of lipids solved in hexane were poured into the columns and the different solvents to elute the different lipids. Neutral lipids were eluted with 4mL of chloroform: isopropanol (2:1), free fatty acids were eluted with 4mL of diethyl ether-acetic acid 2% and finally 4mL of methanol: chlorhydric acid (9:1) were used to elute polar lipids. All fractions were collected in glass tubes and solvents were evaporated under nitrogen stream.

2.10.- Methylation and analysis of fatty acids. Lipids from the subcutaneous adipose tissue were extracted by the De Pedro *et al.*., (1997) method using a microvawe oven. ~20mg of the melted fat were taken for the methylation. Fatty acid methyl esters (FAMEs) were prepared by transesterification with methanol in sulphuric acid (5% of sulphuric acid in methanol). Lipids or lipid fractions obtained following fractionation analysis described previously were placed in screw-top test tubes and mixed with 1mL of 0.5% sodium methylate. They were maintained in an electric stove at 80ºC for 30min, and were vortexed every 10min. Then 1mL of sulphuric acid (5%) in methanol was added, and again, tubes were kept in the stove for 30min. When tubes were cold, 1mL of hexane and 1mL of saturated NaCl were added. After the centrifugation the upper organic phase was taken in glass chromatography vials. FAMEs were analyzed in a Hewlett-Packard model HP-5890A gas chromatograph, equipped with a flame ionization detector (FID). FAMEs were separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0mm film thickness). The injector and detector temperatures were held at 230°C and the oven temperature, at 220°C. The flow rate of the carrier gas (N_2) was set at 1.8 mL/min. Identification of FAMEs was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percent of total FAMEs.

2.11.- Iron-ascorbate induced oxidation. The susceptibility of muscle tissue homogenates to iron ascorbate-induced lipid oxidation was determined by the method of Kornbrust and Mavis (1980). Aliquots of 1mL of homogenates (1g meat in 9mL of KCl 1.15%) were incubated at 37ºC with 80 mM Tris-maleate buffer (pH 7.4), 5mM $FeSO_4$ and 2mM ascorbic acid in a total volume of 10mL. At fixed time intervals, (0, 50, 100 and 200min) aliquots (1mL) were taken for the measurement of 2-thiobarbituric acid-reactive substances (TBA-RS) by the method of Buege and Aust (1978) by adding 2mL of TBA-TCA-ClH and heating it at 100ºC for 15min. The measurements in the spectrophotomer were made at 535nm. TBA-RS were expressed as nmol malondialdehyde (MDA)/mg protein. Protein was measured in muscle homogenates following Lowry procedure (Lowry *et al.*., 1951), adding NaOH (0.1N) and a solution made of cuprum sulphate (0.5%, w/v), with sodium carbonate (2%, w/v) and potassium sodium tartrate (0.2%, w/v) diluted in a basic solution. Finally, Folin-Ciccalteau reagent was added. The measurement was made at 740nm by spectrophotometry. Protein concentration was measured by comparison with a standard of BSA (bovine serum albumin).

2.12.- Lipid oxidation. Lipid oxidation was assessed in duplicate by the 2-thiobarbituric acid (TBA) method of Salih *et al.*., (1987). 2g of sample were homogenized with 7.5mL of percloric acid (3.86%) and 0.25mL of BHT (4.2% in ethanol) at 2000 rpm for 2 min. Homogenisations took place in an ice bath to minimise the development of oxidative reactions during extraction of TBA-RS.

The homogenate was filtered through with a double paper filter and centrifuged at 3000rpm for 2min. After that, 2mL aliquots (in duplicate) were mixed with 2mL of tiobarbituric acid (0.02M). The tubes were heated at 90ºC for 30min in a hot water bath. After cooling, tubes were centrifuged at 3000rpm for 2min and the absorbance was measured at 508nm, 532nm and 600nm. Corrected absorbance at 532nm was calculated following the formula:

$$Abs_{532nm} = Abs_{532nm} – [(Abs_{508nm} - Abs_{600nm}) * (600-532) / (600-508)] - Abs_{600nm}$$

A standard curve was prepared using different dilutions of a solution of 1,1,3,3-tetraethoxypropane (TEP) (0.2268 g) in 1L of 3.86% perchloric acid. TBA-RS values were calculated from the standard curve and expressed as mg malondialdehyde/kg meat.

2.13.- Protein oxidation. Protein oxidation was assessed by measurement of carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl following the method described by Oliver *et al.*., (1987). Homogenates (1g of sample in 10mL of 0.15M KCL buffer) were divided into two 0.1mL aliquots (A and B), and placed in eppendorfs. Proteins were precipitated in both aliquots (A and B) with 1mL 10% TCA and centrifuged at 2240g for 5min. 1mL of 2N HCL was added to pellet A (used to measure protein concentration) and 1mL of 0.2% 2,4-dinitrophenylhydrazine (DNPH) in 2N HCL was added to pellet B (used for carbonyl concentration measurement). Both samples were incubated at room temperature for 1h. Later, samples were again precipitated with 1mL of 10% TCA and washed twice with 1mL ethanol:ethyl acetate (1:1) to eliminate previous reagents. Finally, 1.5mL of 6M guanidine HCL with 20mM sodium phosphate buffer was added. Carbonyl concentration was measured on the treated sample (B) by measuring DNPH incorporated on the basis of absorption of 21.0 mM^{-1} cm^{-1} at 370nm for protein hydrazones. Results were expressed as nmol of DNPH fixed per milligram of protein. Protein concentration was calculated by spectrophotometry at 280nm using bovine serum albumin (BSA) as standard. Protein oxidation was expressed as nmol carbonyls/mg protein.

2.14.- Determination of the activity of lipogenic enzymes. For the measurement of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) activity in subcutaneous fat and in *Longissimus dorsi* and *Biceps femoris* muscles, 2g of subcutaneous fat or muscles –*Longissimus dorsi* and *Biceps femoris*- were homogenized in 10 (adipose tissue) or 5 (muscle) mL of an ice-cold 25mM Tris-HCL buffer containing 9% glycerol, 5mM $MgCl_2$ and 7mM β-mercaptoethanol (pH 7.6). Then, the homogenates were centrifuged at 3000×g at 4ºC for 10min and the supernatant was again centrifuged at 25000×g at 4ºC for 20min. Finally, the supernatant was collected for glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) and malic enzyme (ME) activities (EC 1.1.1.40). Enzyme activities were measured at 340nm for 30min at 30°C with a thermospectrophotometer (Thermo Electron Corp. Helios α. Waltham, MA) and expressed on the basis of nmol NADP reduced to NADPH per min per mg protein, by the extinction coefficient of NADPH at 340nm of 6.22×10^6 M^{-1} cm^{-1}. Determinations were made in triplicate.

Glucose-6-phosphate dehydrogenase (G6PDH) was determined by the method of Bautista *et al.*., (1988). The enzyme extracts were mixed with Tris buffer (1M, pH 8) $NADP^+$ (10 mM) and $MgCl_2$ (0.1 M). The reaction was initiated by the addition of 60 µL of glucose-6-phosphate to a final volume of 1 mL and monitored at 340nm.

Malic enzyme (ME) determination was measured according to the method of Spina *et al.*., (1970). The reaction mixture contained Tris buffer (0.5M, pH 7.4), $NADP^+$ (40mM), $MgCl_2$ (0.1 M), NH_4

(80mM), KCl (2M) and malic acid (200 mM). The reaction was initiated by the addition of 400µL of the enzyme extract to a final volume of 1 mL and monitored at 340nm.

Protein determination was measured spectrophotometrically at 595nm according to the method of Bradford (1976) in microtiter plates. 10µL of enzyme extracts were added in each bowl. Finally, 200µL of Bradford reactive composed of Commassie brilliant blue in ethanol and phosphoric acid (85%) were added in each bowl. The plate was incubated at room temperature for 5 min. At last, the absorbencies were measured in an ELISA lector (595nm) using a curve of BSA (bovine serum albumin) as standard.

2.15.- Weight loss of dry-cured products by refrigerated storage. Dry-cured loins were cut into cubes of $1cm^3$. The pieces were supported with a needle in a Styrofoam plate to allow a similar drying on each face of the cube. The samples were refrigerated at 4ºC for 10 days. The cubes were weighed before (day 0) and after the storage (day 5 and 10). The percentage of loss was calculated by difference of weight as follows:

$$((Wb-Wa)/Wb)x100;$$

Where:

Wb: weight before storage
Wa: weight after storage.

2.16.- Texture analysis. Texture analysis was performed in a texturometer TA XT-2i Texture Analyser (STable Micro Systems Ltd., Surrey, U.K.). For the determination of texture profile analysis (TPA) uniform portions of the dry-cured loins and hams were cut into $1cm^3$ cubes. Samples were axially compressed to 50% of the original height with a flat plunger of 50 mm diameter (P/50) at a crosshead speed of 2mm/s through a 2-cycle sequence. The following texture parameters were measured from force–deformation curves (Bourne, 1978): Hardness (N/cm^2) = maximum force required to compress the sample (peak force during the 1^{st} compression cycle); adhesiveness (N s) = work necessary to pull the compressing plunger away from the sample; springiness (cm) = height that the sample recovers during the time that elapses between the end of the 1^{st} compression and the start of the 2^{nd}; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the 1^{st} compression and A2 the total energy required for the 2^{nd} compression); chewiness (N s) = the work needed to chew a solid sample to a steady state of swallowing (hardness x cohesiveness x springiness). For Warner-Bratzler (W-B) analyses, samples were prepared in 2 x 30 x 15mm slices (thickness x length x width) using a slicing machine. Samples were cut with a Warner-Bratzler blade (HDP/BS) in perpendicular direction to the muscle fibres. Determinations were repeated 8 times per sample and were averaged.

2.17.- Sensory analysis of dry-cured meat products. Fourteen trained panellists formed the tasting panel. Analyses were developed in tasting rooms with the conditions specified in UNE regulation. FIZZ Network (version 1.01: Biosystemes, France) software was used for the sessions and the recording data obtained. M. *Biceps femoris,* dissected from hams, and dry-cured loins were sliced (2mm thick) parallel and transversal to muscle fibre direction, respectively, with a slicing machine. Three slices of each dry-cured product were given to panellists who evaluated different

parameters (Table 2) in a quantitative-descriptive analysis with a non structured scale 0-10. Three samples were analyzed in each tasting session, which were randomly presented to the panellists. In addition, about 100 ml of mineral water at room temperature was provided to panellists. All sessions were conducted at ~20ºC in a 6-booth sensory panel room equipped with white fluorescent lighting. In each session, the panel average for each sample was recorded.

Table 2. Sensory attributes evaluated by the tasting panel in the sensory analyse of dry-cured products.

Appearance	
lean colour	Intensity of red colour in the lean (light pink-dark brown)
Marbling	Level of visible intramuscular fat (very lean-intense marbled)
Aroma	
Odour	Intensity of odour before eating (odourless - very intense odour)
Texture	
Hardness	Firmness perception during chewing (very tender- very firm)
Fibrousness	Perception of fibres during chewing (not fibrous-very fibrous)
Juiciness	Impression of juiciness during chewing (not juicy - very juicy)
Flavour	
Flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
sweet taste	Intensity of sweet taste (not sweet - very sweet)
salty taste	Intensity of salt taste (not salty - very salty)
acid taste	Intensity of acid taste (not acid- very acid)
bitter taste	Intensity of bitter taste (not bitter- very bitter)

2.18.- Volatile analyses of dry-cured meat products. A SPME fiber (Supelco Co. Canada) coated with divinilbenzene-carboxen-poly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm was used for the extraction of volatiles. Prior to analysis, the SPME fiber was preconditioned at 270ºC for 50min in the gas chromatograph injection port. The sampling technique to extract volatile compounds from headspace was the following: ~0.5g of meat were minced and placed in 5mL vials with a silicone stopper which were previously deodorized heating them in a electric stove at 80ºC at least for 2 hours. Each sample was analyzed in duplicate. The fiber was exposed to the headspace of the sample for 30 min, immersing it in water at 37ºC. The analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector Agilent model 5973. Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (30m x 0.25mm i.d., 1.0mm film thickness; Restek). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6mL/ min at 40ºC. The injection port was in the *splitless* mode and the temperature program was isothermal at 40ºC for 10min and then raised at a rate of 7ºC seg^{-1} to 250ºC and held for 5min. The GC-MS transfer line temperature was 270ºC. The MS operated in the electron impact mode with electron impact energy of 70eV; a multiplier voltage of 1650V and a collected data at a rate of 1scan s^{-1} over a range of *m/z* 40-300. n-Alkanes (Sigma R-8769) were analyzed under the same conditions to calculate the Retention Indices (RI) values for the volatiles. RI were calculated by the formula of Van Den Dool and Kratz (1963):

$$RI = [100\ (tr_i - tr_z)/(tr_{z+1} - tr_z)] + 100z$$

Where:

RI= Relative index of the compound i.
tr_i= Retention time of the compound i.
tr_z= Retention time of the alkane z

tr_{z+1}= Retention time of the alkane z+1
z= number of carbon atoms in alkane z

Volatile compounds were identified by comparison with reference compounds (Sigma, Aldrich), by comparison of RI with those described by Kondjoyan and Berdagué (1996) and by comparison of their mass spectra with those contained in Wiley library.

2.19.- Statistical analysis. The effects of genotype and sex were analyzed by the Analysis of Variance (ANOVA) procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. Means were used to compare differences. Tukey's test was applied to compare the mean values of the genotypes. The relationships between traits were analyzed by the calculation of Pearson's coefficient and by the principal component analysis (PCA).

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Chapter I

CARCASS COMPOSITION AND MEAT QUALITY OF 3 DIFFERENT IBERIAN X DUROC GENOTYPE PIGS[1]

ABSTRACT

Carcass composition and meat quality of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles from 3 different Iberian x Duroc genotype pigs were studied: GEN1: ♂ Iberian x ♀ Duroc1; GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. Duroc1 (DU1) were selected for manufacture dry-cured meat products while Duroc2 (DU2) were pigs selected for meat production, with high percentages of meat cuts and low carcass fat. Genotype had a significant effect on the differences found while sex had not. GEN2 showed the highest weights at days 180 and 238 of weaning and the highest slaughter weights (day 316) followed by GEN3, while the lowest weights were found in GEN1. GEN3 had well conformed carcasses in comparison with GEN1 and GEN2, since GEN3 showed the highest percentages of ham and loin and the highest weight of loin as well as the lowest back and ham fat thickness. However, the use of DU2 pigs in the cross with Iberian had negative effects on meat quality, as GEN3 gave the worst meat quality in both muscles analyzed, in *postmortem* pH, cook and drip loss, and colour and the lowest percentages of intramuscular fat (IMF). In subcutaneous fat (SCF), GEN3 had higher percentages of polyunsaturated fatty acids (PUFA) than GEN2, while GEN2 had higher saturated fatty acids (SFA) levels. In LD, IMF from GEN3 showed the highest percentage of MUFA and PUFA; while the fatty acid profile of GEN2 was more saturated. BF muscle showed similar trend, but not significantly. On the other hand, few differences were found between reciprocal crosses (GEN1 vs. GEN2). GEN2 showed higher IMF in LD than GEN1, agreeing with their carcass weight. As a result, GEN1 had a fatty acid profile of IMF in the LD more unsaturated.

Keywords: meat quality, carcass, Iberian, Duroc, crossbreeding, pH.

1.- INTRODUCTION

The number of Iberian pigs (pure and crossed) has increased in recent years, mainly as a consequence of the increase in consumption of dry-cured meat products and fresh meat, which has improved the productive and feeding systems of the Iberian pig. Nowadays, one of the alternatives applied to improve productive parameters is to cross with the Duroc breed at 50%. These crosses increase the number of piglets per sow and the weight at weaning and at the end of fattening (Aparicio, 1987), without changes on the adaptation capacity of the Iberian pig to the environment and without reducing the quality of the cured meat products (Antequera, García, López, Ventanas, Asensio, & Córdoba, 1994; López-Bote, 1998; Tejeda, Gandemer, Antequera, Viau, & García, 2002). Iberian x Duroc crosses are so popular that it is estimated that among the pigs slaughtered as "Iberian", less than the 25% are pure (Sierra, 1992). This makes it important to assess the selection of the Duroc lines to cross with Iberian, because the Duroc breed cannot be considered a homogeneous breed as a consequence of its vast distribution (Jonnes, 1988), which has favoured genetic selection according to the different criteria of production and meat quality.

Some authors (Castellanos, Barragán, Rodríguez, Toro, & Silió, 1997) have warned that the high frequency of Iberian x Duroc crosses could be a danger for the genetic purity of the Iberian breed. As a consequence, a specific law for Iberian products was passed in 2001 in Spain (B.O.E., 15th October 2001). One of the most important aspects of this law is the genotypes that can be used in the manufacture of dry-cured meat products (hams, shoulders and loins) labelled as "Iberian". The

[1] Ramírez, R., & Cava, R. (2006). Carcass composition and meat quality of 3 different Iberian x Duroc genotype pigs. doi:10.1016/j.meatsci.2006.08.003

law allows the use of pure Iberian pigs as well as Iberian x Duroc crosses. However, in the crosses it is necessary to use Iberian females to preserve the genetic purity and the biodiversity of the Iberian breed. In this sense, Morcuende, Estévez, Ramírez, & Cava, (in press) have reported on the existence of productive improvements such as higher slaughter weight and meat yields in the crosses that use Duroc sows rather than Iberian ones.

Previous research (Morcuende, Estévez, Ramírez, & Cava, 2004; Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst, 2001) have assessed the differences between Duroc genetic lines. Cilla *et al*., (2006) and Soriano, Quiles, Mariscal and García, (2005) found significant differences between sire Duroc lines in crossbreedings which affected carcass composition and meat quality for the manufacture of dry-cured meat products. The selection carried out in some Duroc lines was to improve lean growth efficiency yielding leaner and less fat pigs and higher growth rates. Different authors have reported that when strong emphasis is placed on high lean conversion efficiency, unfavourable changes in pork quality can occur (Cameron, Nute, Brown, Enser,& Wood, 1999; Lonergan *et al*., 2001; McPhee & Trout, 1995 and Oksbjerg *et al*., 2000). Therefore, the cross of Iberian with these Duroc lines could affect productive parameters and meat quality. The limited amount of previous research on the consequences of the use of different Duroc lines in crosses with Iberian pigs, as well as the consequences of using the Iberian line as maternal or paternal line shows the need to develope studies to clarify these aspects.

2. - MATERIALS AND METHODS

2.1.- Animals.

3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening. The genotype Duroc2 (DU2) corresponded to animals selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were castrated, as is traditionally done and they were raised together in a semi-intensive system and were fed *ad libitum* with the diet shown in Table 1. Pigs were randomly slaughtered after 316 days of rearing at 150-165 Kg live weight.

Table 1.- Proximate composition (%) and fatty acid composition (% total fatty acids) of the pig diets

	Mixed diet	
	I	II
Proximate composition		
Crude protein	16.0	13.5
Crude fat	3.3	5.0
Crude fiber	4.8	3.7
Ash	6.9	6.2
Lysine	0.9	0.5
Metabolizable energy (Kcal/Kg)	3071.5	3183.7
Fatty acid composition		
C14:0	0.1	0.1
C16:0	14.6	21.0
C18:0	4.4	5.6
C18:1n-9	23.3	31.3
C18:2n-6	34.7	35.1
C18:3n-6	2.0	2.3

Mixed diet I: from 60 to 100kg live weight
Mixed diet II: from 100 to 165 kg live weight

2.2.- Growth performance and carcass composition.

The weights of hams, shoulders and loins were taken 5 hours post-mortem. The backfat thickness (BFT) and ham fat thickness (HFT) were measured in the 5th rib and in the m. *Gluteo-biceps* in the carcass and ham, respectively. Samples of subcutaneous fat (taken from backfat at 5th-6th rib) and *Biceps femoris* (BF) and *Longissimus dorsi* (LD) muscles were dissected from the carcasses and stored at -80ºC until analyses.

2.3.- Muscles composition.

pH values 45 min (pH_{45}) and 24h (pH_U) after slaughter in the BF and LD muscles were measured with a puncture pHmeter Crisol mod. 507. Lipids were extracted from 5g of meat with chloroform/methanol (1:2), according to Bligh and Dyer (1959). Protein content was determined by the Kjieldalh method (AOAC, 2000), moisture was determined by drying the samples (~5 g) at 102ºC (AOAC, 2000) and haem pigments were assessed following the method of Hornsey (1956).

2.4.- Fatty acid profile determination.

Fatty acid methyl esters (FAMEs) were prepared by transesterification using methanol in the presence of sulphuric acid (5% of sulphuric acid in methanol). FAMEs were analyzed in a Hewlett-Packard model HP-5890A gas chromatograph, equipped with a flame ionization detector (FID). They were separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0mm film thickness). The injector and detector temperatures were held at 230 °C and the oven temperature, at 220°C. The flow rate of the carrier gas (N_2) was set at 1.8mL/min. Identification of FAMEs was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percent of major FAMEs.

2.5.- Instrumental colour.

Colour measurements were made following the recommendations on colour determination of the American Meat Science Association (Hunt *et al*., 1991). The following colour coordinates were determined: lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue). Colour parameters were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a white tile. In addition, hue angle, which describes the hue or colour was calculated ($H^º$=arctg b*/ a* · 360/2π) as well as the saturation index or chroma (C*) (C= $(a^{*2}+b^{*2})^{0.5}$), which describes the brightness or vividness of colour. The measurements were repeated at 5 randomly selected places on each slice and averaged.

2.6.- Drip and cook loss.

Drip loss was measured following the method of Honikel (1998). The samples were weighed and suspended in two plastic bags, the inner bag perforated, and the exudates was collected in the outer bag. Meat was stored for 10 days at refrigerated storage (4ºC). Drip loss (%) was calculated by difference in weight between day 0 and day 10 of storage. For cook loss (%), each chop was placed in a plastic bag and cooked by immersion at 80ºC for 60 min. The difference of weight before and after cooking was used to calculate cook loss percentage.

2.7.- Statistical analysis.

The effects of genotype and sex were analyzed by the Analysis of Variance (ANOVA) procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. Means were used to compare differences. HSD Tukey's test was applied to compare the mean values of the genotypes. The relationships between traits were analyzed by the calculation of Pearson's coefficient.

3.- RESULTS AND DISCUSSION

3.1.- Growth performance and carcass composition.

Genotype significantly affected live weight and average daily gain (ADG) during the whole rearing period while sex had no effect on growth and ADG (Table 2). Pigs from GEN2 showed the highest live weights at day 180 and 238 and at the end of rearing (day 316), in contrast animals from GEN1 had the lowest weights. Animals from GEN3 showed intermediate weights. Animals from GEN2 and GEN3 had higher ADG than GEN1 from day 180 to 238 (GEN1: 674, GEN2: 753, GEN3: 782g/day); however, between days 238-316, ADG was similar in the 3 genotypes (GEN1: 390, GEN2: 397, GEN3: 396g/day).

Table 2.- The weight of the different genotypes and the males and females during the growth.

	Genotype			Sex			Significance		
Day of rearing	GEN1	GEN2	GEN3	♂	♀	Sem	genotype	sex	interaction
180	80.1b	91.2a	84.0ab	86.6	84.0	1.7	*	ns	ns
238	119.2b	134.9a	129.4ab	131.1	125.4	2.3	*	ns	ns
316	149.7b	165.9a	160.3ab	162.3	155.8	2.4	*	ns	ns

ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001

Carcass composition was significantly affected by genotype whereas sex only affected the loin and shoulder weights; these were significantly higher in males than in females (Table 3). Carcass weight was significantly higher in GEN2 than in GEN1, while GEN3 presented an intermediate weight, agreeing with the results for slaughter weight (day 316). In contrast, killing out percentage (KOP) was significantly lower in GEN3 than in GEN1 and GEN2. Genotype had a major effect on the weight and percentage of loin and ham, being higher in carcasses from GEN3 than in the other two genotypes. The level of carcass fattening, measured as BFT and HFT, was significantly higher in GEN2 than in GEN3, while the fattening of GEN1 was intermediate.

Table 3.- Production parameters of the genotypes and sexes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Carcass (Kg)	124.6b	135.8a	125.9ab	132.0	125.5	2.04	*	ns	ns
Killing Out (%)	82.8a	81.9a	78.6b	81.5	80.7	0.62	**	ns	ns
Backfat thickness (cm)	6.3ab	6.4a	5.3b	5.9	6.1	0.18	*	ns	ns
Ham fat thickness (cm)	3.1ab	3.4a	2.7b	3.2	2.9	0.11	**	ns	ns
Meat pieces weight									
Ham(Kg)[1]	13.9b	15.3a	15.1ab	15.2	14.3	0.23	ns	ns	ns
Shoulder (Kg)[1]	9.6	10.4	9.9	10.3	9.6	0.16	ns	**	ns
Loin (Kg)	2.5b	2.7b	3.2a	2.9	2.7	0.09	***	*	**
Percentages (%)									
Ham percentage (%)	11.2b	11.3b	11.9a	11.5	11.4	0.10	**	ns	ns
Shoulder percentage (%)	7.7	7.6	7.9	7.8	7.7	0.05	ns	ns	ns
Loin percentage (%)	2.0b	2.0b	2.5a	2.2	2.1	0.07	***	ns	*

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB. [1]: Weight without shaping.
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001. a,b: Different letters in the same row indicate significant statistical differences (Tukey's test, p<0.05)

Results show an important effect of the Duroc sire line on carcass composition. Therefore, the use of Duroc lines selected on the basis of their productive parameters (DU2) produce hybrids (GEN3) with high weights and percentages of the main cuts and low levels of carcass fat. Nonetheless, for the manufacture of high quality dry-cured meat products (hams, shoulders, loins...) a high level of fattening is required to provide correct ripening during maturation for the development of their sensory characteristics (Gandemer, 2002).

As regards the differences between reciprocal crosses (GEN1 vs GEN2), only small differences were found, which suggests that maternal effects did not play an important role on productive and carcass parameters. These results contrast with those reported by Morcuende *et al*. (in press) in a study with Iberian x Duroc reciprocal crosses, who found better productive parameters in animals from Duroc sows than in animals from Iberian sows.

3.2.- Meat quality parameters.

In general, differences found in meat quality were mainly due to pig genotype, sex did not affect them (Table 4). Regarding differences between muscles, the effect of the genotype on the parameters analyzed was more marked in LD than in BF muscle.

The rate of pH decline was significantly higher in GEN3 than in GEN1 and GEN2. In this respect, the pH value at 45min post-mortem (pH_{45min}) was significantly lower in BF from GEN3 than in the other two genotypes, and the ultimate pH values (pH_U) were the lowest in LD and BF from GEN3.

Muscles from GEN3 tended to have a higher loss of water during cooking and storage than those from GEN1 and GEN2. Thus, drip loss during refrigerated storage was the highest in the LD from GEN3 and significantly higher than GEN1 in the BF. Similarly, water loss during cooking was higher in muscles from GEN3 than in the other two genotypes, although differences were only significant in the LD.

Regarding instrumental colour, lightness (CIE L*-value) was highest in LD and BF from GEN3, which indicates that meat from GEN3 was paler in comparison with the other genotypes. No significant differences were found in CIE a*-value and CIE b*-value due to genotype. The effect of sex on instrumental colour was small and only redness (CIE a*-value) of the BF muscle was significantly higher in females than in males. The content of heme pigments was highest in GEN1 in both muscles, although the differences were only significant in LD.

The incidence of PSE meat is one of the main quality problems in pork (Oliver, Gisperr, & Diesdre, 1993). The appearance of this type of meat can be monitored by measurements of pH, instrumental colour and exudate loss (Kauffman *et al*., 1993) as PSE meat is characterized by its intense paleness, low pH, low consistency and intense exudation (Briskey, 1964). Findings indicate that LD and BF from GEN3 showed the characteristics of PSE meat since they were paler (CIE-L*), had high acidification post-mortem (pH_{45} and pH_U) and were associated with high drip and cook losses. Post-mortem pH is important since it is normally associated to muscle paleness. In this respect, significant negative correlations ($p<0.01$) were found ($r=-0.624$) between pH_U and L*.

The IMF level is one of the muscle parameters that influence meat and meat products quality. GEN2 showed higher IMF contents than GEN3 in both muscles, whereas in the reciprocal crosses (GEN1

vs GEN2); IMF of the LD muscle was higher in GEN2 than in GEN1, according to the higher fat and carcass weights of GEN2. Differences due to genotype were only significant in LD, although BF showed the same trend. Therefore, meat from GEN2 because of the higher content of IMF has the best characteristics for the manufacture of high quality meat products, in which an increase in IMF supposes a marked enhancement of quality (Gandemer, 2002). However, the lower IMF content in LD and BF of the GEN3 could be negative for the sensorial characteristics of meat and meat products, such as taste, aroma, tenderness and/or juiciness (Essén-Gustavson, Karlsson, Lundström, & Enfalt, 1994; Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000).

Table 4.- Moisture, intramuscular fat (IMF) and protein contents (g/1000g), haem pigments (mg/100g) pH, drip loss (% DL) cook loss (%CL) and instrumental colour of the LD and BF muscles.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	Interaction
pH_{45} LD	5.7	5.8	5.8	5.7	5.8	0.05	ns	ns	ns
pH_{45} BF	5.8a	5.9a	5.6b	5.7	5.7	0.05	**	ns	ns
pH_U LD	5.7a	5.5ab	5.1b	5.4	5.4	0.07	*	ns	ns
pH_U BF	5.8a	5.7a	5.2b	5.5	5.6	0.06	***	ns	ns
Drip and cook loss									
DL LD (%)	4.0b	4.3b	6.0a	4.8	4.8	0.30	**	ns	ns
DL BF (%)	3.4b	4.0ab	5.1a	3.9	4.4	0.28	*	ns	ns
CL LD (%)	6.0b	6.5b	10.4a	8.1	7.3	0.54	***	ns	ns
CL BF (%)	9.7	11.0	11.6	10.9	10.7	0.35	ns	ns	ns
Instrumental colour									
Cie L* LD	48.9b	53.1a	52.8a	51.8	51.6	0.52	***	ns	ns
Cie L* BF	41.9b	42.4b	46.8a	44.7	42.9	0.74	*	ns	ns
Cie a* LD	10.2	10.0	9.9	9.9	10.2	0.26	ns	ns	ns
Cie a* BF	18.3	17.4	17.9	17.2	18.5	0.31	ns	*	ns
Cie b* LD	4.3	4.9	5.0	4.7	48	0.17	ns	ns	ns
Cie b* BF	6.2	6.2	7.2	6.7	6.4	0.23	ns	ns	ns
Haem pigments									
Hematine LD (mg/Kg)	48.7a	38.3b	37.9b	41.0	41.7	1.42	**	ns	ns
Hematine BF (mg/Kg)	93.1	86.8	82.4	80.6	93.4	2.26	ns	***	**
Chemical composition									
Moisture LD (g/100g)	71.4ab	70.3b	71.7a	71.0	71.2	0.24	*	ns	ns
Moisture BF (g/100g)	74.1a	73.3b	73.6b	73.6	73.5	0.16	*	ns	ns
Protein LD (g/100g)	23.4a	21.3b	21.3b	22.2	21.7	0.34	*	ns	ns
Protein BF (g/100g)	19.2b	19.3b	20.5a	19.9	19.5	0.23	*	ns	ns
IMF LD (g/100g)	3.8b	5.9a	3.5b	4.4	4.4	0.28	***	ns	ns
IMF BF (g/100g)	3.4	3.7	3.0	3.4	3.4	0.17	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
LD: *Longissimus dorsi*; BF: *Biceps femoris,* DL drip loss, CL cook loss
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001. a,b: Different letters in the same row indicate significant statistical differences (Tukey's test, p<0.05)

Sex of animals had no effect on the fatty acid composition of SCF and IMF of LD and BF muscles, however, genotype significantly affected fatty acid profiles of both locations (Table 5).

SCF from GEN3 had a higher content of unsaturated fatty acids than GEN1, mainly due to a higher content of C18:2n-6 and polyunsaturated fatty acids (PUFA), and a lower content of C18:0 than GEN2. The differences between genotypes GEN2 and GEN3 are due to the different paternal lines of Duroc (DU1 and DU2); whereas no significant differences were found between reciprocal crosses (GEN1 vs. GEN2).

The genotype significantly affected fatty acid composition of IMF from the LD muscle, while the BF muscle did not show significant differences among genotypes, although the trend was similar to LD (Table 5). PUFA content was significantly higher in LD from GEN1 and GEN3 than from GEN2,

which showed the highest percentages of C16:0, C18:0 and saturated fatty acids (SFA). In LD, the Duroc sire line (DU1, DU2) caused significant differences in the fatty acid composition of the IMF, since IMF from GEN3 was more unsaturated with high contents of C18:1n-9, monounsaturated fatty acids (MUFA), C18:2n-6, C20:4n-6, PUFA and a low content of C16:0, C18:0 and SFA. Similarly, some differences in the fatty acid profile of IMF were found between LD from reciprocal crosses (GEN1 vs. GEN2). IMF from GEN1 was more unsaturated than GEN2 as a consequence of a higher content of C18:2n-6, C18:3n-6, C20:4n-6, PUFA, and a lower content of C16:0 and SFA, which agrees with the higher IMF in GEN2 than in GEN1 (5.9 vs 3.8g/100g, respectively).

Table 5.- Major fatty acids composition (%total fatty acids) in subcutaneous and intramuscular fat in *Longissimus dorsi* and in *Biceps femoris* muscles as related genotype and sex.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Subcutaneous fat									
C16:0	24.1	24.7	24.6	24.4	24.5	0.10	ns	ns	ns
C18:0	13.3ab	13.7a	12.3b	13.2	13.0	0.19	*	ns	ns
C18:1n-9	46.9	45.7	46.1	46.1	46.2	0.21	ns	ns	ns
C18:2n-6	9.7b	10.1ab	10.7a	10.1	10.2	0.14	*	ns	ns
C18:3n-6	0.5	0.5	0.5	0.5	0.5	0.02	ns	ns	ns
C20:4n-6	0.1b	0.1b	0.2a	0.1	0.1	0.00	**	ns	ns
SFA	38.0	39.0	37.7	38.3	38.2	0.22	ns	ns	ns
MUFA	50.9	49.7	50.3	50.2	50.3	0.25	ns	ns	ns
PUFA	11.0b	11.3ab	12.0a	11.4	11.5	0.14	*	ns	ns
Longissimus dorsi									
C16:0	23.8b	25.2a	23.6b	24.1	24.2	0.20	***	ns	*
C18:0	12.8a	13.6a	11.7b	12.5	12.9	0.21	***	ns	ns
C18:1n-9	48.8ab	48.0b	49.1a	48.7	48.6	0.20	*	ns	ns
C18:2n-6	6.1a	4.9b	6.4a	5.9	5.7	0.19	***	ns	ns
C18:3n-6	0.3a	0.2b	0.3a	0.3	0.3	0.01	***	ns	ns
C20:4n-6	1.0b	0.8c	1.3a	1.1	1.0	0.07	***	ns	ns
SFA	38.3b	40.6a	36.9b	38.3	38.9	0.40	***	ns	**
MUFA	53.6ab	53.0b	54.4a	53.8	53.6	0.23	*	ns	ns
PUFA	8.1a	6.4b	8.6a	7.8	7.5	0.27	***	ns	ns
Biceps femoris									
C16:0	22.7	22.6	22.0	22.4	22.4	0.17	ns	ns	ns
C18:0	11.8	11.4	10.8	11.3	11.4	0.16	ns	ns	ns
C18:1n-9	49.0	48.9	49.1	48.9	49.1	0.24	ns	ns	ns
C18:2n-6	7.3	7.3	8.2	7.6	7.6	0.22	ns	ns	ns
C18:3n-6	0.4	0.5	0.5	0.5	0.5	0.02	ns	ns	ns
C20:4n-6	1.6	1.5	1.9	1.7	1.6	0.08	ns	ns	ns
SFA	36.2	35.8	34.5	35.4	35.5	0.32	ns	ns	ns
MUFA	53.8	54.2	54.2	54.0	54.1	0.27	ns	ns	ns
PUFA	10.0	10.0	11.4	10.6	10.4	0.34	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001
a,b,c: Different letters in the same row indicate significant statistical differences (Tukey's test, p<0.05).
SFA: saturated fatty acids (C12:0, C14:0, C16:0, C17:0, C18:0, C20:0), MUFA: monounsaturated fatty acids (C16:1n-7, C17:1n-7, C18:1n-9, C20:1n-9), PUFA: polyunsaturated fatty acids (C18:2n-6, C18:3n-6, C20:2 n-6, C20:4n-6, C22:2n-6, C22:4n-6).

Morcuende *et al*., (in press) did not find significant differences in the quality parameters of LD and BF muscles from Iberian x Duroc reciprocal crosses. Nevertheless, reciprocal crosses of this study (GEN1 and GEN2) showed some differences, principally, in the chemical composition of the muscles and in the fatty acid composition of the LD. Probably, some of the differences found could be reduced with more homogeneous slaughter weights between these 2 groups (149.6 vs. 165.8Kg).

Differences in fatty acid profiles between genotypes could be attributed to different causes: i) a different content of IMF; ii) a different potential for endogenous synthesis of fatty acids. On the one hand, the lowest content of PUFA in GEN2 could be caused by the dilution effect of phospholipids and/or PUFA due to a high content of IMF. Similar results have been found by other authors in breeds or genotypes with high IMF contents (Cameron & Enser, 1991; Suzuki, Shibata, Kadowaki, Abe, & Toyoshima, 2003; Morcuende *et al*., in press). On the other hand, differences in the thickness of back and ham fat and the different fatty acid compositions, especially of SFA, suggest a different adipogenic potential for the genotypes, as a result of a different capacity for the synthesis and storage of fat related to genetic characteristics. This was supported by the significant correlations between BFT and HFT and the percentages of the major fatty acids found (Table 6). BFT and HFT correlated positively with C16:0, C18:0 and SFA, whereas they correlated negatively with C18:1n-9, C18:2n-6, PUFA and MUFA. It could indicate a higher adipogenic character of GEN2 than GEN3, while GEN1 would show an intermediate adipogenic potential. In pigs, endogenous synthesis of fatty acids takes place from acetyl CoA and malonil CoA molecules to produce palmitic acid (C16:0), from which stearic acid (C18:0) is synthesized. The highest proportion of SFA in GEN2 could suggest a high endogenous synthesis capacity of this genotype, as a consequence of the increase of the activity of the metabolic routes in which the enzymes involved in the synthesis of "de novo" fatty acids take part (Mourot & Kouba, 1998). In a parallel study, Ramírez, Morcuende and Cava (2006) determined the activity of lipogenic enzymes in LD and BF muscles and in SCF, finding a higher activity of both enzymes in GEN1 and GEN2 than in GEN3.

Table 6. Pearson's coeficient correlation (r) between fatty acid composition of the subcutaneous and intramuscular fat of the *L. dorsi* and *B. femoris* and back and ham fat thickess (BFT, HFT).

Subcutaneous fat	BFT	HFT
C16:0	0.120	-0.140
C18:0	0.286	0.374*
C18:1n-9	0.194	-0.05
C18:2n-6	-0.631**	-0.189
SFA	0.347	0.318
MUFA	0.076	-0.149
PUFA	-0.636**	-0.237
Longissimus dorsi	BFT	HFT
C16:0	0.467 *	0.279
C18:0	0.433	0.042
C18:1n-9	-0.255*	0.126
C18:2n-6	-0.462*	-0.374*
SFA	0.491**	0.187
MUFA	-0.260	0.155
PUFA	-0.497**	-0.403*
Biceps femoris	BFT	HFT
C16:0	0.362	0.249
C18:0	0.147	0.111
C18:1n-9	0.047	0.195
C18:2n-6	-0.322	-0.398*
SFA	0.300	0.227
MUFA	0.096	0.223
PUFA	-0.342	-0.377*

SFA: saturated fatty acids (C12:0, C14:0, C16:0, C17:0, C18:0, C20:0), MUFA: monounsaturated fatty acids (C16:1n-7, C17:1n-7, C18:1n-9, C20:1n-9), PUFA: polyunsaturated fatty acids (C18:2n-6, C18:3n-6, C20:2n-6, C20:4n-6, C22:2n-6, C22:4n-6).
*: Bilateral significant Pearson's correlation at level p<0.05 **: Bilateral significant Pearson's correlation at level p<0.01.

The differences in the fatty acid profile of SCF and IMF due to the genotype could have important consequences. Firstly, the fatty acid profile of the SCF determines Iberian pig carcass value (De Pedro, Casillas, & Miranda, 1997; García-Olmo *et al*., 2002), so differences in the fatty acid profile between genotypes can modify carcass classifications with regard to quality, which as a consequence can change its economic value. In addition, high proportions of PUFA reduce the oxidative stability of lipids, which could have negative effects on the sensory characteristics of fresh

meat and meat products (Nawar, 1996). From a point of view of the implication of the fatty acid profile for the quality of chilled meat and dry-cured meat products, meat from GEN3, due to its higher PUFA content, could be more susceptible to lipid oxidation, and therefore, to the development of rancidity.

3.3.- Pearson's correlation coefficient.

Table 7 shows Pearson's correlation coefficients between carcass composition and meat quality parameters. Significant positive correlations ($p<0.01$) were found between carcass weight and ham ($r= 0.857$) and shoulder weight ($r= 0.907$), ham fat thickness ($r= 0.482$) and IMF content of the LD ($r= 0.423$). In contrast, killing out percentage was negatively correlated ($p<0.05$) with loin ($r=-0.440$), ham ($r=-0.489$) and shoulder ($r=-0.382$) percentage and positively ($p<0.05$) with the thickness of backfat ($r= 0.370$) and ham fat ($r= 0.473$). Loin weight correlated positively with loin ($r= 0.880$, $p<0.01$) and ham percentage ($r= 0.488$, $p<0.01$) and ham weight ($r= 0.469$, $p<0.01$), whereas it correlated negatively with BFT ($r=-0.514$, $p<0.01$). Similar correlations were observed in ham and shoulder weights, since they showed significant positive correlations with percentages of other meat cuts and negative correlations with fat measurements, such as back and ham fat depths and IMF content. These correlations are evidence that high percentages of lean cuts in pork are associated with increases of lean growth efficiency and with decreases in fat depots such as the IMF content. IMF is a determinant factor for the manufacture of dry-cured meat products (Ruiz-Carrascal *et al*., 2000; Gandemer, 2002). Lonergan *et al*., (2001), in a study of different lines of Duroc pigs, found that the selection of Duroc in terms of lean growth efficiency causes important reductions in pork quality. Finally, significant positive correlations ($p<0.05$) were found between BFT and IMF content of LD ($r= 0.416$) and BF ($r= 0.457$) as has been previously reported by Hovenier, Brascamp, Kanis, Van Der Werf and Wassenber (1993) and Suzuki *et al*., (2003), who showed the importance of BFT measurements because of their close correlation with IMF levels.

Table 7. Pearson's correlation coefficient (r) between some production parameters and the chemical composition of *L. dorsi* and *B. femoris* of the pigs.

	KOP	Loin[1]	Loin[2]	Ham[1]	Ham[2]	Shoulder[1]	Shoulder[2]	BFT	HFT	IMF LD	IMF BF
Carcass[1]	0.345	0.214	-0.268	0.857**	-0.233	0.907**	-0.131	0.227	0.482**	0.423*	0.031
KOP		-0.283	-0.440*	0.087	-0.489**	0.181	-0.382*	0.370*	0.473*	0.269	-0.007
Loin[1]			0.880**	0.469**	0.488**	0.247	0.109	-0.514**	-0.220	-0.264	-0.180
Loin[2]				0.052	0.595**	-0.185	0.183	-0.620**	-0.465*	-0.448*	-0.201
Ham[1]					0.301	0.885**	0.142	-0.086	0.342	0.161	-0.007
Ham[2]						-0.012	0.502**	-0.586**	-0.256	-0.449*	-0.081
Shoulder[1]							0.298	0.082	0.393*	0.237	-0.026
Shoulder[2]								-0.309	-0.183	-0.377*	-0.142
BFT									0.269	0.416*	0.457*
HFT										0.354	0.324
IMF LD											0.277

1: weight, 2: percentage, KOP: killing out percentage. BFT: backfat thickness. HFT: ham fat thickness. IMF: intramuscular fat.
*** Bilateral significant Pearson's correlation at level $p<0.001$. ** Bilateral significant Pearson's correlation at level $p<0.01$. * Bilateral significant Pearson's correlation at level $p<0.05$.

4.- CONCLUSIONS

Iberian x Duroc reciprocal crosses did not show important differences in the carcass composition and meat quality parameters analysed. However, the different paternal lines of Duroc crossed with Iberian had marked influences on the production characteristics and meat quality. The use of Duroc males with selected genotypes (DU2) increases the lean meat content, although it reduces meat quality, since low post-mortem pH, intense paleness, low fatness and high cook and drip losses were found in the meat from this cross (GEN3), which could reduce the acceptability and shelf-life of the meat as well as its suitability for the manufacture of cured meat products. By contrast, the cross of Iberian females with Duroc males selected for the production of meat products (DU1) increases the carcass weight, though it reduces the lean meat content. Moreover, it produces high percentages of intramuscular fat and better quality characteristics in the meat, making it suitable for both cured meat production and for fresh consumption.

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Chapter II

FATTY ACID COMPOSITION AND ADIPOGENIC ENZYME ACTIVITY OF MUSCLE AND ADIPOSE TISSUE AS AFFECTED BY IBERIAN X DUROC PIG GENOTYPE[1]

ABSTRACT

The adipogenic potential of subcutaneous fat (SCF) and muscles *Longissimus dorsi* (LD) and *Biceps femoris* (BF) were evaluated in 3 different Iberian x Duroc pig genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. Reciprocal crosses (GEN1 vs GEN2) showed similar traits, while the genotype of the Duroc sire line (GEN2 vs GEN3) significantly influenced the adipogenic character. GEN3 had lower fat depths and a more unsaturated SCF than GEN2. The intramuscular fat (IMF) content of the LD was higher in GEN2 than in GEN3, while BF showed a similar trend. The fatty acid composition of IMF and neutral lipid fraction (NL) in LD and BF was more unsaturated in GEN3 than in GEN2. Glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) activities in SCF and in both muscles analysed were higher in GEN2 than in GEN3. The highest fat depths and IMF of GEN1 and GEN2 was in accordance to the highest G6PDH and ME activities, which indicates that the lipid synthesis and depot in muscles and in SCF were higher in GEN1 and GEN2 than in GEN3; being these differences associated with the Duroc sire line. Therefore, the use of Duroc selected genotypes reduces the adipogenic character of Iberian x Duroc crosses which could have important repercussions on the quality of meat and dry-cured products.

Keywords: lipogenic enzymes; glucose-6-phosphate dehydrogenase; malic enzyme; intramuscular fat; fatty acid; Duroc line; Iberian pig.

1.- INTRODUCTION

Nowadays, the Iberian pig breed has increased its popularity, because the meat and meat products traditionally obtained from this breed are highly appreciated by consumers for their excellent quality. Different studies have shown that meat from Iberian pigs has better quality (colour, fatty acid profile and sensory characteristics) than that obtained from industrial genotype pigs (Estévez, Morcuende, Cava, 2003). As a result, the census of Iberian pigs has increased in the last years, which has conducted to an improvement of the feeding and productive systems. As Iberian is a rustic breed with a slow growth rate and low prolificity (Dobao, García, De Pedro, Rodrigáñez, & Silió, 1986), to improve productive parameters, one of the alternatives more often applied is the cross of Iberian pigs with Duroc at 50%. A high proportion of Iberian pigs are crossed with Duroc since it increases the prolificity in 2-3 piglets, improves the growth rate, the feed efficiency and the lean content (Dobao *et al*., 1986) without a significant reduction of the quality of the meat and meat products (López-Bote, 1998). These crosses are so frequent that it is estimated that between the animals slaughtered as "Iberian", less than the 25% are pure Iberian (Sierra, 1992).

Despite of the fact that the consumption of Iberian pig fresh meat has increased in the last years, the production of Iberian pigs is mainly focused on obtaining raw meat to manufacture dry-cured meat products. For this purpose, the industry requires fat carcasses from castrated heavy pigs slaughtered at around 160kg of live weight. A high intramuscular fat content is important to aid a slow dehydratation during the curing process (Gandemer, 2002). Fat content and fatty acid composition

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are determinant factors for the quality of Iberian dry-cured meat products (Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000). Cava, Ruíz, Ventanas, and Antequera (1999) observed a marked influence of intramuscular fatty acid composition of fresh meat on the flavour of Iberian dry-cured meat products. Besides, fatty acid composition of intramuscular fat with high levels of linoleic acid can affect water migration, as a very unsaturated fat avoids water migration within the piece, and as a consequence, retards drying process (López-Bote, 1998; Girard, Bucharles, Berdague, & Ramihone, 1989).

