EFFECTS OF BREED AND PRKAG3 AND CAST GENETIC POLYMORPHISMS ON THE QUYALITY OF SERRANO DRY-CURED HAM

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Dipòsit legal: Gl. 1430-2012
http://hdl.handle.net/10803/7xxx

Philosophiae Doctor (PhD) Dissertation

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PhD Programme in Technology

Thesis supervisors: Dr. Pere Gou i Botó

Dissertation presented to compete for
the Philosophiae Doctor degree at the University of Girona

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I HEREBY CERTIFY:

That this work, entitled “*Effects of Breed and PRKAG3 and CAST Genetic Polymorphisms on the Quality of Serrano Dry-cured Ham*”, presented by **ZONGYUAN ZHEN** to obtain the title of doctor, has been carried out under the direction of Dr. **PERE GOU i BOTÓ**, and meets the requirements to obtain the Philosophiae Doctor degree at the University of Girona.

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Girona, June 29, 2012

This work has been carried out at the Food Technology Programme at the research centre IRTA-Monells (Institut de Recerca i Tecnologies Agroalimentàries) within the framework of the following research projects:

- TRUEFOOD - “Traditional United Europe Food” - European Commission Integrated Project within the 6th RTD Framework Programme (Contract No.: FOOD-CT-2006-016264).

- Q-PorkChains - 6th European Union Framework Integrated Project Q-PorkChains (Contract No.: FOOD-CT-2007-036245).

- SABRE - Cutting Edge Genomics for Sustainable Animal Breeding (funded by the European Commission under the 6th Framework Programme for RTD; contract No.: FOOD-CT-2006-016250).

- SSSAP - State-Sponsored Study Abroad Programs (SSSAP) (State Scholarship fund organized by China Scholarship Council).

Acknowledgement

My sincere gratitude to Dr. Pere Gou, my supervisor, who has been giving me much more and far better guidance than I could have expected in the past three year. From him, I learned not only solid academic knowledge, but also conscientiousness, assiduousness and concentration, which will be my most precious treasure in future.

I am deeply grateful to Dr. Jacint Arnau, as a head of the Operative Programme of Food Processing and Engineering, for all his deep concern and great support since the very first place when I contacted with him from China.

Grateful thanks to all my colleagues at CENTA and IRTA in Monells, who have helped me in all kinds of ways and shared joy and love with me, especially to Dr. Israel Muñoz and MariaJose Bautista, two of the best and kindest drivers in the world.

I greatly appreciate the opportunity and all the assistance given by IRTA, the best institute I have ever seen. Thank UdG for accepting me as a doctor candidate and supporting me all through the course.

Special acknowledgment to China Scholarship Council (CSC), Nanjing Agricultural University (NAU) and my former supervisor at NAU, Dr. Guanghong Zhou for the chance, backing and encouragement that they have provided to me.

I couldn’t have made it without the love and support from my family, especially, my mother Jifang Deng, father Zaiquan Zhen, elder sister Zongli Zhen and my beloved wife, Jie Wang. They are the reason and goal, the motivation and inspiration for me to walk hard and come through till the end.

My apology for not being able to mention here everyone who ever helped me. You and what you have done for me will always be kept and appreciated in the bottom of my heart.

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Resum

En aquesta tesi s'han realitzat dos estudis complementaris sobre la importància dels factors genètics sobre la qualitat del pernil curat "Jamón Serrano". El primer compara pernils curats elaborats a partir de línies genètiques comerciales de quatre races representatives (Duroc, Landrace, Large White y Piétrain) i sotmesos a un procés industrial estàndard i actual. Es van estudiar 120 mascles provinents de quatre races, formades per 21 Duroc, 44 Landrace, 44 Large White i 13 Piétrain, representant les línies genètiques que actualment s'estan utilitzant a Espanya dins de cada raça. Es van analitzar les característiques dels pernils frescos i les característiques físico-químiques així com sensorials dels pernils curats. Es van estudiar les correlacions entre caràcters i les diferències entre races. Les majors diferències en els pernils frescos i en la composició i reologia final es van observar entre Piétrain i les altres tres races. Les principals diferències en color instrumental i aparença sensorial es van observar entre la raça Duroc i les altres tres races, probablement degut al alt contingut de greix intramuscular del Duroc. No es van detectar diferències importants entre races respecte a textura sensorial, flavor i qualitat sensorial global. Les diferències en característiques sensorials entre les quatre races van ser massa petites per a poder discriminar entre elles en base a característiques individuals. No obstant, considerant les anàlisis multivariades (PCA), la raça Large White va mostrar les característiques sensorials més apropiades per al pernil curat en les condicions de elaboració aquí utilitzades, mentres que Piétrain, per contra, va mostrar les menys apropiades.