In Spain, a specific law for Iberian products was passed in 2001 to regulate the Iberian market (B.O.E., 15th October 2001). One of the most important aspects that this law regulates is the genotype used for the manufacture of dry-cured meat products (hams, forelegs and loins) labelled as "Iberian". The law allows using pure Iberian pigs as well as Iberian x Duroc crosses, but in the crosses obliges always to employ Iberian females to preserve the genetic patrimony and the biodiversity of the Iberian breed. This fact makes it really important the assess and selection of the Duroc paternal line for the cross with Iberian, because Duroc breed is so widespread, that cannot be considered an homogeneous breed since important differences in productive and carcass parameters and in the quality of meat and meat products have been reported (Cilla *et al*., 2006; Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst 2001). However, concerning differences between Iberian x Duroc reciprocal crosses, Morcuende, Estévez, Ramírez, & Cava (2003) did not find important differences between them.

By contrast to other species in which the lipogenesis of fatty acids takes place in the liver, in pigs, it mainly takes place *in situ*, in the adipose tissues (O'Hea & Leveille, 1969). The synthesis of triglycerides of the adipose tissue comes from fatty acid from circulating triglycerides as a result of adipose lipoprotein lipase activity (Steffen, Brown, & Mersmann, 1978) and from fatty acid the synthesised *de novo* (mainly from diet starch) in that tissue (O'Hea & Leveille, 1969). The NADPH for synthesis of *de novo* fatty acids is supplied by malic enzyme (ME) and by glucose-6-phosphate dehydrogenase (G6PDH) (Young, Sharago, & Lardy, 1964; Wise & Ball, 1964). The G6PDH takes part in the route of pentose phosphate while the ME transforms malic acid in pyruvic acid. Endogenous synthesis of fatty acids comes from acetyl CoA and malonil CoA molecules to produce palmitic acid (C16:0), from which can be synthesized stearic acid (C18:0) by elongation, being these fatty acids unsaturated, by means of desaturase enzymes, in palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9). Differences in the lipogenic enzyme activity are mostly caused by animal genotype and to a less extent by the diet (Morales, Pérez, Baucells, Mourot, & Gasa, 2002). In this respect, higher lipogenic activity has been found in rustic breeds with high intramuscular fat levels than in industrial ones (Mourot & Kouba, 1998; Morales *et al*., 2002). Moreover, we have found important differences among genotypes in a previous study (Ramírez & Cava, 2006) in meat quality. The objectives of this study were to assess the consequences of the use of different Duroc paternal lines in Iberian x Duroc crosses as well as the differences between Iberian x Duroc reciprocal crosses on the adipogenic character and fatty acid composition of subcutaneous and intramuscular fat.

2.- MATERIALS AND METHODS

2.1.- Animals.

In order to develop this work, 3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype Duroc1 (DU1) were pigs selected for the manufacture of dry-cured meat products (hams, loins, forelegs), with a high level of fattening. The genotype Duroc2 (DU2) were animals selected for meat production, with high percentages of meat cuts and with low carcass fattening. Pigs were raised all together in an intensive system and were fed ad libitum with the concentrate feeds shown in table 1. Pigs were randomly slaughtered after 316 days of rearing with 150-165Kg live weight.

Table 1.- Proximate composition (%) and fatty acid composition (% total fatty acids) of the pig diets

	Mixed diet	
	I	II
Proximate composition		
Crude protein	16.0	13.5
Crude fat	3.3	5.0
Crude fiber	4.8	3.7
Ash	6.9	6.2
Lysine	0.9	0.5
Metabolizable energy (Kcal/Kg)	3071.5	3183.7
Fatty acid composition		
C14:0	0.1	0.1
C16:0	14.6	21.0
C18:0	4.4	5.6
C18:1n-9	23.3	31.3
C18:2n-6	34.7	35.1
C18:3n-6	2.0	2.3

Mixed diet I: from 60 to 100kg live weight
Mixed diet II: from 100 to 165 kg live weight

2.2.- Back fat and ham thickness.

The backfat thickness (BFT) and ham fat thickness (HFT) were measured in the 5^{th} rib and in the *Biceps femoris* muscle in the carcass and ham, respectively. *Biceps femoris* (BF) and *Longissimus dorsi* (LD) muscles were removed from the carcasses and stored at -80ºC until their analyses. For the analyses, the central part of the muscles was taken. Subcutaneous fat (SCF) was taken from the inner layer of the backfat in the $5\text{-}6^{th}$ thoracic rib and also was stored at -80ºC until being analysed.

2.3.- Fat extraction, lipid fractionation and fatty acid analysis.

Intramuscular lipids were extracted from 5g of meat with chloroform/methanol (1:2) according to the method described by Bligh and Dyer (1959) and quantified by weighing after solvent evaporation. Moisture content was determined by drying samples at 102ºC until constant weight according AOAC method (AOAC, 2000). Total lipid extracts from intramuscular fat were fractionated by solid phase extraction on 100mg aminopropyl minicolumns (Varian, CA), following the procedure described by Monin, Hortós, Díaz, Rock, and García-Regueiro (2003). Fatty acid methyl esters (FAMEs) from neutral lipids (NL), total IMF and SCF were prepared by acidic esterification (5% sulphuric acid in methanol). FAMEs were analysed in a Hewlett-Packard model HP-5890A gas chromatograph equipped with a flame ionization detector (FID). The FAMEs were separated on a semicapillary

column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0 µm film thickness). The injector and detector temperatures were held at 230°C. The column oven temperature was maintained at 220°C. The flow rate of the carrier gas (N_2) was set at 1.8mL/min. Identification of FAMEs was based on retention times of reference compounds (SIGMA). Fatty acid composition was expressed as percentage of FAMEs analysed.

2.4.- Tissue enzyme activity of lipogenesis enzymes.

1g of SCF or 2g of muscles –LD and BF- were homogenized in 10mL of an ice-cold 25mM Tris-HCL buffer containing 9% glycerol, 5mM $MgCl_2$ and 7mM β-mercaptoethanol (pH 7.6). The homogenate was centrifuged at 3000×*g* at 4ºC for 10 min and the supernatant was recentrifuged at 25000×*g* at 4ºC for 20 min. The resulting supernatants were analyzed for glucose-6-phosphate dehydrogenase (G6PDH) (EC 1.1.1.49) and malic enzyme (ME) (EC 1.1.1.40) activities.

Enzyme activities were measured at 340 nm at 30 °C for 3 min with a thermospectrophotometer (Thermo Electron Corp. Helios α. Waltham, MA) and expressed on the basis of nmol $NADP^+$ reduced to NADPH per min per mg protein, using the extinction coefficient of NADPH at 340 nm of $6.22 \times 10^6\ M^{-1}\ cm^{-1}$. Glucose-6-phosphate dehydrogenase (G6PDH) was determined by the method of Bautista, Garrido-Pertierra, & Soler (1988). 400 *µ*L of the enzymatic extract were mixed with 540 *µ*L of a buffer at pH 8 (10mL of Tris 1M, 2ml of $NADP^+$ 10 mM, 10 ml of $MgCl_2$ 0.1 M and 63 mL of mili-Q water). The reaction was initiated by the addition of 60 *µ*L of glucose-6-phosphate 20mM. The rate of reduction of $NADP^+$ was monitored at 340nm. Malic enzyme (ME) determination was measured according to the method of Spina, Bright & Rosenbloom. (1970). The reaction mixture contained 25*µ*L of $NADP^+$ 40 mM, 252 *µ*L of mili-Q water and 323 *µ*L of a buffer with pH 7.4 (200 *µ*L of Tris 0.5 M, 10 *µ*L of $MgCl_2$ 0.1M, 12.5 *µ*L of NH_4 80mM, 50 *µ*L of KCl 2M, 50 *µ*L of malic acid 200mM). The reaction was initiated by the addition of 400*µ*L of the enzyme extract to a final volume of 1 mL and monitored at 340 nm. Protein determination was measured spectrophotometrically at 595nm according to the method of Bradford (1976). All enzymes determinations were carried out in triplicates.

2.5.- Statistical analysis.

The effects of genotype and sex were analysed by the Analysis of Variance procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was applied. Means were used to compare differences. When means were significantly different a HSD Turkey's test was applied to compare the mean values of the genotypes. The relationships between traits were analysed by the calculation of Pearson's coefficient. Principal component analysis (PCA) was applied to determine relationships between variables and samples.

3.- RESULTS AND DISCUSSION

3.1.- Fat depths and fatty acid composition of subcutaneous fat.

Fat depths and fatty acid composition were significantly influenced by genotype, while they were not affected by animal sex (Table 2). Backfat thickness –BFT- and ham fat thickness –HFT- were significantly higher in GEN2 than in GEN3, while GEN1 carcasses had an intermediate degree of fattening. There was no effect of animal sex on fatty acid composition of SCF, whereas important

differences were found between genotypes. SCF of GEN3 was the most unsaturated, since it showed the highest percentages of C18:2n-6, C20:4n-6 and PUFA and the lowest of C18:0.

Table 2. Fat depths (mm) and fatty acid composition (g/100g FA) of subcutaneous fat from 3 different Iberian x Duroc genotypes.

	Genotype			Sex		Significance		
	GEN1	GEN2	GEN3	♂	♀	gen	sex	interaction
Fat depths(mm)								
BFT	63^{ab}±7.7	64^{a}±8.8	53^{b}±9.1	59±9.9	61±9.6	*	ns	ns
HFT	31^{ab}±4.2	34^{a}±5.2	27^{b}±5.8	32±5.1	29±6.3	**	ns	ns
Fatty acid composition								
C12:0	0.05±0.00	0.05±0.01	0.13±0.01	0.11±0.01	0.05±0.00	ns	ns	ns
C14:0	0.04±0.01	0.04±0.00	0.05±0.01	0.05±0.01	0.05±0.01	ns	ns	ns
C16:0	24.10±0.47	24.68±0.59	24.61±0.37	24.41±0.52	24.54±0.57	ns	ns	ns
C16:1 n-7	2.14^{b}±0.19	2.24^{b}±0.21	2.68^{a}±0.26	2.30±0.27	2.42±0.36	***	ns	ns
C17:0	0.31±0.03	0.30±0.04	0.32±0.05	0.31±0.04	0.31±0.04	ns	ns	ns
C17:1 n-7	0.31±0.02	0.30±0.04	0.36±0.08	0.32±0.04	0.33±0.07	ns	ns	ns
C18:0	13.35^{ab}±0.56	13.71^{a}±0.83	12.35^{b}±1.02	13.23±0.94	13.03±1.07	*	ns	ns
C18:1 n-9	46.96±0.87	45.71±0.94	46.07±1.29	46.18±1.38	46.26±0.92	ns	ns	ns
C18:2 n-6	9.70^{b}±0.69	10.07^{ab}±0.41	10.67^{a}±0.77	10.12±0.86	10.20±0.64	*	ns	ns
C18:3 n-6	0.47±0.11	0.49±0.11	0.57±0.04	0.48±0.14	0.53±0.04	ns	ns	ns
C20:0	0.21±0.02	0.22±0.02	0.21±0.02	0.22±0.02	0.21±0.02	ns	ns	ns
C20:1n-9	1.49^{a}±0.17	1.40^{ab}±0.17	1.20^{b}±0.20	1.44±0.22	1.28±0.18	**	ns	ns
C20:2 n-6	0.67±0.06	0.63±0.08	0.54±0.16	0.62±0.15	0.61±0.07	ns	ns	ns
C20:4 n-6	0.15^{b}±0.02	0.15^{b}±0.01	0.17^{a}±0.01	0.16±0.02	0.15±0.01	**	ns	ns
C22:4 n-6	0.07±0.04	0.01±0.03	0.06±0.04	0.06±0.04	0.03±0.04	ns	ns	ns
SFA	38.05±0.76	39.00±1.08	37.68±1.31	38.33±1.06	38.18±1.34	ns	ns	ns
MUFA	50.90±0.98	49.65±1.10	50.30±1.61	50.24±1.55	50.29±1.14	ns	ns	ns
PUFA	11.05^{b}±0.74	11.34^{ab}±0.47	12.01^{a}±0.78	11.44±0.88	11.53±0.68	*	ns	ns

GEN:genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
BFT: backfat thickness. HFT: ham fat thickness. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

Results indicate a higher fattening in GEN2 carcasses than in those from the other two genotypes, while no significant differences were found between the reciprocal crosses (GEN1 vs. GEN2). However, the differences found between the genotypes from the 2 paternal lines of Duroc (GEN2 vs. GEN3) indicate that the cross of a selected Duroc genotype (DU2) with Iberian reduced carcass fat and increased the degree of unsaturation of SCF. Differences in fatty acid composition are explained by differences in carcass fattening due to genotype. As more developed is the backfat in the carcass, higher is the proportion of fatty acids stored in the adipose tissue arising from *de novo* fatty acid synthesis, specifically SFA and MUFA, and less is the content in PUFA, since the proportion of PUFA provided by dietary lipids is lower (Gandemer, 2002). In terms of meat and technological quality, fatty acid composition has an important effect on softness and oxidative stability of fat and meat, being associated high PUFA level with a softer fat and with an increase in rancidity (Wood *et al*., 2003).

Table 3.- Intramuscular fat content (g/100g) and fatty acid composition (g/100g FA) of intramuscular fat and neutral lipid of m. *Longissimus dorsi* from 3 different Iberian x Duroc genotypes.

	Genotype			Sex		Significance		
	GEN1	GEN2	GEN3	♂	♀	gen	sex	interaction
IMF	3.84^{b}±0.72	5.87^{a}±1.42	3.32^{b}±1.03	4.39±1.63	4.33±1.47	***	ns	ns
IMF (dm)	13.39^{b}±2.30	19.61^{a}±3.80	11.69^{b}±3.67	14.99±5.14	14.91±4.33	***	ns	ns
Intramuscular fat fatty acid composition								
C12:0	0.04^{b}±0.00	0.06^{a}±0.01	0.05^{ab}±0.01	0.05±0.01	0.05±0.01	***	ns	*
C14:0	1.16^{b}±0.10	1.36^{a}±0.10	1.18^{b}±0.10	1.24±0.17	1.23±0.09	***	ns	*
C16:0	23.79^{b}±0.77	25.16^{a}±0.54	23.59^{b}±1.13	24.14±1.39	24.25±0.76	***	ns	*
C16:1 n-7	3.65^{b}±0.32	3.94^{ab}±0.33	4.16^{a}±0.39	4.00±0.42	3.86±0.38	**	ns	ns
C17:0	0.17^{ab}±0.03	0.14^{b}±0.02	0.18^{a}±0.03	0.17±0.04	0.16±0.02	**	ns	ns
C17:1 n-7	0.21^{ab}±0.02	0.18^{b}±0.02	0.21^{a}±0.04	0.20±0.03	0.20±0.04	*	ns	ns
C18:0	12.85^{a}±0.66	13.63^{a}±0.69	11.75^{b}±1.05	12.50±1.29	12.96±0.94	***	ns	ns
C18:1 n-9	48.82^{ab}±0.93	48.03^{b}±0.99	49.16^{a}±1.12	48.70±1.09	48.63±1.14	*	ns	ns
C18:2 n-6	6.15^{a}±0.47	4.95^{b}±0.60	6.38^{a}±1.12	5.90±1.21	5.73±0.79	***	ns	ns
C18:3 n-6	0.29^{a}±0.05	0.23^{b}±0.03	0.34^{a}±0.06	0.29±0.08	0.28±0.04	***	ns	ns
C20:0	0.26^{a}±0.01	0.25^{ab}±0.02	0.23^{b}±0.05	0.23±0.04	0.26±0.02	*	*	**
C20:1n-9	0.97±0.10	0.88±0.09	0.87±0.09	0.92±0.11	0.88±0.09	ns	ns	ns
C20:2 n-6	0.35^{a}±0.19	0.23^{b}±0.02	0.26^{ab}±0.04	0.31±0.17	0.25±0.03	*	ns	ns
C20:4 n-6	1.05^{b}±0.25	0.76^{c}±0.17	1.35^{a}±0.35	1.09±0.43	1.02±0.28	***	ns	ns
C20:5 n-3	0.04^{ab}±0.03	0.02^{b}±0.01	0.05^{a}±0.02	0.04±0.03	0.03±0.01	**	ns	ns
C22:2 n-6	0.03±0.01	0.02±0.01	0.02±0.00	0.02±0.01	0.02±0.00	ns	ns	ns
C22:4 n-6	0.19^{ab}±0.07	0.15^{b}±0.03	0.23^{a}±0.07	0.19±0.09	0.19±0.04	**	ns	**
SFA	38.27^{b}±1.28	40.60^{a}±1.00	36.97^{b}±2.06	38.33±2.68	38.90±1.52	***	ns	**
MUFA	53.64^{ab}±0.90	53.03^{b}±1.05	54.41^{a}±1.37	53.82±1.25	53.57±1.25	*	ns	ns
PUFA	8.09^{a}±0.74	6.37^{b}±0.81	8.63^{a}±1.58	7.85±1.78	7.52±1.15	***	ns	ns
Neutral lipids								
C12:0	0.07±0.02	0.07±0.00	0.08±0.02	0.08±0.02	0.07±0.01	**	ns	ns
C14:0	1.35^{b}±0.08	1.51^{a}±0.10	1.30^{b}±0.12	1.40±0.15	1.37±0.09	***	ns	*
C16:0	25.33^{ab}±0.57	26.06^{a}±0.47	24.61^{b}±0.81	25.16±1.05	25.50±0.56	***	ns	*
C16:1 n-7	3.88^{b}±0.39	4.08^{ab}±0.33	4.40^{a}±0.44	4.22±0.48	4.04±0.39	*	ns	ns
C17:0	0.17±0.02	0.15±0.02	0.18±0.03	0.17±0.03	0.16±0.02	ns	ns	ns
C17:1 n-7	0.20±0.06	0.17±0.05	0.23±0.05	0.20±0.05	0.20±0.05	ns	ns	ns
C18:0	12.98^{ba}±0.72	13.46^{a}±0.70	11.64^{b}±1.14	12.32±1.29	13.02±0.94	***	*	ns
C18:1 n-9	49.55^{a}±0.63	48.45^{b}±0.93	50.46^{a}±1.28	49.64±1.62	49.34±0.94	***	ns	**
C18:2 n-6	4.33±0.45	3.98±0.47	4.71±0.51	4.48±0.56	4.21±0.49	ns	ns	ns
C18:3 n-6	0.25^{ab}±0.02	0.22^{b}±0.02	0.29^{a}±0.04	0.26±0.04	0.25±0.03	***	ns	ns
C20:0	0.27±0.02	0.29±0.03	0.28±0.03	0.28±0.03	0.28±0.03	ns	ns	*
C20:1n-9	0.95^{a}±0.08	0.87^{b}±0.08	0.89^{ab}±0.08	0.93±0.09	0.88±0.07	*	ns	ns
C20:2 n-6	0.26^{ab}±0.03	0.24^{b}±0.02	0.29^{a}±0.03	0.27±0.04	0.25±0.03	*	ns	ns
C20:4 n-6	0.23^{b}±0.04	0.28^{ab}±0.07	0.42^{a}±0.09	0.37±0.12	0.26±0.05	**	*	ns
C20:5 n-3	0.05^{b}±0.01	0.03^{c}±0.01	0.07^{a}±0.02	0.05±0.02	0.05±0.01	***	ns	*
C22:2 n-6	0.04±0.02	0.05±0.01	0.05±0.02	0.05±0.01	0.05±0.02	ns	ns	ns
C22:4 n-6	0.08±0.01	0.10±0.02	0.10±0.02	0.11±0.02	0.08±0.02	ns	*	ns
SFA	40.16^{a}±1.07	41.54^{a}±0.98	38.10^{b}±1.86	39.40±2.33	40.41±1.34	***	*	*
MUFA	54.58^{b}±0.82	53.57^{b}±0.96	55.98^{a}±1.53	54.99±1.89	54.45±1.14	***	ns	*
PUFA	5.25^{ab}±0.53	4.89^{b}±0.57	5.93^{a}±0.63	5.60±0.68	5.13±0.60	*	ns	ns

GEN:genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
IMF: intramuscular fat. IMF(dm): intramuscular fat (dry matter). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

3.2.- Intramuscular fat content and fatty acid composition.

In general, the genotype was the main factor for the differences in IMF content and fatty acid composition of total IMF and NL of LD (Table 3) and BF (Table 4), while animal sex had no effect or it was negligible.

The IMF content of the LD muscle was affected by the genotype, as GEN2 had significantly higher IMF content than GEN1 and GEN3. BF showed a similar trend, GEN3 contained less IMF than the other two genotypes, although the differences were not significant. IMF content is a highly variable parameter and its variability is dependent on the genetic of the pig breed (McLaren, Buchanan, & Johnson, 1987). Intensive pig production and selection based on lean growth rate have led to a significant decrease in the IMF content of commercial breeds (Karlsson, Enfält, Essén-Gustavsson, Lundström, Rydhmer, & Stern, 1993). Important differences have been reported by Cilla *et al*. (2006) in the IMF and BFT of crosses with different Duroc sire lines. Increased IMF levels reduce the force for chewing and promote saliva secretion helping chewing and increasing juiciness and tenderness perception (Wood *et al*., 2003). Moreover, an adequate level of IMF is a determinant factor for the manufacture of dry-cured meat products (Gandemer, 2002; López-Bote, 1998). Therefore, the highest IMF content of GEN2 could contribute to improve the sensory quality of the meat and meat products.

In general, the fatty acid composition of LD was more affected by the genotype than BF, although fatty acid composition of the latter showed a trend similar to the former.

In LD, IMF from GEN3 was more unsaturated than that from GEN2, with higher contents of C18:1n-9, MUFA, C18:2n-6, C20:4n-6 and PUFA and lower contents of C16:0, C18:0 and SFA. These differences between GEN2 and GEN3 were associated with the Duroc sire line (DU1 or DU2). Concerning differences between reciprocal crosses (GEN1 vs. GEN2) in the IMF of this muscle, fatty acid profile of GEN1 was more unsaturated as a result of the higher percentages of C18:2n-6, C18:3n-6, C20:4n-6 and PUFA and the lower percentages of C16:0 and SFA. It agrees with the lowest IMF content of this genotype, since PUFA, which are mostly located in the cell membranes phospholipids, are relatively constant; therefore, a higher IMF level would give a higher level of triglycerides and a relative decrease in the phospholipids content (Leseigneur-Meynier & Gandemer, 1991).

Similarly, NL of the LD muscle showed important differences depending on the genotype of the Duroc paternal line, with highest levels of C16:0, C18:0 and SFA in GEN2 and highest levels of C18:3n-6, C20:2n-6, C20:4n-6, C20:3n-3 and PUFA in GEN3. However, reciprocal crosses (GEN1 and GEN2) showed similar percentages of SFA, MUFA and PUFA. In addition, significant differences in some fatty acid percentages were due to the sex of the animals. Females showed highest percentages of C18:0 and SFA, while males showed highest percentages of some PUFA, such as C20:4n-6 and C22:4n-6.

Table 4. Intramuscular fat content (g/100g) and fatty acid composition (g/100g FA) of intramuscular fat and neutral lipid of m. *Biceps femoris* from 3 different Iberian x Duroc genotypes.

	Genotype			Sex		Significance		
	GEN1	GEN2	GEN3	♂	♀	gen	sex	interaction
IMF	3.40±1.19	3.66±0.92	3.02±0.72	3.36±1.10	3.35±0.80	ns	ns	ns
IMF (dm)	13.07±4.34	13.66±3.15	11.21±3.33	12.67±3.93	12.60±2.78	ns	ns	ns
Intramuscular fat fatty acid composition								
C12:0	0.06±0.01	0.06±0.01	0.05±0.01	0.06±0.01	0.06±0.01	ns	ns	ns
C14:0	1.16^{ab}±0.08	1.24^{a}±0.11	1.12^{b}±0.07	1.19±0.12	1.16±0.07	*	ns	ns
C16:0	22.65±1.06	22.60±0.73	21.98±0.71	22.40±1.02	22.41±0.74	ns	ns	ns
C16:1 n-7	3.70±0.23	4.12±0.30	3.95±0.42	3.95±0.36	3.92±0.37	ns	ns	ns
C17:0	0.22±0.07	0.23±0.06	0.24±0.05	0.24±0.05	0.23±0.06	ns	ns	ns
C17:1 n-7	0.26±0.08	0.27±0.08	0.26±0.05	0.27±0.07	0.26±0.06	ns	ns	ns
C18:0	11.83±1.04	11.41±0.47	10.81±0.80	11.28±0.98	11.39±0.80	ns	ns	ns
C18:1 n-9	48.98±1.26	48.91±1.50	49.14±1.17	48.90±1.33	49.12±1.26	ns	ns	ns
C18:2 n-6	7.27±1.31	7.32±1.09	8.21±1.07	7.64±1.27	7.58±1.16	ns	ns	ns
C18:3 n-6	0.42±0.13	0.46±0.11	0.48±0.08	0.45±0.12	0.45±0.10	ns	ns	ns
C20:0	0.24±0.10	0.26±0.06	0.24±0.07	0.25±0.07	0.24±0.08	ns	ns	ns
C20:1n-9	0.86±0.32	0.85±0.08	0.83±0.12	0.90±0.08	0.79±0.25	ns	ns	ns
C20:2 n-6	0.35±0.06	0.34±0.06	0.35±0.05	0.35±0.06	0.33±0.05	ns	ns	ns
C20:4 n-6	1.58±0.51	1.51±0.42	1.85±0.41	1.65±0.48	1.64±0.44	ns	ns	ns
C20:5 n-3	0.07±0.04	0.07±0.04	0.08±0.03	0.08±0.04	0.07±0.03	ns	ns	ns
C22:2 n-6	0.04±0.04	0.02±0.01	0.03±0.01	0.02±0.01	0.03±0.03	ns	ns	ns
C22:4 n-6	0.31±0.07	0.32±0.10	0.38±0.09	0.36±0.11	0.32±0.07	ns	ns	ns
SFA	36.16±2.07	35.81±1.00	34.45±1.33	35.42±1.89	35.48±1.43	ns	ns	ns
MUFA	53.80±1.36	54.15±1.58	54.18±1.54	54.02±1.53	54.08±1.43	ns	ns	ns
PUFA	10.04±2.07	10.04±1.74	11.36±1.59	10.56±1.96	10.44±1.80	ns	ns	ns
Neutral lipids								
C12:0	0.05^{b}±0.01	0.07^{ab}±0.02	0.08^{a}±0.02	0.07±0.02	0.06±0.01	**	*	ns
C14:0	1.13±0.09	1.23±0.13	1.15±0.14	1.16±0.14	1.18±0.12	ns	ns	ns
C16:0	23.50±0.74	23.37±0.56	22.90±0.73	23.24±0.83	23.25±0.59	ns	ns	ns
C16:1 n-7	3.93±0.23	4.23±0.33	4.15±0.41	4.12±0.35	4.11±0.35	ns	ns	ns
C17:0	0.19±0.05	0.21±0.04	0.21±0.04	0.21±0.05	0.20±0.04	ns	ns	ns
C17:1 n-7	0.19±0.05	0.19±0.06	0.22±0.06	0.20±0.06	0.20±0.07	ns	ns	ns
C18:0	11.54^{a}±0.90	11.34^{ab}±0.52	10.56^{b}±0.87	11.08±1.14	11.19±0.54	*	ns	ns
C18:1 n-9	51.80±1.16	50.76±1.07	51.63±1.28	51.20±1.42	51.56±1.03	ns	ns	ns
C18:2 n-6	5.24^{b}±0.56	5.94^{ab}±0.77	6.29^{a}±0.91	5.99±0.85	5.70±0.88	*	ns	ns
C18:3 n-6	0.30±0.05	0.31±0.05	0.34±0.05	0.33±0.05	0.31±0.05	ns	ns	ns
C20:0	0.25^{b}±0.05	0.42^{a}±0.19	0.39^{ab}±0.07	0.38±0.17	0.34±0.10	*	ns	ns
C20:1n-9	0.97±0.09	0.87±0.09	0.92±0.09	0.94±0.11	0.90±0.08	ns	ns	ns
C20:2 n-6	0.28±0.07	0.31±0.04	0.31±0.09	0.33±0.05	0.28±0.08	ns	ns	ns
C20:4 n-6	0.39^{b}±0.15	0.60^{a}±0.17	0.60^{a}±0.10	0.58±0.18	0.50±0.15	**	ns	ns
C20:5 n-3	0.06±0.01	0.04±0.03	0.06±0.02	0.05±0.03	0.06±0.02	ns	ns	ns
C22:2 n-6	0.06^{ab}±0.02	0.04^{b}±0.03	0.08^{a}±0.01	0.06±0.03	0.06±0.02	**	ns	ns
C22:4 n-6	0.10±0.02	0.14±0.05	0.11±0.04	0.12±0.05	0.12±0.03	*	ns	ns
SFA	36.67^{a}±1.51	36.63^{a}±0.88	35.28^{b}±1.45	36.15±1.81	36.21±0.98	**	ns	ns
MUFA	56.89±1.14	56.05±1.03	56.93±1.56	56.45±1.55	56.76±1.01	ns	ns	ns
PUFA	6.45^{b}±0.74	7.38^{ab}±0.99	7.79^{a}±1.11	7.45±1.08	7.03±1.09	*	ns	ns

GEN:genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
IMF: intramuscular fat. IMF(dm): intramuscular fat (dry matter). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

In BF, neither the genotype nor the sex of the pigs affected the levels of any major fatty acids of the IMF. Nevertheless, the fatty acid composition of BF showed similar a trend as LD, with the highest percentages of unsaturated fatty acids in GEN3. Moreover, the fatty acid composition of NL of this muscle was significantly affected by the genotype. So, the highest levels of unsaturated fatty acids, such as C18:2n-6, C22:2n-6 and PUFA were found in GEN3 while GEN1 and GEN2 had significantly highest percentages of SFA, being the highest level of C18:0 in GEN1 and C20:0 in GEN2.

The higher PUFA in BF could be due to the different metabolic pattern of LD (glycolytic) and BF (intermediate oxidative) (Leseigneur-Meynier & Gandemer, 1991) and the higher PUFA content in more oxidative muscles than in glycolytic ones as a consequence of their abundance of membranes, rich in polyunsaturated phospholipids.

Fatty acid profiles high in oleic acid and low in linoleic acid have been associated with better flavour in Iberian dry-cured meat products as an excess of oxidation development increases the appearance of rancid flavours (Cava *et al*., 1999). However, meat with abundant PUFA levels can cause undesirable technological and sensory consequences since PUFA are more sensitive to oxidation, leading to meat texture, flavour and colour alterations (Morrissey, Sheehy, Galvin, Kerry, & Buckley, 1998). Although the meat from GEN3 had the highest MUFA levels, which is desirable in Iberian products (Ruiz-Carrascal *et al*., 2000), it also had the highest percentages of PUFA, such as C18:2n-6 and C18:3n-6 and the lowest IMF content. Therefore, the IMF content and the fatty acid profile of GEN3, although has a better profile from a nutritional point of view (Simopoulos, 2003), it could also reduce the oxidative stability of chilled fresh meat and dry-cured meat products.

3.3.- Lipogenic enzyme activity of subcutaneous fat and muscles.

There were neither effect ($p>0.05$) of the sex nor significant interaction between the sex and the genotype on lipogenic enzyme activity of SCF (Table 5). However, the genotype caused important differences in the activity of both enzymes.

G6PDH and ME activities were higher in SCF than in muscles (Table 5), which is in accordance with the literature consulted (Gondret *et al*., 2005; Kouba & Maurot 1999; Martínez-Puig *et al*., 2006, Morales *et al*., 2002; Morcuende *et al*., in press). G6PDH activity was higher than ME activity in SCF, whereas an opposite behaviour was found in muscles. The higher activity of ME than G6PDH in LD and BF could be due to the fact that NADPH in the muscle is mostly provided by the ME activity and not by the pentose phosphate pathway in which G6PDH is involved (Mourot & Kouba, 1998). Some authors (Martínez-Puig *et al*., 2006) have suggested that this fact would make muscle less sensitive than adipose tissue to variations of peripheral glucose, since the regulation of lipogenic enzymes is very different in both tissues, as numerous regulating factors are involved in these processes (Azain, 2004).

In SCF, GEN3 had the lowest G6PDH activity and ME activity. Similar results were found in LD muscle, as lower G6PDH activity and ME activity were found in GEN3 than in GEN1 and GEN2, despite of the fact that these differences were not significant in the latter. BF muscle showed the same trend, GEN1 and GEN2 showed the highest G6PDH activity and ME activity.

Results revealed no differences in the lipogenic potential in SCF and LD and BF between reciprocal crosses (GEN1 vs GEN2) agreeing with findings of Morcuende *et al*., (in press). These authors reported no differences in G6PDH and ME activities in muscle and SCF of pigs from the reciprocal cross Iberian x Duroc. However, the activities of both enzymes in the different tissues were strongly influenced by the Duroc sire genotype (GEN2 vs GEN3). G6PDH and ME activities in SCF were 1.3-fold and 1.5-fold higher in GEN2 than in GEN3, respectively; and G6PDH and ME activities in BF and LD were 3-fold and 1.5-fold higher in GEN2 than in GEN3, respectively. The influence of genetic on lipogenic activity has been reported by different authors (Morales *et al*., 2002; Mourot & Kouba 1998; Mourot, Kouba, & Bonneau, 1996) who stated a higher enzyme lipogenic activity in rustic pig breeds such as Iberian or Meishan than in industrial pig genotypes, such as Landrace or Large White. However, as far as we know, no studies have previously reported differences in the lipogenic activity due to the genotype of pigs within the same breed. Nonetheless, different authors (Cilla *et al*., 2006; Lonergan *et al*., 2001) previously described important differences between lines of Duroc in the IMF content, carcass composition and meat quality parameters, which can support the differences on the adipogenic character and fatty acid composition found between the genotypes in the present study.

Table 5. Glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) activity in subcutaneous fat, *L. dorsi* and *B. femoris* muscles (expressed as nmol of NADPH formed min^{-1} mg of $protein^{-1}$) from 3 different Iberian x Duroc genotypes.

	Genotype			Sex		Significance		
	GEN1	GEN2	GEN3	♂	♀	gen	sex	interaction
Subcutaneous fat								
G6PDH	1282.3^{a}±132.9	1332.7^{a}±269.4	1005.1^{b}±164.6	1156.0±276.9	1249.0±206.2	***	ns	ns
ME	837.6^{a}±154.2	707.6^{ab}±228.9	561.0^{b}±199.5	632.5±215.3	757.9±218.2	*	ns	ns
Longissimus dorsi								
G6PDH	1.7^{a}±0.6	1.7^{a}±0.6	0.5^{b}±0.5	1.3±0.8	1.2±0.8	***	ns	ns
ME	7.5±1.5	6.6±1.3	5.5±1.9	6.2±1.2	6.7±1.3	ns	ns	ns
Biceps femoris								
G6PDH	2.1^{a}±1.5	2.9^{a}±0.8	0.8^{b}±0.8	1.8±1.6	2.0±1.9	***	ns	ns
ME	7.3^{a}±1.5	7.7^{a}±1.3	5.5^{b}±1.0	6.5±1.7	7.2±1.4	***	ns	ns

GEN:genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
ME: malic enzyme, G6PDH: glucose 6 phosphate dehydrogenase.
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

The higher activity in both lipogenic enzymes (ME and G6PDH) is consistent with the higher BFT and HFT and IMF content of the muscles. The highest fat depths and IMF of GEN1 and GEN2 agrees with the highest G6PDH and ME activities, which indicates that the net production of NADPH, and as a consequence, the lipid synthesis and storage in muscle and in subcutaneous fat were higher in GEN1 and GEN2 than in GEN3. Different studies have related the lipogenic enzyme activity with the number or volume of adipocytes from different adipose tissues in bovine (Eguinoa, Brocklehurst, Aranal, Mendizabal, Vernon, & Purroy, 2003) and also in pigs (Mourot *et al*., 1996) as the increase in adipocite size was associated with a higher lipogenic enzyme activity and backfat thickness.

3.4.- Correlations and multivariate analysis.

Correlations between lipogenic enzyme activity and fatty acid composition of SCF and IMF of LD and BF are shown in Table 6.

Significant correlations were found between BFT and G6PDH (r= +0.564, p<0.001) and ME activities (r= +0.411, p<0.05) in SCF. However, no correlations were found between HFT and the enzyme lipogenic activity in SCF, maybe because SCF to evaluate enzyme lipogenic activity was taken from the backfat and not from the ham fat. In this respect, some authors (Mourot *et al*., 1996) found significant differences between the enzyme activities of SCF from different tissues, such as neckfat and backfat. These authors also suggested that pigs have certain adipose tissues in which fat is deposed preferentially and other adipose tissues where the lipogenesis is higher and which deliver triacylglycerides to the bloodstream. IMFdm content positively correlated with G6PDH in LD (r= +0.416, p<0.05) and with ME in BF (r= +0.388, p<0.05). The positive relationship between G6PDH and ME and BFT, HFT and IMF indicate that these traits are closely related with the different adipogenic potential of the genotypes which is consistent with differences in BFT, HFT and IMF content of the studied genotypes.

Table 6.- Correlations between lipogenic enzyme activity and fatty acid composition of subcutaneous and intramuscular fat of m. *L. dorsi* and m. *B. femoris* from 3 different genotypes Iberian x Duroc.

Subcutaneous fat				*Longissimus dorsi*				*Biceps femoris*		
	G6PDH	ME			G6PDH	ME			G6PDH	ME
BFT	0.564 (***)	0.411(*)		IMF	0.405 (*)	0.046		IMF	0.224	0.361
HFT	0.320	0.267		IMF (dm)	0.416(*)	0.022		IMF (dm)	0.239	0.388 (*)
C16:0	-0.035	-0.156	IMF	C16:0	0.27	0.02	IMF	C16:0	0.12	0.458 (*)
C18:0	0.414(*)	0.400(*)		C18:0	0.491(**)	0.328		C18:0	0.098	0.414(*)
C18: 1 n-9	0.117	0.081		C18: 1 n-9	-0.329	-0.385(*)		C18: 1 n-9	0.172	-0.195
C18:2 n-6	-0.492(**)	-0.297		C18:2 n-6	-0.248	0.173		C18:2 n-6	-0.335	-0.351
SFA	0.364(*)	0.266		SFA	0.406(*)	0.184		SFA	0.156	0.504(**)
MUFA	-0.003	-0.037		MUFA	-0.384(*)	-0.441(*)		MUFA	0.228	-0.14
PUFA	-0.537(**)	-0.338		PUFA	-0.267	0.103		PUFA	-0.317	-0.337
			NL	C16:0	0.411(*)	0.039	NL	C16:0	0.108	0.509(**)
				C18:0	0.513(**)	0.344		C18:0	0.105	0.451(*)
				C18: 1 n-9	-0.406(*)	-0.353(*)		C18: 1 n-9	0.101	-0.254
				C18:2 n-6	-0.356	0.201		C18:2 n-6	-0.325	-0.403(**)
				SFA	0.503(**)	0.212		SFA	0.13	0.504(**)
				MUFA	-0.437(*)	-0.383(*)		MUFA	0.12	-0.23
				PUFA	-0.391(*)	0.152		PUFA	-0.286	-0.397(*)

GEN:genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
BFT: backfat thickness. HFT: ham fat thickness. IMF: intramuscular fat. NL: neutral lipids. ME: malic enzyme, G6PDH: glucose 6 phosphate dehydrogenase. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
* p<0.05, ** p <0.01, ***p<0.001

In SCF and LD, G6PDH activity correlated positively with those fatty acids related with *de novo* synthesis of fat, C16:0, C18:0 and SFA, while the correlations were negative in fatty acids more influenced by the diet as polyunsaturated fatty acids, C18:2n-6 and PUFA. In LD, relations between G6PDH and saturated fatty acids were clearer due to the fact that NL are less rich in PUFA than total fat. In BF, ME activity correlated positively with the percentage of C16:0, C18:0, and SFA of IMF. In NL, ME activity correlated positively with C16:0, C18:0 and SFA and negatively with C18:2n-6 and PUFA. As endogenous synthesis of fatty acids produces C16:0 and C18:0, positive correlations

between enzyme activities and SFA and negative correlations with unsaturated fatty acids are expected, as the relative level of PUFA and MUFA decrease when SFA percentage and lipogenic activity increase.

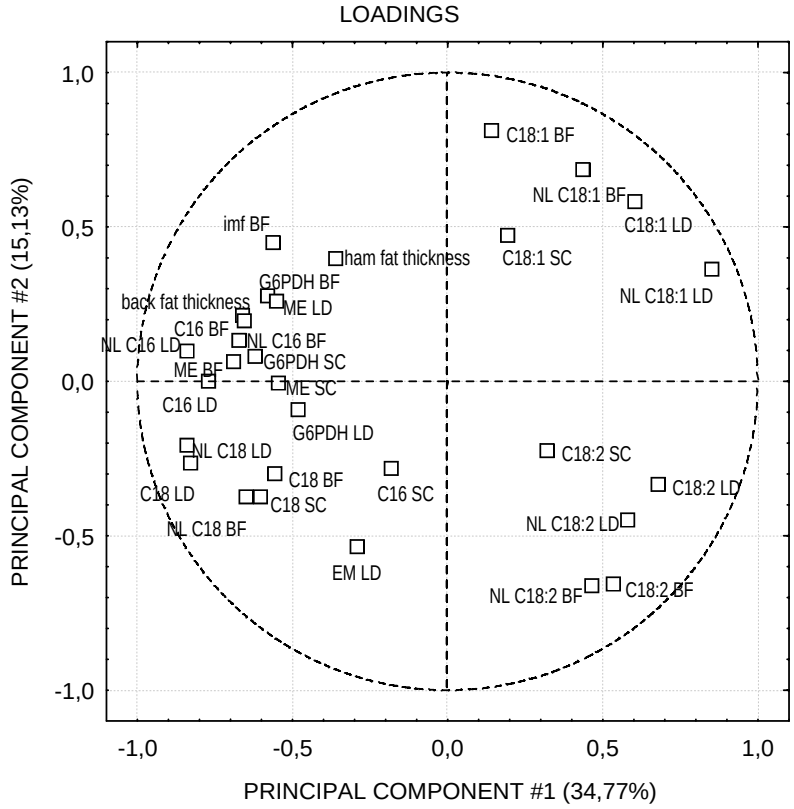

Figure 1.- Loadings plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2).

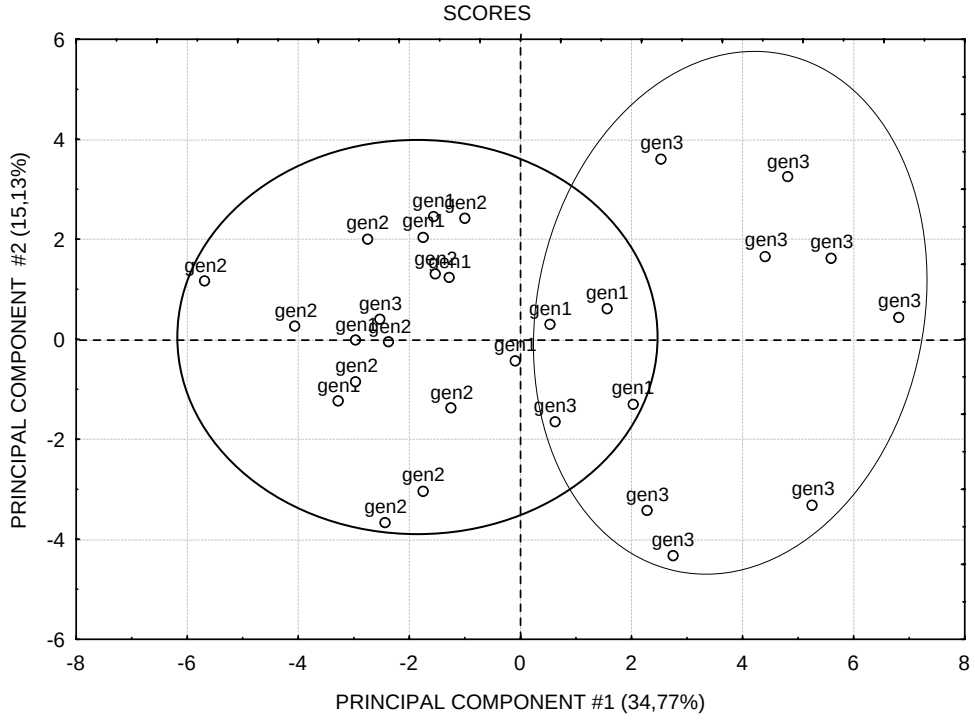

Figure 2.- Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1 and PC2).

PCA of the variables resulted in 5 significant factors that accounted for 75.95% of the variability. Principal components, PC1 and PC2, explained the 34.77% and 15.13% of the variation of the data, respectively. The loadings of this PCA are shown in Fig. 1. In this plot, it is possible to differentiate 2 groups of variables. In the positive *x* axis of the PC1 are located MUFA and PUFA from SCF, LD and BF muscles (from total IMF and NL fraction); while in the negative *x* axis are placed SFA, fat depths, IMF content and the activity of the lipogenic enzymes. The PC2 differentiates the group of MUFA, located in the positive *y* axis, from the group of PUFA, located in the negative *y* axis. The distribution of the data on the PC1 and PC2 (Fig. 2) shows 2 separate groups of points, corresponding one of them to the reciprocal crosses (GEN1 and GEN2) and the other, to the GEN3. Results show a close connection between high lipogenic enzyme activity, which generates SFA, and as a consequence increase adipose tissues in SCF and IMF, and GEN1 and GEN2. The lowest lipogenic potential of GEN3 decreased the fatness of the pigs, which increased the relative level of PUFA and MUFA, the main variables associated to this genotype.

4.- CONCLUSION

Iberian x Duroc reciprocal crosses had a similar lipogenic character. Nonetheless, the differences between paternal lines of Duroc crossed with Iberian pigs had a marked influence on the adipogenic potential. The use of Duroc sires with selected genotypes (DU2) reduces G6PDH and ME enzyme activities and the IMF content, which has negative consequences for the manufacture of dry-cured meat products. Moreover, it also modifies fatty acid profile increasing unsaturated fatty acids, which has positive nutritional effects, although it can also decrease the shelf life of the meat and make it more prone to suffer oxidation processes during refrigerated storage and during the manufacture of dry-cured meat products.

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Chapter III

THE CROSSBREEDING OF DIFFERENT DUROC LINES WITH IBERIAN PIG AFFECTS COLOR AND OXIDATIVE STABILITY OF MEAT DURING STORAGE[1]

ABSTRACT

Colour and oxidative stability of *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles from 3 different Iberian x Duroc genotypes (GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian) were analysed during 10 days of refrigerated storage. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype Duroc1 was selected for the manufacture of dry-cured meat products while the genotype Duroc2 was selected for meat production. BF showed more intense colour and oxidative changes during storage than LD, which is in accordance with their different metabolic pattern, since BF is an intermediate oxidative muscle whereas LD is a glycolitic one. Important differences were found between crosses due to the genotype of the Duroc sire line; however, reciprocal crosses (GEN1 and GEN2) showed a similar pattern. The lowest post-mortem pH of GEN3 could be a deciding factor of the oxidative and colour stability during storage as GEN3 had higher drip and cook loss as well as higher susceptibility to iron ascorbate-induced oxidation. In addition, chops from GEN3 were paler (lower L*) and showed a higher discolouration (higher decrease of a*) besides higher lipid (TBA-RS) and protein oxidation (carbonyl content) after the storage.

Keywords: crossbreeding, Duroc, Iberian, colour, pH, TBA-RS, protein oxidation, storage.

1.- INTRODUCTION

Meat colour is the main factor which affects the acceptability at the time of consumer purchase (Faustman & Cassens 1989). Meat discolouration is closely connected with the development of oxidation processes (Faustman & Cassens, 1990). Lipid oxidation reduces the shelf-life of meat, decreasing nutritive and sensory quality of meat (Gray & Crackel, 1992). Changes associated with lipid oxidation include the development of unpleasant tastes and odours, as well as changes in colour, rheological properties, solubility and potential formation of toxic compounds (Baron & Andersen, 2002). On the other hand, several authors have evaluated protein oxidation by carbonyl measurements (Mercier, Gatellier, Viau, Remignon & Renerre, 1998; Mercier, Gatellier & Renerre, 2004; Ventanas, Estevez, Tejeda & Ruiz, 2006). Protein oxidation reduces the quality of meat and meat products promoting protein fragmentation or aggregation and a decrease in protein solubility (Decker, Xiong, Calvert, Crum & Blanchart, 1993). Moreover, some studies (Juncher ***et al***., 2001) have reported on final pH as one the most important parameters to explain the differences in colour, lipid oxidation and drip loss during chill storage of pork. The meat industry has great interest in optimizing the water holding capacity, limiting loss of fluids (drip loss) during slaughter and subsequent manipulations as it implies a financial loss (Maribo, Olsen, Barton-Gade, Møller & Karlsson, 1998). It is widely recognized that one of the major causes of the development of poor quality meat in pigs is the susceptibility to stress which is associated with the pig genotype, i.e. pigs carrying one or two copies of the Halothane gene although have increased lean content, they are more likely to produce pale, soft, and exudative (PSE) meat which has poor sensory quality characteristics and excessive drip loss.

[1] Ramírez, M.R. & Cava, R. (2006). The crossbreeding of different Duroc lines with Iberian pig affects color and oxidative stability of meat during storage. Submitted for publication to Meat Science

The Iberian pig production is mainly focused on the manufacture of dry-cured-meat products such as shoulders, loins and hams. However, recently the importance of the consumption of fresh meat has increased, being considered Iberian pork as a high quality alternative to meat from lean pigs commonly consumed in Spain. Nowadays, in order to improve the productive parameters, Iberian pigs are crossed with Duroc. By this way, the hybrids have better productive parameters, such as more piglets per sow and a higher weight at weaning and at the end of fattening (Aparicio, 1987), without a serious reduction of the high adaptability to the environment of the Iberian pig and the high quality of the dry-cured meat products (López-Bote, 1998). However, due to the large distribution of the Duroc pigs, they have been object of different genetic selections. Cilla ***et al***., (2006) and Soriano, Quiles, Mariscal and García-Ruiz (2005) have reported important differences between Duroc lines crossed with industrial pigs. Moreover, the legislation in Spain (B.O.E., 15th October, 2001) for the production of dry-cured products labelled as "Iberian", only allows Iberian x Duroc crosses if the Iberian pig is the maternal line, in order to preserve the Iberian breed purity. Nevertheless, the limited amount of previous research about the influence of different lines of Duroc in the cross with Iberian, a very rustic breed, and the influence of the reciprocal cross show the need to develop new studies. Therefore, the two main objectives of this study are to assess different Duroc sire lines crossed with Iberian pigs as well as the differences derived of the use of Iberian breed as maternal or paternal line on the colour and oxidative stability of meat during refrigerated storage.

2.- MATERIALS AND METHODS

2.1.- Experimental design.

The material used in this trial was obtained from the same pigs as those used in a previous paper (Ramírez & Cava, 2006). Three groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype Duroc1 (DU1) was selected for the production of dry-cured meat products, with a high level of fattening. The genotype Duroc2 (DU2) was selected for meat production, with high percentages of meat cuts and with low carcass fat. Pigs were raised all together in an intensive system and were fed *ad libitum* with a cereal based diet. They were randomly slaughtered after 316 days of rearing at 150-165 Kg live weight. After slaughter, *Longissimus dorsi* (LD) and *Biceps femoris* (BF) were removed from carcass and sliced (1.5 cm of thickness). They were placed on Styrofoam meat trays and over-wrapped in PVC oxygen permeable films and stored under fluorescent light at +4ºC for 10 days. A solution of chloramphenicol (4mg/ml)/ciclohexamide (1mg/ml) was sprayed on the surface of the chops to avoid microbial growth. At sampling times instrumental colour, lipid and protein oxidation were measured. After instrumental colour determination, samples were stored at -80ºC.

2.2.- pH.

The value of pH at 45 min (pH_{45min}) and 24 h (pHu) *post mortem* was measured in the m. *Longissimus dorsi* and m. *Biceps femoris* with a portable puncture pH-meter Crison mod. 507 (Crison Instruments, Barcelona, Spain).

2.3.- Iron-ascorbate induced oxidation.

The susceptibility of muscle tissue homogenates to iron ascorbate-induced lipid oxidation was determined by the method of Kornbrust and Mavis (1980). Homogenates were incubated at 37ºC in 40nM tris-maleate buffer (pH 7.4) with 1mM $FeSO_4$ and 2mM ascorbic acid in a total volume of 10 ml. At fixed time intervals (0, 50, 100 and 200 min), aliquots were taken for the measurement of 2-thiobarbituric acid-reactive substances (TBA-RS) by the method of Buege and Aust (1978). TBA-RS were expressed as nmol malondialdehyde (MDA)/mg protein. Protein content was measured in muscle homogenates following the Lowry method (Lowry, Rosebrough, Farr, & Randall, 1951).

2.4.- Drip and cook loss.

Drip loss was measured following the method of Honikel (1998). The samples were weighed and suspended in two plastic bags, the inner bag perforated, and the exudate was collected in the outer bag. Meat was stored for 10 days at refrigerated storage (4ºC). Drip loss (%) was calculated by difference in weight between day 0 and day 10 of storage. For cook loss (%), each chop was placed in a plastic bag and cooked by immersion at 80ºC for 60 min. The difference of weight before and after cooking was used to calculate cook loss percentage.

2.5.- Instrumental colour.

Colour parameters were determined with a Minolta CR-300 colorimeter (Minolta Camera Co., Osaka, Japan) with illuminant D65, a 0º viewing angle and a 2.5 cm port/viewing area. Before use, the colorimeter was standardized with a white tile (mod CR-A43). The following colour coordinates were determined: lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue). In addition, hue angle [$h^o = \tan^{-1}(b^*/a^*)\ (360/2\pi)$] and saturation index or Chroma [$C^* = (a^{*2}+b^{*2})^{0.5}$] were calculated. The measurement was repeated at five randomly selected locations on each chop and averaged.

2.6.- Lipid oxidation.

Lipid oxidation was assessed in duplicate by the 2-thiobarbituric acid (TBA) method of Salih, Smith, Price and Dawson (1987) in 2g of meat. TBA-RS values were calculated from a standard curve of TEP (1,1,3,3-tetraethoxypropane) and expressed as mg malondialdehyde/kg meat.

2.7.- Protein oxidation.

Protein oxidation was measured by estimation of carbonyl groups formed during incubation with 2,4-dinitrophenylhydrazine (DNPH) in 2N HCl following the method described by Oliver, Ahn, Moerman, Goldstein and Stadtman (1987). Carbonyl concentration was measured on the treated sample by measuring DNPH incorporated on the basis of absorption of 21.0 mM^{-1} cm^{-1} at 370 nm for protein hydrazones. Results were expressed as nmol of DNPH fixed per milligram of protein. Protein oxidation was expressed as nmol carbonyls/mg protein. Protein concentration was calculated by spectrophotometry at 280 nm using bovine serum albumin (BSA) as standard.

2.8.- Statistical analysis.

The effects of genotype and sex were analysed by the Analysis of Variance procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. Means were used to compare differences. HSD Tukey's test was used to compare the mean values of the genotypes. Mean values and standard errors of the means (SEM) are reported. The relationships between traits were analysed using Pearson's correlation.

3.- RESULTS

3.1.- pH, cook and drip loss.

Genotype significantly affected pH of LD and BF, whereas no effect of sex on pH values was found (Table 1). In general, muscle acidification was more intense in GEN3 than in the other genotypes. pH_{45min} in BF and pHu in LD and BF were significantly lower ($p<0.05$) in GEN3 than in GEN1 and GEN2. On the other hand, meat from GEN3 showed higher loss of water than the other 2 genotypes, as drip loss during refrigerated storage was significantly highest in LD and BF from GEN3. A similar trend was found for cook loss, being higher in LD and BF from GEN3, although in the BF the differences were not significant.

Table 1.- pH values, cook loss (%) and drip loss (%) of *Longissimus dorsi* and *Biceps femoris* during storage as related to genotype and sex (Data from Ramírez and Cava, 2006).

Effects	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
pH									
$pH_{45}LD$	5.7	5.8	5.8	5.7	5.8	0.05	ns	ns	ns
$pH_{45}BF$	5.8a	5.9a	5.6b	5.7	5.7	0.05	**	ns	ns
$pH_{U}LD$	5.7a	5.5ab	5.1b	5.4	5.4	0.07	*	ns	ns
$pH_{U}BF$	5.8a	5.7a	5.2b	5.5	5.6	0.06	***	ns	ns
Drip loss (%)									
Drip loss LD	4.0b	4.3b	6.0a	4.8	4.8	0.30	**	ns	ns
Drip loss BF	3.4b	4.0ab	5.1a	3.9	4.4	0.28	*	ns	ns
Cook loss (%)									
Cook loss LD	6.0b	6.5b	10.9a	8.1	7.3	0.54	***	ns	ns
Cook loss BF	9.7	11.0	11.6	10.9	10.7	0.35	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
LD: m. *Longissimus dorsi*; BF: m. *Biceps femoris*
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; a,b: Different letters in the same row for the same parameter means differences statistically significant (Tukey's Test, p<0.05).

3.2.- Iron ascorbate-induced oxidation.

The evolution of iron ascorbate-induced lipid oxidation (nmol MDA/mg protein) of LD and BF homogenates showed important differences between muscles and genotypes, whereas sex had no effect (Table 2). BF homogenates were more sensitive to oxidation than LD homogenates. In both locations, LD and BF muscle homogenates from GEN3 were the most oxidized at 200 min of incubation.

Table 2.- Iron-ascorbate induced peroxidation (nmol MDA/mg protein) of *Longissimus dorsi* and *Biceps femoris* during storage as related to genotype and sex.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Longissimus dorsi									
min 0	0.30	0.22	0.39	0.30	0.31	0.030	ns	ns	ns
min 50	0.51a	0.39ab	0.34b	0.43	0.40	0.028	*	ns	ns
min 100	2.05	1.72	2.26	1.92	2.10	0.120	ns	ns	ns
min 200	2.65b	2.63b	3.59a	3.06	2.87	0.133	**	ns	ns
Biceps femoris									
min 0	0.49	0.26	0.43	0.41	0.37	0.047	ns	ns	ns
min 50	0.51	0.38	0.79	0.54	0.59	0.076	ns	ns	ns
min 100	2.62	2.73	3.04	3.03	2.56	0.232	ns	ns	ns
min 200	2.93b	4.57ab	5.06a	4.37	4.10	0.313	*	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; a,b: Different letters in the same row for the same parameter means differences statistically significant (Tukey's Test, p<0.05).

3.3.- Evolution of colour during refrigerated storage.

Marked differences in the evolution of the colour parameters were found between muscles during refrigerated storage, being these changes more intense in BF chops than in those from LD (Tables 3 and 4, respectively). In general, CIE L*-value decreased during the refrigerated storage in LD chops, increasing its darkness, while CIE L*-value increased in BF, being those chops paler at the end of storage. Redness (CIE a*-value) tended to decrease with the storage while yellowness (CIE b*-value) followed an opposite trend, increasing throughout the storage. Chroma (CIE C*-value) did not change significantly, while hue (CIE hº-value) increased during the storage.

Table 3.- Evolution of instrumental colour parameters of *Longissimus dorsi* during storage as related to genotype and sex.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Lightness (CIE L-value)*									
Day 0	48.9b	53.1a	52.7a	51.8	51.6	0.52	***	ns	ns
Day 1	49.8b	53.7a	53.8a	52.4	52.7	0.57	**	ns	ns
Day 3	48.2b	52.8a	53.6a	51.7	51.6	0.56	***	ns	ns
Day 5	48.1b	52.3a	52.3a	50.9	51.1	0.61	**	ns	ns
Day 7	47.6b	51.6a	50.5a	49.4	50.5	0.59	**	ns	ns
Day 10	46.5b	50.3a	49.3ab	48.0	49.6	0.59	**	ns	ns
Redness (CIE a-value)*									
Day 0	10.2	10.0	9.9	9.9	10.2	0.26	ns	ns	ns
Day 1	10.3	10.4	10.1	10.2	10.4	0.20	ns	ns	ns
Day 3	10.2	9.9	10.3	10.0	10.3	0.24	ns	ns	ns
Day 5	10.2	9.6	9.7	10.1	9.7	0.26	ns	ns	ns
Day 7	10.1	9.6	9.9	9.7	10.0	0.29	ns	ns	ns
Day 10	10.1	8.9	9.7	9.9	9.8	0.49	ns	ns	ns
Yellowness (CIE b-value)*									
Day 0	4.3	4.9	5.0	4.7	4.8	0.17	ns	ns	ns
Day 1	6.2b	7.4a	7.3ab	6.9	7.1	0.19	*	ns	ns
Day 3	6.6	7.3	7.6	6.9	7.4	0.19	ns	ns	ns
Day 5	6.4	6.8	6.9	6.8	6.6	0.17	ns	ns	ns
Day 7	7.0	7.1	7.3	7.1	7.1	0.18	ns	ns	ns
Day 10	7.5	7.0	7.3	7.4	7.1	0.17	ns	ns	ns
Chroma (CIE C-value)*									
Day 0	11.1	11.2	11.1	10.9	11.3	0.30	ns	ns	ns
Day 1	12.0	12.8	12.5	12.3	12.6	0.26	ns	ns	ns
Day 3	12.1	12.3	12.8	12.2	12.7	0.29	ns	ns	ns
Day 5	12.0	11.8	11.9	11.9	11.9	0.27	ns	ns	ns
Day 7	12.3	11.9	12.3	12.1	12.2	0.31	ns	ns	ns
Day 10	12.6a	11.3b	12.1ab	12.1	12.2	0.29	*	ns	ns
Hue (CIE hº-value)									
Day 0	22.7b	26.2a	26.8a	25.2	25.4	0.57	**	ns	ns
Day 1	31.1b	35.5a	35.6a	33.9	34.5	0.55	***	ns	ns
Day 3	32.6b	36.4a	36.5a	34.8	35.7	0.49	***	ns	*
Day 5	32.1b	35.3a	35.4a	34.0	34.4	0.35	***	ns	ns
Day 7	34.7b	36.5a	36.4a	35.4	36.1	0.46	***	ns	ns
Day 10	36.6b	38.2a	37.0a	37.7	37.9	0.49	***	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; a,b: Different letters in the same row for the same parameter means differences statistically significant (Tukey's Test, p<0.05).

Sex had a significant effect on colour coordinates of BF chops. BF from females showed lower CIE L* and hue values and higher CIE a* and Chroma values than those from males. As a result, at the end of storage, BF chops from females were darker and redder and the colour was more intense than those from males.

Table 4.- Evolution of instrumental colour parameters of *Biceps femoris* during storage as related to genotype and sex.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Lightness (CIE L-value)*									
Day 0	41.9b	42.4b	46.8a	44.7	42.9	0.74	**	ns	ns
Day 1	43.9b	42.8b	47.1a	45.8	43.5	0.65	**	*	ns
Day 3	42.4b	42.7b	48.2a	45.6	43.5	0.73	***	ns	ns
Day 5	42.9b	42.6b	47.8a	46.0	43.1	0.87	*	ns	ns
Day 7	46.0	44.5	49.1	48.0	45.2	0.80	*	ns	ns
Day 10	46.5b	45.2b	51.7a	49.6	46.2	0.92	**	*	ns
Redness (CIE a-value)*									
Day 0	18.3	17.4	17.9	17.2	18.5	0.31	ns	*	ns
Day 1	18.4	17.6	16.9	17.0	18.2	0.29	ns	*	*
Day 3	17.3	17.4	15.9	16.0	17.6	0.34	ns	*	ns
Day 5	16.0a	17.0a	13.6b	14.7	16.3	0.43	***	*	ns
Day 7	17.0a	17.4a	13.4b	14.7	17.0	0.55	***	**	ns
Day 10	16.3a	16.2a	13.1b	14.2	16.1	0.51	**	*	ns
Yellowness (CIE b-value)*									
Day 0	6.2	6.1	7.2	6.7	6.4	0.23	ns	ns	ns
Day 1	9.6	9.2	10.3	9.8	9.6	0.20	ns	ns	ns
Day 3	9.4	9.5	10.6	9.9	9.8	0.21	*	ns	ns
Day 5	8.8	9.3	9.5	9.2	9.1	0.17	ns	ns	ns
Day 7	10.0	10.0	10.2	10.2	10.0	0.18	ns	ns	ns
Day 10	9.8	9.7	10.8	10.4	9.9	0.26	ns	ns	ns
Chroma (CIE C-value)*									
Day 0	19.3	18.5	19.3	18.5	19.6	0.34	ns	ns	ns
Day 1	20.7	19.9	19.8	19.7	20.6	0.31	ns	ns	ns
Day 3	19.7	19.8	19.1	18.9	20.1	0.31	ns	*	ns
Day 5	18.2ab	19.3a	16.6b	17.4	18.7	0.38	**	*	ns
Day 7	19.7a	20.1a	17.0b	18.0	19.8	0.31	**	*	ns
Day 10	19.1a	19.0a	17.2b	17.7	19.0	0.37	*	ns	ns
Hue (CIE h°-value)									
Day 0	18.9	19.4	21.8	21.1	19.1	0.56	ns	ns	ns
Day 1	27.6b	27.6b	31.3a	30.1	27.8	0.49	***	**	ns
Day 3	28.8b	28.8b	33.8a	31.8	29.3	0.72	***	*	ns
Day 5	29.0b	28.8b	35.1a	32.6	29.5	0.88	***	*	ns
Day 7	30.8b	30.1b	37.8a	35.2	30.9	0.66	**	*	ns
Day 10	31.4b	31.1b	39.9a	36.6	32.0	1.41	**	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; a,b: Different letters in the same row for the same parameter means differences statistically significant (Tukey's Test, p<0.05).

Genotype had an important effect on colour changes of LD and BF during the storage, thus, meat from GEN3 showed lower colour stability than meat from GEN1 and GEN2. In this respect, after 10 days of storage, LD from GEN2 and GEN3 and BF from GEN3 were paler than those from the other genotypes, as reflected their higher CIE L*-values. Similarly, BF chops from GEN3 showed lower CIE

a*-values than GEN1 and GEN2 at day 5, 7 and 10 of storage, which indicate a greater discolouration of GEN3 and a redder colour in muscles from GEN1 and GEN2. However, in LD chops no differences in CIE a*-value were found between genotypes. Chroma and especially hue were also affected by genotype. Chroma was significantly highest in LD chops from GEN1 at day 10 and in BF chops from GEN1and GEN2 at day 7 and day 10. In contrast, LD chops from GEN1 and BF chops from GEN1 and GEN2 had the lowest hue values all the days of storage analysed.

3.4.- Evolution of protein and lipid oxidation during refrigerated storage.

In general, BF was more affected by protein and lipid oxidation than LD (Table 5). Regarding protein oxidation, the genotype significantly affected the accumulation of carbonyls in LD and BF during storage. At days 0 and 10 of refrigerated storage, LD and BF from GEN3 showed higher protein oxidation than those from GEN1 and GEN2, although these differences were only statistically significant in BF at day 10.

Concerning lipid oxidation, both muscles increased TBA-RS values during storage. LD showed significant differences due to the genotype at day 0, but not during the rest of the storage. Nevertheless, BF was significantly affected by the genotype throughout the storage. BF from GEN3 showed significant higher TBA-RS values than those from GEN1 and GEN2, being the increase in TBA-RS twice higher in GEN3 than in GEN1 and GEN2 at the end of storage (ΔTBA-RS$_{d0-d10}$ GEN1: 0.22, GEN2: 0.17 and GEN3: 0.47 mg MDA/kg).

Table 5.- Lipid (mg MDA/kg sample) and protein oxidation (nmol carbonyls/mg protein) of *Longissimus dorsi* and *Biceps femoris* during storage as related to genotype and sex.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
PROTEIN OXIDATION[1]									
Longissimus dorsi									
Day 0	2.33	2.42	2.75	2.79	2.27	0.15	ns	ns	ns
Day 10	2.59	2.76	2.86	2.65	2.84	0.22	ns	ns	ns
Biceps femoris									
Day 0	2.12	3.14	3.02	2.48	3.06	0.18	ns	ns	ns
Day 10	2.56b	4.57b	9.53a	6.17	5.18	0.88	**	ns	ns
LIPID OXIDATION[2]									
Longissimus dorsi									
Day 0	0.05b	0.07ab	0.08a	0.07	0.07	0.004	*	ns	ns
Day 3	0.09	0.09	0.08	0.09	0.09	0.003	ns	ns	ns
Day 5	0.08	0.09	0.08	0.08	0.09	0.003	ns	ns	ns
Day 7	0.11	0.10	0.11	0.11	0.10	0.004	ns	ns	ns
Day 10	0.14	0.17	0.17	0.16	0.16	0.011	ns	ns	ns
Biceps femoris									
Day 0	0.09ab	0.08b	0.10a	0.09	0.09	0.003	*	ns	ns
Day 3	0.13b	0.10b	0.20a	0.16	0.13	0.012	***	ns	ns
Day 5	0.17b	0.13b	0.27a	0.20	0.18	0.019	**	ns	ns
Day 7	0.17b	0.15b	0.37a	0.25	0.22	0.033	**	ns	ns
Day 10	0.31ab	0.25b	0.57a	0.43	0.33	0.056	*	ns	ns

GEN1: IB x DU1; GEN2: DU1 x IB; GEN3: DU2 x IB

[1] nmol carbonyls/mg protein

[2] mg MDA/kg sample.

ns: not significant; *: p<0.05; **: p<0.01; ***: p<0.001; a,b: Different letters in the same row for the same parameter means differences statistically significant (Tukey's Test, p<0.05).

4.- DISCUSSION

The low values of pH_{45min} and pHu in muscles from GEN3 could explain the higher release of water during cooking or storage of meat from this genotype. The rate of *post-mortem* pH decline and the ultimate pH (pHu) determine the paleness as well as the water holding capacity of meat, measured in our study as drip and cook loss. This fact is supported by the negative correlations between pHu and drip (r = -0.539; p<0.001) and cook loss (r = -0.480; p<0.001) (Table 6). Thus, a higher rate of pH decline and low pHu favour a paler meat with a low water holding capacity (Juncher *et al*., 2001) as has been found in GEN3, which could reduce the quality of the meat from this genotype.

Iron-induced lipid peroxidation (I-ILP) assays showed that muscle homogenates from GEN3 were more sensitive to oxidative stress compared with those from GEN1 and GEN2, and therefore, an enhanced lipid oxidation during storage would be expected. In this respect, a significant positive correlation was found between $I\text{-}ILP_{min200}$ values and TBA-RS values (r = +0.454, p<0.001) at day 10 of storage (Table 6).

The evolution of colour during the refrigerated storage showed changes in colour coordinates of BF and LD consistent with those reported by Juncher *et al*., (2001), Brewer, Shu, Bidner, Meisinger and McKeith (2001), Lindahl, Lundström and Tornberg (2001), Rosenvold and Andersen (2003), and Hansen, Juncher, Henckel, Karlsson, Bertelsen and Skibsted (2004) who found increases of lightness (CIE L*-value) and yellowness (CIE b*-value) and decreases of redness (CIE a*-value) during the storage of meat.

On the other hand, metabolic and compositional characteristics of muscles (lipid and PUFA content, heme pigments, etc) related to the oxidative/glycolytic activity determine colour stability of meat during storage (Brewer *et al*., 2001). The inside part of BF is a red muscle while the outside part is a white muscle, whereas LD is a glycolytic muscle. This determines a higher myoglobin content in red oxidative muscles than in white glycolytic ones, and consequently, a lower CIE L*-value and higher CIE a*-value as well as a higher oxidative susceptibility. It has been widely reported (Cava, Estévez, Ruiz & Morcuende, 2003; Morcuende, Estévez, Ruiz & Cava, 2003) that oxidative muscles are more prone to oxidation reactions, as these muscles have more haem pigment and other prooxidants and higher amounts of polyunsaturated fatty acids, which increase their susceptibility to lipid oxidation. This agrees with the higher increases of lipid and protein oxidation values found in BF than in LD.

BF and LD from GEN3 were paler (higher CIE L*-value) than those from GEN1 and GEN2. Although the evolution of CIE L*-values was opposite in LD and BF, the initial differences of CIE L*-value remained unchanged at the end of the storage. Initial and ultimate differences of the lightness between genotypes could be caused by the low *post-mortem* pH of muscles from GEN3 and its effect on CIE L*-values and water release from raw muscle, measured as drip loss. Both factors may contribute to increase the paleness of the muscles during storage, keeping the initial differences in lightness and/or increasing reflectance due to an increase of the exudation of meat (Lindahl *et al*., 2001; Juncher *et al*. 2001; Brewer *et al*., 2001). In addition, Lindahl, Heckel, Karlsson and Andersen (2006) reported in pigs from different crossbreeds a significant increase of lightness in muscles as a consequence of low pH values early *post-mortem*. In the last years, the intensive selection carried out in some pig genotypes to improve the lean growth efficiency is associated with a decrease of

meat quality, increasing the problems of susceptibility to stress as well as the problems in the *rigor mortis*, such as PSE meat.

Redness is related to myoglobin content and its chemical state in meat (reviewed by Mancini & Hunt, 2005). The oxidative state of muscle pigments has an important role on the colour of meat, so the relative proportions of the three forms of myoglobin -Mb, MbO_2, MetMb- determine a more red, brown or grey colour of meat. The development of oxidation processes causes a discolouration of meat during the storage. Therefore, a decrease of a* during the storage implies a reduction of oxymyoglobin content, the main pigment responsible of the red colour of fresh meat. The higher discolouration of BF from GEN3 and in a lesser extent in LD from GEN3 indicate a more intense oxidation of myoglobin in this genotype, which is in agreement with the higher TBA-RS and carbonyls accumulation in this genotype at the end of the storage. Several authors (Rosenvold & Andersen, 2003, Hansen *et al*., 2004) have found a close connection between lipid and pigment oxidation (measured as the reduction of a*) due to the oxidation of myoglobin pigments of the meat (reviewed by Baron & Andersen, 2002). Additionally, low ultimate pH values have been related not only to higher paleness of meat, as was previously mentioned, but also to a more intense decrease of CIE a*-value during the storage (Juncher *et al*., 2001; Huff-Lonergan & Lonergan, 2005) since the rate of oxidation of oxymyoglobin to metmyoglobin is accelerated at low pH (Andersen, Bertelsen, & Skibseted, 1988).

Changes in Chroma and hue are linked with changes in CIE a* and b*-values and those factors that affect them. Consequently, after refrigerated storage LD and BF from GEN1 and GEN2 kept a redder and a more intense colour (higher Chroma) that diverged less from the true red axis (lower hue) than those from GEN3.

Genotype clearly affected the oxidative deterioration of proteins and the accumulation of carbonyls during the storage. Very little is known about oxidative changes of proteins during storage and the factors which regulate these processes in different types of meat and meat products (Mercier *et al*., 1998; Mercier *et al*., 2004; Ventanas *et al*. 2006). However, it is well established that protein carbonyls have their origin mainly in the reaction between aldehydes from lipid oxidation and protein oxidation compounds. Consequences of protein oxidation on meat quality are not completely clear. Several authors have measured protein oxidation in meat and meat products and have related this parameter to lipid oxidation (i.e. Mercier *et al*., 1998, 2004; Ventanas *et al*, 2006). Although in the present study a non-significant correlation was found between both parameters (r = +0.204; p>0.05) it correlated significantly with I-ILP_{200} (r = +0.295; p<0.05) (Table 6). The marked increase of carbonyl content in GEN3 muscles, especially in BF, could be caused by its low pHu, which agrees with the negative correlation found between both parameters (r = -0.268, p<0.05).

The development of oxidation products reactive to TBA in pork during refrigerated storage is widely referred in the bibliography since it is one of the primary mechanisms of quality deterioration in meat products (reviewed by Gray, Gomaa, & Buckley, 1996). Similarly to protein oxidation, BF from GEN3 was the most susceptible to lipid oxidation during refrigerated storage and had the highest TBA-RS values. As in protein oxidation, pHu negatively correlated with I-ILP_{200} (r = -0.616; p<0.001) as well as with TBA-RS_{day10} (r = -0.275; p<0.05). The significant correlations found between pHu and most of

the parameters analysed could indicate a higher oxidative susceptibility of meat with lower *post-mortem* pH. This relation has been previously reported by Juncher *et al*. (2001), since the autoxidation of oxymyoglobin is acid-catalyzed, and consequently, a decrease of pH accelerates the autoxidation of oxymyoglobin, decreasing color stability and increasing lipid and also protein oxidation (Huff-Lonergan & Lonergan, 2005). Therefore, pH seems to be an important factor for the colour and the oxidative stability during storage. On the other hand, results suggest that the intensive selection of some pig genotypes has negative consequences in pork quality under storage. Similar results have been reported by Lonergan, Huff-Lonergan, Rowe, Kuhlers and Jungst (2001) who found that the intensive selection of a Duroc line for lean growth efficiency reduced early *post-mortem* pH and caused softer and more exudative pork.

Table 6.- Pearson's correlation coefficients (*r*) between CIE a*-value, pH, lipid and protein oxidation, iron induced lipid peroxidation, cook loss and drip loss.

	I-ILP $_{min\ 200}$	TBA-RS $_{day\ 10}$	Carbonyls $_{day\ 10}$	CL (%)	DL (%)	pH_{45min}	pHu
CIE a*-value $_{day\ 10}$	0.113	0.068	0.198	-0.355**	-0.337**	0.075	0.059
I-ILP $_{min\ 200}$		0.454***	0.295*	0.465***	0.223	-0.189	-0.616***
TBA-RS $_{day\ 10}$			0.204	0.238	0.162	-0.233	-0.275*
Carbonyls $_{day\ 10}$				0.270*	0.096	-0.141	-0.268*
CL (%)					0.204	0.047	-0.539***
DL (%)						-0.035	-0.480***
pH_{45min}							0.179

I-ILP $_{min\ 200}$: iron-induced lipid peroxidation min 200; DL (%): drip loss; CL (%): cook loss
*: p<0.05; **: p<0.01; ***: p<0.001

5.- CONCLUSIONS

Iberian x Duroc reciprocal cross did not affect the colour and the oxidative stability of chilled meat. Nevertheless, the Duroc sire line had important consequences on the meat quality of Iberian x Duroc crosses, especially on the ultimate pH, which is a parameter associated with the genotype and which seems to be an important factor for the colour and the oxidative stability of meat during storage. The cross of the Iberian breed with selected Duroc genotypes (DU2) has negative consequences for meat quality, since it decreases *post-mortem* pH as well as the colour and oxidative stability of meat during storage, and as a result, its shelf-life and visual appearance.

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Chapter IV

CHANGES IN FATTY ACID COMPOSITION OF 2 MUSCLES FROM 3 IBERIAN x DUROC GENOTYPES AFTER REFRIGERATED STORAGE[1]

ABSTRACT

The evolution of the fatty acid profile of 2 muscles *Longissimus dorsi* (LD) and *Biceps femoris* (BF) from 3 Iberian x Duroc genotypes was studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype Duroc1 (DU1) was selected for the production of dry-cured meat products while the genotype Duroc2 (DU2) was selected for meat production. LD and BF from the reciprocal cross showed similar fatty acids evolution after refrigerated storage. However, the Duroc sire line significantly affected the fatty acid profile of IMF and lipid fractions since important differences were found between GEN2 and GEN3. Meat from GEN3 had the highest level of PUFA in IMF and lipid fractions as well as the lowest rate of plasmalogens in PL fraction. After storage, meat from GEN3 showed a large increase of long chain PUFA in free fatty acids fraction (FFA) and the highest increase in the ratio DMA/FA ((dimethylacetals/fatty acid)x100) after the refrigerated storage, which is indicative of a higher deterioration of this genotype. Therefore, the crossbreeding of Iberian pigs with Duroc selected genotypes (DU2) reduced the fatty acids profile stability of meat during refrigerated storage.

Keywords: fatty acid, intramuscular fat, lipid fractions, genotype, storage, Duroc, Iberian.

1.- INTRODUCTION

Iberian breed is an autochthonous breed traditionally reared in the south-west of the Iberian Peninsula. The Iberian pig is mainly destined to the manufacture of dry cured-meat products such as shoulders, loins and hams, and recently, to fresh meat consumption. Meat and dry-cured meat products are highly appreciated by the consumers due to their sensory and nutritive quality. The high demand of meat and dry-cured meat products from Iberian pigs has increased the number of Iberian pigs in Spain. As Iberian pig has slow growth rate and low prolificacy (Dobao *et al*., 1986), it is very common the use of the Duroc breed in cross with the Iberian breed to improve productive parameters (Dobao *et al*., 1986); without a significant reduction of the quality of meat and meat products (Antequera *et al*., 1994; López-Bote, 1998; Tejeda *et al*., 2002).

Because of the increase of the market of Iberian pigs in the last years in Spain, a specific law to regulate this production was passed in 2001 (B.O.E., 15 October 2001). One of the most important aspects that this law regulates is the genotype used for the manufacture of cured meat products (hams, shoulders and loins) labelled as “Iberian”. The law allows using pure Iberian pigs as well as Iberian x Duroc crosses, but it is necessary the use of Iberian females in the crossbreeding with Duroc. Therefore, the differences between reciprocal crosses and the characteristics of the Duroc sire lines for crossbreeding with Iberian pig are of prime importance for Iberian pig producers.

For the manufacture of high quality dry-cured Iberian meat products, the industry requires heavy pigs with a high content of subcutaneous and intramuscular fat (IMF) (Ruiz-Carrascal *et al*., 2000). Cava *et al*., (1999) reported a marked influence of the fatty acid composition of IMF on the volatile aldehyde profile of Iberian dry-cured ham. Furthermore, the fatty acid composition of intramuscular fat is determinant for the shelf life of refrigerated meat, because that could favour the development of

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oxidation, and as a result, the development of rancidity and the loss of nutritive value (Kanner 1994; Gray *et al*., 1996; Morrissey *et al*., 1998).

Lipolytic enzymes remain active during refrigerated or frozen storage (Motilva *et al*., 1992) and maturation of dry-cured products (Toldrá *et al*., 1997). Lipolysis is considered as a promoter of lipid oxidation because released free fatty acids are very prone to lipid oxidation; particularly long chain unsaturated fatty acids (Nawar, 1996). Lipid oxidation modifies sensory and nutritional characteristics of meat, causing a yellow coloration of fat, loss of water, texture alteration and off-flavour generation (Gray *et al*., 1996). This reaction mainly depends on lipid content, fatty acid composition and the balance between prooxidants and antioxidants factors (Morrissey *et al*., 1998).

Significant differences have been previously reported in meat quality from different Duroc lines (Lonergan *et al*., 2001; Soriano *et al*., 2005; Cilla *et al*., 2006). Moreover, there are important differences in the lipolytic activities of lipase, phospholipase and cathepsin associated to the pig genotype (Toldrá *et al*., 1996; Rosell and Toldrá 1998; Hernández *et al*., 2004; Cava *et al*., 2004). Therefore, the evaluation of the changes during the refrigerated storage of the meat related to the pig genotype is of prime importance to a further understanding of the degradation processes of meat. The objective of this paper was to determine the effect of the crossbreeding of different Iberian x Duroc genotypes on fatty acid profile changes from intramuscular lipid fractions during refrigerated storage.

2.- MATERIAL AND METHODS

2.1.- Animals.

3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening. The genotype Duroc2 (DU2) corresponded to animals selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were castrated, as is traditionally done and they were raised together in a semi-intensive system and were fed *ad libitum* with the diet shown in Table 1. Pigs were randomly slaughtered after 316 days of rearing at 150-165 Kg live weight.

2.2.- Refrigerated storage.

Longissimus dorsi (LD) and *Biceps femoris* (BF) were sliced (1.5 cm of thickness). They were placed on Styrofoam meat trays, over-wrapped in PVC oxygen permeable films, and stored under fluorescent light at +4º C for 10 days. A solution of chloramphenicol (4mg/ml)/ciclohexamide (1mg/ml) was sprayed on the surface of the chops to avoid microbial growth. Samples were stored at -80ºC.

2.3.- Fat extraction, lipids extract fractionation and fatty acid analyses.

Lipids were extracted from 5g of meat samples with chloroform/methanol (1:2) according to the method described by Bligh and Dyer (1959). Total lipid extracts from intramuscular fat –IMF- were fractionated by solid phase extraction on 100mg aminopropyl minicolumns (Varian, CA) into neutral lipids (NL), free fatty acids (FFA) and polar lipids (PL), following the procedure described by Monin *et al*., (2003). Fatty acid methyl esters (FAMEs) from fatty acids and dimethylacetals (DMA) from fatty

aldehydes were prepared by acidic-trans-esterification in the presence of sulphuric acid (5% sulphuric acid in methanol). FAMEs and DMA were analyzed in a Hewlett-Packard model HP-5890A gas chromatograph equipped with a flame ionization detector (FID). The derivatives were separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0mm film thickness). The injector and detector temperatures were held at 230°C. The column oven temperature was maintained at 220°C. The flow rate of the carrier gas (N_2) was set at 1.8mL/min. Identification of FAMEs was based on retention times of reference compounds (SIGMA). Fatty acid composition was expressed as g/100g of FAMEs analyzed.

Table 1.- Proximate composition (%) and fatty acid composition (% total fatty acids) of the pig diets

	Mixed diet	
	I	II
Proximate composition		
Crude protein	16.0	13.5
Crude fat	3.3	5.0
Crude fiber	4.8	3.7
Ash	6.9	6.2
Lysine	0.9	0.5
Metabolizable energy (Kcal/Kg)	3071.5	3183.7
Fatty acid composition		
C14:0	0.1	0.1
C16:0	14.6	21.0
C18:0	4.4	5.6
C18:1n-9	23.3	31.3
C18:2n-6	34.7	35.1
C18:3n-6	2.0	2.3

Mixed diet I: from 60 to 100kg live weight
Mixed diet II: from 100 to 165 kg live weight

2.4. - Statistical analysis.

The effects of genotype and sex were analyzed using the Analysis of Variance procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. When ANOVA detected significant differences between mean values, means were compared using HSD Turkey's test.

3.- RESULTS

3.1.- Fatty acid profiles of total lipids.

The genotype significantly affected the fatty acid composition of IMF from LD muscle; the effect of the sex of animals was negligible (Table 2). GEN2 had higher content of saturated fatty acids (SFA) and lower content of polyunsaturated fatty acids (PUFA) than GEN1 and GEN3. Significant differences between reciprocal crosses (GEN1 vs. GEN2) were found, as the IMF lipids from GEN1 were more unsaturated than those from GEN2 because of the higher percentages of C18:2*n*-6, C18:3*n*-6, C20:2*n*-6, C20:4*n*-6 and PUFA and the lower percentages of C16:0 and SFA. Important differences were found between GEN2 and GEN3 because of the Duroc sire line (DU1 or DU2). IMF from GEN3 was richer in MUFA and PUFA than that from GEN2, which was more saturated due to the high content of SFA. In the BF muscle, neither the genotype nor the sex of the pigs affected fatty

acid profiles of the IMF. Nevertheless, the fatty acid composition of the BF followed a similar trend than that previously described for LD.

After 10 days of refrigerated storage of LD, a decrease in PUFA was observed, especially in GEN3, while remained unchanged in GEN2 and slightly decreased in GEN1. IMF lipids from GEN2 were more saturated, with higher contents of C16:0 and C18:0, than those from GEN3, while GEN1 had an intermediate content of SFA. In BF, little differences were found between genotypes. GEN2 had higher contents of C16:0 and SFA than the other two genotypes, and lower C20:4*n*-6 and C20:5*n*-3 contents than GEN3.

3.2. - Fatty acid profiles in neutral lipids from intramuscular fat.

FA profiles of NL showed a similar pattern than that described for total lipids of intramuscular fat. Genotype significantly affected fatty acid composition of NL fraction in both muscles, whereas the sex of the animals only affected FA profiles of LD (Table 3). In this sense, NL from females were more saturated due to higher SFA and lower PUFA content than those from males. Reciprocal cross (GEN1 vs. GEN2) did not affect the sum of SFA, MUFA and PUFA in both muscles, although in LD GEN2 had lower contents of C18:1*n*-9, C20:1*n*-9 and higher C14:0 and C16:0 than GEN1. Duroc sire line (GEN2 vs. GEN3) significantly affected FA profiles of NL from LD and BF. In LD, NL from GEN3 were more unsaturated than those from GEN2 which had higher contents of C16:0 and C18:0 and lower contents of C18:1*n*-9, C18:3*n*-6, C20:2*n*-6, C20:4*n*-6, C20:5*n*-3. Similarly, NL of BF from GEN3 had more C18:2*n*-6, C20:4*n*-6, C22:2*n*-6 and less C18:0 than those from GEN2.

In general, storage did not produce great changes in FA profiles of NL, although diminished differences found between genotypes at day 0. Thus, differences between reciprocal crosses (GEN1 vs. GEN2) were negligible and slight differences between genotypes with different Duroc sire line (GEN2 vs. GEN3) were found. In LD, differences between genotypes in SFA and MUFA were maintained and FA profiles showed a similar pattern than that described for day 0. However, no differences were found in PUFA after storage between genotypes, mainly due the higher decrease of long chain PUFA (C20:2*n*-6, C20:4*n*-6, C20:5*n*-3) in GEN3. In BF, after storage, NL from the 3 genotypes showed slight differences in their fatty acid profiles, and only SFA differed between them. GEN2 had higher levels of C16:0 and C18:0 than GEN3 while GEN1 had intermediate levels.

3.3.- Fatty acid profile of polar lipids

No significant differences were found in SFA, MUFA and PUFA before the storage in both muscles (Table 4). In LD, GEN1 and GEN2 had highest levels of C18:3*n*-6, GEN2 and GEN3 had the highest percentage of C18:0 and GEN3 had the highest percentages of C20:4*n*-6. In BF, some PUFA (C18:2*n*-6, C18:3*n*-6 and C20:5*n*-3) were significantly highest in GEN1; the levels of C18:0, C18:2*n*-6 and C22:2*n*-6 were significantly highest in GEN2 while C18:0 and long chain PUFA (C20:4*n*-6, C20:5*n*-3, C22:2*n*-6) were significantly highest in GEN3.

Table 2.- Evolution of fatty acid composition of intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Longissimus dorsi																		
C12:0	0.04b	0.06a	0.05ab	0.05	0.05	0.00	**	ns	*	0.05	0.05	0.05	0.05	0.05	0.00	ns	*	ns
C14:0	1.16b	1.36a	1.18b	1.24	1.23	0.02	***	ns	*	1.24	1.36	1.44	1.45	1.26	0.06	ns	ns	ns
C16:0	23.79b	25.16a	23.59b	24.14	24.25	0.20	***	ns	*	24.86b	25.64a	24.75b	25.20	24.99	0.13	**	ns	*
C16:1 n-7	3.65b	3.94ab	4.16a	4.00	3.86	0.07	*	ns	ns	3.54b	3.84ab	4.13a	3.91	3.79	0.07	**	ns	ns
C17:0	0.17ab	0.14b	0.18a	0.17	0.16	0.01	**	ns	ns	0.17	0.15	0.16	0.16	0.16	0.00	ns	ns	ns
C17:1 n-7	0.21ab	0.18b	0.21a	0.20	0.20	0.01	*	ns	ns	0.25	0.25	0.21	0.25	0.23	0.01	ns	ns	ns
C18:0	12.85a	13.63a	11.75b	12.50	12.96	0.21	***	ns	ns	13.36a	13.70a	12.27b	12.99	13.21	0.18	**	ns	ns
C18:1 n-9	48.82ab	48.03b	49.16a	48.70	48.63	0.20	*	ns	ns	47.84	47.27	48.55	47.72	48.05	0.25	ns	ns	ns
C18:2 n-6	6.15a	4.95b	6.38a	5.90	5.73	0.19	***	ns	ns	5.95	5.26	5.77	5.65	5.65	0.16	ns	ns	ns
C18:3 n-6	0.29a	0.23b	0.34a	0.29	0.28	0.01	***	ns	ns	0.25	0.21	0.23	0.23	0.23	0.01	ns	ns	ns
C20:0	0.26a	0.25ab	0.23b	0.23	0.26	0.01	*	*	**	0.23	0.23	0.22	0.22	0.23	0.01	ns	ns	ns
C20:1n-9	0.97	0.88	0.87	0.92	0.88	0.02	ns	ns	ns	0.95a	0.88ab	0.84b	0.90	0.88	0.02	*	ns	ns
C20:2 n-6	0.35a	0.23b	0.26ab	0.31	0.25	0.02	*	ns	ns	0.28a	0.24b	0.24b	0.25	0.25	0.01	**	ns	ns
C20:4 n-6	1.05b	0.76c	1.35a	1.09	1.02	0.07	***	ns	ns	0.78	0.72	0.93	0.80	0.82	0.04	ns	ns	ns
C20:5 n-3	0.04ab	0.02b	0.05a	0.04	0.03	0.00	***	ns	ns	0.05	0.03	0.05	0.04	0.05	0.00	ns	ns	ns
C22:2 n-6	0.03	0.02	0.02	0.02	0.02	0.00	ns	ns	ns	0.01	0.01	0.00	0.01	0.01	0.00	ns	ns	ns
C22:4 n-6	0.19ab	0.15b	0.23a	0.19	0.19	0.01	*	ns	**	0.17	0.14	0.17	0.16	0.16	0.01	ns	ns	ns
SFA	38.27b	40.60a	36.97b	38.33	38.90	0.40	***	ns	**	39.69ab	40.91a	38.66b	39.85	39.67	0.29	**	ns	ns
MUFA	53.64ab	53.03b	54.41a	53.82	53.57	0.23	*	ns	ns	52.59	52.24	53.73	52.78	52.94	0.29	ns	ns	ns
PUFA	8.09a	6.37b	8.63a	7.85	7.52	0.27	***	ns	ns	7.49	6.62	7.38	7.14	7.16	0.20	ns	ns	ns

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Table 2.- Evolution of fatty acid composition of intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Cont.																		
Biceps femoris																		
C12:0	0.06	0.06	0.05	0.06	0.06	0.00	ns	ns	ns	0.05	0.05	0.16	0.12	0.06	0.03	ns	ns	ns
C14:0	1.16ab	1.24a	1.12b	1.19	1.16	0.02	*	ns	ns	1.11b	1.23a	1.12b	1.18	1.13	0.02	**	ns	ns
C16:0	22.65	22.60	21.98	22.40	22.41	0.16	ns	ns	ns	22.54ab	23.12a	22.38b	22.72	22.65	0.12	*	ns	ns
C16:1 n-7	3.70	4.12	3.95	3.95	3.92	0.07	ns	ns	ns	3.79	4.06	3.86	3.96	3.85	0.06	ns	ns	ns
C17:0	0.22	0.23	0.24	0.24	0.23	0.01	ns	ns	ns	0.19	0.17	0.19	0.18	0.18	0.01	ns	ns	ns
C17:1 n-7	0.26	0.27	0.26	0.27	0.26	0.01	ns	ns	ns	0.24	0.23	0.25	0.24	0.24	0.01	ns	ns	ns
C18:0	11.83	11.41	10.81	11.28	11.39	0.16	ns	ns	ns	11.30	11.61	10.97	11.20	11.38	0.12	ns	ns	ns
C18:1 n-9	48.98	48.91	49.14	48.90	49.12	0.24	ns	ns	ns	49.28	48.67	48.97	48.95	48.98	0.20	ns	ns	ns
C18:2 n-6	7.27	7.32	8.21	7.64	7.58	0.22	ns	ns	ns	7.81	7.48	8.33	7.84	7.90	0.16	ns	ns	ns
C18:3 n-6	0.42	0.46	0.48	0.45	0.45	0.02	ns	ns	ns	0.26	0.26	0.27	0.27	0.26	0.01	ns	ns	ns
C20:0	0.24	0.26	0.24	0.25	0.24	0.01	ns	ns	ns	0.23	0.22	0.31	0.28	0.24	0.02	ns	ns	ns
C20:1n-9	0.86	0.85	0.83	0.90	0.79	0.04	ns	ns	ns	0.90	0.86	0.77	0.81	0.88	0.03	ns	ns	ns
C20:2 n-6	0.35	0.34	0.35	0.35	0.33	0.01	ns	ns	ns	0.33	0.31	0.33	0.33	0.32	0.01	ns	ns	ns
C20:4 n-6	1.58	1.51	1.85	1.65	1.64	0.08	ns	ns	ns	1.64ab	1.42b	1.74a	1.58	1.61	0.05	*	ns	ns
C20:5 n-3	0.07	0.07	0.08	0.08	0.07	0.01	ns	ns	ns	0.08ab	0.07b	0.09a	0.08	0.08	0.00	***	ns	ns
C22:2 n-6	0.04	0.02	0.03	0.02	0.03	0.00	ns	ns	ns	0.00	0.00	0.00	0.00	0.00	0.00	ns	ns	ns
C22:4 n-6	0.31	0.32	0.38	0.36	0.32	0.02	ns	ns	ns	0.27	0.23	0.27	0.26	0.25	0.01	ns	ns	ns
SFA	36.16	35.81	34.45	35.42	35.48	0.30	ns	ns	ns	35.41b	36.41a	35.13b	35.68	35.64	0.19	*	ns	ns
MUFA	53.80	54.15	54.18	54.02	54.08	0.27	ns	ns	ns	54.21	53.83	53.85	53.97	53.94	0.23	ns	ns	ns
PUFA	10.04	10.04	11.36	10.56	10.44	0.34	ns	ns	ns	10.38	9.76	11.02	10.35	10.42	0.22	ns	ns	ns

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
LD: *Longissimus dorsi.* BF: *Biceps femoris*. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
sem: standard error mean. gen: genotype; inter: interaction genotype x sex
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

In general, storage did not affect markedly fatty acid profile of this fraction. In LD, the level of SFA, MUFA and PUFA did not change between genotypes after the storage, although the percentage of linoleic acid (C18:2*n*-6) was higher in GEN1 and GEN2 than in GEN3. Similarly, after the refrigerated storage of BF, the sum of SFA, MUFA and PUFA remained unchanged. In GEN3, the levels of some PUFA decreased (C22:2*n*-6) while others remained unchanged (C20:2*n*-6 and C20:4*n*-6).