El segon estudi analitza l'efecte dels polimorfismes genètics *PRKAG3* i *CAST*, prèviament identificats, sobre les característiques de qualitat del pernil curat espanyol "Jamón Serrano". Es va genotipar per a *PRKAG3 Ile199Val*, *CAST Arg249Lys* i *CAST Ser638Arg* una mostra de teixit de 665 porcs encreuats, i es va seleccionar una submostra de 120 pernils curats per a anàlisis químics, reològics, de color instrumental i anàlisi sensorial. Es van estudiar les associacions entre els polimorfismes i característiques de qualitat del pernil curat. Hi va haver associacions significatives ($p<0,05$) i suggerents ($p<0.10$) dels genotips *PRKAG3*, *CAST249* i *CAST638* i del haplotip *CAST* amb varis caràcters de qualitat del pernil curat, principalment relacionats amb flavor i textura. Els genotips *PRKAG3*

Ile/Ile, *CAST249 Arg/Arg*, *CAST638 Arg/Arg* i l'haplotip *CAST 249Arg-638Arg* són els més favorables per a l'elaboració del pernil curat "Jamón Serrano".

Es pot concloure que els pernils curats Serrano elaborats amb les quatre races estudiades en aquesta tesi van mostrar un índex de proteòlisi i unes característiques sensorials, inclosa la qualitat sensorial global avaluada pel panell expert, similars. Això va indicar que l'efecte de la raça no va ser prou fort per a provocar diferències perceptibles entre els pernils curats. Per contra, les diferències entre genotips dels polimorfismes *PRKAG3* i *CAST* poden induir diferències perceptibles en la qualitat sensorial dels pernils curats. En conseqüència, els polimorfismes genètics podrien ser millors indicadors per a la selecció de la matèria prima a les indústries elaboradores de pernil curat que les races.

Paraules clau: pernil curat Serrano; raça pura; polimorfisme genètic; Duroc; Landrace; Large White; Piétrain; *PRKAG3*; *CAST*.

Resumen

En esta tesis se han realizado dos estudios complementarios sobre la importancia de los factores genéticos sobre la calidad del jamón curado “Jamón Serrano”. El primero compara jamones curados elaborados a partir de lineas genéticas comerciales de cuatro razas representativas (Duroc, Landrace, Large White y Piétrain) y sujetos a un proceso industrial estándar y actual. Se estudiaron 120 machos provenientes de cuatro razas, formados por 21 Duroc, 44 Landrace, 44 Large White y 13 Piétrain, representando las lineas genéticas que actualmente se estan usando en España dentro de cada raza. Se analizaron las características de los jamones frescos y las características físico-químicas así como sensoriales de los jamones curados. Se estudiaron las correlaciones entre caracteres y las diferencias entre razas. Las mayores diferencias en los jamones frescos y en la composición y reología final se observaron entre Piétrain y las otras tres razas. Las principales diferencias en color instrumental y apariencia sensorial se observaron entre la raza Duroc y las otras tres razas, probablemente debido al alto contenido de grasa intramuscular del Duroc. No se detectaron diferencias importantes entre razas en cuanto a textura sensorial, flavor y calidad sensorial global. Las diferencias en características sensoriales entre las cuatro razas fueron demasiado pequeñas para poder discriminar entre ellas en base a características individuales. Sin embargo, considerando los analisis multivariados (PCA), la raza Large White mostró las características sensoriales más apropiadas para el jamón curado en las condiciones de elaboración aquí utilizadas, mientras que Piétrain, por el contrario, mostró las menos apropiadas.

El segundo estudio analiza el efecto de los polimorfismos genéticos *PRKAG3* y *CAST*, previamente identificados, sobre las características de calidad del jamón curado español “Jamón Serrano”. Se genotipó para *PRKAG3 Ile199Val*, *CAST Arg249Lys* y *CAST Ser638Arg* una muestra de tejido de 665 cerdos cruzados, y se seleccionó una submuestra de 120 jamones curados para análisis químicos, reológicos, de color instrumental y análisis sensorial. Se estudiaron las asociaciones entre los polimorfismos y características de calidad del jamón curado. Hubo asociaciones significativas ($p<0,05$) y sugerentes ($p<0.10$) de los genotipos *PRKAG3*, *CAST249* y *CAST638* y del haplotipo *CAST* con varios caracteres de calidad del jamón Serrano, principalmente relacionados con flavor y

textura. Los genotipos *PRKAG3 Ile/Ile*, *CAST249 Arg/Arg*, *CAST638 Arg/Arg* y el haplotipo *CAST 249Arg-638Arg* son los más favorables para la elaboración del jamón curado “Jamón Serrano”.