Fatty aldehyde composition and the ratio dimethylacetals/fatty acids (DMA/FA) are shown in Table 5. In general, the analysis of fatty aldehydes released from plasmalogens showed that *n*-hexadecanal was the most abundant fatty aldehyde, followed by *n*-octadecanal and *n*-octadec-9-enal. Dimethylacetals profile differed between muscles and was significantly affected by the genotype. In LD, a significantly higher percentage of *n*-hexadecanal was found in GEN1 and GEN2 than in GEN3, which showed highest levels of *n*-octadecanal and *n*-octadec-9-enal. In contrast, in BF only *n*-octadec-9-enal showed differences between genotypes and GEN1 had the highest percentages. Results suggest a marked effect of both the muscle and the Duroc sire line on dimethylacetals of phospholipids.

After storage, no great changes in dimethylacetals were found, however the storage created some additional differences between genotypes. These changes depended on the muscle studied. So, in LD *n*-hexadecanal tended to increase while *n*-octadecanal and *n*-octadec-9-enal remained unchanged, and differences described for day 0 were maintained for *n*-hexadecanal and *n*-octadec-9-enal. In BF, *n*-hexadecanal tended to decrease and *n*-octadec-9-enal tended to increase. GEN1 and GEN2 had significantly higher percentages of *n*-hexadecanal and *n*-octadec-9-enal and lower of *n*-octadecanal than GEN3.

DMA/FA ratio did not show differences among genotypes neither LD nor BF at day 0. After storage, DMA/FA tended to decrease in both muscles and genotypes, with the exception of LD from GEN3 in which increased. At day 10 of storage, LD from GEN3 had a significantly higher ratio DMA/FA than GEN1 and in BF the ratio DMA/FA was higher in GEN1 and GEN3 than in GEN2.

3.4.- Free fatty acids profile.

Free fatty acid profiles of raw and stored LD and BF are shown in Table 6. No differences in the fatty acid composition were found due to the sex of the animals, whereas important differences were due to the genotype. In general, FFA profiles of both muscles followed the same pattern described for total lipids, so GEN1 and GEN2 had higher percentages of SFA and MUFA and lower of PUFA than GEN3. As a result, GEN3 had the highest contents of C18:2 *n*-6 and C20:4*n*-6 in LD and C18:2*n*-6, C20:4*n*-6, C20:5*n*-3 in BF. Great changes in the profile of FFA were found after storage. Changes followed a similar pattern in LD and BF, thereby SFA and MUFA tended to decrease while PUFA increased, although changes were more intense in LD than in BF.

Table 3.- Evolution of fatty acid composition of neutral lipids from intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	Sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Longissimus dorsi																		
C12:0	0.07	0.07	0.08	0.08	0.07	0.00	*	ns	ns	0.06b	0.06a	0.06a	0.06	0.06	0.00	**	ns	ns
C14:0	1.35b	1.51a	1.30b	1.40	1.37	0.03	***	ns	*	1.32b	1.48a	1.39b	1.44	1.36	0.02	***	*	ns
C16:0	25.33ab	26.06a	24.61b	25.16	25.50	0.19	***	ns	*	25.24b	26.02a	25.30b	25.62	25.45	0.12	**	ns	ns
C16:1 n-7	3.88b	4.08ab	4.40a	4.22	4.04	0.08	*	ns	ns	3.70b	4.01ab	4.33a	4.08	3.97	0.08	**	ns	ns
C17:0	0.17	0.15	0.18	0.17	0.16	0.01	ns	ns	ns	0.16	0.15	0.15	0.15	0.15	0.00	ns	ns	ns
C17:1 n-7	0.20	0.17	0.23	0.20	0.20	0.01	ns	ns	ns	0.21	0.19	0.20	0.20	0.20	0.00	ns	ns	ns
C18:0	12.98a	13.46a	11.64b	12.32	13.02	0.21	***	*	ns	13.43a	13.69a	12.26b	13.00	13.22	0.20	**	ns	ns
C18:1 n-9	49.55a	48.45b	50.46a	49.64	49.34	0.24	***	ns	*	49.26ab	48.57b	50.21a	49.22	49.48	0.26	*	ns	ns
C18:2 n-6	4.33	3.98	4.71	4.48	4.21	0.13	ns	ns	ns	4.55	3.90	4.26	4.23	4.22	0.13	ns	ns	ns
C18:3 n-6	0.25ab	0.22b	0.29a	0.26	0.25	0.01	***	ns	ns	0.25	0.25	0.25	0.24	0.26	0.01	ns	ns	ns
C20:0	0.27	0.29	0.28	0.28	0.28	0.01	ns	ns	*	0.24	0.26	0.21	0.25	0.23	0.01	ns	ns	ns
C20:1n-9	0.95a	0.87b	0.89ab	0.93	0.88	0.02	*	ns	ns	0.99a	0.92ab	0.80b	0.94	0.86	0.03	*	ns	ns
C20:2 n-6	0.26ab	0.24b	0.29a	0.27	0.25	0.01	*	ns	ns	0.27a	0.23b	0.23b	0.24	0.24	0.01	**	ns	ns
C20:4 n-6	0.23b	0.28ab	0.42a	0.37	0.26	0.03	**	*	ns	0.19	0.15	0.22	0.20	0.17	0.01	ns	ns	ns
C20:5 n-3	0.05b	0.03c	0.07a	0.05	0.05	0.00	***	ns	*	0.04	0.04	0.04	0.04	0.04	0.00	ns	ns	*
C22:2 n-6	0.04	0.05	0.05	0.05	0.05	0.00	ns	ns	ns	0.01	0.01	0.00	0.01	0.01	0.00	ns	ns	ns
C22:4 n-6	0.08	0.10	0.10	0.11	0.08	0.01	ns	*	ns	0.10	0.09	0.09	0.10	0.09	0.00	ns	ns	ns
SFA	40.16a	41.54a	38.1b	39.40	40.41	0.38	***	*	*	40.28ab	41.50a	39.22b	40.36	40.32	0.28	**	ns	ns
MUFA	54.58b	53.57b	55.98a	54.99	54.45	0.28	***	ns	*	54.15ab	53.69b	55.55a	54.44	54.51	0.31	*	ns	ns
PUFA	5.25ab	4.89b	5.93a	5.60	5.13	0.18	*	ns	ns	5.40	4.67	5.09	5.06	5.02	0.15	ns	ns	ns

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Table 3.- Evolution of fatty acid composition of neutral lipids from intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	Sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Cont.																		
Biceps femoris																		
C12:0	0.05b	0.07ab	0.08a	0.07	0.06	0.00	**	*	ns	0.07	0.05	0.06	0.06	0.06	0.00	ns	ns	ns
C14:0	1.13	1.23	1.15	1.16	1.18	0.02	ns	ns	ns	1.23	1.31	1.22	1.27	1.24	0.02	ns	ns	ns
C16:0	23.50	23.37	22.90	23.24	23.25	0.13	ns	ns	ns	23.12ab	23.60a	22.84b	23.19	23.19	0.13	*	ns	ns
C16:1 n-7	3.93	4.23	4.15	4.12	4.11	0.06	ns	ns	ns	3.99	4.17	4.15	4.16	4.06	0.07	ns	ns	ns
C17:0	0.19	0.21	0.21	0.21	0.20	0.01	ns	ns	ns	0.17	0.17	0.17	0.17	0.17	0.00	ns	ns	ns
C17:1 n-7	0.19	0.19	0.22	0.20	0.20	0.01	ns	ns	ns	0.24	0.23	0.25	0.24	0.24	0.01	ns	ns	ns
C18:0	11.54a	11.34a	10.56b	11.08	11.19	0.16	*	ns	ns	11.13ab	11.52a	10.70b	11.00	11.22	0.13	*	ns	ns
C18:1 n-9	51.80	50.76	51.63	51.20	51.56	0.23	ns	ns	ns	51.29	50.65	51.49	51.32	50.97	0.22	ns	ns	ns
C18:2 n-6	5.24b	5.94ab	6.29a	5.99	5.70	0.16	*	ns	ns	6.06	5.91	6.52	6.07	6.26	0.14	ns	ns	ns
C18:3 n-6	0.30	0.31	0.34	0.33	0.31	0.01	ns	ns	ns	0.21	0.24	0.26	0.24	0.23	0.01	ns	ns	ns
C20:0	0.25b	0.42a	0.39ab	0.38	0.34	0.03	*	ns	ns	0.24	0.28	0.28	0.28	0.25	0.03	ns	ns	ns
C20:1n-9	0.97	0.87	0.92	0.94	0.90	0.02	ns	ns	ns	0.96	0.81	0.77	0.82	0.86	0.04	ns	ns	ns
C20:2 n-6	0.28	0.31	0.31	0.33	0.28	0.01	ns	ns	ns	0.31	0.30	0.32	0.31	0.30	0.01	ns	ns	ns
C20:4 n-6	0.39b	0.60a	0.60a	0.58	0.50	0.03	**	ns	ns	0.78	0.60	0.79	0.68	0.75	0.04	ns	ns	ns
C20:5 n-3	0.06	0.04	0.06	0.05	0.06	0.00	ns	ns	ns	0.05	0.04	0.05	0.05	0.04	0.00	ns	ns	ns
C22:2 n-6	0.06ab	0.04b	0.08a	0.06	0.06	0.00	**	ns	ns	0.00	0.00	0.00	0.00	0.00	0.00	ns	ns	ns
C22:4 n-6	0.10	0.14	0.11	0.12	0.12	0.01	*	ns	ns	0.15	0.13	0.14	0.14	0.15	0.01	ns	ns	ns
SFA	36.67a	36.63a	35.28b	36.15	36.21	0.26	*	ns	ns	35.96ab	36.93a	35.27b	35.97	36.14	0.24	**	ns	ns
MUFA	56.89	56.05	56.93	56.45	56.76	0.24	ns	ns	ns	56.47	55.86	56.66	56.54	56.12	0.25	ns	ns	ns
PUFA	6.45b	7.38ab	7.79a	7.45	7.03	0.20	*	ns	ns	7.57	7.21	8.08	7.49	7.74	0.19	ns	ns	ns

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
LD: *Longissimus dorsi.* BF: *Biceps femoris*. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
sem: standard error mean. gen: genotype; inter: interaction genotype x sex
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

These changes increased the differences between genotypes previously described, and as a consequence GEN1 and GEN2 had the highest percentages of SFA and MUFA, while GEN3 had the highest of PUFA such as C18:2*n*-6, C20:4*n*-6, C20:5*n*-3 in LD and C18:2*n*-6, C20:4*n*-6, C22:4*n*-6 in BF.

4.- DISCUSSION

The fatty acid composition of the IMF from LD and BF as well as the changes after storage were similar between reciprocal crosses (GEN1 vs. GEN2), although when the fatty acid level between GEN2 and GEN3 was significantly different, GEN1 profile showed a content of fatty acids intermediate to those genotypes. However, the differences found in the fatty acid composition of IMF between reciprocal crosses (GEN1 vs. GEN2) decreased in NL fraction. The similar percentages of SFA, MUFA and PUFA between these genotype found in this lipid fraction suggest that differences in the IMF fatty acid composition between reciprocal crosses could be due to the different of IMF level, since GEN1 had a lower IMF content in comparison with GEN2 (Ramírez *et al*., submitted). The higher IMF content could give a higher content of triacylglycerides, and as a result, a relative decrease in the phospholipids content (Leseigneur-Meynier and Gandemer, 1991) since PUFA, which are mostly located in the membrane, are relatively constant in the cell membranes.

On the other hand, the Duroc sire line (DU1 or DU2) significantly affected the fatty acid composition of IMF and NL fraction since the meat from GEN3 had more PUFA and less SFA. These results could indicate that the differences between genotypes in IMF and NL were due to a different adipogenic potential, which could increase "*de novo*" fatty acids synthesis in GEN1 and GEN2 and as a consequence their level in NL fraction. Endogenous synthesis of fatty acids comes from acetyl CoA and malonil CoA molecules to produce palmitic acid (C16:0), from which can be synthesized stearic acid (C18:0) by elongation, so a higher level of SFA is expected in genotypes with more adipogenic nature. Higher lipogenic enzyme activity has been reported (Ramírez *et al*., submitted) in GEN1 and GEN2 than in GEN3, which is accord with these results. On the other hand, one of the possible consequences of a high PUFA percentage in meat is the reduction of the shelf life of meat, since PUFA are extremely sensitive to oxidation, leading to meat texture, flavour and colour alterations (Morrissey *et al*., 1998).

After storage, a marked reduction of PUFA of IMF and NL from GEN3 was found and no differences between genotypes were found after storage in PUFA contents. These results could be caused by initial differences in the fatty acid composition which could affect the oxidation of PUFA during storage as well as by differences in the hydrolysis of triacylglycerides because of a different lipolytic enzyme activity, which could be more intense in GEN3. In this sense, several authors have reported important differences in muscle lipolytic activities due to the pig genotype (Toldrá *et al*., 1996; Rosell and Toldrá 1998; Hernández *et al*., 2004; Cava *et al*., 2004).

Table 4.- Evolution of fatty acid composition of polar lipids from intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Longissimus dorsi																		
C12:0	0.11	0.10	0.08	0.10	0.10	0.01	ns	ns	*	0.09	0.07	0.12	0.09	0.10	0.01	ns	ns	ns
C14:0	0.11a	0.12a	0.06b	0.10	0.09	0.01	**	ns	*	0.09	0.15	0.13	0.14	0.10	0.02	ns	ns	ns
C16:0	12.23	11.05	10.87	11.35	11.35	0.27	ns	ns	ns	12.44	12.39	12.89	12.44	12.70	0.30	ns	ns	ns
C16:1 n-7	0.65	0.68	0.55	0.64	0.62	0.03	ns	ns	ns	0.60	0.62	0.70	0.67	0.62	0.03	ns	ns	ns
C17:0	0.81	0.81	0.65	0.70	0.80	0.04	ns	ns	ns	0.34	0.28	0.31	0.30	0.32	0.01	ns	ns	ns
C18:0	11.65b	13.64a	13.38a	13.28	12.60	0.23	***	ns	ns	14.32	14.16	15.17	14.44	14.68	0.21	ns	ns	ns
C18:1 n-9	14.85	14.52	14.94	15.02	14.53	0.54	ns	ns	ns	17.78	16.12	16.73	17.46	16.27	0.38	ns	ns	ns
C18:2 n-6	36.81	35.10	34.23	35.10	35.55	0.44	ns	ns	ns	34.38a	34.76a	32.27b	34.17	33.42	0.39	**	ns	ns
C18:3 n-6	0.55a	0.55a	0.45b	0.50	0.53	0.02	*	ns	ns	0.72	0.98	0.97	0.70	1.09	0.09	ns	*	ns
C20:0	0.04	0.10	0.13	0.13	0.05	0.02	ns	ns	ns	0.10	0.16	0.11	0.07	0.18	0.02	ns	*	ns
C20:1n-9	0.52	0.42	0.47	0.57	0.37	0.07	ns	ns	ns	0.38a	0.31ab	0.28b	0.32	0.33	0.02	*	ns	ns
C20:2 n-6	0.57	0.53	0.48	0.53	0.52	0.03	ns	ns	ns	0.55	0.53	0.52	0.52	0.54	0.01	ns	ns	ns
C20:4 n-6	16.40b	17.84b	18.98a	17.49	18.06	0.30	***	ns	ns	15.28	16.38	16.79	15.79	16.54	0.30	ns	ns	ns
C20:5 n-3	0.44	0.47	0.40	0.40	0.47	0.02	ns	ns	ns	0.54	0.62	0.49	0.52	0.59	0.04	ns	ns	ns
C22:2 n-6	0.10	0.08	0.26	0.24	0.07	0.08	ns	ns	ns	nd	nd	nd	nd	nd				
C22:4 n-6	2.39	2.46	2.35	2.36	2.44	0.08	ns	ns	ns	2.39	2.44	2.52	2.37	2.53	0.04	ns	ns	ns
SFA	24.94	25.81	25.17	25.66	25.00	0.24	ns	ns	ns	27.37	27.23	28.73	27.48	28.08	0.44	ns	ns	ns
MUFA	16.02	15.63	15.96	16.24	15.52	0.56	ns	ns	ns	18.77	17.05	17.71	18.45	17.22	0.40	ns	ns	ns
PUFA	57.26	57.03	57.16	56.62	57.64	0.51	ns	ns	ns	53.86	55.72	53.56	54.07	54.71	0.52	ns	ns	ns

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Table 4.- Evolution of fatty acid composition of polar lipids from intramuscular fat (g/100g FA) of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Cont.																		
Biceps femoris																		
C12:0	0.07b	0.09a	0.08ab	0.08	0.07	0.00	*	ns	ns	0.13	0.12	0.14	0.13	0.14	0.00	ns	ns	*
C14:0	0.05	0.06	0.07	0.05	0.07	0.01	ns	ns	ns	0.14a	0.11ab	0.09b	0.12	0.11	0.01	*	ns	ns
C16:0	11.56	10.96	11.01	11.38	10.97	0.21	ns	ns	ns	12.21a	11.51ab	10.85b	11.59	11.41	0.19	*	ns	ns
C16:1 n-7	0.60	0.57	0.66	0.63	0.59	0.03	ns	ns	ns	0.63ab	0.73a	0.60b	0.65	0.65	0.02	*	ns	ns
C17:0	0.57	0.49	0.59	0.54	0.56	0.02	ns	ns	ns	0.35	0.33	0.32	0.32	0.35	0.01	ns	ns	ns
C18:0	14.53b	15.98a	16.50a	15.58	15.83	0.21	***	ns	ns	17.08ab	16.58b	17.55a	17.19	16.95	0.15	*	ns	ns
C18:1 n-9	16.58	16.61	15.69	16.40	16.17	0.29	ns	ns	ns	18.52a	18.50a	16.79b	17.79	18.03	0.30	*	ns	ns
C18:2 n-6	33.66a	32.05a	30.97b	32.17	32.18	0.30	***	ns	ns	29.99	30.62	29.81	29.83	30.44	0.17	ns	ns	ns
C18:3 n-6	0.40a	0.36ab	0.28b	0.33	0.37	0.02	**	ns	ns	0.42b	0.52a	0.38b	0.43	0.45	0.02	***	ns	ns
C20:0	0.08	0.40	0.38	0.27	0.32	0.06	*	ns	ns	0.03	0.06	0.04	0.05	0.04	0.01	ns	ns	ns
C20:1n-9	0.38b	0.43ab	0.56a	0.45	0.47	0.03	*	ns	ns	0.37	0.35	0.35	0.36	0.36	0.02	ns	ns	ns
C20:2 n-6	0.67	0.58	0.57	0.59	0.62	0.02	ns	ns	ns	0.61a	0.52b	0.57a	0.57	0.55	0.01	***	ns	ns
C20:4 n-6	16.76b	17.26ab	18.59a	17.35	17.76	0.25	**	ns	ns	17.40b	17.87b	19.23a	18.46	17.95	0.22	***	ns	ns
C20:5 n-3	0.51a	0.43b	0.50a	0.48	0.48	0.01	**	ns	ns	0.46	0.52	0.53	0.51	0.50	0.01	ns	ns	ns
C22:2 n-6	0.00b	0.21a	0.26a	0.13	0.20	0.03	***	ns	ns	0.00	0.25	0.00	0.16	0.02	0.07	ns	ns	ns
C22:4 n-6	2.15	2.10	1.96	2.03	2.10	0.07	ns	ns	ns	2.23	1.80	2.32	2.09	2.13	0.10	ns	ns	ns
SFA	26.85	27.99	28.63	27.90	27.82	0.29	ns	ns	ns	29.94	28.70	28.86	29.30	28.99	0.22	ns	ns	ns
MUFA	17.55	17.61	16.90	17.48	17.22	0.29	ns	ns	ns	19.53	19.58	18.14	19.09	19.05	0.26	ns	ns	ns
PUFA	54.15	53.00	53.12	53.07	53.71	0.33	ns	ns	ns	51.11	52.08	52.62	51.88	52.05	0.26	ns	ns	ns

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
LD: *Longissimus dorsi.* BF: *Biceps femoris*. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
sem: standard error mean. gen: genotype; inter: interaction genotype x sex
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

Table 5.- Evolution of fatty aldehyde composition in polar lipids fraction from the m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10									
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Longisimus dorsi																		
C16:0AL	48.74a	47.61a	45.52b	47.18	47.30	0.41	**	ns	ns	48.99ab	50.00a	47.85b	48.79	49.09	0.34	*	ns	ns
C18:0AL	34.65b	36.54ab	37.01a	35.72	36.49	0.41	*	ns	ns	34.47	34.64	34.92	34.09	35.24	0.33	ns	ns	ns
C18:1AL	16.60ab	15.85b	17.47a	17.10	16.21	0.28	*	ns	*	16.54ab	15.36b	17.23a	17.12	15.67	0.34	*	*	ns
DMA/FA	17.47	16.27	15.69	15.58	17.25	0.42	ns	*	ns	12.33b	14.64ab	18.07a	14.95	15.24	0.74	**	ns	ns
Bíceps femoris																		
C16:0AL	45.86	47.64	45.68	46.05	46.75	0.41	ns	ns	ns	46.72a	45.20a	40.45b	43.74	44.31	0.78	***	ns	ns
C18:0AL	32.64	32.39	33.72	33.08	32.79	0.31	ns	ns	ns	32.25b	32.38b	36.12a	33.83	33.44	0.56	**	ns	ns
C18:1AL	21.50a	19.56b	20.59ab	20.88	20.19	0.31	*	ns	ns	21.03b	22.42ab	23.43a	22.43	22.25	0.32	**	ns	ns
DMA/FA	14.35	13.77	15.90	14.38	14.97	0.41	ns	ns	ns	15.53a	12.03b	14.27a	14.21	13.59	0.42	***	ns	ns

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
LD: *Longissimus dorsi.* BF: *Biceps femoris*. sem: standard error mean. gen: genotype; inter: interaction genotype x sex
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).
DMA/FA=(dimethylacetals/fatty acid)x100.

Fatty acid composition of PL influences the oxidative stability of meat, since a high proportion of PUFA increases the susceptibility to oxidation (Wood ***et al***., 2004). However, no differences were found in the sum of SFA, MUFA and PUFA either before or after storage. Changes in the specific content of some fatty acids in PL may not have affected IMF FA evolution in comparison with changes in NL fraction which has very high proportions in comparison with PL fraction due to the high level of fatness of pigs (IMF content: 3.5-6 % in LD, 3-3.7% in BF).

DMA profile is in agreement with previous studies (Estévez and Cava, 2004; Morcuende ***et al***., 2003). Our results showed that the genotype affected the DMA profile in BF and LD. Estévez and Cava (2004) reported important differences in the levels of the different fatty aldehydes, which could be associated to differences in the plasmalogen synthesis because of genetic or breeding factors. Singh ***et al***., (1993) reported dihydroxyacetone phosphate acyltransferase is the enzyme involved in the first step of plasmalogens biosynthesis, however, the regulation of the enzymes related to plasmalogens synthesis or degradation is not well known. Reciprocal crosses (GEN1 and GEN2) had similar profiles of plasmalogens, while important differences were found between GEN2 and GEN3 because of the differences in the Duroc sire line (DU1 vs. DU2). Estévez and Cava (2004) found higher percentages of *n*-hexadecanal in Iberian pigs than in industrial ones, which agree with the results found in the present study, in which GEN2 showed the highest level of hexadecanal while GEN3, which come from a Duroc selected line (DU2), showed the highest percentages of octadecanal and octadecenal. Plasmalogens are a special subclass of glycerophospholipids distinguished by their vinyl–ether bonds at the s*n*-1 position of the glycerol backbone. These authors (Estévez and Cava, 2004) have previously suggested that they could play an important role on the oxidative stability of meat during the refrigerated storage. The ratio DMA/FA ranged from 15.69 to 17.47% in LD and from 13.77 to 15.90% in BF, which is lower than those reported by Morcuende ***et al***., (2003) and Estévez and Cava (2004). Differences could probably be due to the higher IMF content of the pigs in the present study. The increases in the ratio DMA/FA from PL during storage could be caused by the release of FA while the level of DMA remains constant because of their resistance to lipolysis. As other authors have previously reported (Estévez and Cava, 2004; Morcuende ***et al***., 2003), the increase of this ratio can be explained by the resistance of the ether links between glycerol and fatty aldehydes to lipases, which increases the relative proportion of fatty aldehyde while the amount of fatty acids linked to glycerol molecule decreases. The lowest ratio DMA/FA after storage in GEN2 could indicate a less intense lipolysis in PL from this genotype. In this respect, Estévez and Cava (2004) found differences in the ratio DMA/FA between meat from commercial pigs and Iberian pig during storage, being higher in the former than in the latter. On the other hand, plasmalogens act *in vivo* protecting cells against oxidative stress (Engelmann, 2004), since their vinyl–ether bond is preferentially oxidized, which protects PUFA at the s*n*-2 position from oxidation. This antioxidant activity could continue *postmortem* according to Estévez and Cava (2004), who reported lower oxidation levels in meat with higher fatty aldehyde proportions in its PLs.

Table 6.- Evolution of fatty acid composition of free fatty acids of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Longissimus dorsi																		
C12:0	0.32	0.31	0.36	0.31	0.35	0.02	ns	ns	ns	0.31	0.26	0.30	0.26	0.32	0.02	ns	ns	ns
C14:0	1.28	1.10	1.06	1.09	1.19	0.05	ns	ns	ns	0.51b	0.71a	0.49b	0.61	0.54	0.04	**	ns	ns
C16:0	22.21ab	23.19a	21.95b	22.52	22.40	0.21	*	ns	ns	19.91	21.13	20.02	20.85	19.92	0.30	ns	ns	ns
C16:1 n-7	3.18a	2.77b	2.19c	2.63	2.76	0.09	***	ns	ns	1.60b	2.07a	1.67ab	1.94	1.65	0.09	*	ns	ns
C17:0	0.40a	0.28b	0.38ab	0.31	0.38	0.02	*	ns	ns	0.46	0.35	0.36	0.35	0.42	0.03	ns	ns	ns
C17:1 n-7	0.40	0.37	0.66	0.40	0.55	0.11	ns	ns	ns	0.17	0.14	0.19	0.18	0.16	0.01	ns	ns	ns
C18:0	14.41	15.60	15.98	15.40	15.33	0.45	ns	ns	ns	15.04ab	17.52a	12.61b	15.38	14.75	0.69	**	ns	ns
C18:1 n-9	38.73a	39.03a	31.55b	35.88	36.81	0.91	***	ns	ns	27.68ab	33.35a	25.64b	31.09	26.92	1.30	**	ns	ns
C18:2 n-6	12.14b	13.02b	18.51a	15.05	14.25	0.82	***	ns	ns	18.99ab	14.34b	25.67a	18.47	20.82	1.40	***	ns	ns
C18:3 n-6	0.64	0.49	1.03	0.63	0.82	0.12	ns	ns	ns	5.05	3.18	2.22	2.51	4.29	0.52	ns	ns	ns
C20:0	0.42	0.33	0.36	0.37	0.36	0.03	ns	ns	ns	1.09a	0.61ab	0.27b	0.59	0.70	0.11	**	ns	ns
C20:1n-9	0.33	0.53	0.38	0.43	0.41	0.04	ns	ns	ns	0.55	0.63	0.44	0.61	0.47	0.04	ns	ns	ns
C20:2 n-6	0.23	0.12	0.06	0.15	0.11	0.04	ns	ns	ns	0.51a	0.35ab	0.28b	0.34	0.41	0.03	**	ns	ns
C20:4 n-6	2.72b	2.86b	4.8a	3.60	3.42	0.26	***	ns	ns	5.79ab	4.17b	8.28a	5.34	6.80	0.52	**	ns	ns
C20:5 n-3	0.25	0.00	0.31	0.03	0.33	0.09	ns	ns	ns	0.07b	0.08b	0.36a	0.16	0.19	0.03	***	ns	ns
C22:2 n-6	0.56	0.00	0.14	0.05	0.38	0.12	ns	ns	ns	0.36	0.31	0.00	0.05	0.37	0.13	ns	ns	ns
C22:4 n-6	0.06	0.00	0.20	0.07	0.11	0.04	ns	ns	ns	1.92	0.80	1.20	1.29	1.28	0.20	ns	ns	ns
SFA	39.05	40.81	40.09	40.01	40.02	0.59	ns	ns	ns	37.32ab	40.58a	34.06b	38.03	36.65	0.90	**	ns	ns
MUFA	42.65a	42.70a	34.78b	39.34	40.52	0.94	***	ns	ns	29.99ab	36.19a	27.94b	33.81	29.20	1.41	*	ns	ns
PUFA	16.60b	16.49b	25.10a	19.58	19.42	1.17	***	ns	ns	32.69ab	23.23b	38.02a	28.17	34.15	2.01	**	ns	ns

Continue next page

Table 6.- Evolution of fatty acid composition of free fatty acids of m. *L. dorsi* and *B. femoris* during 10 days of refrigerated storage.

	day 0									day 10								
	Genotype			Sex			Probabilities			Genotype			Sex			Probabilities		
	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	inter
Cont.																		
Biceps femoris																		
C12:0	0.29b	0.38a	0.40a	0.38	0.35	0.02	**	ns	ns	0.24	0.24	0.19	0.20	0.25	0.02	ns	ns	ns
C14:0	0.78	1.20	1.47	1.21	1.12	0.15	ns	ns	ns	0.57a	0.56a	0.38b	0.51	0.49	0.03	***	ns	ns
C16:0	23.58a	23.68a	22.33b	23.15	23.21	0.21	**	ns	ns	21.77	21.67	21.20	21.59	21.50	0.16	ns	ns	ns
C16:1 n-7	1.97ab	2.21a	1.87b	2.01	2.02	0.05	*	ns	ns	2.05a	1.99a	1.51b	1.84	1.85	0.07	***	ns	ns
C17:0	0.43	0.39	0.42	0.38	0.44	0.02	ns	ns	ns	0.49a	0.42b	0.40b	0.44	0.43	0.01	***	ns	ns
C17:1 n-7	0.28b	0.31ab	0.34a	0.32	0.30	0.01	*	ns	ns	0.23a	0.20b	0.21ab	0.22	0.21	0.01	*	ns	ns
C18:0	15.65a	15.70a	13.36b	14.59	15.15	0.33	**	ns	ns	14.31a	14.84a	11.79b	13.14	14.07	0.39	***	ns	ns
C18:1 n-9	29.24ab	31.66a	26.18b	28.41	29.59	0.64	***	ns	ns	30.14a	30.61a	24.83b	27.89	29.02	0.67	***	ns	ns
continued																		
C18:2 n-6	19.61b	18.81b	24.25a	22.01	19.93	0.68	***	ns	ns	21.45b	21.35b	27.58a	24.18	22.91	0.75	***	ns	ns
C18:3 n-6	0.73	0.71	0.70	0.70	0.73	0.04	ns	ns	ns	0.62	0.66	0.65	0.66	0.63	0.01	ns	ns	ns
C20:0	0.70a	0.21b	0.19b	0.21	0.49	0.08	**	*	*	0.16ab	0.20a	0.12b	0.16	0.16	0.01	***	ns	ns
C20:1n-9	0.80a	0.48b	0.33b	0.43	0.62	0.07	**	ns	*	0.68a	0.62a	0.38b	0.53	0.58	0.04	***	ns	ns
C20:2 n-6	0.31	0.26	0.23	0.24	0.29	0.02	ns	ns	ns	0.35	0.35	0.29	0.33	0.33	0.01	ns	ns	ns
C20:4 n-6	4.47b	3.57b	7.06a	5.12	4.98	0.36	***	ns	ns	5.91b	5.48b	9.19a	7.25	6.55	0.41	***	ns	ns
C20:5 n-3	0.16b	0.18b	0.42a	0.25	0.26	0.03	***	ns	ns	0.31	0.26	0.35	0.30	0.31	0.02	ns	ns	ns
C22:2 n-6	0.49	0.10	0.00	0.11	0.27	0.09	ns	ns	ns	nd	nd	nd	nd	nd				
C22:4 n-6	0.41	0.14	0.47	0.43	0.25	0.08	ns	ns	ns	0.71b	0.56b	0.94a	0.77	0.71	0.04	***	ns	ns
SFA	41.44a	41.56a	38.17b	39.92	40.75	0.54	**	ns	ns	37.54a	37.93a	34.07b	36.03	36.90	0.50	***	ns	ns
MUFA	32.30a	34.66a	28.73b	31.17	32.54	0.71	***	ns	ns	33.10a	33.42a	26.93b	30.47	31.65	0.75	***	ns	ns
PUFA	26.18b	23.78b	33.12a	28.85	26.71	1.05	***	ns	ns	29.36b	28.66b	39.00a	33.50	31.45	1.15	***	ns	ns

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
LD: *Longissimus dorsi*. BF: *Biceps femoris*. SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
sem: standard error mean. gen: genotype; inter: interaction genotype x sex
ns: not significant, * p<0.05, ** p <0.01, ***p<0.001. a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's test, p<0.05).

PUFA proportions in FFA increased at day 10 of storage; especially long chain PUFA, agreeing with previous studies (Alasnier ***et al***., 2000; Morcuende ***et al***., 2003). Muscle lipolytic enzymes are responsible for the hydrolysis of the lipids, releasing FFA during meat storage. Some authors (Rosell and Toldrá, 1998) have reported higher phospholipase activity in selected than in no selected genotypes. The highest increase of PUFA free fatty acids was found in GEN3, being most of them long chain PUFA. Differences in the fatty acid composition of FFA fraction could result in a different concentration of substrates for the oxidative reactions, which could decrease meat stability during storage. In a previous study (Ramírez and Cava, submitted), we found lower oxidative and colour stability in meat from GEN3, in comparison with GEN1 and GEN2.

Lipolysis was the cause of the changes in the fatty acid profiles of PL and NL and the release of FFA. The lack of differences in SFA, MUFA and PUFA in PL of refrigerated muscles suggests that lipolysis was similar in the 3 genotypes. However, the increase of the long chain PUFA in FFA and the concomitant decrease in PL suggest a greater hydrolysis of some fatty acids of PL fraction, which could not modify the sum of PUFA after the storage. It is also possible that NL fraction had contributed importantly to the increase of PUFA in FFA fraction after storage, as PUFA in NL were significantly higher in GEN3 than in the other genotypes and they were reduced after storage. Therefore, small reductions of the relative proportion of some PUFA in this fraction could produce consequently important increases in the FFA.

CONCLUSIONS

The evolution of the fatty acid composition during the storage of the meat was affected by the pig genotype. Reciprocal cross did not show important differences in the changes of fatty acids after refrigerated storage of LD and BF. However, the Duroc sire line significantly affected the fatty acid profile of IMF and lipid fractions since important differences were found between GEN2 and GEN3. The use of Duroc selected genotypes (DU2) in the crossbreeding with Iberian pigs could reduce the shelf life of meat during refrigerated storage, due to this meat showed some characteristics before storage, which could make meat more prone to suffer oxidation processes, such as (1) the highest level of PUFA in IMF and lipid fractions and (2) the lowest rate of plasmalogens in PL fraction. On the other hand, GEN3 showed some signs of deterioration of the meat during the storage, such as (3) the large increase of long chain PUFA in FFA and (4) the highest increase in the ratio DMA/FA after the refrigerated storage. These factors could make the meat from GEN3 especially susceptible to suffer oxidation processes during the storage, which could favour the appearance of rancid flavours after cooking or during the manufacture process of dry cured meat products.

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Chapter V

EFFECT OF IBERIAN X DUROC GENOTYPE ON DRY-CURED LOIN QUALITY[1]

ABSTRACT

Dry-cured loins from 3 different pig genotypes were studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The genotype Duroc1 were pigs selected for the manufacture of dry-cured meat products, while the genotype Duroc2 were pigs selected for meat production. Significant differences were found in chemical composition of dry-cured loins specially the intramuscular fat (IMF) content ($p<0.05$) which was highest in GEN2. Dry-cured loins from GEN3 showed less intense colour, with lower CIE a* and chroma values. IMF from GEN2 was more saturated whereas GEN3 had higher percentages of PUFA and MUFA. GEN1 had lower levels of SFA and higher PUFA than GEN2, although it had intermediate contents of MUFA and C18:1 n-9. Loins from GEN3, showed a more intense lipid oxidation with higher TBA-RS values ($p>0.05$) and hexanal content ($p<0.05$). Loins from GEN3 had higher Warner-Bratzler shear force ($p>0.05$) and instrumental hardness ($p<0.05$) than those from GEN2, being intermediate in GEN1. Panellists considered that loins from GEN2 were more marbled ($p<0.05$), more odorous ($p<0.05$), juicier ($p<0.05$) and sweeter ($p<0.05$) and less salty ($p<0.05$) than loins from GEN3. Dry-cured loins from GEN1 had scores intermediate between the other 2 genotypes.

Keywords: dry-cured loin, Iberian, reciprocal cross, Duroc line, crossbreeding.

1.- INTRODUCTION

Iberian dry-cured loin is one of the Iberian meat products most appreciated by consumers. The demand of Iberian dry-cured products has grown in the last years. For that reason, Iberian pig census has increased in one million of pigs between 1998 and 2004 (MAPA, 2004). Iberian pig production has advanced in the last years, since new feeding diets and crosses have been developed. In this respect, Iberian breed has been frequently crossed with Duroc pigs. The Duroc breed was introduced in Europe mainly due to its higher intramuscular fat content compared to other breeds (Barton-Gade, 1987) and because the cross of industrial genotypes with the Duroc breed results in higher intramuscular lipid content (Oliver *et al*., 1994), which is considered beneficial for meat products sensory quality. However, in the Iberian breed, which is a rustic breed with an elevated adipogenic potential, the introduction of the Duroc breed has specifically improved productive parameters, such as more piglets per sow and a higher weight at weaning and at the end of fattening (Aparicio, 1987), keeping the high adaptability to the environment characteristic of the Iberian pig and without a significant decrease in the quality of cured meat products (López-Bote, 1998).

Duroc is a widespread breed object of different genetic selections which has lead to important differences between lines. Some of them aimed to the meat production, which tends to animals with more lean meat, less fat content and higher growth rates. Lonergan, Huff-Lonergan, Rowe, Kuhlers, & Jungst (2001) reported that the intensive selection carried out in some Duroc lines to improve lean growth efficiency had negative consequences on meat quality. Besides, Cilla *et al*., (2006) found important differences in the quality of dry-cured hams from pigs finished with different lines of Duroc. On the other hand, since 2001 the legislation (B.O.E. 15th October, 2001) only allows Iberian x Duroc crosses, when the maternal line is pure Iberian to preserve the purity of the Iberian breed.

[1] Ramírez, M.R. & Cava, R. (2006). Effect of Iberian x Duroc genotype on dry-cured loin quality. Accepted for publication in Meat Science.

Many parameters have been assessed to characterize dry-cured meat products. Colour is the most outstanding characteristic of appearance (Gandemer, 2002). It could influence consumers' choice, especially when the products are presented in the supermarket sliced and packaged. Another aspect with great importance during the processing is the lipid oxidation, which is influenced by several factors such as intramuscular fat content and fatty acid profile (Cava, Ruiz, Ventanas, & Antequera, 1999). On the other hand, texture characteristics constitute one of the main attributes perceived by consumers. IMF content also has a positive influence on some texture and appearance traits of dry-cured ham (Ruiz-Carrascal, Ventanas, Cava, Andrés & García, 2000). All these parameters are influenced by the characteristics of the raw meat, which was significantly affected by the Iberian x Duroc pig genotype (Ramírez & Cava, 2006).

The objectives of this paper are to evaluate in 3 Iberian x Duroc pig genotypes the consequences derived of the use of different sire lines of Duroc as well as the differences between reciprocal crosses in the quality of the dry-cured loins.

2.- MATERIALS AND METHODS

2.1.- Animals.

3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) were pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening. The genotype Duroc2 (DU2) were animals selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were castrated, as is traditionally done and they were raised together in a semi-intensive system and were fed *ad libitum* with commercial diet. Pigs were randomly slaughtered after 316 days of rearing at 150-165 Kg live weight.

2.2.- Dry-curing process.

The right *Longissimus dorsi* (LD) muscle of each carcass (10loins/genotype) was removed and processed to obtain dry-cured loins. Loins were seasoned by rubbing a mixture of salt, nitrite, olive oil and spices such as Spanish paprika (*Capsicum annuum, L.),* oregano (*Origanum vulgare L*.) and garlic (*Altium sativum, L*.). Loins were kept at 4°C for 4 days to allow the seasoning mixture to penetrate. Then, loins were stuffed into collagen casings and held at 4°C for 30 days at ~80% relative humidity. Finally, loins were ripened at ~12°C and at ~70% relative humidity for 90 days. Loins were processed for a total dry-curing time of 4 months.

2.3.- Chemical composition.

Lipids were extracted from 5g of loin with chloroform/methanol (1:2), according to Bligh and Dyer (1959). Protein content was determined by the Kjieldalh method (AOAC, 2000) and moisture was determined by drying the samples (~5 g) at 102ºC (AOAC, 2000).

2.4.- Weight loss during refrigerated storage.

To perform the assay, dry-cured loin chops were cut into 1 x 1 x 1 cm cubes. The dry-cured loin cubes were supported with a needle on Styrofoam plates to allow a similar drying on each face of the cube. Dry-cured loin cubes were refrigerated at 4ºC for 10 days. Sample cubes were weighed at day

5 and day 10 of storage. The percentage of loss was calculated by difference in weight as follows: ((Wb-Wa)/Wb)x100; Wb: weight before storage, Wa: weight after storage.

2.5.- Instrumental colour.

Colour measurements were made following the recommendations on colour determination of the American Meat Science Association (Hunt ***et al***., 1991). The following colour coordinates were determined: lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue). Colour parameters were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a white tile. In addition, hue angle, which describes the hue or colour was calculated (H^o=atan b*/ a* · 360/2π) as well as the saturation index or chroma (C*) (C= $(a^{*2}+b^{*2})^{0.5}$), which describes the brightness or vividness of colour. The measurements were repeated at 5 randomly selected places on each slice and averaged.

2.6.- Fatty acid profile determination.

Fatty acid methyl esters (FAMEs) were prepared by esterification using methanol in the presence of sulphuric acid (5% of sulphuric acid in methanol). FAMEs were analysed in a Hewlett-Packard model HP-5890A gas chromatograph, equipped with a flame ionization detector (FID). They were separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0mm film thickness). The injector and detector temperatures were held at 230°C and the oven temperature, at 220°C. The flow rate of the carrier gas (N_2) was set at 1.8mL/min. Identification of FAMEs was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percentage of major FAMEs.

2.7.- Lipid oxidation.

Lipid oxidation was assessed in duplicate by the 2-thiobarbituric acid (TBA) method of Salih, Smith, Price, & Dawson (1987) using 2 g of loin. TBA-RS values were calculated from a standard curve of malondialdehyde and expressed as mg malondialdehyde kg^{-1} meat (mg MDA/Kg).

Hexanal content was assessed with a SPME fibre (Supelco Co. Canada) coated with divinilbenzene-carboxen-poly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm. The sampling technique to extract volatile compounds from headspace was the following: 0.5g of sample were minced and placed in a 5mL vial with a silicone stopper which were previously deodorized by heating at 80ºC for 2 hours. Each sample was analysed in duplicate. Headspace volatile compounds were extracted at 37°C for 30 min.

Analyses were performed in a gas chromatograph (Hewlett-Packard HP5890GC series II gas chromatograph) coupled to a mass selective detector (Agilent 5973N MSD, mod. G2577A). Volatiles were separated in a 5% phenyl-95% dimethyl polysiloxane column (30m x 0.25mm i.d., 1.0mm film thickness; Restek). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6mL/ min at 40ºC. Prior to analysis, the SPME fiber was preconditioned at 270ºC for 50min in the gas chromatograph injection port. The injection port was in the splitless mode and the temperature program was isothermal at 40ºC for 10 min and then raised at the rate of 7ºC seg^{-1} to 250ºC and held for 5min. The GC-MS transfer line temperature was 270ºC. The MS operated in the electron impact mode with electron impact energy of 70 eV; a multiplier voltage of 1650V and a collected data at a rate of 1scan

s^{-1} over a range of m/z 40-300. n-Alcanes (Sigma R-8769) were analysed under the same conditions to calculate the Retention Indices (RI) values for the volatiles. Hexanal was identified by comparison with reference compounds (Sigma, Aldrich), by comparison of RI with those described by Kondjoyan and Berdagué (1996) and by comparison of their mass spectra with those contained in Wiley library.

2.8.- Texture analysis.

Texture analysis was performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, U.K.). For the determination of texture profile analysis (TPA) uniform portions of the loin were cut into $1cm^3$ cubes. Samples were axially compressed to 50% of the original height with a flat plunger 50 mm in diameter (P/50) at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from force–deformation curves (Bourne, 1978): Hardness (N/cm^2) = maximum force required to compress the sample (peak force during the 1^{st} compression cycle); adhesiveness (N s) = work necessary to pull the compressing plunger away from the sample; springiness (cm) = height that the sample recovers during the time that elapses between the end of the 1^{st} compression and the start of the 2^{nd}; cohesiveness (dimensionless) = extent to which the sample could be deformed before rupture (A1/A2, A1 being the total energy required to for the 1^{st} compression and A2 the total energy required for the 2^{nd} compression); chewiness (N s) = the work needed to chew a solid sample to a steady state of swallowing (hardness x cohesiveness x springiness). For Warner-Bratzler (W-B) analyses, samples were prepared in 2 x 30 x 15mm slices (thickness x length x width) with a slicing machine. Samples were cut with a Warner-Bratzler blade (HDP/BS) in perpendicular direction to the muscle fibres. Determinations were repeated 8 times per sample and were averaged.

2.9.- Sensory analysis.

Fourteen trained panellists formed the tasting panel. A profile of 11 sensory attributes of dry-cured loin grouped in appearance, aroma, texture, flavour and taste were analysed (Table 1).

Table 1. Sensory attributes evaluated by the tasting panel in the sensory analyses of dry-cured loin.

Appearance	
lean colour	Intensity of red colour in the lean (light pink-dark brown)
marbling	Level of visible intramuscular fat (very lean-intense marbled)
Aroma	
odour	Intensity of odour before eating (odourless - very intense odour)
Texture	
hardness	Firmness perception during chewing (very tender- very firm)
fibrousness	Perception of fibres during chewing (not fibrous-very fibrous)
juiciness	Impression of juiciness during chewing (not juicy - very juicy)
Flavour	
flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
sweet taste	Intensity of sweet taste (not sweet - very sweet)
salty taste	Intensity of salt taste (not salty - very salty)
acid taste	Intensity of acid taste (not acid- very acid)
bitter taste	Intensity of bitter taste (not bitter- very bitter)

Analyses were developed in tasting rooms with the conditions specified in UNE regulation. All sessions were conducted at room temperature in a sensory panel room equipped with white fluorescent lighting. The software used for record scores in sensory sessions was FIZZ Network (version 1.01: Biosystemes, France). The loins were cut into 2mm thick slices, perpendicularly to

muscle fibre direction, with a slicing machine. Slices were served on plates to panellists. The panel sessions were held mid-morning, about 3 h after breakfast. Panellists evaluated different parameters by means of a quantitative-descriptive analysis in a non structured scale 0-10. Three samples randomly presented to the panellist were analysed in each tasting session. About 100 ml of water at room temperature was provided to the panellists. In each session the panel average for each sample was recorded.

2.10.- Statistical analysis.

The effects of genotype and sex were analysed by the Analysis of Variance (ANOVA) procedure of SPSS, version 12.0 (SPSS, 2003). A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. Means were used to compare differences. HSD Tukey's test was applied to compare the mean values of the genotypes. Mean values and standard errors of the means (SEM) are reported. The relationships between traits were analysed by the calculation of Pearson's correlation coefficient. A principal component analysis (PCA) was performed to determine relationships between variables and samples.

3.- RESULTS

Proximate composition of dry-cured loins was significantly affected by genotype (Table 2). Moisture content was significantly lower ($p<0.05$) in GEN1 and GEN2 than in GEN3. Protein content was significantly higher ($p<0.05$) in dry-cured loins from GEN1 than in GEN2, and GEN3 had an intermediate content. Concerning IMF, dry-cured loins from the three genotypes showed high contents of IMF which ranged from 8,5 to 12.4g/100g. Dry-cured loins from GEN2 contained significantly higher content of IMF than those form GEN1 and GEN3.

Table 2.- Chemical composition of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Moisture (g/100g)	33.97b	36.14b	38.66a	37.12	35.72	0.69	0.001	0.197	0.126
IMF (g/100g)	9.75b	12.37a	8.49b	10.38	10.07	0.48	0.001	0.722	0.102
Protein (g/100g)	50.84a	46.01b	48.00ab	47.00	49.30	0.73	0.014	0.119	0.228

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB

a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$).

Loins were stored in order to assess weight loss during 10 days (Table 3). Weight loss was higher in the first 5 days of storage (Δday0-5) accounting ~20% of the initial weight of the samples, while during the rest of the trial (Δday5-10) weight loss was lower, around 7% of the initial weight. Weight loss was affected by sex, being significantly higher in males than in females in the first 5 days of storage (Δday0-5) and total weight loss (Δday0-10).

No differences between genotypes were found in the weight loss in the period day0-5, while in the second period of storage loins from GEN2 and GEN3 showed significantly higher loss than GEN1. At the end of the storage, cumulative weight loss (Δday0-10) in GEN3 was significantly higher than in GEN1, being intermediate in GEN2.

Table 3.- Weight loss after refrigerated storage of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Δ_{day0-5}	20.1	19.4	21.7	21.4	19.4	0.47	0.089	0.023	0.308
$\Delta_{day5-10}$	5.8b	7.9a	7.7a	7.4	7.0	0.22	0.001	0.355	0.501
$\Delta_{day0-10}$	24.6b	25.8ab	27.8a	27.1	25.0	0.51	0.036	0.025	0.240

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05).

Genotype significantly affected the instrumental colour of dry-cured loins (Table 4). Lightness (CIE L*-value) was significantly higher in GEN2 than in GEN1 and GEN3. Moreover, loins from GEN1 and GEN2 were redder (a*) than those from GEN3. Similarly, CIE b*-value and chroma were significantly highest in GEN2 than in GEN3, while GEN1 showed intermediate values. Nevertheless, no differences between genotypes were found in hue.

Table 4.- Instrumental colour of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
CIE L*-value	36.0b	40.5a	36.8b	37.8	37.9	0.55	0.001	0.807	0.695
CIE a*-value	13.0a	13.7a	11.5b	12.6	12.9	0.30	0.002	0.626	0.148
CIE b*-value	5.7ab	6.3a	5.1b	5.6	5.9	0.18	0.013	0.362	0.573
Chroma	14.2ab	15.1a	12.6b	13.8	14.1	0.34	0.002	0.542	0.208
Hue	23.4	24.8	23.9	23.8	24.4	0.33	0.257	0.380	0.560

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05)

The genotype significantly affected fatty acid composition of IMF of dry-cured loins (Table 5). Compared to IMF from GEN1 and GEN3, IMF from GEN2 had a larger proportion of saturated fatty acids (SFA), such as C12:0, C14:0, C16:0 and C18:0. In contrast, dry-cured loins from GEN3 had a higher proportion of polyunsaturated fatty acids (PUFA), such as C18:2 n-6 and C20:4 n-6, and monounsaturated fatty acids (MUFA) such as C18:1 n-9 than that from GEN1 and GEN2. IMF from GEN1 showed lower percentages of SFA and higher of PUFA than GEN2, and intermediate contents of MUFA and C18:1 n-9.

The extent of lipid oxidation of dry-cured loins was measured as TBA-RS numbers and hexanal content (Table 6). TBA-RS numbers did not show significant differences between genotypes, however, the loins from GEN3 tended to be more oxidized (p>0.05). Hexanal content in dry-cured loin showed a similar trend to TBA-RS. Hexanal content in the headspace of samples from GEN3 was significantly higher (p<0.05) than in those from GEN1 and GEN2.

Texture profile analysis and Warner-Bratzler shear force (WBSF) of dry-cured loins from the 3 genotypes studied are shown in Table 7. Texture profile analysis (TPA) reflected significant differences due to genotype in some instrumental texture parameters. Genotype significantly affected (p<0.05) hardness, springiness and chewiness of dry-cured loins while cohesiveness and adhesiveness did not show differences between genotypes. Thus GEN2 had a significant lower hardness, springiness and chewiness values (p<0.05) than those from GEN3, while samples from GEN1 had intermediate values between GEN2 and GEN3.

Table 5.- Fatty acid composition (g/100g FA) intramuscular fat of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
C12:0	0.04b	0.04a	0.03b	0.04	0.03	0.00	0.001	0.010	0.035
C14:0	1.08b	1.25a	1.08b	1.19	1.09	0.02	0.001	0.008	0.060
C16:0	23.27b	24.59a	23.10b	23.77	23.57	0.16	0.001	0.440	0.152
C16:1 n-7	3.47	3.63	3.80	3.74	3.55	0.07	0.159	0.171	0.785
C17:0	0.18	0.15	0.17	0.16	0.17	0.00	0.049	0.987	0.625
C17:1 n-7	0.20	0.19	0.22	0.21	0.20	0.01	0.035	0.653	0.827
C18:0	13.26ab	14.04a	12.40b	13.01	13.44	0.19	0.001	0.152	0.516
C18:1 n-9	48.81ab	47.65b	49.20a	48.43	48.65	0.20	0.002	0.544	0.822
C18:2 n-6	6.37a	5.58b	6.63a	6.24	6.14	0.14	0.001	0.550	0.115
C18:3 n-3	0.21	0.22	0.26	0.24	0.22	0.01	0.055	0.119	0.237
C20:0	0.09	0.23	0.17	0.20	0.12	0.03	0.232	0.278	0.476
C20:1 n-9	1.06	0.89	1.00	0.97	0.99	0.04	0.196	0.848	0.459
C20:2 n-6	0.32a	0.27b	0.29ab	0.30	0.28	0.01	0.001	0.083	0.056
C20:4 n-6	1.15a	0.80b	1.13a	0.99	1.05	0.05	0.003	0.581	0.655
C22:2 n-6	0.09	0.10	0.10	0.10	0.10	0.00	0.205	0.812	0.599
C22:4 n-6	0.20	0.22	0.25	0.22	0.23	0.01	0.376	0.677	0.120
C22:5 n-3	0.21a	0.15b	0.18ab	0.19	0.17	0.01	0.047	0.273	0.962
SFA	37.90b	40.30a	36.94b	38.38	38.42	0.35	0.001	0.820	0.122
MUFA	53.55ab	52.35b	54.22a	53.34	53.40	0.23	0.002	0.930	0.745
PUFA	8.54a	7.34b	8.84a	8.28	8.19	0.20	0.001	0.673	0.150

GEN: genotype. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB.
SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
a,b: Different letters in the same row indicate significant statistical differences within the same day of analysis (Tukey's Test, $p<0.05$).

No significant differences were found in WBSF test between dry-cured loins from the genotypes studied. However, a higher force to cut loin slices was needed in samples from GEN3 and GEN1 than in those from GEN2 as reflected the higher values ($p>0.05$) from the WBSF test. The higher values of hardness in TPA and WBSF indicated a higher instrumental hardness of dry-cured loins from these two genotypes.

Table 6.- TBA-RS (mg MDA/Kg) and hexanal content (area units-AU- $x10^6$) of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
TBA-RS	0.27	0.29	0.32	0.30	0.29	0.01	0.201	0.607	0.579
Hexanal	201.0b	307.9b	651.2a	384.8	400.8	61.3	0.006	0.767	0.863

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, $p<0.05$).

Important differences were found by panellist in dry-cured loins according to the pig genotype (Figure 1). Regarding the visual appearance of the loins, no differences were found in the lean colour, in spite of the differences found in the instrumental colour analyses. However, panellists considered that loins from GEN2 were more marbled that those from GEN3, while GEN1 tended to present intermediate values between GEN2 and GEN3.

No differences were found in some sensory texture parameters such as hardness (p>0.05) and fibrousness (p>0.05) of the loins, contrasting with results from instrumental texture analysis, although both traits followed a similar trend described for hardness and WBSF with higher scores in loins from GEN1 and GEN3 than in GEN2. In contrast, panellists found that loins from GEN2 were juicier than those from GEN3. Similar results were found in odour intensity trait, loins from GEN2 were more odorous than loins from GEN1 and GEN3. Despite of the differences found in odour, no differences were found in flavour intensity. Concerning taste descriptors, loins showed a similar intensity of acid and bitter tastes between genotypes, however, loins from GEN1 and GEN2 were sweeter than those from GEN3, and contrarily, loins from GEN3 were saltier than loins from GEN2.

Table 7.- Texture analyses of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			P-value		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
WBSF (N)	8.28	5.19	7.19	6.25	7.55	0.61	0.125	0.347	0.723
Texture profile analysis									
Hardness (N)	211.83ab	189.49b	219.91a	201.79	211.70	5.21	0.049	0.329	0.879
Adhesiveness (N*s)	-0.87	-1.12	-0.92	-1.02	-1.08	0.06	0.238	0.802	0.952
Springiness (cm)	0.38b	0.38b	0.43a	0.43	0.47	0.01	0.011	0.455	0.143
Cohesiveness	0.39	0.37	0.40	0.45	0.45	0.01	0.207	0.921	0.364
Chewiness (N)	30.25ab	26.51b	37.56a	31.80	31.10	1.64	0.013	0.876	0.273

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05)

4.- DISCUSSION

Chemical composition of dry-cured loins was significantly affected by pig genotype. Loins from GEN2 had the highest IMF content, which is one of the determinant factors of the final quality of meat products, especially in salting and drying processes as well as for their sensory quality (Ruiz *et al*., 2002).

Weight loss has important economical consequences for industry, especially when the product is sliced, packaged and stored. Large weight loss in dry-cured hams has been associated with high lean meat content (Chizzolini *et al*., 1996) and low fatness (Candek-Potokar, Monin, & Zlender, 2002). The higher moisture content of loins from GEN3 could contribute to their higher weight loss, as showed the positive correlation between both parameters (r = +0.686, p<0.001). However, other factors could influence the water loss during storage, so Ramírez and Cava (2006) in a previous study with the fresh *L. dorsi* from the same animals found a higher drip loss and cook loss in muscles from GEN3 which coincides with the pattern showed in the dry-cured loin from this genotype.

Instrumental colour parameters of dry-cured loins showed a close relationship with chemical composition. Thus, the higher lightness (CIE L*-value) in dry-cured loins from GEN2 than in those from GEN1 and GEN3 could be related with their higher marbling in agreement with findings reported by Carrapiso and García (2005), who found positive correlations between L* and marbling in dry-cured Iberian ham. In the present study, positive correlations between CIE L*-value and IMF content (r = +0.711, p<0.001) and marbling scores (r = +0.624, p<0.001) were found. In other type of dry-cured product such as dry-cured ham, redness (CIE a*-value) has been associated with the myoglobin concentration, the salt content and the formation of nitrosomyoglobin (Pérez-Álvarez,

Sayas-Barberá, Fernández-López, Gago-Gago, Pagán-Moreno, Aranda-Catalá, 1998). Colour plays a determinant role in the appearance and overall acceptability of dry-cured meat products; because a defective colour is rejected by consumers due to its association with a shorten ripening (García-Rey., Quiles-Zafra & Luque de Castro, 2006). Therefore, loins from GEN3 showed a more inadequate colour which could decrease the consumer's acceptability of these loins.

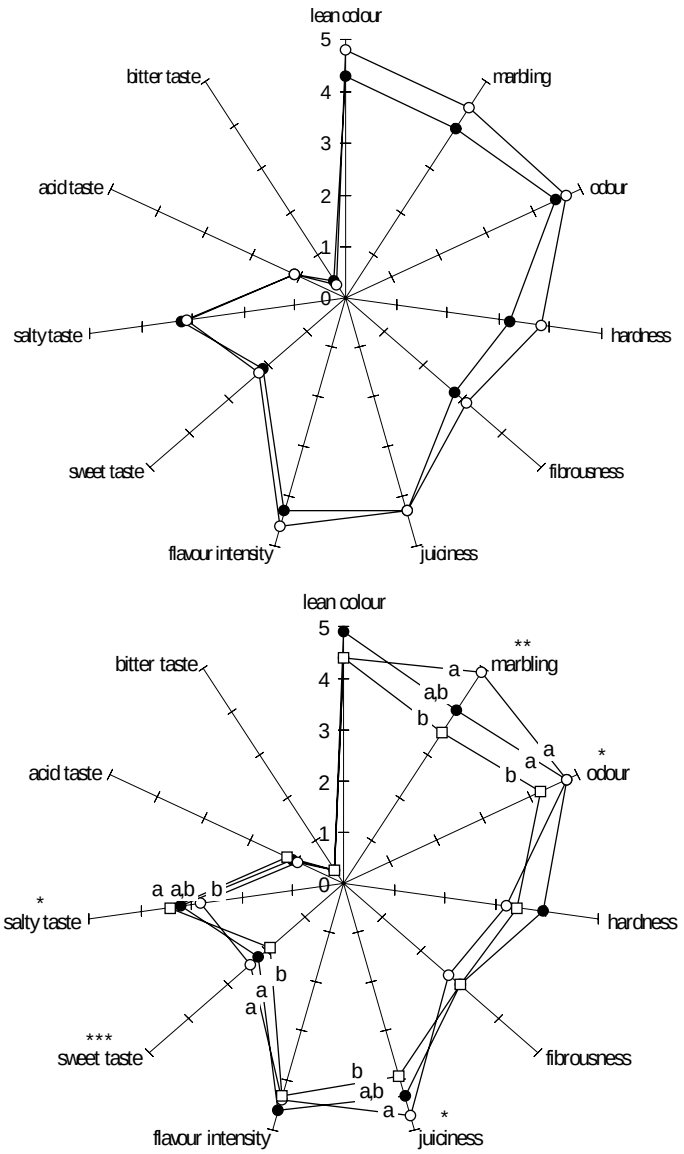

Figure 1.- Sensory analysis of dry-cured loins from three different Iberian x Duroc genotypes. Upper figure effect of animal sex (○: males, ●: females). Bottom figure: effect of genotype (●: GEN1, ○: GEN2, □: GEN3). *: p<0.05: **: p<0.01; ***: p<0.001; a,b,c: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05).

Fatty acid composition of IMF of dry-cured loins was affected by the IMF content as result of the different genotype. High IMF contents imply high contents of triacylglycerides and a decrease in the phospholipid content. Therefore, as higher is the IMF content; lower is their relative PUFA content and higher is the SFA content, as showed the correlations of IMF with SFA (r = +0.701, p<0.001) and PUFA (r = -0.871, p<0.001). On the other hand, the development of oxidation processes depends on

part of the content and composition of the IMF as high levels of PUFA increase the susceptibility of meat to oxidative processes and rancidity (Cava ***et al***., 1999). In this respect, loins from GEN3, which were more unsaturated, showed a more intense extent of lipid oxidation as reflected the higher TBA-RS and hexanal content of this genotype**.** Hexanal has been widely reported as a good indicator of lipid deterioration in raw, cooked and stored meat and in dry-cured meat products (Shahidi & Pegg, 1993; Morales & Aparicio, 1997; Cava ***et al***., 1999). In the later, hexanal contributes to their characteristic flavour, although high concentrations favour the appearance of rancidity and the flavour deterioration (Cava ***et al***., 1999). Thus, in a study in dry-cured Iberian hams, Cava ***et al***., (1999) found that in hams with less rancid flavour, the hexanal content and TBA-RS were lower.

Instrumental and sensory texture parameters followed the same pattern to that described for the previous parameters, as dry-cured loins from GEN3 were less juicy and had a higher instrumental hardness, springiness and chewiness. This is supported by the positive correlations between instrumental hardness and WBSF (r = +0.449, $p<0.05$), the same as sensory hardness and instrumental hardness (r = +0.373, $p<0.05$). This trend is in agreement with Szczesniak (2002) who reported similar results between instrumental and sensory rates for hardness. An excessive hardness is considered negative for the quality of dry-cured meat products (Guerrero, Gou, Arnau, 1999).

These trends could be caused by the different characteristics of the meat and particularly those related to the moisture and specially the intramuscular fat content of the muscle. So, in other dry-cured meat products such as dry-cured hams, some authors (Ruiz-Ramírez ***et al***., 2005, Serra, Ruiz-Ramírez, Arnau, & Gou, 2005) have related a lower moisture content with an increase in hardness, which coincides with the negative correlations found between moisture content and WBSF (r = -0.489, $p<0.01$) and with sensory hardness (r = -0.411, $p<0.05$). However, other authors (Ruiz-Carrascal ***et al***., 2000; Ruiz, García, Muriel, Andrés, & Ventanas, 2002) have reported a more relevant influence of the IMF content in textural characteristics of Iberian dry-cured hams, as increasing levels of IMF enhance the juiciness while reduce fibrousness perception which has a positive influence on the overall acceptability of dry-cured ham. We have found similar results, since IMF content significantly correlated with some instrumental texture parameters such as hardness (r = -0.541, $p<0.01$), WBSF (r = -0.380, $p<0.05$) and with chewiness (r = -0.431, $p<0.05$), as well as with sensory texture parameters such as fibrousness (r = -0.398, $p<0.05$) and juiciness perception (r = +0.706, $p<0.001$). Therefore, due to their different IMF content, loins from GEN2 might show the most adequate textural characteristics, followed by loins from GEN1 while the loins from GEN3 might have the worst textural quality.

Different studies on dry-cured products have related sensory and instrumental texture with animal genetic and with physico-chemical parameters influenced by the genetic such as i. the ultimate pH and ii. the intramuscular fat content (Guerrero, Gou, Alonso, & Arnau, 1996; Ruiz-Ramírez, Arnau, Serra & Gou, 2005; García-Rey ***et al***., 2006). In this sense, García-Rey ***et al***. (2006) reported the influence of low *postmortem* pH and genotype in the appearance of defective texture in dry-cured ham. Similarly, Ruiz-Ramírez ***et al***., (2005) found a close relationship between low *postmortem* pH and the increases in instrumental textural traits such as hardness, cohesiveness and springiness of

hams. The differences between genotypes previously reported in instrumental and sensory texture parameters concurs with the lowest *postmortem* pH values in the *Longissimus dorsi* muscle from GEN3, as previously reported Ramírez and Cava, (2006).

Panellist perceived important differences in the appearance of the loins. Loins from GEN2 were considered more marbled, which is in accordance with the highest IMF content of the loins from this genotype. A previous study (Ruiz-Carrascal *et al*., 2000) related marbling to the IMF content in Iberian ham, being in agreement with the results of the current paper (r = +0.837, $p<0.001$).

Sensory perceptions related to odour constitute one of the sensory attributes with a higher influence on the acceptability of dry-cured products from Iberian pigs (Ruiz *et al*., 2002). IMF content was also related to odour intensity, which is supported by the positive correlations found between odour and IMF content (r = +0.489, $p<0.01$). Numerous papers have studied the influence of the lipids on the formation of aroma compounds since lipid oxidation has great importance in the development of the dry-cured meat products flavour (reviewed by Gandemer, 2002). On the other hand and despite the differences found by panellists in the odour intensity, no differences were found in the flavour intensity between genotypes. This fact could be due to the use of a rubbing mixture of curing agents (salt and nitrite) and spices as ingredients which could have disguised the aroma compounds produced in a different extent.

Taste of meat and meat products is mainly due to sodium chloride, amino acids, peptides and nucleotides (MacLeod, 1986), so differences in the concentration of these compounds could be the cause of different taste perceptions. In this sense, loins from GEN1 and GEN2 were perceived sweeter than those from GEN3, while loins from GEN3 were scored saltier than loins from GEN2. Results are in accordance with Ruiz, García, Díaz, Cava, Tejeda, & Ventanas (1999), who reported an opposite relationship between sweetness and saltiness, as sweet notes were not easily perceived in salty hams. Salt is the main responsible of salty taste in low moisture content meat products, although others factors such as certain aminoacids could contribute to saltiness (Ruiz *et al*., 1999). Loins with lower IMF content (GEN3) were perceived saltier than loins with high IMF content, so positive correlations were found between IMF content and sweetness (r = +0.514, $p<0.01$) and negative with saltiness (r = -0.467, $p<0.05$).

Principal component analysis (PCA) resulted in four significant factors (principal components –PC-) that accounted for 72.45% of the variability. Principal components, PC1 and PC2, explained the 34.13% and 16.37% of the variation of the data, respectively. The loadings of this PCA (Fig. 2) can be divided in two groups. In the positive x-axis of PC1 are located far from the origin those variables related to the instrumental colour coordinates such as CIE a*-value, and b*-value and the fat component such as IMF and SFA contents which are close to the sensory traits marbling, juiciness, odour intensity and sweetness; while in the negative x-axis are placed PUFA percentage, hexanal content and saltiness and some sensory texture parameters such as hardness and fibrousness and instrumental texture traits such as WBSF and hardness and chewiness.

The distribution of the data on the two first PC variables is shown in Figure 3. The score-plots showed clusters of samples related to the different instrumental and sensory parameters analysed. Dry-cured loins from GEN2 were located in the positive *x*-axis of PC1; in the area corresponding to

high values of IMF, marbling, juiciness, odour intensity and sweetness, whereas dry-cured loins from GEN3 were in the negative *x*-axis of PC1 with high values of hexanal content, saltiness and sensory and instrumental hardness.

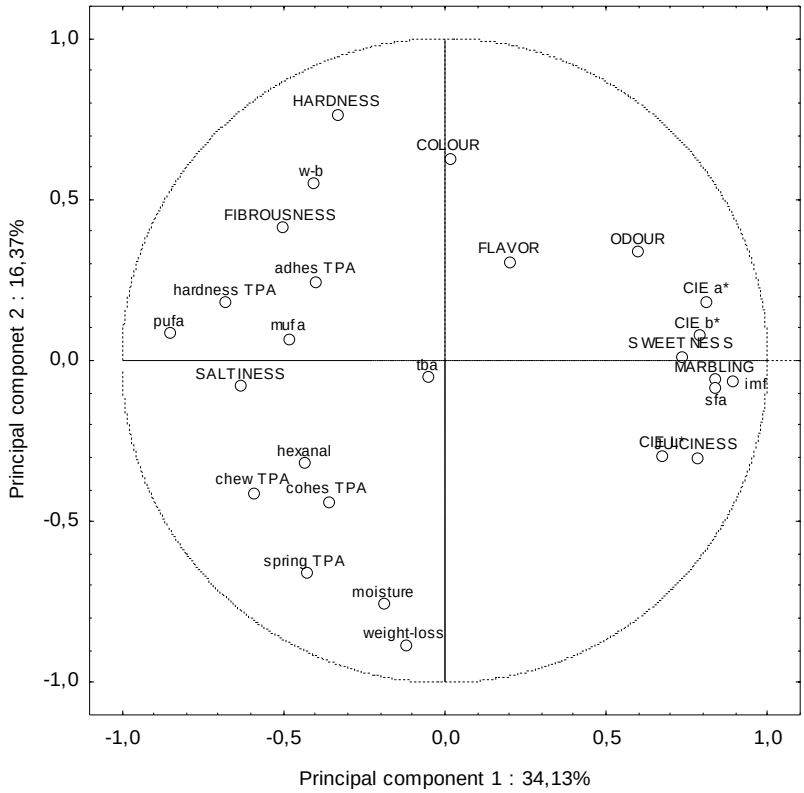

Figure 2.- Loadings plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2).

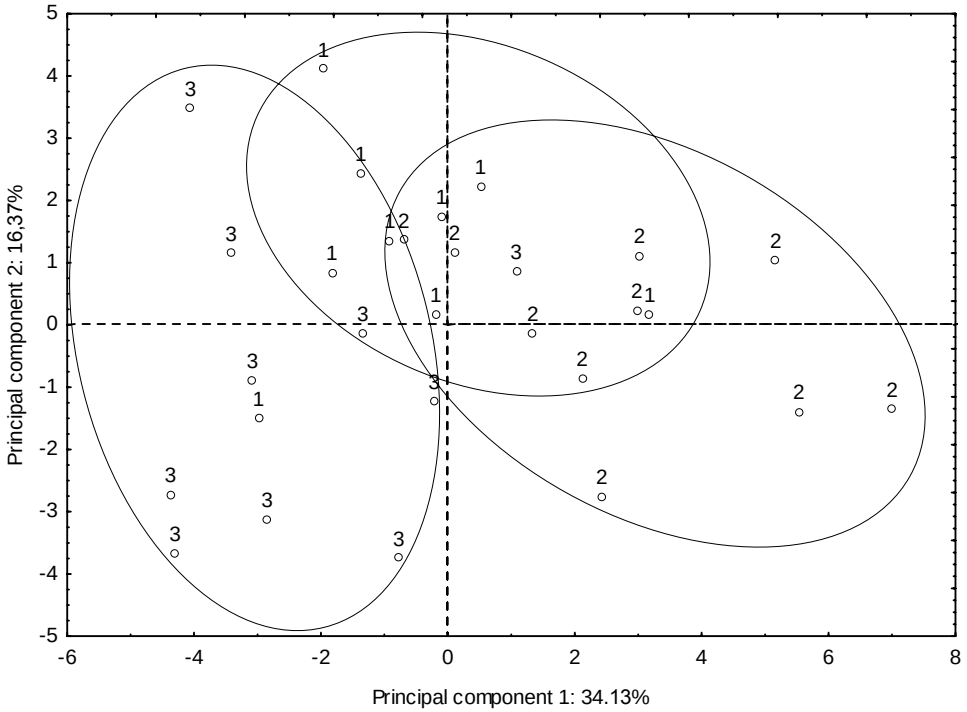

Figure 3.- Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1 and PC2). (1:GEN1; 2: GEN2; 3: GEN3)

5.- CONCLUSION

Iberian x Duroc reciprocal cross scarcely affected dry-cured loin quality, although loins from Iberian mother presented a better quality. However, the cross of selected Duroc genotypes with Iberian pigs significantly reduced the quality of dry-cured loins, as they had lower marbling and IMF content, less intensity of colour and odour, worse textural characteristics, higher PUFA content and more susceptibility to oxidation and rancidity as well as a saltier taste. Therefore, the characterization of the Duroc lines crossed with Iberian could be necessary to avoid damages in the quality of dry-cured loins.

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Chapter VI

COMPOSITION, INSTRUMENTAL AND SENSORY ANALYSIS OF DRY-CURED HAMS AS AFFECTED BY THE IBERIAN X DUROC GENOTYPE[1]

ABSTRACT

Dry-cured ham quality from 3 different Iberian x Duroc genotypes was studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The line Duroc1 (DU1) was selected for the manufacture of dry-cured meat products, whereas the line Duroc2 (DU2) was selected for meat production with low carcass fat. Dry-cured hams from all genotypes had similar chemical composition. However, intramuscular fat from dry-cured hams from GEN3 was more unsaturated than GEN2, while GEN1 was intermediate. Lipid oxidation degree, measured as TBARS number and hexanal content, was similar between genotypes, although it tended to be higher in GEN3. Colour of hams was affected by the genotype, hams from GEN2 showed higher lightness (CIE L*) while hams from GEN3 had a less intense colour (lower a* and C). Instrumental texture did not differ between genotypes, however, in the sensorial analysis panellists considered the hams from GEN3 more fibrous, while those from GEN2 were considered juicier. Hams from GEN3 also were perceived saltier and more acid. Therefore, hams from GEN3 had lower quality, and this genotype was considered less suitable for the manufacture of Iberian dry-cured hams.

Key words: dry-cured ham, Iberian, Duroc, crossbreeding, proximate composition, sensory.

1.- INTRODUCTION

Iberian dry-cured ham market has spectacularly increased in the last years, with around 2.700.000 Iberian hams produced in Spain in 2003[1]. Iberian dry-cured ham is a traditional product which has excellent sensory quality that makes it very appreciated by consumers. Sensory quality of dry-cured Iberian ham is mainly due to the high percentage of intramuscular fat rich in monounsaturated fatty acids (MUFA) that provide juiciness and contribute to its particular and intense flavour. An adequate intramuscular fat level is one of the factors that mainly contributes to the sensory quality of Iberian dry-cured ham[2,3]. Beside this, the degree of unsaturation of fatty acids (FA) influences lipolytic and oxidative processes that occur during the curing process. Lipolysis and oxidation can get undesirable high if the meat contains a high proportion of polyunsaturated fatty acids (PUFA)[4]. On the other hand, dry-cured ham quality is strongly affected by its textural and mechanical properties, which are mainly determined by the characteristics of the raw material and the genotype[5].

The market for dry-cured products from Iberian pig has been recently regulated[6]. One of the aspects that this law regulates is the pig genotype for the manufacture of Iberian meat products. It obliges to use pure Iberian pigs or crosses with Duroc breed when the maternal line is pure Iberian. Traditionally, in crossbreeding the Duroc line was used as maternal since it was generally believed that this was leading to some production improvements. However, the consequences of this change in the traditional production pattern on the quality of meat and meat products have not been sufficiently assessed. Moreover, although in the Iberian breed, the inclusion of Duroc breed increases production parameters[7] without a significant decrease of the quality of meat products[8,9], the Duroc breed can not be considered to be a homogeneous breed due to its widespread distribution. In this respect, different studies have shown important differences between different

[1] Ramírez, M.R. & Cava, R. (2006). Composition, instrumental and sensory analysis of dry-cured hams as affected by the Iberian x Duroc genotype. Submitted to Journal of the Science of Food and Agriculture for publication.

Duroc lines which could reduce Iberian ham quality[10,11]. In addition an important effect of the Duroc sire line on meat quality was found in a previous study[12].

Therefore, the objectives of this paper are to evaluate the consequences of the use of different lines of Duroc in crosses with Iberian breed as well as the differences between Iberian x Duroc reciprocal crosses on the quality of dry-cured hams.

2.- MATERIALS AND METHODS

2.1.- Animals.

3 groups of 10 pigs each (5 males and 5 females) from different genotypes were studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of carcass fat. The genotype Duroc2 (DU2) corresponded to animals selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were castrated, as traditionally, raised together in a semi-intensive system, and fed *ad libitum* with a commercial mixed diet. Pigs were slaughtered after 316 days of rearing at 150-165 Kg live weight.

2.2.- Dry-curing process.

One leg from each animal was processed (10 hams/genotype). The weight of the fresh ham was 14.0-15.5 Kg, with pH values<6.0. After salting, hams were kept at 0–3ºC and 80–90% relative humidity for ~6 months. Then, the hams were ripened for ~18 months in at 10–25ºC and 60–80% relative humidity. Hams were processed for a total dry-curing time of 24 months. After the ripening process the *Biceps femoris* muscles were removed from the hams. After instrumental colour and texture measurements and sensory analysis of the *Biceps femoris* muscle, they were vacuum-packaged and kept at −80 °C for further chemical analyses.

2.3.- Proximate composition.

Lipids were extracted from 5g of loins with chloroform/methanol (1:2), according to Bligh and Dyer[13]. Protein content was determined by the Kjieldahl method[14] and moisture was determined by drying the samples (~5 g) at 102ºC[14].

2.4.- Instrumental colour.

Colour measurements were made following the recommendations on colour determination of the American Meat Science Association[15]. The colour parameters lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue) were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardized before use with a white standard tile. In addition, the hue angle, describing the hue or colour was calculated (H^o=arctg b*/ a* · 360/2π) as well as the saturation index or chroma (C*) (C= $(a^{*2}+b^{*2})^{0.5}$), describing the brightness or vividness of colour. The measurements were repeated at 5 randomly selected places on each slice and averaged.

2.5.- Fatty acid profile determination.

Fatty acid methyl esters (FAMEs) were prepared by transesterification using methanol in the presence of sulphuric acid (5% of sulphuric acid in methanol). FAMEs were analysed in a Hewlett-Packard gas chromatograph (HP-5890A), equipped with a flame ionization detector (FID). They were

separated on a semicapillary column (Hewlett-Packard FFAP-TPA fused-silica column, 30m length, 0.53mm i.d., and 1.0mm film thickness). Injector and detector temperatures were held at 230°C and oven temperature, at 220°C. Flow rate of the carrier gas (N_2) was set at 1.8mL/min. Identification of FAMEs was based on retention times of reference compounds (Sigma). Fatty acid composition was expressed as percent of major FAMEs.

2.6.- Lipid oxidation.

Lipid oxidation was analyzed following a 2-thiobarbituric acid (TBA) method by Salih ***et al***.[16] using 2g of ham. TBA-RS values were calculated from a standard curve of malondialdehyde and expressed as mg malondialdehyde kg^{-1} meat (mg MDA/kg).

Hexanal content was assessed by headspace GCMS. For extraction, 0.5g of sample were minced and placed in a 5mL vial with a silicone stopper which were previously deodorized by heating at 80ºC for 2 hours. The volatile compounds from headspace were extracted at 37°C for 30 min with a SPME fiber (Supelco Co. Canada) coated with divinylbenzene-carboxen-poly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm. Analyses were performed with a gas chromatograph (Hewlett-Packard HP5890GC series II gas chromatograph) coupled to a mass selective detector (Agilent 5973N MSD, mod. G2577A). Prior to analysis, the SPME fiber was preconditioned at 270ºC for 50min in the gas chromatograph injection port. Volatiles were separated on a 5% phenyl-95% dimethyl polysiloxane GC-column (30m x 0.25mm i.d., 1.0mm film thickness; Restek) with helium as carrier gas at 18.5 psi, resulting in a flow of 1.6mL/ min at 40ºC. The injection port was in the splitless mode and the temperature program was isothermal at 40ºC for 10 min and then raised at the rate of 7ºC sec^{-1} to 250ºC and held for 5min. The GC-MS transfer line temperature was 270ºC. The MS operated in the electron impact mode with electron impact energy of 70 eV; a multiplier voltage of 1650V and a collected data at a rate of 1scan s^{-1} over a range of m/z 40-300. n-Alcanes (Sigma R-8769) were analysed under the same conditions to calculate the Retention Indices (RI) values for the volatiles. Hexanal was identified by comparison with reference compounds (Sigma, Aldrich), by comparison of RI with those described by Kondjoyan and Berdagué[17] and by comparison of their mass spectra with those contained in Wiley library.

2.7.- Texture analysis.

Texture analysis was performed in a texturometer Stable Microsystem TA XT-2i. For Warner-Bratzler shear force (WBSF) determinations, samples were prepared in slices of dimensions 2mm x 30mm x 15mm (thickness x length x width). In the analyses samples were cut with a Warner-Bratzler blade in perpendicular direction to the muscle fibres. For the development of the textural profile analyses (TPA) samples were cut in cubes of $1cm^3$ (1cm x 1cm x 1cm). In each analysis the cube was axially compressed twice until reach at 50% of the initial height. The following texture parameters were measured from force–deformation curves: hardness, adhesiveness, springiness, cohesiveness and chewiness. Hardness (N) was defined by the maximum peak force during first compression cycle. The adhesiveness (N*s) was the negative area under the curve obtained between following cycles. Cohesiveness was calculated as the ratio of the area under the second curve to the area under the first curve. Springiness was defined as ‘‘the height that the food recovers during the time that elapses between the end of the first bite and the start of the second bite’’[18] or the time recorded between the

start of the second area and the second probe reversal to the time recorded between the start of the first area and the first probe reversal. Chewiness (N) was calculated by multiplying hardness, cohesiveness and springiness. Determinations were repeated 8 times per sample and averaged to their statistical analysis.

2.8.- Sensory analysis.

Fourteen trained panellists formed the tasting panel. Analyses were developed in tasting rooms with the conditions specified in UNE regulation. FIZZ Network (version 1.01: Biosystemes, France) software was used for the sessions and the recording data obtained. M. *Biceps femoris* were dissected from the dry-cured hams and sliced (2mm thick) parallel to muscle fibre direction with a slicing machine. Three thin slices of each dry-cured ham were given to panellists who evaluated different parameters (Table 1) in a quantitative-descriptive analysis with a non structured scale 0-10. Three hams, which were randomly presented to the panellists were analysed in each tasting session. In addition, about 100 ml of mineral water at room temperature was provided to panellists. All sessions were conducted at ~20ºC in a 6-booth sensory panel room equipped with white fluorescent lighting. In each session, the panel average for each sample was recorded.

Table 1.- Descriptors of the sensory analysis of the dry-cured ham.

Descriptors	Definition
Appearance	
lean lightness	Luminosity of the lean (dark-light)
lean redness	Intensity of red colour in the lean (pink-red)
marbling	Level of visible intramuscular fat (very lean-intense marbled)
Aroma	
odour	Level of overall odour before eat the sample (odourless - very intense odour)
Texture	
hardness	Firmness perception during chewing (very tender- very firm)
fibrousness	Perception of fibres during chewing (not fibrous-very fibrous)
juiciness	Impression of juiciness during chewing (not juicy - very juicy)
Flavour	
flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
sweet taste	Intensity of sweet taste (not sweet - very sweet)
salty taste	Intensity of salt taste (not salty - very salty)
acid taste	Intensity of acid taste (not acid- very acid)
bitter taste	Intensity of bitter taste (not bitter- very bitter)

2.9. - Statistical analysis.

The effects of genotype and sex were analysed with the Analysis of Variance (ANOVA) procedure of SPSS, version 12.0[19]. A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. Means were used to compare differences. HSD Tukey's test was applied to compare the mean values of the genotypes. In addition Pearson's correlation coefficient for the correlation between treatments and a principal component analysis (PCA) to determine relationships between variables and samples was performed.

3.- RESULTS AND DISCUSSION

The chemical composition of dry-cured hams is shown in Table 2. No differences in moisture, intramuscular fat or protein contents were detected. The values obtained for the proximate composition are within the range considered as acceptable in *Biceps femoris* (BF) from dry-cured

Iberian ham[2]. No differences in composition were found between genotypes; agreeing with the results for raw m. *Biceps femoris*[12]. IMF content is one of the main factors for the quality of dry-cured meat products and their technological aptitude for drying and maturation processes, since an adequate level of fat is determinant for the regulation of salt/water diffusion and drying processes[20, 21].

Table 2.- Proximate composition (g/Kg) of dry-cured hams from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
Moisture	548.8	567.1	546.8	554.2	554.2	5.5	ns	ns	ns
IMF	70.7	78.2	70.7	73.5	73.5	5.5	ns	ns	ns
Protein	298.5	299.6	306.1	297.7	304.7	3.4	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: non significant (p>0.05).

Intramuscular FA profile of dry-cured hams was significantly affected by the genotype (Table 3). Compared to IMF from GEN3, GEN2 had a larger proportion of saturated fatty acids (SFA), such as palmitic and stearic acids. IMF from GEN3 had a higher content of unsaturated FA such as linoleic acid (C18:2 n-6) and oleic acid (C18:1 n-9) than that from GEN2. Intramuscular fat from GEN1 had intermediate levels of SFA, stearic and oleic acid between GEN2 and GEN3.

Table 3.- Fatty acid composition (g/Kg FA) of IMF of dry-cured hams from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
C12:0	0.2	0.2	0.2	0.2	0.2	0.0	ns	ns	ns
C14:0	7.4b	8.5a	7.8ab	8.2	7.7	0.2	*	ns	ns
C16:0	204.7	210	205.4	207.0	206.5	1.2	ns	ns	ns
C16:1 n-7	28.8	31.8	32.9	31.3	30.9	0.7	ns	ns	ns
C17:0	2.1	2	2	2.0	2.0	0.0	ns	ns	ns
C17:1 n-7	2.3	2.1	2.4	2.2	2.3	0.1	ns	ns	ns
C18:0	129.8ab	135.1a	123.9b	128.1	131.2	2.2	*	ns	ns
C18:1 n-9	514.0ab	499.8b	509.1a	505.3	509.5	2.2	*	ns	ns
C18:2 n-6	71.6b	70.2b	76.7a	74.5	71.2	1.3	*	ns	ns
C18:3 n-3	2.4	2.4	2.5	2.4	2.4	0.0	ns	ns	ns
C20:0	2	2.7	2	2.1	2.3	0.2	ns	ns	ns
C20:1 n-9	13.6a	12.7ab	11.8b	12.9	12.6	0.3	*	ns	ns
C20:2 n-6	2.8	4	4	3.8	3.4	0.3	ns	ns	ns
C20:4 n-6	12.9	12.8	14	14.0	12.5	0.4	ns	ns	ns
C22:2 n-6	1.4ab	1.6a	1.0b	1.3	1.3	0.1	*	ns	ns
C22:4 n-6	2.5	2.4	2.4	2.6	2.3	0.1	ns	ns	ns
C22:5 n-3	1.7	1.6	1.8	1.8	1.6	0.1	ns	*	ns
SFA	346.1ab	358.5a	341.4b	347.8	350.0	2.8	*	ns	ns
MUFA	558.6	546.5	556.1	551.8	555.2	2.4	ns	ns	ns
PUFA	95.3	95.1	102.4	100.5	94.8	1.9	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids.
ns: non significant (p>0.05), *: p<0.05. a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05).

Differences in the FA composition of IMF of dry-cured hams could be caused by i) differences between genotypes for FA profile of fresh muscles[12] and/or ii) changes in FA during ham processing. However it can be assumed, that processing affected all hams similarly, as differences found in fresh were maintained in dry-cured hams[12]. Both, a low proportion of PUFA and a high content of IMF, like in dry-cured hams from GEN1 and GEN2, influence sensory attributes of Iberian dry-cured ham positively, increasing marbling and juiciness and decreasing rancidity, resulting in a increased consumer preference [3,22].

TBA-RS numbers and hexanal content (Table 4) showed no differences in the extent of oxidative processes in BF of dry-cured hams from the 3 genotypes, although both parameters tended to be higher ($p>0.05$) in GEN3. This indicates that oxidative processes at the end of the ripening were similar in the 3 genotypes. However, the increased ($p>0.05$) TBA-RS and hexanal content in samples from GEN3 could indicate a higher susceptibility to oxidative stress during slicing, packaging and storage of the product, due to the higher contents of linoleic acid (C18:2 n-6) in IMF in these samples. Malondialdehyde and hexanal, which arise from the oxidation of n-6 FA, mainly from linoleic and arachidonic acid[23], are widely used to monitor oxidative changes of lipids in dry-cured hams[24, 25, 26]. The relatively high amount of hexanal found in dry-cured Iberian ham leads to the faintly rancid aroma, which is part of the typical flavour of this product, in which other volatile compounds counteract the negative effect of hexanal. However, an excess in such aromatic notes leads to an overall unpleasant flavour and a loss of quality[2,3]. In agreement with the previous studies[24,25] TBA-RS and hexanal content in BF of Iberian dry-cured hams showed a similar pattern and had a positive correlation (r = +0.397; $p<0.05$).

Table 4.- TBA-RS (mg MDA/Kg) and hexanal content (area units-AU- x 10^6) of dry-cured loins from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
TBA-RS	1.76	1.79	1.85	1.77	1.82	0.10	ns	ns	ns
Hexanal	646.60	563.29	809.43	641.47	694.26	48.65	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: non significant ($p>0.05$)

Instrumental colour parameters were significantly affected by the Iberian x Duroc genotype (Table 5). BF from GEN2 had different colour characteristics to GEN3. BF from GEN2 had a more intense (higher Chroma), redder (higher CIE a*-value) and lighter colour (higher CIE L*-value) compared to those from GEN3, while GEN1 had intermediate values. Yellowness (CIE b*) and Hue angle (CIE Hº) showed similar values between genotypes. In dry-cured ham, colour is one of the most outstanding characteristics of appearance[3], and it is accepted that it could influence consumers' choice of sliced and packaged ham in the supermarket. Thus, the darker and less intense red colour of the BF of dry-cured hams from GEN3 is expected to affect its acceptability among consumers that prefer dry-cured hams with an intense red colour[5]. The colour of the dry-cured ham is influenced by several factors related to muscle composition and oxidation intensity such as moisture, fat and heme pigment contents, oxidative status and nitrite content[20]. Since no significant differences in CIE a* values were found in the BF from the same pigs before processing[12] and the chemical composition of dry-cured

BF did not differ between genotypes in the present paper, the differences in the colour dry-cured BF could be caused by other factors than chemical composition, such as oxidative processes of heme pigments.

Table 5.- Instrumental colour of dry-cured hams from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
CIE L*-value	37.84b	40.65a	37.76b	38.88	38.70	0.43	**	ns	ns
CIE a*-value	17.67ab	18.18a	16.53b	17.37	17.61	0.25	*	ns	ns
CIE b*-value	6.47	7.13	6.49	6.84	6.56	0.14	ns	ns	ns
Chroma (C)	18.83ab	19.53a	17.76b	18.68	18.81	0.26	*	ns	ns
Hue (Hº)	20.15	21.39	21.48	21.47	20.51	0.37	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: non significant (p>0.05), *: p<0.05: **: p<0.01; a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05).

The results from WBSF and TPA (Table 6) suggest that BF from dry-cured hams of all three genotypes had similar instrumental texture characteristics since similar values of hardness, adhesiveness, springiness, cohesiveness and chewiness were detected. Texture has earlier been related to physicochemical characteristics of dry-cured ham such as ultimate pH, moisture and IMF contents[22,27]. The authors[27] found a significant influence of the moisture content on instrumental texture traits such as hardness, cohesiveness and springiness. In line with this, we found negative correlations between moisture content and WBSF (r = -0.462, p<0.05) and instrumental hardness (r = -0.393, p<0.05) in the present study. Also in dry-cured loins from the same pigs as the present study, Ramírez and Cava, (unpublished[28]) found a marked effect of the IMF content on instrumental texture parameters. However, in the present paper only adhesiveness (r = +0.598, p<0.001) and cohesiveness (r = +0.544, p<0.05) were related to IMF content. The similar proximate composition of dry-cured hams from the different genotypes could partly explain the lack of differences in instrumental analysis.

Table 6.- Texture profile of dry-cured hams from 3 different Iberian x Duroc genotypes.

	Genotype			Sex			Significance		
	GEN1	GEN2	GEN3	♂	♀	sem	genotype	sex	interaction
WBSF (N)	11.11	10.80	12.45	11.80	11.03	0.83	ns	ns	ns
TPA									
Hardness (N)	146.54	102.66	157.49	119.04	150.41	11.33	ns	ns	ns
Adhesiveness (N*s)	-0.39	-0.43	-0.37	-0.42	-0.38	0.02	ns	ns	ns
Springiness (cm)	0.45	0.43	0.43	0.44	0.43	0.01	ns	ns	ns
Cohesiveness	65.37	45.21	66.14	50.72	66.53	5.52	ns	ns	ns
Chewiness (N)	0.45	0.43	0.43	0.44	0.43	0.01	ns	ns	ns

GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB
ns: non significant (p>0.05)

Twelve descriptors were evaluated in the sensory analysis of the dry-cured hams (Figure 1). Differences due to the sex of animals were negligible and not statistical, except for acid taste, where differences between hams from males and females were found. Sensory panel was more effective than instrumental methods in assessing differences between dry-cured hams. The slight differences in both moisture and IMF contents might have influenced the sensory perception of panellists in

certain traits such as lightness, juiciness, fibrousnesses and saltiness, while the technical measurements were unaffected.

Lean redness and marbling scores were similar in dry-cured hams from the 3 genotypes while scores for lightness were higher in GEN2. These results are consistent with data reported for instrumental colour determination and IMF and moisture chemical contents. In line with this, also positive correlations between lightness of the lean and CIE L*-value (r = +0.541, p<0.05) and moisture content (r = +0.455, p<0.05) were found. Similarly, marbling and CIE L*-value showed significant positive correlations (r = +0.367, p<0.05). These results agree with Carrapiso and García[29] who also obtained a significant correlation between L*and marbling and suggested that colour of Iberian ham is more influenced by fat distribution than by chemical IMF content of the muscle.

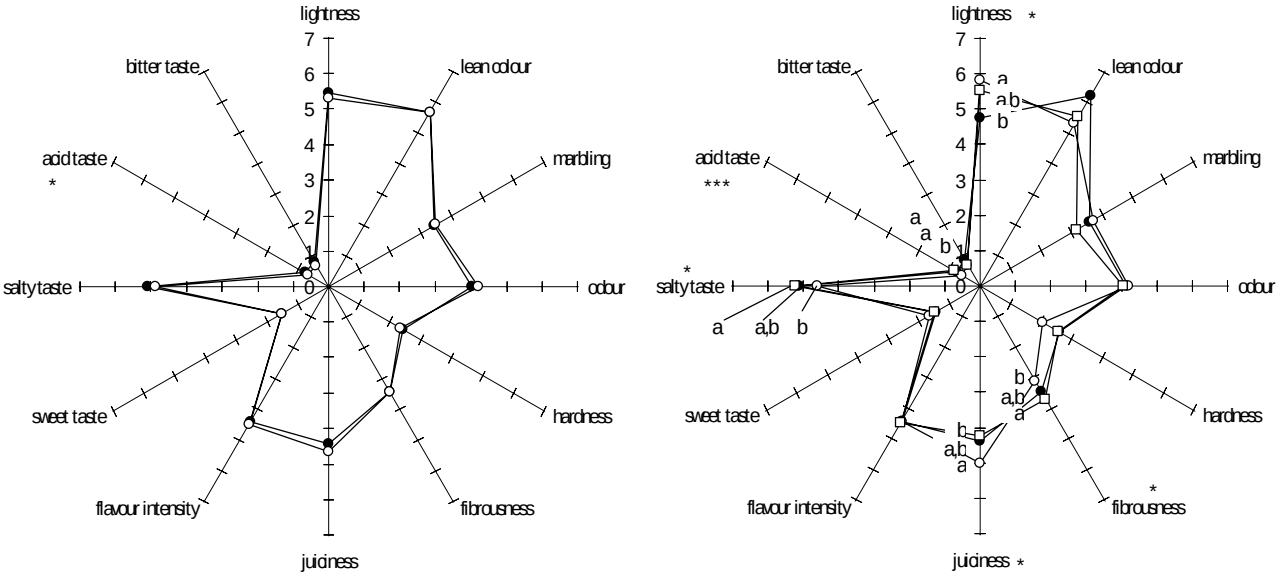

Figure 1.- Sensory analysis of dry-cured hams from 3 different Iberian x Duroc genotypes. Left figure: effect of animal sex (○: males, ●: females). Right figure: effect of genotype (●: GEN1, ○: GEN2, □: GEN3). *: p<0.05: **: p<0.01; ***: p<0.001; a,b: Different letters in the same row indicate significant statistical differences (Tukey's Test, p<0.05).