Se puede concluir que los jamones Serrano elaborados con las cuatro razas estudiadas en esta tesis mostraron un índice de proteolisis y unas características sensoriales, incluida la calidad sensorial global evaluada por el panel experto, similares. Esto indicó que el efecto de la raza no fue suficientemente fuerte como para producir diferencias perceptibles entre los jamones curados. Por el contrario, las diferencias entre genotipos de los polimorfismos *PRKAG3* y *CAST* pueden inducir diferencias perceptibles en la calidad sensorial de los jamones curados. En consecuencia, los polimorfismos genéticos podrían ser mejores indicadores para la selección de la materia prima en las industrias elaboradoras de jamón curado que las razas.

Palabras clave: jamón curado Serrano; raza pura; polimorfismo genético; Duroc; Landrace; Large White; Piétrain; *PRKAG3*; *CAST*

Abstract

Two pieces of complementary research dealing with the study of the importance of genetic factors on the quality of the Spanish dry-cured ham “Jamón Serrano” were carried out for this thesis. The first one compared Serrano dry-cured hams made from commercial lines of four representative breeds (Duroc, Landrace, Large White and Piétrain) subjected to the current standard industrial process. One hundred and twenty-two male pigs from four pure breeds were studied; 21 Duroc, 44 Landrace, 44 Large White and 13 Piétrain, representing the genetic lines within each of the breeds currently used in Spain. Physiochemical traits of raw and dry-cured hams, as well as the sensory traits of dry-cured hams were tested. Correlations between traits and differences between breeds were analyzed. Most of the differences in raw material, final composition and rheology were between Piétrain and the other three breeds. Differences in instrumental colour and appearance were mainly between the Duroc breed and the other three breeds, which were probably caused by the high intramuscular muscle of the Duroc breed. No important differences between breeds were found in the sensory texture, flavour or overall quality. The differences between the four breeds in dry-cured ham sensory quality were too small to discriminate them on single traits. However, according to multivariate analyses (PCA), the Large White showed the most appropriate sensory characteristics for dry-cured ham production of the four pure breeds under the processing conditions used, while to the contrary, the Piétrain showed the least appropriate ones.

The second piece of research studied the effect of the previously identified *PRKAG3* and *CAST* genetic polymorphisms on the quality traits of the Spanish dry-cured ham Jamón Serrano. A tissue sample from 665 crossbreed pigs was genotyped for *PRKAG3 Ile199Val*, *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms, and a subsample of 120 dry-cured hams was selected to perform the physicochemical, rheological, instrumental colour and sensory analyses. Associations between the polymorphisms and quality traits of dry-cured ham were studied. There were significant ($p<0.05$) and suggestive associations ($p<0.10$) between the *PRKAG3*, *CAST249*, *CAST638* genotypes or *CAST* haplotypes and several quality traits of the Spanish dry-cured ham Jamón Serrano, mainly related to flavour and texture. The *PRKAG3 Ile/Ile* genotype, the *CAST249 Arg/Arg*

genotype, the *CAST638 Arg/Arg* genotype and the haplotype *CAST 249Arg-638Arg* are the most favourable for the production of the Spanish dry-cured ham Jamón Serrano.

It can be concluded that the Serrano hams produced from the four pure breeds in this study showed similar proteolysis index and sensory characteristics including the global sensory quality tested by an expert panel. This indicated that the effect of different pure breeds was not strong enough to produce significant perceivable differences between dry-cured hams. In contrast, differences between genotypes of *PRKAG3* and *CAST* polymorphisms on proteolysis and sensory traits indicated that *PRKAG3* and *CAST* polymorphisms could induce perceivable differences in the sensory quality of dry-cured hams. Accordingly, genetic polymorphisms could be better indicators of material screening in the dry-cured ham industry than breeds.

Keywords: Serrano dry-cured ham; Pure breed; Genetic polymorphism; Duroc; Landrace; Large White; Piétrain; *PRKAG3*; *CAST.*

1. Introduction

The production of dry-cured ham has been established since time immemorial as a process of preservation through salting and subsequent drying. However, the use of refrigeration has reduced this need while the product has increased in consumer acceptance. As a result, the process has been modified and improved in order to obtain meat products with flavourful and attractive characteristics.

Serrano dry-cured ham, one of Spain's most outstanding meat products, is produced from different crossbred white pigs. Serrano ham is characterized by a red colour, firm texture, marbling, and a typical flavour. The hams are processed by means of an increasing temperature and a decreasing air relative humidity during the drying stage, which takes from 9 to 24 months depending on desired final quality and intense flavour.

Numerous biochemical reactions take place throughout the dry-curing process, especially during the ripening period contributing to the development of an adequate texture and a characteristic flavour. An extensive knowledge of these reactions has been acquired during the last decade. However, it has not been sufficiently implemented in the dry-curing process.