As mentioned above, panellists found differences between genotypes in certain texture traits, not detected by instrumental techniques. However, results from the sensory analysis are consistent with results from TPA and WBSF as positive correlations were found between hardness scores and TPA hardness (r =0.429, p<0.05) and WBSF (r = +0.613, p<0.001). Panellist scored hams from GEN3 more fibrous (p<0.05), harder (p>0.05) and less juicy (p<0.05) than those from GEN2, while GEN1 showed intermediate values. Some authors found that instrumentally determined tenderness only resembles bite resistance to shearing, whereas human textural perception in general is more complex[30]. Sensory tenderness is affected by different factors such as the different deformations, the deformation rates, and the intrinsic heterogeneity of the biological material [31]. Increasing IMF levels reduce the force for chewing, ease the separation of muscle fibres and cause an enhanced perception of meat tenderness[32]. Differences in sensory texture attributes could be caused by several factors: i.) ultimate pH in fresh BF, ii.) IMF content, and iii.) moisture content in the dry-cured product. Better sensory texture parameters have been reported in Iberian hams with higher IMF

content and significant positive correlations between marbling and juiciness [2,22]. García-Rey *et al*.[5,33] showed that low *postmortem* pH and some pig genotypes resulted in defective texture in dry-cured hams. In a previous paper[12] significant lowest *postmortem* pH values were reached in *B. femoris* from GEN3, which corresponds with the higher fibrousness and lower juiciness in hams from GEN3 in the present paper. According to this, significant positive correlations between marbling and juiciness (r = +0.634, $p<0.001$) and negative between moisture content and hardness (r = -0.565, $p<0.01$) were found. Ruiz *et al*.[3] suggested the negative influence of some textural traits like high fibrousness could reduce the acceptability of the dry-cured hams.

Among descriptors associated with the taste, only salty and acid taste differed between genotypes. Hams from GEN3 were scored saltier than those from GEN2 while those from GEN1 were intermediate. As also a negative correlation between moisture and saltiness was found (r = -0.641, $p<0.001$), this suggests a different salt content in the former and a differences in sodium chloride uptake during salting. The final salt content might be influenced by the lower subcutaneous fat thickness in green hams[12] and/or to the less moisture content in the matured ham. Hams from GEN3 were scored more acid than those from GEN2 which could be related to the lower ultimate pH in fresh BF from the former[12].

Dry-cured hams from the 3 genotypes had similar odour and flavour characteristics. In line with this no differences in TBA-RS and hexanal contents were found. However, hexanal content and aroma intensity were positively correlated (r = +0.396, $p<0.05$). In spite of this, hexanal, is in a high extent related to the development of rancid flavours in Iberian ham and it could not alone be considered as an indicator for aroma intensity[25]. We suggest a close relationship between IMF/marbling, aroma and odour intensity scores as positive correlation between marbling, the intensity of the odour and aroma (r =+0.596, $p<0.001$ and +0.649, $p<0.001$ respectively) and between odour intensity and IMF (r = +0.501, $p<0.01$) were found.

A principal component analysis (PCA) was performed to examine the relationships between some selected traits in the samples. PCA showed 5 significant factors that accounted for 66.92% of the variability. Principal components, PC1 and PC2, explained 26.50% and 14.55% of the variation, respectively. Figure 2 shows the score plot of the different variables (coefficients of the eigenvectors) for PC1 and PC2, presenting the quality parameters measured in the dry-cured ham. The distribution of the data on PC1 and PC2 (Figure 3) showed 2 separate groups of points. On the positive x axis of the PC1 we can find hams from GEN1 and GEN3 associated with fibrousness, acid and salty tastes, lightness, hardness and PUFA percentage, while hams from GEN2 were located in the negative x axis near to visual, texture and odour features such as marbling, juiciness, sweetness and odour intensity, all of them related to IMF content.

4.- CONCLUSIONS

Iberian x Duroc reciprocal crosses scarcely affected the physicochemical and instrumental characteristics quality of dry-cured hams whereas the Duroc genotype had a significant effect. The use of Duroc sire lines with non adequate characteristics will negatively contribute to the quality of dry-cured Iberian hams, especially on their texture and colour.

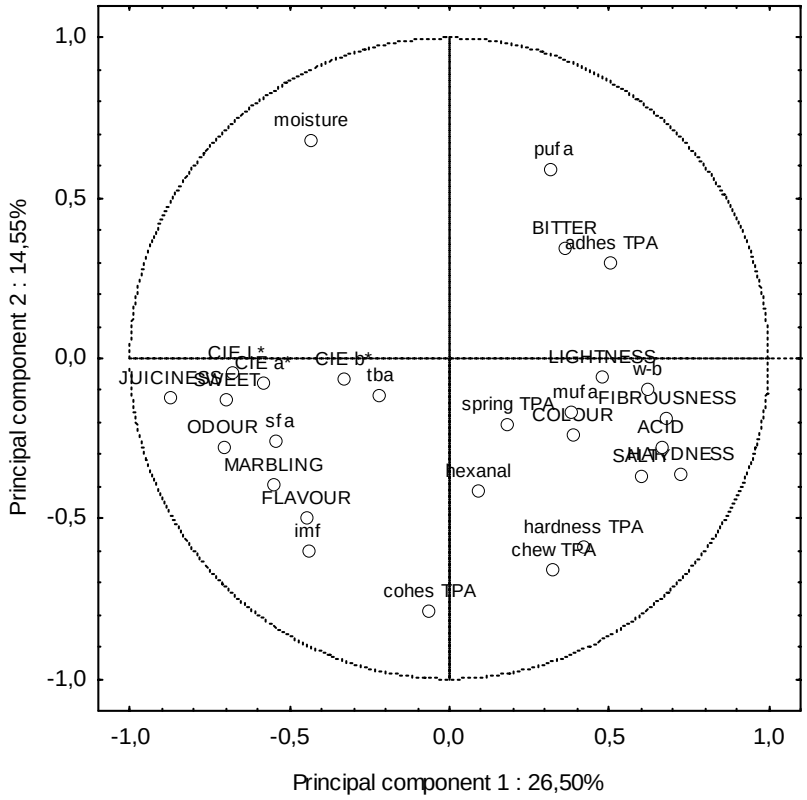

Figure 2.- Loadings plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2).

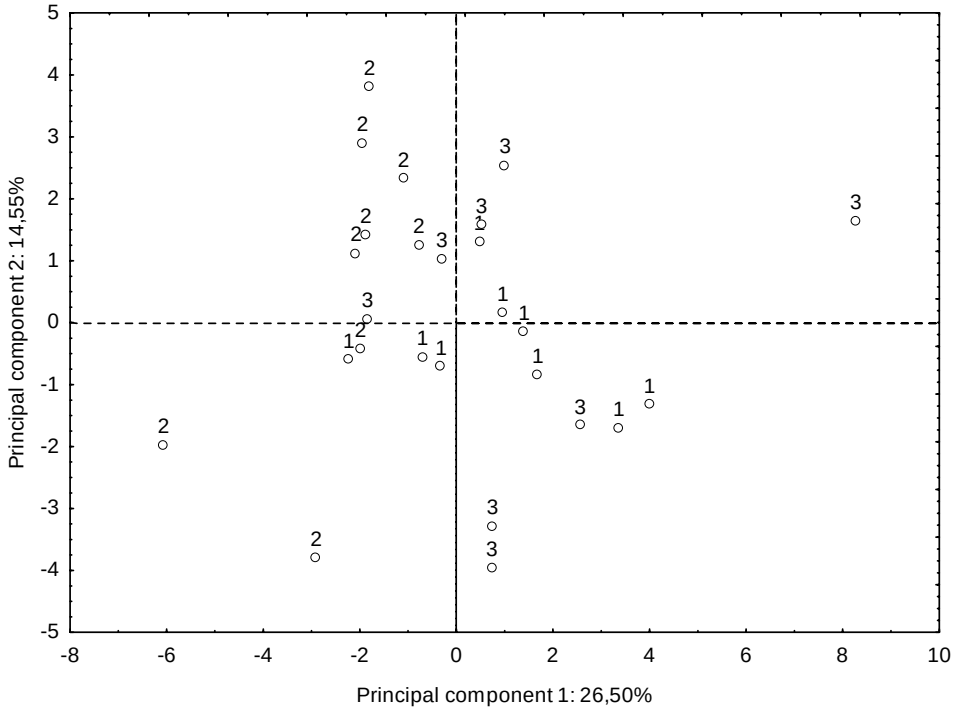

Figure 3.- Loadings plot after principal component analysis of the variables in the plane defined by the two first principal components (PC1 and PC2).

Table 7.- Pearson correlation coefficients (*r*) between chemical composition, instrumental colour and texture and sensory traits.

	Chemical comp.		Instrumental texture						Sensory analysis											Lipid oxidation	
	moist	IMF	wbsf	hard	adhe	spring	cohesi	chewi	lightness	redness	marbling	odour	hardness	fibrous.	juiciness	aroma	sweetness	saltiness	acid	TBA	hexanal
L	0.455*	0.330	-0.498**	-0.090	0.552**	0.163	0.154	0.006	0.541*	-0.551**	0.367*	0.165	-0.375	-0.305	0.481*	0.202	0.412*	-0.302	-0.563**	0.061	-0.137
a*	0.235	0.103	-0.211	-0.281	0.169	-0.293	0.131	-0.210	-0.085	0.161	0.451*	0.425*	-0.259	-0.464*	0.652***	0.346	0.300	-0.401*	-0.297	0.277	0.071
b*	0.018	0.098	-0.053	0.033	0.107	0.214	0.229	0.038	0.423*	-0.336	0.084	-0.055	-0.126	-0.426*	0.252	0.196	0.172	-0.091	-0.165	0.157	-0.055
moisture		-0.152	-0.462*	-0.393*	0.194	-0.11	-0.441*	-0.375	0.186	-0.388	-0.024	0.069	-0.565**	-0.192	0.212	-0.276	0.176	-0.641***	-0.539**	0.078	-0.349
IMF			-0.080	0.178	0.598***	0.129	0.544*	0.269	0.178	-0.037	0.317	0.501**	-0.217	-0.292	0.356	0.270	0.414*	-0.129	-0.083	0.066	-0.048
wbsf				0.151	-0.566**	-0.010	-0.090	0.090	0.394*	0.476*	-0.296	-0.298	0.613***	0.234	-0.466*	-0.271	-0.275	0.351	0.434*	-0.231	0.106
hardness					0.021	0.453*	0.515**	0.955***	-0.129	0.033	-0.146	-0.237	0.429*	0.407*	-0.339	-0.067	-0.268	0.329	0.326	0.121	0.157
chewiness									0.047	-0.013	-0.047	-0.156	0.386	0.395*	-0.273	0.026	-0.248	0.280	0.211	0.015	0.198
marbling												0.596***	-0.021	-0.152	0.634***	0.649***	0.345	-0.171	-0.25	-0.039	0.074
odour													-0.459*	-0.402*	0.685***	0.526**	0.666***	-0.452*	-0.323	0.235	0.134
hardness														0.678***	-0.547**	-0.066	-0.508**	0.593***	0.521**	-0.279	0.137
fibrousness															-0.562**	-0.132	-0.429*	0.360	0.410*	-0.183	0.226
juiciness																0.477*	0.587**	-0.562**	-0.470*	0.282	0.038
aroma																	0.521**	0.120	-0.242	0.104	0.396*
sweetness																		-0.321	-0.420*	0.226	0.158
saltiness																			0.429*	-0.064	0.141
acid																				0.214	0.306
TBA-RS																					0.397*

*: p<0.05: **: p<0.01; ***: p<0.001

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Chapter VII

VOLATILE PROFILES OF DRY-CURED MEAT PRODUCTS FROM 3 DIFFERENT IBERIAN X DUROC GENOTYPES[1]

ABSTRACT

Volatile profile of 2 Iberian dry-cured products, dry-cured loin and ham, from 3 different Iberian x Duroc genotypes was assessed. 3 groups of 10 pigs each one (5 males and 5 females) from different genotypes were studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening, while the genotype Duroc2 (DU2) corresponded to animals selected for meat production. Genotype slightly affected volatile profile of both dry-cured meat products, although dry-cured loin from GEN3 showed higher hexanal content. Dry-cured loin showed a volatile profile very different to that found in dry-cured ham. Volatile compounds of dry-cured meat products were mainly originated by lipid and protein degradation. Most of the volatile detected in both meat products came from lipid oxidation such as acids, aldehydes, ketones, alcohols and hydrocarbons. In addition, high proportion of volatile compounds from Maillard reaction was found. Branched aldehydes and some sulfur and nitrogen compounds have their origin in the aminoacids degradation by Strecker reaction, while branched alcohols and acids come from the lipid oxidation of branched aldehydes. Dry-cured ham showed higher number and a higher level of compounds with origin in protein and lipid degradation than dry-cured loin, which agrees with the longer ripening of the hams (24 months) respect to the loins (4months). In dry-cured loins, apart from these compounds, seasoning mixture provides high amount of volatiles, such as terpenes (from paprika and oregano) and sulfur compounds (from garlic), which have great importance in the overall aroma of this product.

Keywords: volatile, SPME, Duroc, Iberian, dry-cured loin, dry-cured ham.

1.- INTRODUCTION

Iberian dry-cured loin and ham are the most valuable dry-cured meat products, with an extraordinary consumer acceptance because of their sensory quality, specially their unique and characteristic flavour. The main factors that conduct to the characteristic and intense flavour of these products are the meat quality as well as the special features of the ripening process such as its length (14-36 months for dry-cured hams and 3-6 months for dry-cured loins).

The quality of the dry-cured meat products is closely related to the characteristics of the raw material, especially those related to the degree of marbling and the fatty acid composition of fat such as feeding characteristics, age of animals and pig breed (*1*). One of the alternatives more often applied to improve productive parameters of Iberian pig is by the cross with Duroc breed (*2*). However, different studies have demonstrated differences between Duroc lines that affect productive characteristics and meat and meat products quality (*3*). Therefore, the Duroc line could affect the aroma of Iberian dry-cured meat products.

The manufacture process and ripening of loins and hams influence their flavour. Iberian dry-cured loin is manufactured by rubbing a mixture of curing agents (salt and nitrite) and spices (i.e. Spanish paprika, oregano and garlic) on the surface of the loin pieces. Then, they are stuffed into casings and subsequently ripened. Previous researchers have established that the spices rubbed onto the surface play an important role on the flavour of dry-cured loin (*4*,*5*). Moreover, the antioxidant effect of the spices and nitrites added to dry-cured sausages has been probed by Aguirrezábal *et al*., (*6*),

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which could affect the development of lipid oxidation reactions. Nonetheless, dry-cured hams are only covered with salt and nitrites, and undergo to a much longer ripening process; therefore, greater development of lipid and protein degradation reactions would be expectable.

A considerable amount of studies have been devoted to describe the volatile flavour compounds of Iberian hams (i.e. *7*, *8*), while there a few previous studies about Iberian loin flavour (*4, 5*). In addition, there are several researches about reactions that provide volatile compounds such as lipolysis, lipid oxidation, Maillard reaction and protein and aminoacids degradation and the contribution of microorganisms to the flavour development in Iberian products. However, the comparison between the volatile profile of Iberian dry-cured loin and dry-cured ham has never been carried out.

Lipid derived compounds are the most abundant compounds in dry-cured meat products (*9*,*10*). The extent of lipolysis and lipid oxidation determines the final flavour of dry-cured meat products (*11*). On the other hand, Maillard reaction, which occurs between amino compounds and reducing sugars, is one of the most important routes of flavour compounds in dry-cured products (*12*). Besides, compounds from Maillard reaction can also react with other components of meat such as aldehydes and other carbonyls formed during lipid oxidation, which react readily with Maillard intermediates. Such interactions contribute to the achievement of the optimum and characteristic flavour of meat products (*13*).

Therefore, the main objectives of this paper are the characterization of volatile compounds of dry-cured loin and ham and to determine the effect of the genotype on the volatile compounds profile extracted from dry-cured meat products from different Iberian x Duroc genotypes.

2.- MATERIAL AND METHODS

2.1.- Animals.

3 groups of 10 pigs each one (5 males and 5 females) from different genotypes were studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening. The genotype Duroc2 (DU2) corresponded to animals selected for meat production, with high percentages of meat cuts and with low carcass fattening. Pigs were intensively raised all together and fed *ad libitum* with a cereal-based commercial fodder. Pigs were randomly slaughtered after 316 days of rearing with 150-165 Kg live weight.

2.2.- Dry-curing process.

For the manufacture of the dry-cured loins, the *Longissimus dorsi* muscle (10loins/genotype) was removed from the carcass and processed into dry-cured loin. The weight of the fresh muscle was 2.5-3.2 Kg, and pH values were lower than 6.0. Loins were seasoned by rubbing a mixture of salt, nitrite, olive oil and spices such as Spanish paprika (*Capsicum annuum, L.*), oregano (*Origanum vulgare L.*) and garlic (*Altium sativum, L.*). Loins were kept for 4 days at 4°C to allow the seasoning mixture to penetrate. Then, loins were stuffed into collagen casings and held for 30 days at 4°C at a relative humidity of ~80%. Finally, loins were ripened for 90 days at ~12°C and at ~70% relative

humidity. Loins were processed for a total dry-curing time of 4 months. For the analysis of volatile compounds in this product, the surface (3mm) with the pickling mixture was removed.

For the manufacture of dry-cured hams, one ham from each animal was processed (10ham/genotype). The weight of the fresh ham was 14.0-15.5 Kg and pH values were lower than 6.0. After salting, hams were kept at 0–3ºC and 80–90% relative humidity for ~6 months. Then, the hams were ripened for ~18 months at 10–25ºC and 60–80% relative humidity. Hams were processed for a total dry-curing time of 24 months. After the ripening process, the *Biceps femoris* muscles were removed from the hams for the analysis.

2.3.- Volatile analyses.

A SPME fiber (Supelco Co. Canada) coated with divinilbenzene-carboxen-poly (dimethylxilosane) (DVB/CAR/PDMS) 50/30 μm was used. The sampling technique to extract volatile compounds from headspace was the following: 0.5g of meat were minced and placed in 5mL vials with a silicone stopper which were previously deodorized heating them in an electric stove at 80ºC for at least 2 hours. Each sample was analyzed in duplicate. The vial was maintained in temperature controlled water bath at 37ºC. The fiber was exposed to the headspace of the sample for 30 min. The analyses were performed on a HP5890GC series II gas chromatograph (Hewlett-Packard) coupled to a mass selective detector Agilent model 5973. Volatiles were separated using a 5% phenyl-95% dimethyl polysiloxane column (30m x 0.25mm i.d., 1.0 mm film thickness; Restek). The carrier gas was helium at 18.5 psi, resulting in a flow of 1.6mL/ min a 40ºC. Prior to analysis, the SPME fiber was preconditioned at 270ºC for 50min in the gas chromatography injection port. The injection port was in *splitless* mode and the temperature program was isothermal at 40ºC for 10 min and then raised at the rate of 7ºC seg^{-1} to 250ºC and held for 5min. The GC-MS transfer line temperature was 270ºC. The MS operated in the electron impact mode with electron impact energy of 70 eV; a multiplier voltage of 1650V and a collected data at a rate of 1scan s^{-1} over a range of *m/z* 40-300. n-Alcanes (Sigma R-8769) were analyzed under the same conditions to calculate the Retention Indices (RI) values for the volatiles. The compounds were identified by comparison with reference compounds (Sigma, Aldrich), by comparison of RI with those described by Kondjoyan and Berdagué (*14*) and by comparison of their mass spectra with those contained in Wiley library.

2.4.- Statistical analysis.

The effects of genotype and sex were analyzed using the Analysis of Variance (ANOVA) procedure of SPSS, version 12.0. A two-way analysis of the variance (genotype and sex) with interaction (genotype x sex) was carried out. HSD Tukey's test was applied to compare the mean values of the genotypes. Mean values and standard errors of the means (SEM) are reported. The relationship between parameters was assessed by the principal component analysis (PCA).

3.- RESULTS AND DISCUSSION

3.1.- Origin of the volatile compounds of Iberian dry-cured meat products.

41 compounds were isolated in dry-cured loin (Table 1): 5 acids, 4 ketones, 3 alcohols, 11 aldehydes, 3 esters, 2 nitrogen compounds, 3 sulfur compounds, 3 terpenes, 4 lineal hydrocarbons and 3 aromatic hydrocarbons. In dry-cured ham (Table 2) a higher number of volatiles than in dry-

cured loin was detected (55 compounds): 7 acids, 11 ketones, 8 alcohols, 12 aldehyes, 7 lineal hydrocarbons, 2 esters, 3 nitrogen compounds, 3 sulfur compounds, 1 terpen and 1 aromatic hydrocarbon. The most abundant compounds in dry-cured loin were aldehydes (45.7%), followed by terpenes (17.0%) and ketones (10.1%). Similarly, aldehydes (47.7%) had the highest chromatographic area in dry-cured ham, followed by alcohols (24.7%) and ketones (16.7%).

The relative amount of volatile compounds was twice higher in dry-cured ham than in dry-cured loin (Figure 1) since volatile profile of dry-cured ham showed larger amount of lipid derived compounds (aldehydes, ketones and alcohols) and Maillard compounds than dry-cured loin. In general, differences in the volatile profile of dry-cured hams and loins are attributable to the different manufacture process and ripening length. So, the shorter ripening process (4 months vs 24 months) and the lower temperatures during the dry-cured loin processing compared to that of dry-cured hams, could probably contribute to a more limited development of the chemical reactions involved in the flavour compounds generation in dry-cured loin. As a consequence, the formation of lipid and protein derived compounds and the reaction between them to form Maillard volatile compounds could be enhanced in dry-cured ham. On the other hand, dry-cured loin had higher content of certain volatile compounds from the pickling mixture, such as sulfur compounds and terpenes, which show strong aromatic notes and play an important role in the overall aroma notes of this meat product.

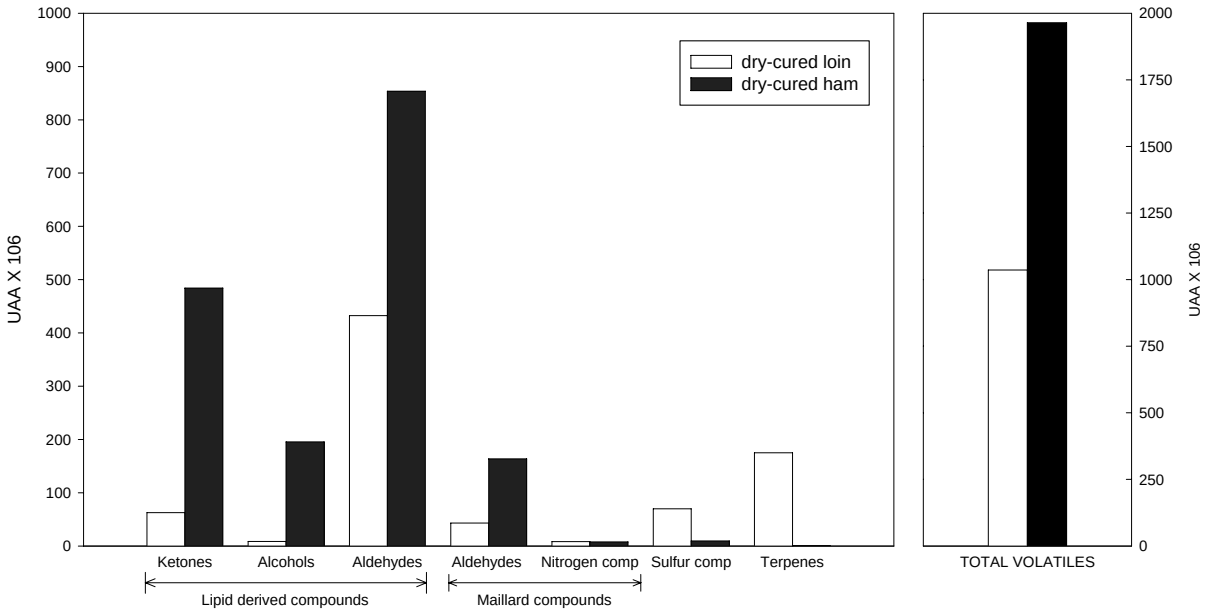

Figure 1.- Volatile compounds (total volatile compounds and classified according to their origin and chemical nature) detected in dry-cured loin and ham.

Volatile compounds were classified according to their most likely origin in spite of the difficulties to establish the origin of some compounds. Volatile compounds were divided in lipid derived volatile compounds which comprise acids, ketones, alcohols, aldehydes and hydrocarbons, whereas volatile compounds formed by the Maillard reaction include heterocyclic nitrogen and sulfur compounds and non-heterocyclic compounds, such as Strecker aldehydes and hydroxyketones as well as aliphatic disulfides. Besides, in dry-cured loin, volatile compounds from spices were detected, mainly terpenes and some sulfur compounds.

Some of these compounds detected have been previously described by Carrapiso ***et al***., (*8*) as odour active compounds in Iberian dry-cured ham such as 2-methyl propanal, 3-methyl-butanal, 2-methyl-butanal, 2-pentanone, pentanal, hexanal, 2-heptanone, heptanal, 2-heptenal, oct-1-en-3-ol and octanal.

Lipid-derived compounds were the main group of compounds in dry-cured loins and hams. In dry-cured loins, lipid derived compounds accounted for more than 55% of the total volatiles and more than 80% in dry-cured ham.

In general, acids are generated by lipid oxidation reactions. The origin of acetic acid, which was the most abundant acid compound detected in dry-cured ham, is not clear. Some authors have reported that it is originated by the fermentation of sugars by microorganisms (*15*) and others by Maillard reaction (*16*). However, the importance of acetic acid in dry-cured ham may be limited as this compound was not described as odour active compound of Iberian ham (*8*). In dry-cured loin, the importance of acetic acid could be higher than in dry-cured ham since it could participate in its spiced characteristic aroma. Mateo ***et al***., (*17*) have detected a wide variety of acids in Spanish paprika (an ingredient of the rubbing mixture) and especially acetic acid, which was also one the most abundant compound detected in dry-cured loin.

The most abundant ketone detected in both products was propan-2-one. 2- Ketones have been abundantly isolated in dry-cured products including dry-cured loin (*4*) and dry-cured ham (*9*). They have also been associated with the aroma of moulded-surface cheeses (*18*), so their contribution to the overall cured flavour could be important. 2-ketones may arise from fatty acids by chemical (autooxidation) or enzymatic (β-oxidation) oxidation of free-fatty acids by moulds.

Aliphatic linear alcohols generally result from the degradation of lipid hydroperoxides (*19*). Dry-cured ham showed a wide variety of these compounds, being detected in higher number and content than in dry-cured loin. Muriel ***et al***., (*4*) reported that alcohols were the most important chemical group in dry-cured loin; in contrast to the present study in which 2 alcohols from lipid oxidation were detected.

Straight chain aliphatic aldehydes are typical products of lipid oxidation. They have low odour threshold values and play an important role in the flavour of dry-cured ham and loin (*4*, *11, 20*). Hexanal was the most abundant compound detected in both products. It is considered the main volatile derived from oxidation of n-6 fatty acids such as linoleic and arachidonic acids. The large amount of hexanal found in matured hams is considered a distinctive trait of these products, but in a high extent this compound has been related to the development of rancid flavours in Iberian ham (*11*). The level of hexanal in dry-cured ham was approximately twice higher than in dry- cured loins ($673x10^6$ UA vs. 380 $x10^6$ UA), which is in accordance with the longer ripening time and the development in a higher extent of the lipid oxidation reactions in the former. Conversely, heptanal and octanal which arise from oleic acid add pleasant notes to the aroma of dry-cured products (*8*).

Aliphatic and aromatic hydrocarbons with origin in lipid oxidation reactions (*21*) were isolated in both dry-cured products. The contribution of these compounds to the aroma of dry-cured products is different. Aliphatic hydrocarbons, due to their high threshold value, are not important contributors to the aroma of these products, while aromatic hydrocarbons because of their abundance could play an important role in the aroma of dry-cured loin and ham (*22*). Several authors have reported the

presence of aromatic hydrocarbons compounds in dry-cured loin (*4*) and ham (*23*). They may play an important role in the overall flavour of meat products as some of them such as ethyl benzene and methyl benzene have been reported to be important to distinguish volatile profile from different types of dry-cured hams (*23*).

Volatiles arising from Maillard reaction accounted for ~18% (dry-cured loin) and ~13% (dry-cured ham) of the total volatiles. Most of them were branched short-chain aldehydes and their corresponding alcohols. The origin of branched aldehydes in dry-cured products is in Strecker degradation reactions of amino acids (*12*), which is an important pathway associated to Maillard reaction. In Strecker reaction, amino acids are decarboxylated and deaminated forming aldehydes, while dicarbonyls formed in Maillard reaction are converted to aminoketones or aminoalcohols, which can also react with themselves or with other compounds providing a wide variety of aromatic compounds (*13*).

Although many studies have been carried out about Iberian products flavour, volatile compounds with origin in Maillard reaction have never been quantified. The high proportion of volatiles from Maillard reaction could be related with the high acceptability of these products, since Maillard compounds, which had very low threshold values, add pleasant aroma notes (*13*). Previous authors (*10*) have reported the importance of these compounds in the reduction of the lipid oxidation compounds in dry-cured hams throughout ripening, due to the reaction of lipid oxidation products with other compounds increasing the complexity of the aroma profile. This could be related with the intricacy of overall aroma and with the different aroma notes of the flavour of these products (*8*). On the other hand, the higher amounts and number of compounds with origin in protein degradation in dry-cured ham respect to the loin show the more implication of Maillard compounds in the aroma formation of the latter respect to the former.

2- and 3-methylbutanal are products of the Strecker degradation of the amino acids isoleucine and leucine while benzeacetaldehyde come from the amino acid phenylalanine (*24*) and acetaldehyde from the aminoacid cysteine (*13*). 2- and 3-methylbutanal, which were abundantly isolated in dry-cured ham, are linked to long ripening processes, and because of their low threshold values and pleasant “cured” flavours, contribute positively to the dry-cured ham flavour (*10*).

Some branched chain acids detected in both meat products, such as 2-ethylpropanoic acid, 3-methylbutanoic acid and 2-methylbutanoic acid, have been identified as products of the microbial metabolism of valine, leucine and isoleucine, respectively (*25*). Some authors have attributed the origin of these compounds to the action of moulds, such as in dry-fermented meat products (*26*). The contribution of moulds to the flavour of dry-cured ham has been previously reported (*27*) since different moulds strive on the surface of dry-cured loin and ham. Proteolysis and lipolysis by the endogenous and microbial enzymes seem to play a decisive role in the generation of flavor precursors in dry-cured meat products (*27*). However, these compounds could be also originated by oxidation from their respective Strecker aldehydes (i.e. 2-methyl-butanal would come from the degradation of the aminoacid isoleucine, and by oxidation, 2-methyl-butanoic acid would be formed). Ventanas *et al*., (*28*) reported that, under ripening conditions of dry-cured products, the formation of Strecker compounds is possible without the participation of microorganisms.

Alcohols such as propan-2-ol have their origin in amino acids by means of Strecker degradation reactions (*29*). 3-methyl-1-butanol seems to play an important role in the overall and characteristic flavour of dry- cured hams (*23*). Its origin is controversial; some authors (*30*) have reported that this compound comes from 3-methylbutanal, a Strecker aldehyde, or from the microbial metabolism of leucine. However, Sánchez Peña ***et al***., (*23*) postulated that its origin was from lipid oxidation since they detected high levels of 3-methyl-1-butanol in muscle and also in subcutaneous fat where proteins are not abundant. Alcohols and aldehydes derived from Maillard reactions are relatively abundant and characteristic compounds of Iberian hams (*23*). In this sense, some of the most remarkable volatiles for the characterization of Iberian hams against hams from other breeds were 3-methyl-1-butanol and 3-methyl-butanal (*23*), which are Strecker aldehydes.

Nitrogen and sulfur compounds have great importance in the overall flavour of meat products because of their very low threshold values. Except those from spices, nitrogen compounds come from the breakdown of proteins, free amino acids and nucleic acids, while sulfur volatile compounds are derived from sulfur-containing amino acids. Some nitrogen compounds like pyrazines, have been abundantly isolated from dry-cured Iberian products (*4, 8*). In addition, some furans were detected in dry-cured ham. Furans, due to their very low threshold value and their pleasant aroma, should contribute importantly to the desirable aroma of the dry-cured products.

Esters accounted for a small proportion of volatiles in dry-cured loin (0.8%) and in dry-cured ham (0.3%). Most of them were ethyl esters, formed from ethanol and carboxilic acids by the action of microorganisms. Esters add fruity aroma notes (*31*). They have been isolated in Iberian dry-cured loin (*4*), in Iberian dry-cured ham (*9, 10*) and in other dry-cured meat products such as in dry-fermented sausages (*26, 31, 32, 33*).

In dry-cured loin, compounds derived from added spices lessen the volatiles derived from lipid oxidation, microbial metabolism and Maillard reactions. Consequently, the flavour of the dry-cured loin is the result of a complex equilibrium between volatile compounds derived from both origins. In dry-cured loin, ~25% of the volatiles had origin in spices (Spanish paprika, oregano and garlic), which were mainly terpenes and to a lesser extent, aliphatic sulfur compounds. 3 sulfur-derived compounds were detected, being dipropenyl -disulfide (diallyl disulfide) the most abundant. A wide variety of sulfur compounds derived from allicin, are characteristic of garlic aroma (*34*), so it is likely that those have an important contribution to the overall aroma of dry-cured loin, since garlic is a potent aromatic ingredient. In addition, sulfur derivatives of propene have also been abundantly detected in dry-cured loin (*4*) and in other dry-fermented products manufactured with garlic (*32, 33*).

Terpenes were abundant in dry-cured loin, while they were scarcely detected in dry-cured ham. They have well-defined odours in the literature, so alpha pinene has been described to add a pine odour, while limonene and carene add lemon notes. In dry-cured ham, the presence of limonene has been associated with the pig diet (*9,23*). However, in dry-cured loin, the high content of terpenes suggests the origin in the spices added during the manufacture process (*4*). Taking into account the compounds of the seasoning mixture of dry-cured loin, neither have terpenes been detected in dried Spanish paprika (*17*) nor in garlic (*32*); whereas they have been abundantly detected in oregano (*35*).

Table 1.- Volatile compounds (area units-AU- x10^6) detected in the headspace of dry-cured loins from 3 different Iberian x Duroc genotypes.

			Genotype			Sex			Probabilities		
RI[x]	Id. method	Compound	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	interaction
Lipid oxidation (55.8%)											
Acids											
970	b	hexanoic acid	11.2	12.7	16.0	14.5	12.3	1.8	0.615	0.595	0.980
Ketones											
502	b	propan-2-one	17.2	27.9	34.0	24.8	28.5	8.4	0.727	0.795	0.560
891	a	heptan-2-one	2.0	2.2	3.8	3.0	2.4	0.5	0.216	0.574	0.188
983	b	octane-2,3-dione	14.3b	28.0ab	55.9a	31.7	34.9	5.6	0.008	0.651	0.939
Alcohols											
868	a	1-hexanol	2.3	2.9	3.1	2.6	2.9	0.2	0.366	0.580	0.534
898	c	2-heptanol	6.2	5.2	6.4	6.5	5.3	0.5	0.584	0.266	0.824
Aldehydes											
696	c	pentanal	4.4	8.5	10.1	6.9	8.6	1.1	0.104	0.392	0.441
799	a	hexanal	201.0b	307.9b	651.2a	384.8	400.8	61.3	0.006	0.767	0.863
901	a	heptanal	7.1b	7.1b	14.7a	11.2	8.4	1.2	0.006	0.206	0.582
960	a	hepten-2-al	0.6	2.4	1.8	1.9	1.4	0.4	0.204	0.600	0.160
1004	a	octanal	4.7	3.6	4.9	4.7	4.2	0.6	0.676	0.681	0.911
1107	b	nonanal	17.4	14.7	14.2	15.9	14.9	1.1	0.493	0.614	0.903
1208	b	decanal	0.9	0.3	0.4	0.9	0.2	0.2	0.413	0.142	0.341
Lineal hydrocarbons											
556	c	2-methyl-pentane	1.8ab	3.1a	1.2b	2.3	1.8	0.3	0.039	0.449	0.780
576	c	3-methyl-pentane	1.8	7.9	1.5	2.3	5.2	1.7	0.229	0.397	0.408
699	a	heptane	4.3	5.1	3.4	4.1	4.4	0.4	0.100	0.730	0.052
999	a	decane	1.2	2.1	1.5	1.9	1.4	0.3	0.546	0.491	0.988
Aromatic hydrocarbons											
-	c	methyl-benzene	15.1	44.7	50.8	49.8	26.3	11.5	0.458	0.382	0.728
875	c	1,3-dimethyl-benzene	17.2	13.9	14.8	15.5	15.0	0.8	0.258	0.697	0.706
976	c	1,2,3-trimethyl-benzene	1.3	1.9	1.1	1.6	1.4	0.4	0.745	0.863	0.277
Maillard reaction (18.1%)											
Acids											
-	c	2-methyl-propanoic acid	3.7	4.2	3.9	4.9	3.1	1.0	0.990	0.397	0.720
839	b	2-methyl-butanoic acid	18.9	21.2	19.1	23.2	16.6	3.3	0.969	0.333	0.556

Table 1.- Volatile compounds (area units-AU- x10^6) detected in the headspace of dry-cured loins from 3 different Iberian x Duroc genotypes.

			Genotype			Sex			Probabilities		
RI[x]	Id. method	Compound	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	interaction
Cont.											
843	b	3-methyl-butanoic acid	6.2	3.8	4.3	5.7	3.9	0.9	0.455	0.279	0.331
Alcohols											
-	c	propan-2-ol	46.8	98.2	43.1	69.3	57.6	14.3	0.222	0.714	0.338
Aldehydes											
-	c	acetaldehyde	40.2	6.9	11.3	6.5	30.2	10.8	0.511	0.276	0.347
647	a	3-methyl-butanal	21.7	23.1	15.8	15.3	24.7	2.7	0.488	0.098	0.310
657	a	2-methyl-butanal	3.2	1.0	2.5	1.0	3.3	1.0	0.736	0.282	0.478
1055	c	benzene acetaldehyde	3.5	3.1	1.7	2.3	3.1	0.6	0.434	0.614	0.390
Ketones											
712	b	3-hydroxy-butan-2-one	78.6	27.5	20.2	67.7	15.8	23.8	0.469	0.229	0.270
Nitrogen compounds											
915	b	2,6-dimethyl-pyrazine	5.0	4.1	4.5	4.5	4.5	0.4	0.701	0.911	0.196
-	c	ethamine-NN-diethyl	5.0	3.1	3.7	5.0	2.9	0.9	0.682	0.253	0.735
Microorganisms esterification (0.8%)											
856	c	butanoic acid 3-methyl-ethyl ester	3.2	4.2	3.2	4.1	3.1	0.4	0.424	0.210	0.423
1034	c	hexanoic acid-1-methylethyl ester	4.0	4.4	4.3	4.4	4.1	0.4	0.948	0.761	0.993
1195	c	octanoic acid methyl ester	0.2	1.3	0.5	0.8	0.6	0.2	0.083	0.762	0.466
Spices (25.2%)											
Acids											
581	c	acetic acid	19.7	10.7	11.1	15.8	11.6	3.9	0.546	0.533	0.259
Sulfur compounds											
864	c	prop-1-ene-3, 3´thiobis	17.0	15.4	12.3	14.9	14.8	1.6	0.520	0.921	0.733
923	b	2- propenyl- methyl-disulfide	3.5	3.9	4.0	4.1	3.5	0.3	0.850	0.316	0.537
1085	c	dipropenyl -disulfide (diallyl disulfide)	51.8	57.9	44.1	48.3	54.0	3.2	0.182	0.418	0.135
Terpenes											
935	b	alpha pinene	106.3	98.6	76.2	93.7	92.9	7.6	0.293	0.913	0.914
1014	b	delta 3 carene	79.8	76.6	68.2	78.2	71.5	7.6	0.823	0.658	0.911
1039	b	1-limonene	7.8	6.8	6.2	7.7	6.1	0.7	0.528	0.198	0.361

[x]RI: retention index in agreement with literature values. [y]SEM: standard error of the mean. [z]: Method of identification: a, mass spectrum and retention time identical with a reference compound; b, mass spectrum and retention index from literature in accordance; c, tentative identification by mass spectrum. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB a,b: Different letters in the same row indicate significant statistical differences (Tukey's test, $p<0.05$)

Table 2.- Volatile compounds (area units-AU- x10^6) detected in the headspace of dry-cured hams from 3 different Iberian x Duroc genotypes.

			Genotype			Sex			Probabilities		
RI[x]	Id. method	Compound	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	interaction
Lipid oxidation (81.6%)											
Acids											
779	b	butanoic acid	13.76	9.35	11.38	11.80	11.20	1.59	0.503	0.813	0.387
970	b	hexanoic acid	36.89	24.56	26.12	27.40	31.22	3.39	0.333	0.695	0.901
1179	b	octanoic acid	0.53	1.03	1.95	1.42	0.86	0.66	0.686	0.647	0.272
Ketones											
502	c	propan-2-one	385.50	388.00	345.92	380.75	367.63	33.47	0.903	0.785	0.022
600	a	butan-2-one	11.18	8.60	33.92	11.86	22.70	5.89	0.194	0.357	0.749
685	a	pentan-2-one	39.15	32.74	31.54	36.14	33.04	4.11	0.772	0.624	0.314
792	b	hexan-2-one	8.83	7.26	3.70	6.25	7.16	1.15	0.228	0.762	0.772
893	a	heptan-2-one	37.44	22.75	22.12	24.81	30.47	3.40	0.155	0.511	0.916
985	b	octane-2,3-dione	19.62	14.77	17.18	16.54	17.84	3.53	0.846	0.872	0.375
991	b	octan-2-one	1.80	0.00	0.00	0.33	0.92	0.42	0.172	0.605	0.758
1068	c	1-etoxi-heptan-2-one	2.20a	0.00b	0.00b	0.66	0.86	0.34	0.008	0.959	0.997
1088	c	non-8-en-2-one	1.80	0.00	0.00	0.38	0.87	0.45	0.228	0.717	0.872
1093	b	nonan-2-one	10.40	0.00	3.15	2.50	6.64	2.53	0.291	0.532	0.788
Alcohols											
482	c	ethanol	70.32	174.53	83.77	117.13	103.93	24.75	0.220	0.920	0.963
680	b	pent-1-en-3-ol	3.90	3.26	4.47	4.05	3.65	0.43	0.496	0.622	0.307
772	a	pentan-1-ol	32.29	19.34	27.82	26.98	25.88	2.43	0.093	0.680	0.940
865	a	hexan-1-ol	70.44a	24.84b	34.72b	43.92	43.40	7.78	0.044	0.779	0.781
900	c	heptan-2-ol	1.64	2.31	1.51	1.95	1.72	0.33	0.552	0.743	0.331
982	c	oct-1-en-3-ol	10.90	6.31	10.77	9.11	9.44	1.14	0.206	0.997	0.821
Aldehydes											
696	b	pentanal	51.00	46.20	54.89	47.39	53.68	5.71	0.871	0.635	0.952
800	a	hexanal	646.60	563.29	809.43	641.47	694.26	48.65	0.160	0.635	0.771
903	a	heptanal	56.05	41.74	49.26	49.48	48.53	5.03	0.519	0.871	0.614
956	a	hepten-(E)-2-al	4.92a	1.65b	2.65ab	3.24	2.94	0.49	0.012	0.560	0.632
1004	a	octanal	46.26a	23.40b	31.24ab	36.56	30.90	3.85	0.039	0.325	0.898

Table 2.- Volatile compounds (area units-AU- x10^6) detected in the headspace of dry-cured hams from 3 different Iberian x Duroc genotypes.

			Genotype			Sex			Probabilities		
RI[x]	Id. method	Compound	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	interaction
Cont.											
1107	b	nonanal	61.94	39.56	44.51	53.08	44.59	4.96	0.151	0.307	0.912
1202	b	decanal	1.17	0.30	0.00	0.51	0.51	0.22	0.094	0.878	0.988
Lineal hydrocarbons											
560	c	2-methyl-pentane	0.97	1.75	1.28	1.59	1.09	0.46	0.685	0.547	0.016
578	c	3-methyl-pentane	0.37	0.61	1.12	1.13	0.24	0.32	0.671	0.166	0.300
698	c	heptane	7.69	2.02	9.36	6.84	5.64	1.50	0.093	0.499	0.117
1002	a	decane	0.00	5.90	1.14	1.94	2.85	1.55	0.247	0.697	0.553
1215	c	2,6-dimethyl-undecane	1.43	1.20	0.00	0.88	0.94	0.32	0.165	0.957	0.510
-	c	dodecane-4-methyl	2.64	2.86	0.00	2.30	1.51	0.56	0.098	0.501	0.876
-	c	nonane-2,6-dimethyl	15.37	9.95	6.26	11.04	10.35	1.68	0.092	0.776	0.549
Aromatic hydrocarbons											
877	c	1,3-dimethyl-benzene	2.00	3.51	7.33	4.31	4.01	1.13	0.175	0.987	0.417
Maillard reaction (12.7%)											
Acids											
758	c	2-methyl-propanoic acid	5.97	5.35	10.80	8.38	6.10	1.42	0.289	0.415	0.827
838	b	2-methyl-butanoic acid	8.15	11.30	26.27	17.40	12.23	3.42	0.088	0.435	0.641
843	b	3-methyl-butanoic acid	1.29b	0.00b	12.56a	6.29	2.33	2.05	0.021	0.243	0.407
Ketones											
711	b	butan-2-one-3-hydroxy	1.21	3.08	0.45	2.04	1.21	0.56	0.168	0.517	0.213
Alcohols											
-	c	propan-2-ol	0.38	30.02	53.49	18.92	35.04	13.10	0.278	0.441	0.340
739	b	3-methyl-butan-1-ol	18.13	6.94	10.24	15.72	7.94	3.87	0.420	0.288	0.444
Aldehydes											
553	c	2-methyl-propanal	10.16	11.07	19.73	12.25	14.59	2.52	0.301	0.623	0.745
647	a	3-methyl-butanal	126.05	72.67	99.12	111.72	86.84	18.00	0.424	0.451	0.301
657	a	2-methyl-butanal	30.67	30.15	37.29	31.48	33.57	2.44	0.511	0.688	0.688
1055	c	Benzeneacetaldehyde	8.77	22.92	6.84	18.94	7.21	5.54	0.539	0.375	0.629
910	c	3-methyl-thio-propanal	1.95	4.16	10.18	1.50	8.99	2.76	0.488	0.167	0.544
Nitrogen compounds											
751	b	pyridine	0.63	4.75	1.24	0.40	4.09	1.29	0.231	0.113	0.153

Table 2.- Volatile compounds (area units-AU- x10^6) detected in the headspace of dry-cured hams from 3 different Iberian x Duroc genotypes.

			Genotype			Sex			Probabilities		
RI[x]	Id. method	Compound	GEN1	GEN2	GEN3	♂	♀	sem	gen	sex	interaction
Cont.											
913	b	2,6 dimethyl-pyrazine	1.47	3.84	0.00	2.08	1.60	0.66	0.081	0.821	0.900
1010	c	trimethyl-pyrazine	0.00	9.70	1.19	0.96	6.49	2.42	0.097	0.174	0.078
Sulfur compounds											
920	c	dihydro-2(3h)-furanone	1.76	1.24	7.97	3.24	3.75	1.24	0.069	0.877	0.663
1065	c	5-ethyldihydro-2(3h)-furanone	4.89a	1.80b	0.00b	2.76	1.87	0.53	0.000	0.116	0.096
1099	c	dipropenyl-disulfide	2.28	6.33	1.93	2.92	4.23	1.01	0.152	0.402	0.735
Microorganisms esterification (0.3%)											
843	c	butanoic acid-1-methylethylester	4.77a	5.33a	0.00b	3.37	3.62	0.83	0.017	0.851	0.681
1034	c	hexanoic acid-1-methylethylester	5.06a	0.55b	2.49ab	1.96	3.46	0.71	0.032	0.367	0.468
Unknown origin (5.4%)											
615	c	acetic acid	121.43	85.20	110.91	108.35	102.96	18.09	0.693	0.870	0.664
1039	b	1-limonene	2.37	0.00	0.00	0.37	1.27	0.64	0.285	0.600	0.752

[x]RI: retention index in agreement with literature values. [Y]sem: standard error of the mean. [z]: Method of identification: a, mass spectrum and retention time identical with a reference compound; b, mass spectrum and retention index from literature in accordance; c, tentative identification by mass spectrum. GEN1: IB x DU1; GEN2: DU1x IB; GEN3: DU2 x IB a,b: Different letters in the same row indicate significant statistical differences (Tukey's test, $p<0.05$)

Several pyrazines, methyl branched aldehydes and their corresponding acids, with origin in Maillard reaction, have been isolated from manufactured hot Spanish paprika by Mateo *et al*., (*17*). These compounds could also contribute together with those formed from raw material to the pleasant flavour of Iberian dry-cured loin.

3.2.- Volatile profile of Iberian dry-cured products as affected by pig genotype

A few compounds showed differences between genotypes in both dry-cured products. In dry-cured loin, no differences were found in the amount of compounds derived from the pickling mixture, as all meat products followed the same manufacture process. Consequently, no significant differences were found due to genotypes in the amount of terpenes and sulfur compounds. Some lipid-derived compounds such as ketones (octane-2,3-dione), aldehydes (hexanal, heptanal), and hydrocarbons (2-methyl-pentane) showed significant differences between genotypes. It is outstanding the significant ($p<0.01$) differences in hexanal content between genotypes, being higher in GEN3 than in GEN1 and GEN2. Hexanal has been considered a good indicator of the oxidative state of meat lipids (*24*) and flavour deterioration since it is responsible for the rancid aroma in meat products when it is present at high concentrations (*11*). Thus, those authors found lower hexanal and TBA-RS numbers in Iberian dry-cured hams with less rancid flavours. However, heptanal, which adds pleasant flavours, was higher in GEN3 than in GEN1 and GEN2. The low number of compounds which showed differences between genotypes in dry-cured loin is in accordance with the results of Muriel *et al*., (*4*), who did not find differences in the volatile profiles of dry-cured loins from different Iberian genotypes. The compounds from the seasoning mixture in dry-cured loin have great importance on the overall flavour of this meat product since sulfur and terpenes compounds were a forth of the aroma compounds detected. The spices added in the seasoning could have contributed to disguise the quality differences previously reported in the other parameters analyzed in fresh meat and in dry-cured loins (*36, 37*) as the high level of the compounds from the seasoning may have reduced the differences in the volatile profile among genotypes.

In dry-cured ham, only 8 compounds showed differences between batches. Hams from GEN1 showed significantly highest contents of 1-etoxi-heptan-2-one, hexan-1-ol, hepten-(E)-2-al, octanal, hexanoic acid-1-methylethylester, butanoic acid-1-methylethylester and 5-ethyldihydro-2(3H)-furanone, while hams from GEN2 showed significantly highest content of butanoic acid-1-methylethylester and hams from GEN3 showed significantly highest content of 3-methyl-butanoic acid. The scarce differences between genotypes could be due to the similar fatty acid composition of intramuscular fat of *Biceps femoris* (*36*) as the changes in the lipids during processing are the main contributors to volatile flavour compounds formation (*8,11*).

Changes in fatty acid composition of IMF affect volatile compounds, especially aldehydes formed during the maturing of the piece (*7, 11*). Cava *et al*., (*11*) reported that hams manufactured from raw meat with lower PUFA contents had lower hexanal content and were perceived as less rancid. Consequently, the differences found in hexanal content in dry-cured loin and the lack of differences in dry-cured ham would agree with the more marked differences of the fatty acids composition in the raw material for the manufacture of these meat products analyzed in fresh (*36*).

3.3. Multivariate analysis.

A principal component analysis (PCA) was carried out to determine the relationship between the volatile compounds detected. PCA of these variables resulted in 5 significant factors that accounted for 56.9% of the variability. The 30.48% and 9.81% of the variability is explained by principal components (PC) #1 and #2, respectively.

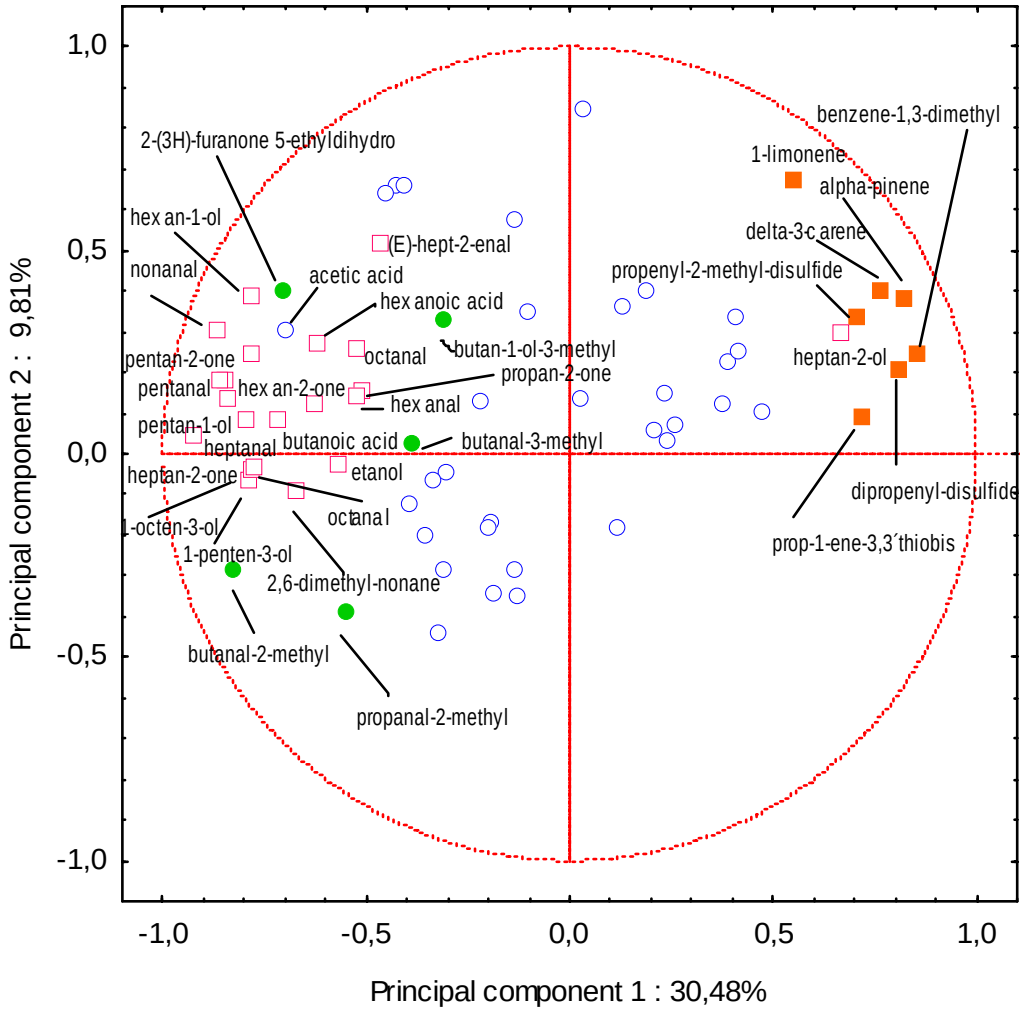

Figure 2.- Loadings plot after principal component analysis of the volatile compounds from dry-cured loin and dry-cured ham in the plane defined by the two first principal components (PC1 and PC2).(○ volatile compounds non identified in the figure, ● volatile compounds with origin in Maillard reaction, ■ volatile compounds with origin in species, □ volatile compounds with origin in lipid oxidation)

Figure 2 shows the score plot of the different variables (coefficients of the eigenvectors) for the two first principal components (PC#1 and PC#2). Although all volatiles were included in the analysis, only volatile compounds which explained more variation of the data have been identified in the figure. In this plot, 2 groups of variables are clearly separated. In the positive axis on the PC#1, far from the origin and explaining an important part of the variation are located volatile compounds isolated in dry-cured loin, mainly those compounds with origin in species such as 1-limonene, alpha-pinene, delta-3-carene, dipropenyl-disulfide, propenyl-2-methyl-disulfide while lipid derived compounds such as aldehydes, ketones and alcohols and Maillard reactions compounds, such as branched aldehydes

and alcohols and furanones had negative loadings. The distribution of the data on the two first PC (Figure 3) shows 2 separate groups of points, corresponding to dry-cured loin and dry-cured ham, while no differences between genotypes were found (data not shown). Loins which had the highest terpenes and sulphur compounds contents were located in the positive area of the PC1, in which terpenes and sulfur compounds showed the highest loadings. Hams were located in the negative area of PC1 in which lipid derived compounds and Maillard compounds had the highest loadings. Therefore, volatile profile seems to characterise adequately dry-cured loins and dry-cured hams and this did not differed between products from different genotypes.

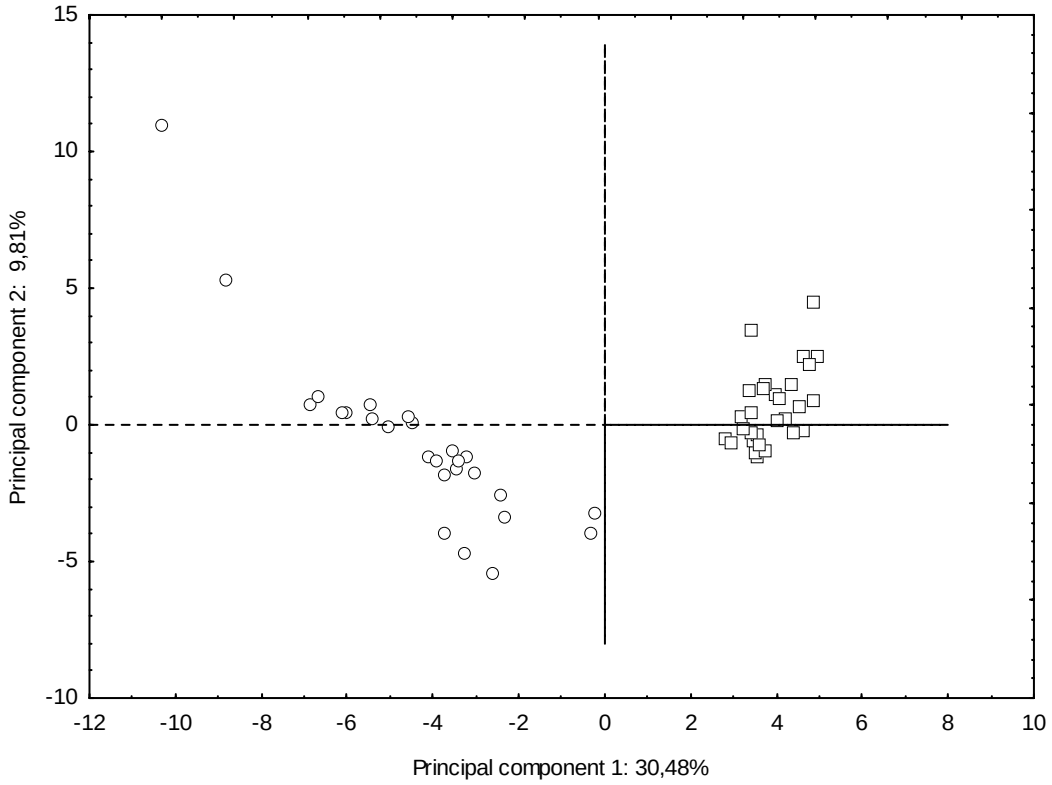

Figure 3.- Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1 and PC2).(○ dry-cured ham □ dry-cured loin)

CONCLUSIONS

Lipid derived volatiles and Maillard compounds were isolated in both meat products although in dry-cured ham were more abundant as a result of the longer ripening process and the greater complexity of the compounds formed. Additionally, volatiles with origin in the seasoning mixture were only isolated in dry-cured loin, therefore, the different manufacture process of these meat products characterize their aromatic profile. However, pig genotype slightly affected the volatile profile of Iberian x Duroc dry-cured meat products.

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Chapter VIII

EFFECT OF PHYSICO-CHEMICAL CHARACTERISTICS OF RAW MUSCLES FROM THREE IBERIAN X DUROC GENOTYPES ON DRY-CURED MEAT PRODUCTS QUALITY[1]

ABSTRACT

The effect of raw material characteristics (*Longissimus dorsi* and *Biceps femoris*) on dry-cured loin and ham quality from 3 different Iberian x Duroc genotypes was studied: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. GEN1 and GEN2 are reciprocal crosses, while the difference between GEN2 and GEN3 is the Duroc sire line. The line Duroc1 (DU1) was selected for the manufacture of dry-cured meat products, whereas the line Duroc2 (DU2) was selected for meat production with low carcass fat. Raw material and dry-cured meat products did not differ between reciprocal crosses (GEN1 vs. GEN2). However, the genotype of the Duroc sire line affected the quality of meat and dry-cured meat products. GEN1 and GEN2 had higher adipogenic nature and higher postmortem pH than GEN3, and as a result, these dry-cured meat products had better instrumental and sensory quality than those from GEN3. By contrast, meat from GEN3 had lower pH, fat content and oxidative stability which decreased the quality of dry-cured meat products. Therefore, there is a close connection between raw material and dry-cured meat products quality as it was affected by characteristics related to the genotype such as the adipogenic character and meat quality traits associated with pH.

Key words: Duroc genotype, Iberian, pH, intramuscular fat, dry-cured ham, dry-cured loin

1.- INTRODUCTION

Different methods are usually utilized in the meat industry to characterize raw materials for the manufacture of dry-cured meat products such as the weight, pH, temperature, intramuscular fat content, etc. However, it is difficult to interpret these measures and to establish a direct effect of them on the quality of dry-cured meat products.

The quality of dry-cured meat products is determined by the quality of the raw material and the manufacture process (Cava and Andrés, 2001). In the literature, there are different studies about the effect of pig genotype on the quality of dry-cured hams (García-Rey *et al*., 2005, 2006). In addition, previous research has related the effect of specific characteristics on fresh meat with the quality of dry-cured meat products. Chemical composition has been related to the sensory properties of dry-cured ham (Ruiz-Carrascal *et al*., 2000), *postmortem* pH affects texture parameters and colour of dry-cured ham (Ruiz-Ramírez *et al*., 2006, Guerrero *et al*., 1999, García-Rey *et al*., 2004), fatty acid profile influences sensory parameters (Cava *et al*., 2000) and instrumental colour in fresh meat is associated with the quality of dry-cured ham (Chizzolini *et al*., 1996). Nevertheless, as far as we concerned the effect of all these parameters together and their relative importance for dry-cured loin and ham quality have never been evaluated.

Duroc breed was introduced in Europe mainly due to its higher intramuscular fat content in comparison with other breeds (Barton-Gade, 1987). In the case of Iberian pig, this breed was introduced to improve productive parameters (López-Bote, 1998). Nowadays, the crosses Iberian x Duroc are so extended that a recent law was passed in 2001 to regulate Iberian market (B.O.E., 15th

[1] Ramírez, M.R. & Cava, R. (2006). Effect of physico-chemical characteristics of raw muscles from three Iberian x Duroc genotypes on dry-cured meat products quality Submitted to Food Science and Technology International for publication.

October 2001). For the manufacture of dry-cured meat products labelled as ''Iberian'', ham and loins must derive from Iberian pure pigs or from Iberian x Duroc crossed pigs (50% or 75%). In the case of crossbreeding, the maternal line must be pure Iberian sows in order to preserve Iberian breed purity. However, Duroc breed is not homogeneous, as due to its widespread distribution, it has been object of different selections. Recently, most pig producers have included genetic selection in their production programmes to improve meat quality (Visscher ***et al***., 2000). Nonetheless, different studies have reported the dissimilarities between Duroc lines and the different quality of dry-cured meat products manufactured from them (Soriano ***et al***., 2005; Cilla ***et al***., 2006). These authors reported that most of the parameters which affected the overall impression of dry-cured ham such as odour, flavour and juiciness were affected by the Duroc sire line in crossbreeding with industrial genotypes. However, this effect would be more marked in the crosses with rustic breeds like Iberian. Therefore, the objectives of this study are characterizing the main quality descriptors in fresh meat which affect dry-cured loin and ham quality and the effect of the genotype in 3 Iberian x Duroc pig genotypes.

2.- MATERIAL AND METHODS

2.1.- Materials.

Animals. 3 groups of 10 pigs each one were studied (5 males and 5 females) from different genotypes: GEN1: ♂ Iberian x ♀ Duroc1, GEN2: ♂ Duroc1 x ♀ Iberian; GEN3: ♂ Duroc2 x ♀ Iberian. The genotype Duroc1 (DU1) corresponded to pigs selected for the production of dry-cured meat products (hams, loins, shoulders), with a high level of fattening. The genotype Duroc2 (DU2) corresponded to animals selected for meat production, with high percentages of meat cuts and low carcass fat. Pigs were castrated, as is traditionally done and they were raised together in a semi-intensive system and were fed *ad libitum.*

2.2.- Methods.

Dry-curing process. For the manufacture of the dry-cured loins, *Longissimus dorsi* (LD) muscle (10loins/genotype) was removed from the carcass and processed into dry-cured loin following a conventional process. The weight of the fresh muscle was 2.5-3.2 Kg, and pH values were lower than 6.0. Loins were processed for a total dry-curing time of 4 months. For the manufacture of dry-cured hams, one ham from each animal was processed (10ham/genotype). The weight of the fresh ham was 14.0-15.5 Kg and pH values were lower than 6.0. Hams were processed for a total dry-curing time of 24 months. After the ripening process, the *Biceps femoris* (BF) muscles were removed from the hams for the analysis.

pH determination. pH values 45 min (pH_{45}) and 24h (pH_U) after slaughter in the BF and LD muscles were measured with a puncture pHmeter Crison mod. 507.

Fat depths. Backfat thickness (BFT) and ham fat thickness (HFT) were measured in the 5^{th} rib and in the m. *Gluteo-biceps* in the carcass and ham, respectively.

Proximate composition. Lipids were extracted, according to Bligh and Dyer (1959). Protein content was determined by the Kjieldahl method (AOAC, 2000) and moisture content was determined by drying the samples (~5 g) at 102ºC (AOAC, 2000).

Instrumental colour. The following colour coordinates were determined: lightness (L*), redness (a*, red±green) and yellowness (b*, yellow±blue). Colour parameters were determined using a Minolta CR-300 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 10º standard observer and a 2.5 cm port/viewing area. In addition, hue angle was calculated (H^o=arctg b*/ a* · 360/2π) as well as the saturation index or chroma (C*) ($C= (a^{*2}+b^{*2})^{0.5}$).

Fatty Acid Profiles. Fatty acid methyl esters (FAMEs) were prepared by transesterification using methanol in the presence of sulphuric acid (5% of sulphuric acid in methanol). FAMEs were analyzed in a Hewlett-Packard model HP-5890A gas chromatograph, equipped with a flame ionization detector (FID) in the conditions described in Cava *et al*., (1997). For the determination of fatty acid profiles of neutral lipid fraction, total lipid extracts from intramuscular fat were fractionated by solid phase extraction on aminopropyl minicolumns (Varian, CA) following the procedure described by Monin *et al*. (2003).

Drip and cook losses. Drip loss was measured following the method of Honikel (1998). Drip loss (%) was calculated by difference in weight between day 0 and day 10 of storage. For cook loss (%), each chop was cooked by immersion at 80ºC for 60 min. The difference of weight before and after cooking was used to calculate cook loss percentage.

Iron-ascorbate induced lipid peroxidation. The susceptibility of muscle tissue homogenates to iron ascorbate-induced lipid oxidation was determined by the method of Kornbrust and Mavis (1980). TBA-RS were expressed as nmol malondialdehyde (MDA)/mg protein. Protein content was measured in muscle homogenates following Lowry method (Lowry *et al*., 1951).

Lipid oxidation. Lipid oxidation was assessed in duplicate by the 2-thiobarbituric acid (TBA) method of Salih *et al*., (1987). TBA-RS values were expressed as mg malondialdehyde/kg meat. Hexanal content was assessed by SPME-GC-MS following the method described by Ramírez and Cava (submitted).

Protein oxidation. Protein oxidation was measured following the method described by Oliver *et al*., (1987). Protein oxidation was expressed as nmol carbonyls/mg protein. Protein concentration was calculated by spectrophotometry at 280 nm using bovine serum albumin (BSA) as standard.

Tissue enzyme activity of lipogenic enzymes*.* Glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) activities were measured in SCF and muscle homogenates at 340 nm at 30 °C for 3 min with a thermospectrophotometer (Thermo Electron Corp. Helios α. Waltham, MA) and expressed on the basis of nmol $NADP^+$ reduced to NADPH per min per mg protein. Glucose-6-phosphate dehydrogenase (G6PDH) was determined by the method of Bautista *et al*., (1988). Malic enzyme (ME) determination was measured according to the method of Spina *et al*., (1970). Protein determination was measured spectrophotometrically at 595nm according to the method of Bradford (1976).

Weight loss during refrigerated storage. To perform the assay, dry-cured loin chops were cut into 1 x 1 x 1 cm cubes. The dry-cured loin cubes were supported with a needle on Styrofoam plates and refrigerated at 4ºC for 10 days. Sample cubes were weighed at day 5 and day 10 of storage. The percentage of loss was calculated by difference of weight.

Texture analysis. Texture analysis was performed in a texturometer TA XT-2i Texture Analyser (STable Micro Systems Ltd., Surrey, U.K.). For the determination of texture profile analysis (TPA) uniform portions of the samples were cut into 1cm^3 cubes. They were axially compressed to 50% of the original height with a flat plunger 50 mm in diameter (P/50) at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from force–deformation curves: Hardness (N/cm^2), adhesiveness (N s), springiness (cm), cohesiveness (dimensionless) and chewiness (N s). For Warner-Bratzler (W-B) analyses, samples were prepared in 2 x 30 x 15mm slices (thickness x length x width) using a slicing machine. Samples were cut with a Warner-Bratzler blade (HDP/BS) in perpendicular direction to the muscle fibres. Determinations were repeated 8 times per sample and were averaged.

Sensory analysis. Fourteen trained panellists formed the tasting panel. A profile of 11 sensory attributes of dry-cured loin and ham grouped in appearance, aroma, texture, flavour and taste were analysed (Table 1). Analyses were developed in tasting rooms with the conditions specified in UNE regulation. The software used was FIZZ Network (version 1.01: Biosystemes, France). Slices of meat products were served on plates to panellists. Panellists evaluated different parameters by means of a quantitative-descriptive analysis in a non structured scale 0-10. Three samples randomly presented to the panellist were analysed in each tasting session.

Table 1. Descriptors of the sensory analysis of the dry-cured meat products.

Descriptors	Definition
Appearance	
colour	Intensity of red colour in the lean (pink-red)
marbling	Level of visible intramuscular fat (very lean-intense marbled)
Aroma	
odour	Level of overall odour before eatingthe sample (odourless - very intense odour)
Texture	
hardness	Firmness perception during chewing (very tender- very firm)
fibrousness	Perception of fibres during chewing (not fibrous-very fibrous)
juiciness	Impression of juiciness during chewing (not juicy - very juicy)
Flavour	
flavour intensity	Intensity of overall flavour (flavourless - very intense flavour)
Taste	
sweet taste	Intensity of sweet taste (not sweet - very sweet)
salty taste	Intensity of salt taste (not salty - very salty)
acid taste	Intensity of acid taste (not acid- very acid)
bitter taste	Intensity of bitter taste (not bitter- very bitter)

Statistical analysis. Pearson correlations coefficients were calculated with the programme SPSS, version 12.0 (SPSS, 2003). Only the parameters with a significant effect are shown in the tables. Parameters which provided more information were selected for the analysis of the principal components (PCA).

3.- RESULTS

3.1.- Effect of fresh *Longissimus dorsi* traits on dry-cured loin quality.

Table 2 shows the correlations between composition, instrumental and sensory parameters from raw *Longissimus dorsi* (LD) and dry-cured loin. Proximate composition of dry-cured loin was closely related to that from LD. IMF contents in raw LD and dry-cured loins were highly positively correlated.

IMF content in fresh LD affected in a great extent the quality of the dry-cured loin. IMF content of LD significantly affected instrumental texture parameters of dry-cured loins as it negatively correlated with hardness and chewiness. In addition, this parameter was related to the sensory characteristics of the dry-cured loin, as suggested the positive correlations found for marbling, juiciness and sweet taste, and the negative ones with fibrousness, acid and salty tastes. The IMF content of the LD positively correlated with instrumental colour coordinates (CIE L*, a*, b) of dry-cured loin, which could indicate that LD with higher IMF content provided also a more intense colour in the dry-cured product.

Significant positive correlations were found between back fat thickness (BFT) and dry-cured loin colour (a* and b*) and sensory parameters (marbling, odour, juiciness and sweetness), while BFT and instrumental hardness were negatively correlated. On the other hand, the activity of the lipogenic enzyme G6PDH in the LD muscle correlated negatively with instrumental hardness, springiness and chewiness, with salty and acid tastes and with hexanal content, and positively, with sweet taste of dry-cured loin. In contrast, ME activity correlated positively with odour intensity of dry-cured loin.

A close relationship between ultimate pH values of raw LD and some traits of dry-cured loins were found. pH_u negatively correlated with weight loss during storage, with certain instrumental texture parameters, such as springiness and chewiness, and with hexanal.

Instrumental colour in raw LD showed close relationships with some parameters in dry-cured loin. In LD, CIE L* correlated positively with springiness and hexanal content of dry-cured loin, while CIE a* correlated positively with IMF content, with CIE a* and CIE b*, with marbling and with sweetness of the final product.

Drip-loss (%) correlated negatively with IMF content and positively with hexanal content in dry-cured loin. Cook loss (%) correlated negatively with instrumental colour (a* and b*), with some sensory characteristics (marbling, colour intensity, juiciness and sweet taste) and positively with instrumental texture parameters (springiness and chewiness), with salty and acid tastes and with hexanal content.

PUFA content of IMF in the raw LD and IMF content of loins were negatively correlated. Furthermore, negative correlations were found between PUFA and instrumental colour parameters (CIE L*, a* and b*), and sensory parameters such as marbling, juiciness and sweet taste; however it was positively correlated with instrumental hardness and chewiness and with salty taste in dry-cured loin.

Parameters related to the oxidative susceptibility of the muscle in fresh such as iron-induced lipid peroxidation values (IILP) were associated with the colour of the dry-cured loin, as show the negative correlations between IILP values and dry-cured loin instrumental colour coordinates (CIE L*, a*, b*).

Texture of the final product was affected by parameters linked to the oxidative susceptibility, since IILP was positively correlated with instrumental springiness and chewiness and with sensory fibrousness. IILP values showed a positive correlation with hexanal content in dry-cured loin. TBA-$RS_{day\ 10}$ correlated positively with weight loss during storage and springiness of dry-cured loin.

Table 2.- Pearson coefficient correlations between selected parameters from raw m. *Longissimus dors*i and dry-cured loin.

		dry-cured loin																		
		Prox.Comp.	Technol. Qual.	Instrumental color			Instrumental texuture					Sensory analysis							Lipid oxidation	
		IMF	WI	L	a*	b	WBSF	hard	adhesiv.	springiness	chewin.	marbling	odour	fibrous.	juicin.	sweetn.	saltin.	acid	TBA	hexanal
m. *Longissimus dorsi*	IMF	0.886***	-0.146	0.840***	0.739***	0.724***	-0.348	-0.574***	-0.251	-0.351	-0.471**	0.774***	0.354	-0.438*	0.614***	0.510**	-0.444*	-0.421**	-0.049	-0.431*
	BFT	0.356	-0.169	0.195	0.533**	0.453*	-0.118	-0.413*	-0.194	-0.225	-0.341	0.513**	0.508**	0.000	0.493**	0.625***	-0.321	-0.332	0.161	-0.301
	G6PDH	0.303	-0.287	0.244	0.344	0.364	-0.015	-0.483**	-0.070	-0.507**	-0.599***	0.331	0.224	-0.282	0.199	0.372*	-0.458*	-0.380*	-0.095	-0.584***
	ME	0.036	-0.201	-0.020	0.233	0.159	-0.044	-0.183	-0.042	-0.053	-0.160	0.282	0.485**	-0.009	0.233	0.333	-0.029	-0.047	0.332	-0.143
	pH_{45min}	0.198	-0.033	0.285	0.216	0.288	-0.168	-0.073	0.027	-0.148	-0.109	0.061	0.193	-0.182	0.170	-0.054	0.053	-0.018	0.153	0.144
	pH_U	0.047	-0.369*	0.034	0.213	0.223	0.096	-0.291	0.078	-0.473**	-0.472*	0.157	0.289	-0.143	0.128	0.214	-0.133	-0.184	0.206	-0.513**
	CIE L*	0.214	0.326	0.246	0.037	0.030	-0.063	0.039	-0.064	0.443*	0.331	0.161	-0.111	0.068	0.139	0.066	-0.020	0.179	0.032	0.542**
	CIE a*	0.391*	-0.047	0.243	0.426*	0.410*	-0.290	-0.257	-0.179	0.100	-0.062	0.421*	0.266	-0.143	0.307	0.460*	-0.200	-0.239	-0.088	-0.042
	DL	-0.410*	0.144	-0.288	-0.263	-0.238	0.099	0.327	0.178	0.331	0.317	-0.348	-0.079	0.340	-0.273	-0.273	0.360	0.345	0.027	0.680***
	CL	-0.353	0.341	-0.252	-0.430*	-0.480**	-0.062	0.363	0.141	0.473**	0.484*	-0.424**	-0.390*	0.193	-0.383*	-0.505**	0.455*	0.409*	0.146	0.673***
	$PUFA_{IMF}$	-0.824***	0.211	-0.571***	-0.661***	-0.612***	0.280	0.476**	0.154	0.292	0.375*	-0.793***	-0.343	0.242	-0.522**	-0.540**	0.531**	0.340	0.073	0.256
	$PUFA_{NL}$	-0.561**	0.287	-0.351	-0.402*	-0.397*	0.095	0.398*	-0.052	0.285	0.344	-0.618***	-0.155	0.199	-0.363	-0.411*	0.423*	0.297	0.160	0.341
	IILP	-0.388*	0.147	-0.434*	-0.451*	-0.574**	-0.110	0.374	0.047	0.388*	0.385*	-0.418*	-0.234	0.410*	-0.344	-0.339	0.354	0.343	0.059	0.653***
	TBA_{d0}	-0.036	0.258	0.083	-0.033	-0.099	-0.419	-0.101	-0.392*	0.406*	0.210	0.047	0.012	-0.078	0.170	0.107	0.041	-0.066	-0.006	0.276
	$TBAd_{10}$	0.126	0.533**	0.301	0.010	0.026	-0.307	-0.096	0.036	0.459*	0.286	0.176	0.004	-0.171	0.187	0.001	0.257	0.261	0.355	0.361
	$Carb_{d10}$	-0.148	0.179	0.007	-0.219	-0.158	-0.200	-0.083	0.010	-0.031	-0.098	-0.239	-0.381*	-0.114	-0.109	-0.166	-0.102	-0.191	-0.055	-0.028

IMF: intramuscular fat; BFT: backfat thickness, G6PDH: glucose 6 phosphate dehydrogenase; ME: malic enzyme; pHu: pH ultimate; DL: drip loss; CL: cook loss, $PUFA_{IMF}$: polyunsaturated fatty acids from intramuscular fat; $PUFA_{NL}$: Polyunsaturated fatty acids from neutral lipid fraction; IILP: iron-induced lipid peroxidation min 200; TBA_{d0}: TBA-RS day 0; TBA_{d10}: TBA-RS day 10; $Carb_{d10}$: carbonyls day 10; prox. Comp: proximate composition; WBSF: Warner-Bratzler shear force, Hard: hardness TPA, wl. weight loss.

* Bilateral significant Pearson's correlation at level p<0.05 ** Bilateral significant Pearson's correlation at level p<0.01; *** Bilateral significant Pearson's correlation at level p<0.001

Table 3.- Pearson coefficient correlations between selected parameters from raw m. *Biceps femoris* and dry-cured ham.

		dry-cured ham															
		Prox. Comp.	Instrumental color			Instrumental texture		Sensory analysis								Lipid oxidation	
		IMF	L	a*	b*	WB	hard	colour	marbling	hardness	fibrous.	juiciness	aroma	sweetness	acid	TBA	hexanal
m. *Biceps femoris*	IMF	-0.124	0.136	0.376	0.685***	0.252	-0.012	-0.040	-0.014	0.267	-0.022	-0.034	-0.009	-0.218	0.016	0.030	-0.067
	BFT	0.159	0.191	0.434*	0.245	0.035	-0.189	0.226	0.536***	0.137	-0.255	0.266	0.230	0.122	-0.255	0.126	-0.141
	HFT	-0.243	0.243	0.469	0.273	-0.113	-0.398*	0.038	0.197	0.029	-0.066	0.298	0.161	-0.042	-0.344*	-0.115	-0.032
	G6PDH	0.003	0.383	0.364**	0.210	-0.188	-0.181	0.019	0.411*	0.026	-0.132	0.249	0.291	-0.073	-0.539	-0.198	-0.366
	ME	-0.019	0.130	0.602***	0.569***	-0.072	-0.165	0.128	0.147	-0.258	-0.569***	0.333	0.157	0.114	-0.393**	0.005	-0.243
	pH_{45min}	-0.026	0.185	0.263	0.084	-0.301	-0.174	-0.031	0.047	-0.225	-0.127	0.155	0.149	-0.008	-0.393*	-0.334	0.051
	pH_U	0.098	0.038	0.244	-0.105	-0.082	-0.078	0.259	0.292	0.096	-0.105	0.029	0.128	-0.110	-0.361	-0.619***	-0.337
	$CIEL^*_{d0}$	-0.083	-0.113	0.026	0.313	0.145	-0.005	-0.188	-0.211	0.002	0.071	-0.065	0.007	0.259	0.332	0.536**	0.309
	$CIEa^*_{d0}$	0.109	-0.026	-0.309	-0.309	0.012	0.332	-0.019	0.192	0.157	0.284	0.085	-0.045	0.109	0.033	0.092	-0.075
	$CIEb^*_{d0}$	0.012	-0.140	-0.201	0.131	0.087	0.070	-0.098	-0.135	0.019	0.209	-0.052	-0.087	0.224	0.273	0.442*	0.104
	$CIEa^*_{d10}$	0.261	0.273	0.137	-0.084	-0.157	0.153	0.070	0.549**	0.053	-0.125	0.220	0.278	-0.074	-0.412*	-0.433*	-0.209
	DL	-0.045	-0.260	-0.165	-0.067	0.310	0.148	0.016	-0.587**	0.001	0.108	-0.200	-0.463*	-0.198	0.317	0.312	-0.090
	CL	-0.024	0.043	-0.245	0.194	0.249	0.060	0.077	-0.080	0.243	0.107	-0.119	0.092	0.123	0.197	0.226	0.153
	$PUFA_{IMF}$	0.116	-0.110	-0.285	-0.579**	-0.121	-0.232	-0.099	-0.033	-0.371	-0.097	0.221	-0.058	0.327	-0.130	0.019	-0.065
	$PUFA_{NL}$	0.267	0.237	-0.226	-0.281	-0.180	-0.373	-0.351	-0.131	-0.477*	-0.171	0.212	-0.166	0.306	-0.308	0.037	-0.206
	IILP	-0.058	-0.008	0.264	0.345	0.165	-0.132	-0.179	-0.159	-0.142	-0.304	0.184	-0.039	0.209	0.127	0.651***	0.296
	TBA_{d0}	-0.256	-0.371	-0.080	-0.118	0.068	0.034	-0.054	-0.378	-0.023	0.093	-0.223	-0.404*	-0.099	0.344	0.351	-0.015
	TBA_{d10}	-0.025	-0.356	-0.113	-0.282	0.143	-0.025	0.188	-0.266	-0.040	0.357	-0.021	-0.066	0.158	0.263	0.413*	0.286
	$Carb_{d10}$	-0.137	-0.065	-0.056	-0.281	0.395*	-0.109	-0.429*	-0.273	0.275	-0.022	-0.204	-0.179	0.073	-0.002	0.011	-0.110

IMF: intramuscular fat; BFT: backfat thickness, G6PDH: glucose 6 phosphate dehydrogenase; ME: malic enzyme; pHu: pH ultimate; DL: drip loss; CL: cook loss, $PUFA_{IMF}$: polyunsaturated fatty acids from intramuscular fat; $PUFA_{NL}$: Polyunsaturated fatty acids from neutral lipid fraction; IILP: iron-induced lipid peroxidation min 200; TBA_{d0}: TBA-RS day 0; TBA_{d10}: TBA-RS day 10; $Carb_{d10}$: carbonyls day 10; prox. Comp: proximate composition; WBSF: Warner-Bratzler shear force, Hard: hardness TPA. colour: colour intensity.

* Bilateral significant Pearson's correlation at level p<0.05 ** Bilateral significant Pearson's correlation at level p<0.01; *** Bilateral significant Pearson's correlation at level p<0.001

3.2.- Effect of *fresh Biceps femoris* traits on dry-cured ham quality.

Correlations between parameters of BF and dry-cured ham followed similar pattern than in dry-cured loin (Table 3), however, they showed a lower number of significant correlations than those previously described.

Surprisingly, no significant correlation was found between IMF content in raw BF and dry-cured BF, although significant positive correlations were found between fat thickness measurements and marbling in ham. HFT correlated negatively with instrumental hardness and with acid taste of dry-cured ham. Among others, the activity of G6PDH lipogenic enzyme correlated positively with marbling.

Negative correlations were found between postmortem pH_{45min} and acid taste and between pH_U and TBA-RS in dry-cured ham. Lightness (CIE L*) and yellowness (CIE b*) of BF correlated positively with TBA-RS of dry-cured ham while CIE a* did not correlated with any parameter of dry-cured ham. However, values of CIE a* after storage correlated positively with marbling, and negatively with acid taste and with TBA-RS numbers.

Drip loss showed negative correlations with marbling and aroma, whereas cook loss did not correlate with other parameters.

PUFAs of NL were negatively correlated with sensory hardness. Lipid oxidation values such as TBA-$RS_{day\ 0}$ were negatively correlated with aroma intensity of dry-cured ham. Besides, TBA-$RS_{day\ 10}$ and IILP in raw material were associated with TBA-RS of dry-cured ham. On the other hand, carbonyl concentration from protein oxidation (day 10) showed positive correlations with instrumental texture parameters such as with WB shear force and negative with the colour intensity perceived by panellists.

3.3.- Multivariate analysis

A principal component analysis (PCA) was carried out to determine the relationship between the selected studied traits of *Longissimus dors*i and dry-cured loin parameters. The PCA of these variables resulted in 5 significant factors that accounted for 62.74% of the variability. Principal components, PC1 and PC2, explained the 27.27% and 15.42% of the data variation, respectively. Figure 1 shows the score plot of the different variables (eigenvectors coefficients) for the two first principal components (PC1 and PC2). The distribution of individuals on the two first PC (Figure 2) shows 2 separate groups of points GEN1 and GEN2 located in the positive *x* axis, while the GEN3 is located in the negative *x* axis. In dry-cured loin, the most important parameters associated with GEN1 and GEN2 and which differentiate those meat and meat products from GEN3 were those parameters related to the adipogenic character of the pigs such as fat thickness, lipogenic enzyme activity, IMF content of the LD as well as marbling and IMF content of LD and dry-cured loin. In addition, GEN1 and GEN2 were associated with postmortem pH, instrumental colour parameters of LD and dry-cured loin and some sensory characteristics of loins such as odour, juiciness and sweetness. In contrast, the parameters associated with GEN3 were cook and drip losses, PUFA content, IILP of LD as well as hexanal content, instrumental texture (hardness, springiness and adhesiveness) and salty and acid tastes of dry-cured loin.

Similarly, another PCA was carried out to determine the relationship between the selected studied traits in *Biceps femoris* and in dry-cured ham parameters. The PCA of these variables resulted in 5 significant factors that accounted for 61.61% of the variability. Principal components, PC1 and PC2, explained the 20.28% and 15.61% of the variation of the data, respectively. Figure 3 shows the score plot of the variables for the two first principal components (PC1 and PC2). The distribution of genotypes on the two first PC (Figure 4) shows 2 separate groups of points GEN1 and GEN2 located in the positive *x* axis, while the GEN3 is located in the negative *x* axis. Parameters associated with GEN1 and GEN2 in raw material were postmortem pH and those related to the adipogenic character of the pigs such as BFT, HFT and lipogenic enzyme activity, while those related to dry-cured ham were instrumental colour values and sensory parameters (marbling, juiciness and aroma intensity). However, the parameters associated with GEN3 were lightness (L*), lipid and protein oxidation, drip and cook losses and PUFA content of BF as well as texture parameters such as hardness, fibrousness and adhesiveness of dry-cured ham.

Therefore, the higher adipogenic characteristics of the pigs from GEN1 and GEN2 and their higher pH value and low cook and drip losses together with higher oxidative stability are the main factors that differentiate raw meat and dry-cured meat products from these genotypes to those from GEN3, which had a poorer quality.

4.- DISCUSSION

Parameters related to the fat content of meat such as fat thickness, IMF content, lypogenic enzymes activity were some of the most determinant parameters of the differences between genotypes in fresh and on dry-cured meat products. The level of fattening of the pieces such as the subcutaneous fat thickness (BFT and HFT) as well as the IMF content affects the drying process, as it is necessary a thick fat covering the ham to avoid an excess of dehydration of the pieces. The positive effect of high IMF content in dry-cured meat products has been previously reported by other authors (Cava *et al*., 2000; Ruiz-Carrascal *et al*., 2000). These reported an improvement of the juiciness in those pieces with higher IMF content. In addition, IMF content had a positive effect on texture and appearance of hams, such as in oiliness, brightness, juiciness and marbling while it was negatively related to dryness, fibrousness and hardness (Ruiz-Carrascal *et al*., 2000). We have found similar results as IMF content enhanced texture and appearance of dry-cured meat products. On the other hand, although IMF did not correlate with odour and flavour intensity, it could affect the formation of volatile compounds with sweet taste, as indicated the positive correlations between both parameters. An adequate IMF content affects the formation of aroma compounds such as lipid derived compounds and Maillard compounds in dry-cured meat products (Shahidi, *et al*., 1986; Mottram, 1998) which could provide sweet taste. In addition, the higher the IMF content, the lower the diffusion rate of sodium chloride through the piece, due to the lower diffusion coefficient of salt in the fat than in the lean tissue, which could favour as a result sweet taste perception (Arnau, *et al*., 1997).

Genotypes with higher IMF content (GEN1 and GEN2) had better colour in both muscles in fresh than GEN3, which had an intense paleness (high CIE L*). This could explain the positive relationship between IMF and instrumental colour values of dry-cured meat products. Previous studies about the

effect of meat quality on dry-cured ham quality (Chizzolini ***et al***., 1996) have found a link between low postmortem pH and colour before curing with the colour of dry-cured hams. Although, they also reported that processing had a positive effect on the colour of pale meat.

The raw material from Iberian pigs does not present usually problems associated with low postmortem pH, in contrast with other selected genotypes such as Pietrain, Landrace, Large White, etc. However, the inclusion of selected genotypes in the crosses with Iberian pig could increase the incidence of problems associated to a low muscle pH.

Low postmortem pH favours the appearance of defectives colour and textures in dry-cured hams (García-Rey ***et al***., 2004; 2006), which affect negatively consumer acceptability (Cilla ***et al***., 2005).

On the other hand, proteolytic activity of the cathepsin enzymes is maintained throughout the dry-curing process (Sárraga ***et al***., 1993; Toldrá and Etherington, 1988) and is favoured at low pH. Hams with increased peptide and free aminoacids concentrations are bitter (Virgili ***et al***., 1998, Ruiz ***et al***., 1999), saltier and more acids (Careri ***et al***., 1993). That could be related to the more intense perception of salt and acid tastes in dry-cured products from GEN3.

The relationship between low postmortem pH and the colour stability of meat products has been broadly studied as oxidation of myoglobin is pH dependent (reviewed by Baron and Andersen, 2002). Low postmortem pH favour the oxidation of myoglobin avoiding their linkage with nitrite compounds which decrease colour intensity in dry-cured meat products. On the other hand, Juncher ***et al***., (2003) reported a significant effect of postmortem pH in the oxidative and colour stability during the storage of nitrite-cured cooked meat products. These results are in accordance with those previously reported by Sárraga and García-Regueiro (1998) who studied in-vitro the microsomal fraction in the processing conditions of dry-cured ham and found higher lipid oxidation development in PSE pigs. Similarly, Cilla ***et al***., (2006) reported that modified-atmosphere-packaged ham slices had intense changes affecting sensory quality, particularly in visual appearance, flavour loss, off-flavour formation, saltiness and rancidity due to oxidation development caused by low pH. This could explain the greater oxidative susceptibility of dry-cured products from pigs with lowest postmortem pH.

The measurement of weight loss of dry-cured meat products is important because the excessive drying of the surface affects negatively their appearance and acceptability when they are sliced and packaged (Ventanas and Córdoba, 1992). The relationship between weight loss with PUFA content and TBA-RS_{day10} values in dry-cured loin could be due to the lower water holding capacity of meat with low postmortem pH (reviewed by Huff-Lonergan and Lonergan, 2005), which could have favoured the exudation of water in the dry-cured meat product. Similar results were found by Isabel ***et al***. (1999) who related weight loss of dry-cured meat products with the extent of lipid oxidation reactions. Some authors (Monahan ***et al***., 1994) have suggested that there is a relationship between the oxidation of membrane phospholipids during storage and weight loss since the integrity of the membrane could inhibit the passage of sarcoplasmic fluid through the muscle and cell membrane.

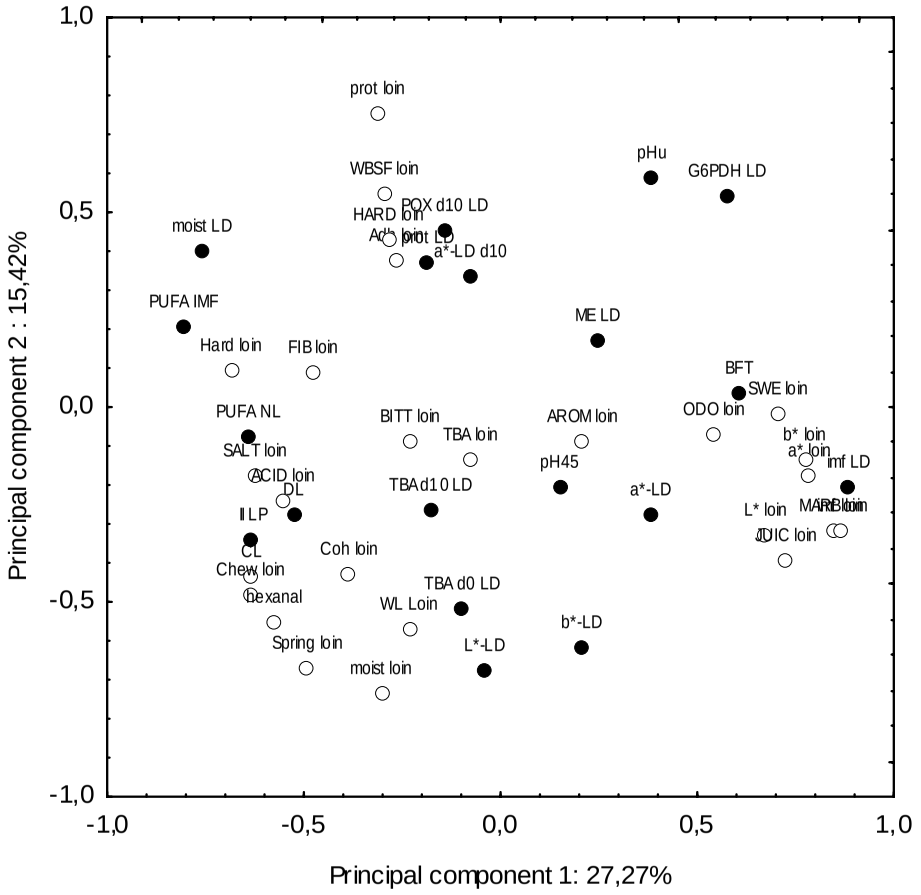

Figure 1.- Loadings plot after principal component analysis of the variables of *L. dorsi* and dry-cured loin in the plane defined by the two first principal components (PC1 and PC2). ● raw LD, ○ dry-cured loin.

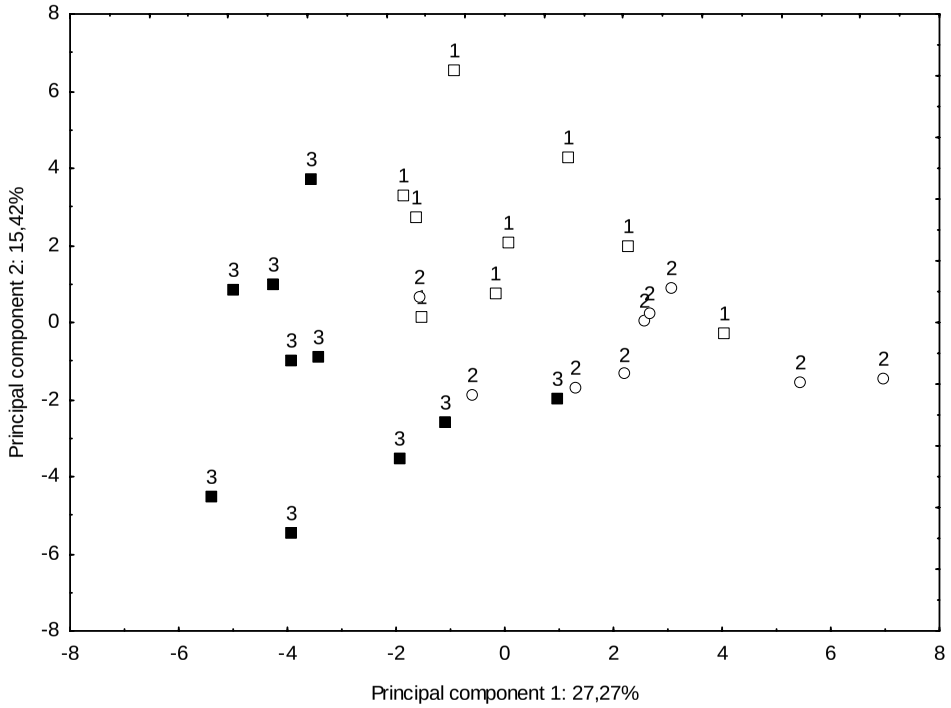

Figure 2.- Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1 and PC2) of the variables of *L. dorsi* and dry-cured loin. (□ GEN1, ○ GEN2, ■ GEN3.)

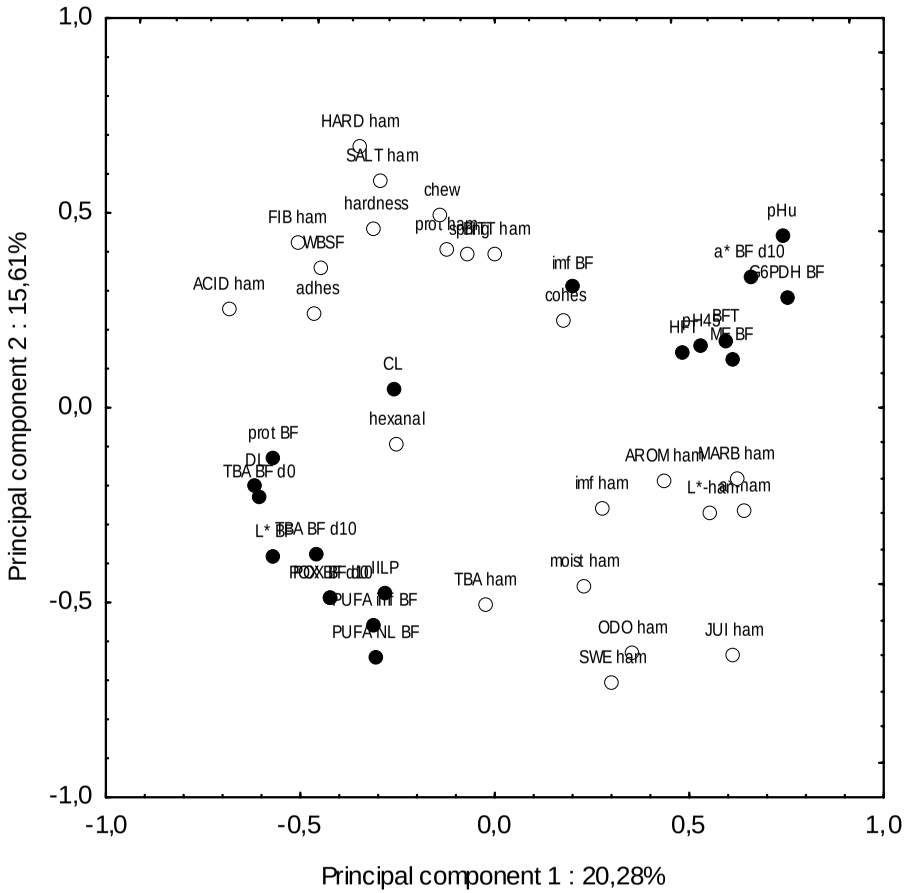

Figure 3.- Loadings plot after principal component analysis of the variables of *B. femoris* and dry-cured ham in the plane defined by the two first principal components (PC1 and PC2). ● raw BF, ○ dry-cured ham.

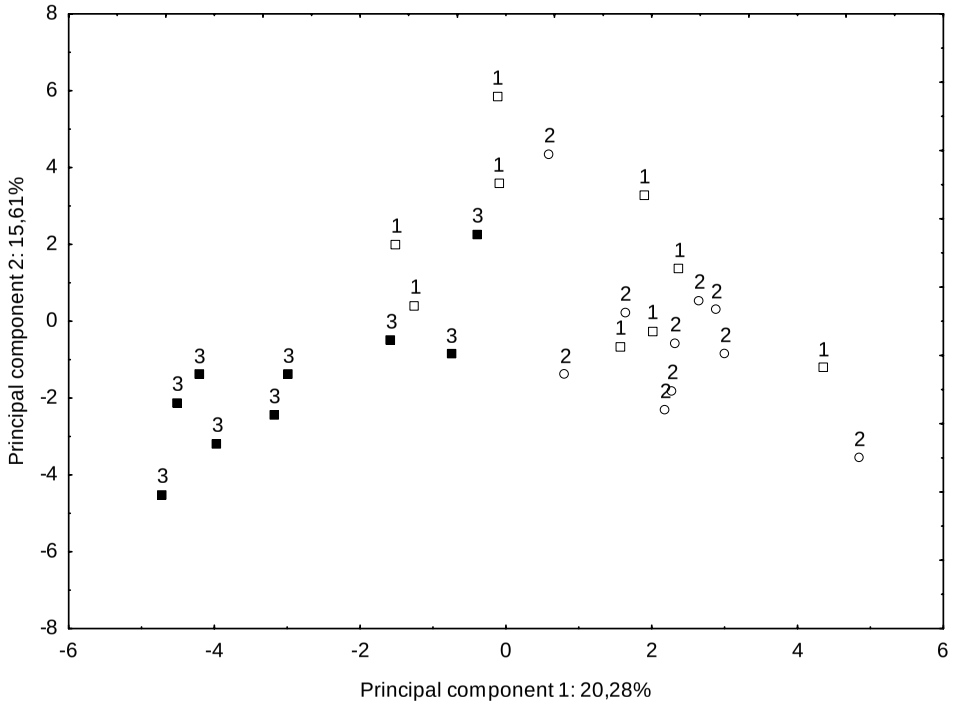

Figure 4.- Scores plot after principal component analysis of the individuals in the plane defined by the two first principal components (PC1 and PC2) of the variables of *B. femoris* and dry-cured ham. (□ GEN1, ○ GEN2, ■ GEN3.)

Fatty acid composition of IMF plays an important role in the hardness, aroma and appearance of dry-cured ham (Ruiz-Carrascal *et al*., 2000; Cava *et al*., 2000). High PUFA content favour the development of oxidation reactions as those fatty acids are which mostly suffer lipolytic reactions during ripening (Martin *et al*., 1999) and they are more susceptible to be peroxidized. Lipid oxidation reactions increase during the maturation of dry-cured meat products. This increase has been monitored by TBA-RS method and by hexanal content (Cava *et al*., 1999). Hams from raw meat with lower linoleic acid, linolenic acid and arachidonic acid had lower hexanal content and were perceived as less rancid by panellists (Cava *et al*., 2000). On the other hand, it is widely recognized the relation between lipid oxidation and myoglobin oxidation (Baron and Andersen, 2002), as high PUFA levels favour the oxidation of myoglobin in the muscle; as the heme group is exposed to the environment favouring peroxidation mechanism (Baron and Andersen, 2002).

Correlations between protein oxidation and colour in dry-cured ham could due to the close relationship between lipid and protein oxidation and oximyoglobin stability (Baron and Andersen, 2002). Ventanas *et al*. (2006) reported a parallel development of lipid and protein oxidation reactions after the ripening of dry-cured loin. They also reported the relation between PUFA content in phospholipids of LD and the extent of lipid and protein oxidation reactions in dry-cured loin. Other authors have reported similar results to ours, as they found that protein oxidation can negatively affect the sensory quality of fresh meat and meat products in terms of texture, tenderness and colour (Rowe, *et al*., 2004). Rowe *et al*., (2004) have reported that increased oxidation of muscle proteins early postmortem could have negative effects on fresh meat colour and tenderness, as protein carbonyl content was positively related with Warner-Bratzler shear force values (Rowe *et al*., 2004).

Genotype clearly affected the quality of the raw material and the dry-cured meat products manufactured from them. Meat and dry-cured meat products quality was not affected by reciprocal cross, however, the Duroc sire line (DU1, DU2) did. Dry-cured meat products from GEN3, due to their lower level of IMF content and their lower pH, intense paleness and drip loss of the raw material from this genotype, had worse quality than those from the other 2 genotypes (GEN1, GEN2).

Therefore, parameters closely related to the genotype, such as postmortem pH and IMF content, could be deciding factors for the quality of dry-cured meat products. As a consequence, the measurement of these parameters in the raw material for the manufacture of dry-cured meat products may provide determinant information to assure the quality of the meat products.

CONCLUSIONS

Parameters related to the adipogenic character of the pigs (fat depths, IMF, lipogenic enzyme activity) affected characteristics of dry-cured products such as those related to texture and taste. However, raw material with poor meat quality characteristics such as low postmortem pH values, high CIE L*-value and drip and cook losses and with a low oxidative stability could favour the appearance of negative texture traits and the development of pale colours and acid tastes in dry-cured meat products. Those quality traits are strongly associated with the pig genotype. Differences in those parameters in the raw material were closely linked with important quality differences of dry-

cured products. As a consequence, the crossbreeding of Iberian pigs with selected Duroc sire lines noticeably decreased the quality of meat and meat products.

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Discussion

GENERAL DISCUSSION

1. – GROWTH PERFORMANCE, PRODUCTION TRAITS AND CARCASS COMPOSITION.

Pig genotype had a significant effect on production traits and carcass composition while sex did not. Reciprocal crosses (GEN1 vs. GEN2) showed small differences in production traits. Pigs from GEN2 were heavier and had a higher carcass and ham weight than GEN1. However, killing-out percentage, main cuts yields and carcass fat were similar in both genotypes, which indicate a similar carcass composition in both genotypes. Results are in contrast with those reported by Morcuende *et al*. (submitted) in a study with Iberian x Duroc reciprocal crosses, who found a substantial advantage of using Iberian pig as the sire breed in production traits of Iberian x Duroc reciprocal cross.

On the other hand, the Duroc sire line importantly affected growth performance and carcass composition. Duroc lines selected on the basis of productive parameters (DU2) gave genotypes (GEN3) with high weights and percentages of the main cuts and low levels of carcass fat (measured as fat thickness at 5^{th}-6^{th} rib and ham). Although this would be desirable in most pig production, it is not desirable in the case of Iberian pig production because of the positive relationship between fat depths thickness and intramuscular fat content (Hovenier *et al*., 1993; Suzuki *et al*., 2003). A high level of subcutaneous and intramuscular fat are required for the manufacture of high quality dry-cured meat products (hams, shoulders, loins...) because fat provides to the meat product an adequate protection against an excessive dehydration during maturation which favour a technological aptitude for long times of ripening, necessary for the development of their sensory characteristics (Gandemer, 2002). Therefore, the use of genotypes from Duroc sire lines selected for the production of dry-cured meat products (DU1) provides heavier carcasses, though it reduces the meat cuts, with a high carcass fat which is more desirable for meat products production.

2. - MEAT QUALITY

Over the past decade, productive systems have been developed to increase the profitability of Iberian pig production. These changes have been oriented towards the use of Duroc breed in the cross with Iberian to increase reproductive and productive traits and less focused on meat quality traits. Although many factors affect meat quality, genetic plays a substantial role in the production of acceptable pork.

In the current study, in terms of meat quality, meat from GEN3 had lower quality than those from the other 2 genotypes (GEN1 and GEN2). However, meat from the reciprocal cross slightly differed in terms of meat quality, indicating that reciprocal crossing had a slight effect on meat quality. Similarly, Morcuende *et al*., (submitted) and Ventanas *et al*., (2006a), did not find significant differences in meat quality traits of both *Longissmus doris* and *Biceps femoris* muscles from Iberian x Duroc reciprocal crosses.

In contrast, the Duroc sire genotype significantly affected meat quality traits. Post-mortem pH values (pH_{45min} and pH_U) were lower in GEN3 than in GEN1 and GEN2 in both *Biceps femoris* and *Longissimus dorsi* muscles. Additionally, muscles from this genotype were paler (lower CIE L*-value) and had higher drip and cook losses in comparison with muscles from the other two genotypes.

Lindahl *et al*., (2006) found a significant increase of lightness in muscles as a consequence of low pH values early *post-mortem*, and Offer (1991) described that low pH values are related to the denaturation of muscle proteins, particularly myosin. These changes are associated with functional properties, such as textural strength and cook loss of meat and meat products (Offer *et al*., 1988). In line with this, in the current study the relationship between low pH values and cook and drip loss was supported by the significant negative correlations between ultimate pH values and drip loss (r = -0.539, $p<0.001$) and cook loss (r = -0.480, $p<0.001$). Although meat from GEN3 can not be considered PSE, physico-chemical characteristics were close to the traits that define PSE condition (Briskey, 1964), and that lead to a poor meat quality of this genotype.

The Iberian pig is characterized for its elevated lipogenic metabolism which produces high subcutaneous and intramuscular fat (López-Bote, 1998). Duroc breed has a higher proportion of intramuscular fat than other industrial pig breeds and for this reason the Iberian pig is usually crossbred with Duroc. Fat content o both subcutaneous and intramuscular, plays an important role in the quality of the meat and meat products from Iberian pig. For that reason, it is crucial that Iberian x Duroc hybrid pigs maintain meat quality traits that characterize Iberian pig breed, such as the high intramuscular fat content. Therefore, a reduction in the adipogenic potential of the hybrids is not desirable, although a reduction in backfat thickness could be desirable.

IMF content is a variable parameter that depends on the genetic of the pig breed. Important differences have been reported by Soriano *et al*., (2005) and by Cilla *et al*. (2006) in the IMF and BFT of crosses with different Duroc sire lines. However, an adequate level of IMF is determinant for the quality of dry-cured meat products (López-Bote, 1998; Gandemer, 2002). In general, muscles from GEN3 had lower fat content than GEN2 while GEN1 had an intermediate content. Results indicate a noticeable effect of both reciprocal cross and Duroc sire line on the deposition of intramuscular fat in muscles. Proximate composition of meat from reciprocal crosses (GEN1 and GEN2) differed between genotypes although these differences depended upon muscle, being more marked in LD than in BF. In general, GEN2 muscles had higher IMF content than those from GEN1; although differences between them could be caused in part by differences in slaughter weights between these 2 groups (149.6 vs. 165.8Kg.). In the case of meat from GEN2 and GEN3, findings suggest a clear effect of the genotype of the Duroc sire line on intramuscular fat content, since average slaughter weights were similar. Intramuscular fat plays an important role on meat and meat products quality because enhances juiciness and thus, their acceptability (Ruiz-Carrascal *et al*., 2000). Therefore, the highest IMF content of GEN2 makes cuts (loins and hams) more suitable for the manufacture of dry-cured meat products compared to GEN3.

Some authors have described a positive relationship between fat depths thickness and intramuscular fat content (Hovenier *et al*., 1993; Suzuki *et al*., 2003) which is line with the positive correlation between backfat thickness and IMF content in muscle *Longissimus dorsi* (r = +0.416, $p<0.05$) and *Biceps femoris* (r = +0.457, $p<0.05$) in the present study.

Enzyme activities of lipogenic enzymes glucose-6-phosphate dehydrogenase (G6PDH) and malic enzyme (ME) in subcutaneous fat and in both muscles (*Longissimus dorsi* and *Biceps femoris*) were significantly affected by genotype. Both enzymatic activities were lower in GEN3 than in GEN1 and

GEN2, which did not show differences in their enzyme activities agreeing with results of Morcuende *et al*., (submitted). On the other hand, results are consistent with the higher fat depths and IMF content in those genotypes in comparison with GEN3. Results suggest a higher lipogenic character in these genotypes, and as a consequence, a higher synthesis and depot of lipids in pig tissues. Previous studies (Mourot *et al*., 1996; Mourot and Kouba 1998; Morales *et al*., 2002) have shown important differences in the adipogenic pattern due to the genotype. These authors found higher lipogenic enzyme activities in non-selected breeds (Iberian or Meishan) than in industrial pig genotypes (Landrace or Large White).

All traits related to fat depot (back fat depths, intramuscular fat content) and lipogenic metabolism (fat and muscle activities of G6PDH and ME) were affected by the Duroc sire line. Some authors (Lonergan *et al*., 2001; Soriano *et al*., 2005; Cilla *et al*., 2006) have previously found important differences between lines of Duroc in the IMF content and carcass fat, which support the differences on the adipogenic character among genotypes of the present study.

Fatty acids are involved in various technological aspects of meat quality such as on firmness or softness of the fat in meat, especially the subcutaneous and intermuscular (carcass fats) but also the intramuscular (marbling) fat (Wood *et al*., 2003). Fatty acid profiles of the three locations (SCF, LD and BF) showed clear differences due to genotype, although these were more pronounced in SCF and LD. In general, lipids from GEN3 were more unsaturated than those from the other genotypes. GEN3 had the highest content of unsaturated fatty acids in SCF and total lipids of IMF and neutral lipid (NL) and free fatty acids (FFA) fractions in both muscles while GEN1 and GEN2 had a higher content of saturated fatty acids. However, small differences were found between genotypes in the fatty acid profile of polar lipids (PL) which had a similar content of SFA, MUFA and PUFA. These differences in the fatty acid profiles among genotypes could be caused by i. the different content of IMF, and the dilution effect of phospholipids and/or PUFA due to a high content of IMF and a different fat deposition in muscles as well as by ii. the different potential for endogenous synthesis of fatty acids. Both hypotheses are supported by the higher lipogenic enzyme activity and the higher SFA content in those genotypes with higher lipogenic activity. As more developed is the backfat in the carcass and the intramuscular fat depots in muscle, higher is the proportion of fatty acids stored in the tissues arising from "*de novo"* synthesis, specifically SFA and less is the content of PUFA.

High PUFA levels can cause undesirable technological and sensory consequences because PUFA enhances lipid oxidation, leading to meat texture, flavour and colour alterations limiting the quality and acceptability of meat and meat products (Morrissey *et al*., 1998). The effect of fatty acids on shelf life is explained by the tendency of unsaturated fatty acids to oxidation, leading to the development of rancidity (Wood *et al*., 2003). Although the fatty acid profile of GEN3 has a better profile from a nutritional point of view (Simopoulos, 2003), it is more susceptible to lipid oxidation due to the higher PUFA and lower SFA, and therefore, to the development of rancidity, so this genotype is the least suitable for the manufacture of meat products.

Therefore, the cross of Duroc selected sires (DU2) with Iberian pigs affected meat quality, since low post-mortem pH, intense paleness and high cook and drip losses were found in the meat from this cross (GEN3). Besides, it reduces the adipogenic activity, the IMF content and modifies fatty acid

profile increasing unsaturated fatty acids. Although it has positive nutritional effects, it could also decrease the shelf life of the meat and make it more prone to suffer oxidation processes under storage and during the manufacture of dry-cured meat products. On the other hand, the cross of Iberian females with Duroc males selected for the production of meat products (GEN2) and its reciprocal cross (GEN1) produces high percentages of intramuscular fat and better quality characteristics in the meat, making it suitable for both cured meat production and fresh consumption.

3. – CHANGES ON MEAT QUALITY DURING REFRIGERATED STORAGE.

In terms of metabolic pattern, the internal part of BF is considered an oxidative muscle while external portion is considered glycolytic, whereas LD is considered a glycolytic muscle. The metabolic pattern of the studied muscles and their characteristics associated (lipid and PUFA content, heme pigments, etc) determined the oxidative susceptibility of muscles under in vitro conditions, the lipid and protein oxidation and the colour changes under retail display. Brewer *et al*., (2001) reported that the metabolic pattern of muscles and the characteristics associated to it affects colour stability of meat during storage. In this sense, red oxidative muscles have higher myoglobin content than white glycolytic ones, and as a result, a lower CIE L*-value and higher CIE a*-value as well as higher oxidative susceptibility. Consequently, muscles with an oxidative metabolism have a lower shelf-life than muscles with a glycolytic or intermediate metabolism (Cava *et al*., 2003; Morcuende *et al*., 2003).

Reciprocal crosses (GEN1 vs GEN2) showed similar changes after the storage, as they had similar oxidative susceptibility, drip loss and colour and fatty acid profile stability during storage. However, the genotype of the Duroc sire line (DU1 or DU2) influenced significantly changes during storage, which were more intense in meat from GEN3.

The low pH_{45min} and pHu values in muscles from GEN3 muscles could explain the higher release of water during cooking or storage of meat from this genotype as a higher rate of pH decline and low pHu favour a paler meat with low water holding capacity (Juncher *et al*., 2001).

According to Andersen (2000), appearance remains the consumer's only criterion to evaluate the product in the purchase situation. Therefore, colour stability is an important quality factor in chilled meat. BF and LD from GEN3 were paler (higher CIE L*-value), which could be caused by their lower *post-mortem* pH and higher drip loss. Both factors may contribute to increase the paleness of the muscles during storage due to an increase of the exudation of meat (Lindahl *et al*., 2001; Brewer *et al*., 2001). In the last years, the intensive selection carried out in some pig genotypes to improve the lean growth efficiency is associated with the decrease of meat quality and the increase of the appearance of PSE meat (Lonergan *et al*., 2001), which also has lower colour and oxidative stability during storage (Juncher *et al*. 2001).

Oxymyoglobin is the main pigment responsible of the red colour of meat. During the storage, the oxidation of oxymyoglobin to metmyoglobin, causes a decrease of redness (CIE a*-value) as well as increases in meat discoloration (reviewed by Baron and Andersen, 2002). The colour stability or the trend to accumulate greyish-brownish metmyoglobin on the meat surface is related to many factors such as animal, age, breed, sex, muscle type, diet, pH, etc. (Renerre, 1990). The higher

discolouration of BF and in a lesser extent of LD from GEN3 suggest a more intense oxidation of myoglobin in this genotype, which is consistent with their higher TBA-RS values and carbonyls accumulation at the end of the storage. Several authors have found that low ultimate pH values in meat increases its paleness and also favour the decrease of CIE a*-value during the storage (Juncher *et al*., 2001; Huff-Lonergan and Lonergan, 2005) which support results found in GEN3.

Lipid oxidation is one of the primary mechanisms of quality deterioration of meat during refrigerated storage (Gray *et al*., 1996). The extent of oxidation of muscle lipids is usually monitored by TBA-RS measurements. On the other hand, protein carbonyls have their origin mainly in the reaction between aldehydes produced by lipid and protein oxidation reactions. In this respect, several authors reported a parallel development of protein oxidation and lipid oxidation in meat and meat products (i.e. Mercier *et al*., 1998, 2004; Ventanas *et al*, 2006b).

Genotype affected both oxidative deterioration of lipids and accumulation of carbonyls during refrigerated storage; although these changes were only significant in *Biceps femoris* muscle. Muscles from GEN3 had the lowest oxidative stability, with higher TBA-RS and carbonyls contents after refrigerated storage. These higher oxidation could be associated to low post-mortem pH values which is supported by the significant negative correlations found between ultimate pH value and induced lipid peroxidation ($r = -0.616$, $p<0.001$), TBA-RS ($r = -0.275$, $p<0.05$) and carbonyls content ($r = -0.268$, $p<0.05$). This relation has been previously reported by Juncher *et al*. (2001), since the autoxidation of oxymyoglobin is acid-catalyzed, and consequently, a decrease of pH accelerates the autoxidation of oxymyoglobin, decreasing color stability and increasing lipid and also protein oxidation (Huff-Lonergan and Lonergan, 2005). Therefore, pH seems to be an important factor for the deterioration of meat during storage. Lonergan *et al*., (2001) found that the intensive selection of a Duroc line for lean growth efficiency reduced early *post-mortem* pH and caused softer and more exudative pork. Consequently and according to our results, low *postmortem* pH could also decrease pork quality under storage.

Meat stored under low temperatures has a limited shelf-life due to biochemical changes developed after slaughter in which lipolysis and lipid oxidation of muscle lipids play an important role (Gray *et al*., 1996; Morrissey *et al*., 1998). Those changes, which affect fatty acid composition, were more intense in GEN3. After storage, PUFA content decreased in IMF and NL fraction, especially in GEN3, probably caused by the higher C18:2n-6 and C20:4n-6 contents and/or a higher lipolytic enzymes activities in this genotype, which could have favoured a more intense hydrolysis and/or oxidation in muscles from GEN3. However, although PL fraction is considered prime targets for lipid oxidation because of their high degree of unsaturation, small differences were found in the fatty acid composition of this fraction either before or after storage.

An increasing ratio DMA/FA in PL fraction during the storage has been previously associated with a more intense hydrolysis of FA, since the level of DMA remains constant because of the resistance of the ether links, between glycerol and aldehyde fatty acids to lipolysis. That increases the relative proportion of fatty aldehydes while the amount of fatty acids linked to glycerol molecule decreases (Morcuende *et al*., 2003; Estévez and Cava, 2004). In addition, Estévez and Cava (2004) found a

ratio DMA/FA significantly higher in industrial than in rustic pig genotypes after storage of meat, which could be related to the higher DMA/FA ratio in GEN3 than in the other genotypes.

Plasmalogens act *in vivo* protecting cells against oxidative stress (Engelmann, 2004), since their vinyl–ether bond is preferentially oxidized, which protects PUFA at the sn-2 position from oxidation. This antioxidant activity could continue *postmortem* according to Estévez and Cava (2004), who reported lower oxidation levels in pigs with higher fatty aldehydes proportion. Results are consistent with iron-induce lipid peroxidation and TBA-RS values after refrigerated storage in LD, although not with results in BF.

In FFA fraction, the level of PUFA increased after the storage; especially long chain PUFA, agreeing with the reduction in PUFA of other lipid fractions and changes in FA profile in previous studies (Alasnier *et al*., 2000; Morcuende *et al*., 2003). Several enzymes (lipases and phospholipases) are involved in the hydrolytic processes of meat lipids during storage which release FFA from both triacylglycerols and phospholipids (Alasnier *et al*., 2000a; Alasnier *et al*., 2000b). Important variations have been reported in the lipolytic activity associated with the pig genotype (Toldrá *et al*., 1996; Rosell and Toldrá 1998; Cava *et al*., 2004), which could be related to the differences in the hydrolisis of fatty acids between genotypes. In this sense, the highest increase of PUFA in FFA fraction was found in GEN3. It is well know that long chain PUFA are very prone to lipid oxidation (Nawar, 1996). The higher PUFA in FFA could increase the amount of substrates for the oxidative reactions and decrease meat stability during storage, which agrees with the lower lipid and protein oxidation and colour stability in meat from GEN3.

4. - DRY-CURED LOIN AND HAM QUALITY AND VOLATILE PROFILE

4.1.- Dry-cured meat products quality.

Chemical composition of dry-cured loins was significantly affected by pig genotype whereas dry-cured ham composition was similar between genotypes, although followed a similar trend as dry-cured loin. Thus, dry-cured loins from GEN2 had the highest content of intramuscular fat while in dry-cured ham no differences were found. Proximate composition of dry-cured hams and loins reflected proximate composition of *Longissimus dorsi* and *Biceps femoris* muscle in fresh, which did not show differences between genotypes in the latter but they did in the former. An adequate level of fat (intramuscular and subcutaneous) is determinant for the sensory quality of meat products (Ruiz *et al*., 2002) as well as for an adequate drying and salt diffusion mechanisms during maturation (Arnau, 1998).

Colour plays an important role in the appearance and overall acceptability of dry-cured meat products; because a non-intense colour is rejected by consumers due to its association with a shorten ripening (García-Rey *et al*., 2006). Both dry-cured loins and hams from GEN3 had a less intense red colour than those from the other genotypes. The redness (CIE a*-value) of dry-cured products has been associated with i. the heme pigments concentration, ii. the sodium chloride content and iii. the formation of nitrosomyoglobin (Pérez-Álvarez *et al*., 1998). Thus, the lower CIE a*-value and chroma in dry-cured meat products from GEN3 could be related to the less intense colour of raw material from this genotype than those from GEN1 and GEN2. However, the higher

lightness (CIE L*-value) in both dry-cured products from GEN2 could be related to the higher marbling in agreement with findings reported by Carrapiso and García (2005), who found positive correlations between L* and marbling in dry-cured Iberian ham.

Fatty acid composition of IMF of dry-cured loins and hams reflected fatty acid profiles of raw *Longissimus dorsi* and *Biceps femoris.* Differences in the fatty acid profile were caused by the genotype as initial differences in the FA profile were maintained after maturation. Thus, GEN3 had higher MUFA and PUFA contents while intramuscular lipids from GEN2 were more saturated. Raw material rich in PUFA had a higher oxidative susceptibility leading to rancidity in dry-cured ham (Cava *et al*., 1999) and in dry-cured loin (Hoz *et al*., in press). As a result, dry-cured meat products from GEN3, which were more unsaturated in fresh, showed a higher lipid oxidation as reflected the higher TBA-RS and hexanal content, although differences were statistically significant in dry-cured loin and not in dry-cured ham. Similarly, a high PUFA content in the final product could favour the development of oxidation reactions, especially in sliced and packaged products during storage. In addition, hexanal has been widely reported as a good indicator of lipid deterioration. In dry-cured meat products, hexanal contributes to their characteristic flavour, although high concentrations favour the appearance of rancidity and the flavour deterioration (Cava *et al*., 1999). Thus, in Iberian dry-cured hams, Cava *et al*., (1999) found that hams with less rancid flavour had less hexanal and TBA-RS.

Previous studies in Iberian dry-cured products have reported a close relationship between marbling degree and overall quality (Ruiz-Carrascal *et al*., 2000). Dry-cured products from GEN2 were more marbled according to panellist perceptions, which agree with the higher IMF content in dry-cured loin ($p<0.05$) and dry-cured ham ($p>0.05$).

Panellists detected more texture differences than those detected by instrumental analysis in dry-cured ham; although both methods followed a similar pattern in both dry-cured meat products. Dry-cured loins from GEN3 were considered less juicy and had a higher instrumental hardness, springiness and chewiness, and similarly, dry-cured hams from GEN3 were more fibrous and less juicy. Texture has been related to chemical composition of dry-cured ham. Some authors (Ruiz-Ramírez *et al*., 2005; Serra *et al*., 2005) have found that the decrease of moisture content increases hardness. However, others (Ruiz-Carrascal *et al*., 2000) have reported a more relevant influence of the IMF content on textural characteristics of Iberian dry-cured hams. Increasing levels of IMF enhance the juiciness while reduce fibrousness perception (Ruiz-Carrascal *et al*., 2000) which has a positive influence on the overall acceptability of dry-cured ham (Ruiz *et al*., 2002). The effect of IMF was more marked in dry-cured loin as they had higher differences in the IMF content in comparison with dry-cured ham. Therefore, texture of dry-cured products from GEN3 could be related to the lower IMF content and marbling of meat products from this genotype. According to Ruiz *et al*., (2002) the negative influence of some textural traits like fibrousness or juiciness could reduce the acceptability of the dry-cured hams from GEN3.

On the other hand, García-Rey *et al*. (2006) reported the influence of low *post-mortem* pH and genotype in the appearance of defective textures in dry-cured ham. Besides, Ruiz-Ramírez *et al*., (2005) found a close relationship between low *post-mortem* pH and the increases in instrumental

textural traits such as hardness, cohesiveness and springiness of hams. The lowest *post-mortem* pH values in *L. dorsi* and *B. femoris* from GEN3 are in accordance with the pattern of dry-cured meat products in instrumental and sensory texture. Therefore, those parameters previously mentioned, such as the ultimate pH and the intramuscular fat content, which are closely linked to the genotype, have an important effect on dry-cured meat products texture.

Numerous papers have studied the influence of lipids on the formation of aroma compounds since lipid oxidation has great importance in the development of the dry-cured meat products flavour (reviewed by Gandemer, 2002). The lowest IMF content of loins from GEN3 could be related to their lowest odour intensity.

Differences in the concentration of taste compounds could be the main reason of the different taste perceptions. In general, dry-cured meat products from GEN1 and GEN2 were perceived sweeter than those from GEN3 which were saltier and more acids (only in dry-cured ham). This could be caused by a different salt concentration and by a different proteolysis of specific aminoacids or to the biochemical reactions in which they are involved (Ruiz *et al*., 1999a; Andrés *et al*., 2004). In addition, there is an opposite relationship between salt perception and IMF content (Ruiz *et al*., 1999a). Loins and hams with lower IMF content, those from GEN3, were perceived as saltier than those with high IMF content, those from GEN2.

Iberian x Duroc reciprocal crosses produced dry-cured products with similar quality, which agrees with previous studies in Iberian dry-cured loin (Ventanas *et al*., in press). However, the use of Duroc genotypes with non adequate characteristics for the cross with Iberian pig has important consequences on the quality of Iberian dry-cured products. Similar results were found by Soriano *et al*., (2005) and by Cilla *et al*., (2006) who reported an important influence of the Duroc line on the quality of dry-cured hams from different crossbreeding.

4.2.- Volatile profile of dry-cured meat products.

Chemical and biochemical changes that take place during ripening of dry-cured meat products from Iberian pig provide a large number of volatile compounds which contribute to their characteristic flavour (Cava *et al*., 1999; Ruiz *et al*., 1999b; Muriel *et al*., 2004).

A great number of volatile compounds were isolated in dry-cured loins (41 compounds) and dry-cured hams (55 compounds) from different chemical families (acids, ketones, alcohols, aldehydes, esters, nitrogen and sulphur compounds, terpenes, hydrocarbons). The number and the relative proportion of each families and compounds differed significantly between dry-cured loins and hams. Most volatile compounds detected in both meat products had their origin in lipid oxidation and in a less extent in Maillard reactions or in the metabolism of microorganisms. The relative amount of volatile compounds was twice higher in dry-cured ham than in dry-cured loin since headspace of dry-cured ham had a higher amount of lipid derived volatiles (aldehydes, ketones and alcohols) and Maillard compounds than dry-cured loin, while dry-cured loins had some exclusive compounds not detected in dry-cured ham such as certain compounds with origin in the pickling mixture (paprika, garlic, oregano …).

In general, differences in the volatile profile of dry-cured hams and loins could be caused by the different manufacture process and ripening conditions (length of ripening, temperatures…). So, the shorter ripening (4 months vs 24 months) and the lower temperatures reached during the dry-cured loin processing compared to that of dry-cured hams, could have contributed to a more limited development of the chemical reactions involved in flavour compounds generation in dry-cured loin. As a consequence, the formation of lipid and protein derived compounds and the reaction between them to form Maillard volatile compounds could be enhanced in dry-cured ham. On the other hand, dry-cured loin had a higher content of certain volatile compounds from the pickling mixture, such as sulfur compounds and terpenes, which have strong aromatic notes and could play an important role in the overall aroma notes of this meat product. Results are in agreement with literature since flavour compounds of dry-cured products have their origin in the oxidation of unsaturated fatty acids and from amino acids degradation as well as in the reactions between compounds from both origins (Ventanas *et al*., 1992; Ruiz *et al*., 1999b; Carrapiso *et al*., 2002a). However, in dry-cured loin, the importance of these compounds is lessened due to both the addition of spices in the manufacture process and the shorter ripening (Muriel *et al*., 2004).

Volatiles from lipid oxidation were higher in dry-cured ham than in dry-cured loin, which agrees with the longer ripening of dry-cured hams respect to loins. Among lipid-derived compounds straight-chain aliphatic acids, aldehydes, alcohols, ketones and some hydrocarbons are most likely derived from the oxidative degradation of unsaturated fatty acids (Shahidi, 1986). Straight chain aliphatic aldehydes have low odour threshold values, and consequently, play an important role especially in dry-cured ham flavour (Martín *et al*., 2000). Hexanal content in dry-cured ham was approximately twice higher than in dry-cured loins, which is in accordance with the longer ripening time in hams and the development in a higher extent of the lipid oxidation reactions.

Most of the volatiles from Maillard reaction were short-chain branched aldehydes and their corresponding alcohols with origin in Strecker degradation reactions of amino acids (Ventanas *et al*., 1992), which is an important pathway associated with Maillard reaction. 2- and 3-methylbutanal, which were abundantly isolated in dry-cured ham, have low threshold values and provide pleasant “cured” flavours to dry-cured ham (Ruiz *et al*., 1999b). The high proportion of volatiles from Maillard reaction could be related to the high acceptability of these products, since Maillard compounds, which had very low threshold values, add pleasant aroma notes (Mottram *et al*., 1998). The higher amounts and number of compounds with origin in protein degradation in dry-cured ham respect to dry-cured loin show the more implication of Maillard compounds in the aroma of the dry-cured ham.

In contrast, esters, which were originated by microorganism’s esterification, had low percentages in both dry-cured products.

In dry-cured loin, nearly a quarter of the volatiles came from spices (Spanish paprika, oregano and garlic), which were mainly terpenes and to a lesser extent, aliphatic sulfur compounds. Sulfur compounds derived from allicin are characteristic of garlic aroma (reviewed by Lanzotti, 2006), while terpenes could come from the oregano in the pickling mixture.

A few compounds showed differences between genotypes in both dry-cured products while sex did not affect volatile compound profiles.

In dry-cured loin, volatile compounds with origin in the pickling mixture (terpenes and sulfur compounds) showed similar contents as all loins underwent the same manufacture process. However, some lipid derived volatile compounds showed differences between genotypes. Hexanal content was highest in GEN3, which is in line with the higher n-6 fatty acids content in IMF since hexanal mainly derives from oxidation of n-6 fatty acids (Tamura *et al*. 1991). Hexanal has been considered an indicator of flavour deterioration in Iberian dry-cured ham (Cava *et al*., 1999). However, the spices added in the seasoning could have contributed to lessen the differences between genotypes in *L. dorsi* and dry-cured loin quality, which agree with results of Muriel *et al*. (2004).

On the other hand, the scarce differences between genotypes in dry-cured ham could be due to the similar intramuscular fat content and fatty acid composition of *Biceps femoris* between genotypes as the changes in the lipids during processing are the main contributors to volatile flavour compounds formation (Ruiz *et al*., 1999; Carrapiso *et al*., 2002a).

Different authors described that the main cause of variation in volatile profiles in Iberian dry-cured products are those related with the feeding regime features (free-range rearing vs. intensive, length of feeding regime, presence and level of compounds with antioxidant activity and type of dietary fat), being the pig genotype (pure Iberian vs. Iberian x Duroc cross, reciprocal cross ..) less determinant in volatile profile of such products (Cava *et al*., 1999; Muriel *et al*., 2004; Carrapiso *et al*., 2002b).

In relation with volatile profiles findings, panellists did not found significant differences in the flavour and odour intensity of dry-cured hams from the three genotypes while detected lower odour intensity in dry-cured loins from GEN3 than in their counterparts.

5.- RELATIONSHIP BETWEEN RAW MATERIAL AND DRY-CURED MEAT PRODUCTS QUALITY

Meat and dry-cured meat products quality was not affected by reciprocal cross, since no differences were found neither in raw material quality traits nor dry-cured meat products. However, Duroc sire line clearly affected the quality of the raw material and dry-cured meat products. The two main factors of the raw material that affected dry-cured meat quality were those related to the adipogenic potential and the IMF content, and the ultimate pH and those traits affected by its value, such as paleness, cook and drip loss; both, closely linked with the genotype.

Parameters related to the fat content of meat such as fat thickness, IMF content or lypogenic enzymes activity were some of the most determinant parameters of the differences between genotypes in fresh and in dry-cured meat products. These parameters mainly affected characteristics of dry-cured products such as those related to texture and flavour. High IMF content in dry-cured meat products increases the juiciness (Cava *et al*., 2000; Ruiz-Carrascal *et al*., 2000), reduces dryness, fibrousness and hardness (Ruiz-Carrascal *et al*., 2000) and favours the formation of lipid derived aroma compounds (Shahidi *et al*., 1986), which is consistent with characteristics found in dry-cured meat products from GEN2 and GEN1.

The less intensity of colour in dry-cured meat products from GEN3 could be due to their less intense colour in raw material. Additionally, the higher PUFA levels of meat from GEN3 could have favoured

the oxidation of myoglobin in the muscle and as a result of the exposition of heme group to the environment enhancing peroxidation mechanism (Baron and Andersen, 2002).

García-Rey *et al*. (2005, 2006) reported the influence of low *post-mortem* pH and genotype in the appearance of defective textures in dry-cured hams. In addition, an excess of proteolysis due to low pH could enhance bitterness (Virgili *et al*., 1995), saltiness, aged or acid tastes (Careri *et al*., 1993) which could be related to the more intense perception of salt and acid tastes in dry-cured products from GEN3. Low *post-mortem* pH could also affect the colour of dry-cured meat products, as the oxidation of myoglobin is pH dependent (reviewed by Baron and Andersen, 2002). So, low *post-mortem* pH favours the oxidation of myoglobin avoiding their linkage with nitrite compounds which decrease colour intensity in dry-cured meat products. Previous studies (Chizzolini *et al*., 1996) have reported the connection between *post-mortem* pH and the colour before curing with the colour of dry-cured hams. In addition, Juncher *et al*., (2003) reported a significant effect of *post-mortem* pH in the oxidative and colour stability during the storage of nitrite-cured cooked meat products.

Lipid oxidation reactions increase during the maturation of dry-cured meat products. Hams manufactured with raw meat rich in PUFA had higher hexanal content and were perceived as more rancid by panellists (Cava *et al*., 1997) which agrees with the higher content of PUFA in raw LD in GEN3 and the higher oxidation development in dry-cured loin. Dry-cured ham followed a similar trend although differences were not statistically significant. Ventanas *et al*. (2006b) reported a parallel development of lipid and protein oxidation reactions in the ripening of dry-cured loin. Other authors (Rowe *et al*., 2004) have reported that protein oxidation modifications can affect negatively the sensory quality of fresh meat and meat products in terms of texture, tenderness and colour which could be related to the higher protein oxidation levels in raw material from GEN3 and the texture and colour of dry-cured meat products from this genotype.

Raw material from GEN3 had low *post-mortem* pH values, high drip and cook loss and low oxidative stability which could favour the appearance of negative texture traits like fibrousness and the development of paler colours and acid tastes in dry-cured meat products. Therefore, dry-cured meat products from GEN3, due to their lower level of IMF content and the low ultimate pH showed worse quality traits. Parameters closely related to the genotype, such as *post-mortem* pH and IMF content, could be predictors for the quality of dry-cured meat products. As a consequence, the measurement of these parameters in the raw material for the manufacture of dry-cured meat products may provide determinant information to assure the quality of the dry-cured products.

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Conclusions

CONCLUSIONS

1. Iberian x Duroc reciprocal cross scarcely affects meat quality; however, pigs from Iberian sows (GEN2) have better production parameters and dry-cured meat products quality, mainly due to their higher intramuscular fat content.
2. Production parameters are significantly affected by the Duroc sire genotype; Duroc genotypes selected for meat production produce leaner hybrids (GEN3) with higher cut yields than those from Duroc sire genotypes selected for the manufacture dry-cured meat products (GEN2), with more carcass fat and lower cuts yields.
3. *Post-mortem* pH and intramuscular fat content, factors closely linked with pig genotype, are determinant factors for the quality and oxidative stability of the meat. Meat from crossbred pigs with Duroc sires selected for meat products manufacture (GEN2) has better quality because of the higher intramuscular fat content, better ultimate pH and colour.
4. Fat depths, intramuscular fat content and lipogenic enzyme activities are positively correlated. These traits were higher in those crossbred pig genotypes (GEN1 and GEN2) from Duroc sire/sow selected for dry-cured meat products manufacture. The measurement of the activity of these enzymes could be used as pig selection criteria in terms of adipogenic character.
5. Meat deterioration during refrigerated storage is significantly affected by the Duroc sire line genotype. Meat from crossbred pigs with Duroc sire lines selected for meat production (GEN3) is more prone to lipid and protein oxidation and discoloration processes.
6. The Duroc sire line significantly affects the quality of dry-cured products. Dry-cured loins and hams from crossbred pigs with Duroc selected for meat production (GEN3) showed a lower quality than those from crossbred pigs with Duroc selected for dry-cured products manufacture (GEN2).
7. Volatile profile of dry-cured loins and hams is scarcely affected by the pig genotype; it is more affected by the characteristics of the manufacture process. Dry-cured ham has highest lipid derived and Maillard compounds, which is in line with its longer ripening and more intense development of lipid oxidation; while dry-cured loin has large amount of compounds from the pickling mixture added in the manufacture process.
8. Raw material characteristics have an important effect on the quality of dry-cured meat products. Intramuscular fat content and *postmortem* pH of raw material are the main factors responsible of the quality of Iberian dry-cured meat products, being both parameters closely related to the pig genotype.

CONCLUSIONES

1. El cruce recíproco ibérico x duroc afecta mínimamente a la calidad de la carne, aunque los híbridos procedentes de hembras ibéricas (GEN2) presentan mejores parámetros productivos así como una mejor calidad en los productos cárnicos, probablemente por su mayor contenido en grasa intramuscular.
2. El genotipo de la línea paterna duroc tiene un gran efecto en los parámetros productivos; las líneas duroc de genotipo carnicero dan lugar a híbridos (GEN3) más magros y con mayores rendimientos cárnicos que los genotipos duroc seleccionados para la producción de productos cárnicos (GEN2), que muestran un mayor contenido de grasa en la canal y menores rendimientos de piezas nobles.
3. Dos parámetros muy relacionados con el genotipo, como el valor del pH *postmortem* y el contenido en grasa intramuscular, son factores determinantes para la calidad y la estabilidad oxidativa de la carne. La carne de cerdos cruzados con líneas de machos duroc seleccionados para la producción de productos cárnicos (GEN2) es de mejor calidad por su mayor contenido en grasa intramuscular, mejor pH *postmortem* y color.
4. Los depósitos grasos en la canal, el contenido en grasa intramuscular y la actividad de las enzimas lipogénicas son factores estrechamente relacionados. Estos parámetros son más elevados en cerdos procedentes de líneas duroc paternas o maternas (GEN1 y GEN2) seleccionados para la elaboración de productos cárnicos curados. La medida de la actividad de estas enzimas podría ser un criterio útil para la selección de cerdos en función de su carácter adipogénico.
5. El genotipo de la línea paterna duroc afecta a la estabilidad oxidativa de la carne durante su almacenamiento a refrigeración. La carne de cerdos cruzados con líneas duroc de genotipo carnicero es más propensa la sufrir reacciones de oxidación de lípidos y de proteínas así como procesos de decoloración.
6. El genotipo de la línea paterna duroc afecta a la calidad de los productos cárnicos curados ya que los lomos y jamones curados procedentes de líneas duroc de genotipo carnicero mostraron peor calidad que los otros genotipos estudiados.
7. El perfil de compuestos volátiles de los lomos y jamones curados apenas se vio afectado por el genotipo del cerdo mientras que las características propias del procesado mostraron un gran efecto. Los jamones curados mostraron un mayor contenido en compuestos derivados de la oxidación de lípidos y de la reacción de Maillard, coincidiendo con el mayor tiempo procesado y el mayor desarrollo de las reacciones de oxidación lipídica, mientras que el lomo curado tiene mayor contenido de compuestos procedentes del adobo.
8. Las características de la carne fresca tienen gran importancia en la calidad de los productos curados. El contenido en grasa intramuscular y el pH *postmortem*, parámetros muy ligados al genotipo, son los principales factores que influyen en la calidad de los productos curados.