There are many factors affecting the quality of dry-cured hams. The raw materials and the processing conditions are the two most influential ones, playing a role in the final texture, flavour and homogeneity of the products. The raw ham properties have been widely studied as a decisive factor for dry-cured ham quality, in particular those related to genetic type of pigs (autochthonous or modern crossbreeds), the age at slaughter (5-18 months), the type of feeding (composition, extensive or intensive) and recently, genetic markers.

Several studies have demonstrated the importance of raw ham meat quality, fat quantity and composition on dry-cured ham characteristics. For instance, a negative effect of hams from a heavily-muscled line on the quality of dry-cured ham can be seen in different studies. Many studies have shown differences in fatness, meat quality and conformation between breeds. However, no recent studies of breed effects on Serrano dry-cured ham characteristics have been found.

The most frequently used modern breeds in Spain are Duroc, Large White, Landrace and Piétrain. The crossbreds containing Duroc have a higher percentage of intramuscular fat, a certain amount of which is needed for the manufacture of Serrano hams. The Large White breed offers meat similar to that of the Duroc breed but with less intramuscular fat, less dry matter and a higher protein content. The Belgium Landrace and Piétrain breeds are used as highly muscled pig genotypes.

Recent studies have shown a certain connection between genetic markers and both pig carcass and meat quality characteristics such as fatness, ham weight, pH and water-holding capacity. The manufacture of dry-cured ham requires raw material with a lower proteolytic capacity, which could be selected using genetic markers to obtain adequate raw ham for salt reduction and/or prolonged maturation. Amongst previously investigated genes, two have been considered as promising by scientists: the *PRKAG3* and the *CAST*. The *PRKAG3* gene encodes a specific isoform of γ subunit of the adenosine monophosphate dependant protein kinase (AMPK), an enzyme playing a key role in cell energy metabolism regulation. The mutation of the *PRKAG3* gene has been shown to affect muscle glycogen content, carcass leanness, ultimate muscle pH and water-holding capacity. The second gene (*CAST*) encodes for calpastatin, a physiological inhibitor of calpain enzymes which are responsible for early *post mortem* muscle proteolysis and has been proved to affect *post mortem* tenderization. *CAST* polymorphisms have been associated with pork texture, water content, ham weight, salt content and colour in dry-cured hams, as well as backfat thickness, meat colour, leanness and pH value.

2. Objectives

The effects of breeds and genetic markers on the quality of raw ham were studied individually in different ways. The results showed they could affect some traits of raw hams, which makes them potentially useful for pig selection in order to obtain appropriate hams for the dry-cured ham industry. Selection based on breeds of pigs represents a group-evaluation method while selection based on genetic markers is an individual-evaluation method.

The aim of this study was to evaluate the effects of current breeds and the *PRKAG3* and *CAST* genotypes on the Spanish dry-cured ham "Jamón Serrano" subjected to the current standard industrial process, and assess their relative importance.

The following specific objectives were carried out within the framework of the main objective:

1. To study the relationships between characteristics of raw and dry-cured hams when using hams from animals of pure breeds, in order to better understand the differences between breeds.
2. To estimate the effect of pure breeds (Duroc, Landrace, Large White and Piétrain) on Serrano dry-cured ham attributes: chemical composition, appearance, texture, flavour and global quality.
3. To study the relationships between the characteristics of raw and dry-cured hams when using hams from commercial crossbred animals, currently used for dry-cured ham production, in order to better understand the effect of the *PRKAG3* and *CAST* genotypes.
4. To estimate the effect of the *PRKAG3* and *CAST* genotypes on Serrano dry-cured ham attributes (chemical composition, appearance, texture, flavour and global quality) using animals from a commercial crossbreed.
5. To compare the relative importance of the effects of breed and the *PRKAG3* and *CAST* genotypes on Serrano dry-cured ham.

3. Experimental framework and report structure

To achieve the objectives, two studies were performed. The first one dealt with the pure breed effects, whereas the second one dealt with the *PRKAG3* and *CAST* genotypes effects.

Framework of first study:

The differences in boar taint and the quality of pork meat between different breeds (Duroc, Landrace, Large White, Belgian Landrace and Piétrain) were recently studied within the frame of an Integrated Project funded by the European Commission under the 6th Framework Programme for RTD (Project SABRE; contract FOOD-CT-2006-016250). A group of animals from each breed were taken from different Spanish pure breed nucleus herds to represent the genetic lines within each breed currently used in Spain.

Hams obtained from some of the animals in this project were used in the pure breeds study of this thesis (Chapter I). Hams were processed in the frame of another Integrated Project funded by the European Commission under the 6th Framework Programme for RTD (Project Q-PorkChains; contract FOOD-CT-2007-036245), which aimed to improve the quality of pork and pork products for the consumer.

Framework of second study:

Within the frame of an Integrated Project funded by the European Commission under the 6th Framework Programme for RTD (Project TRUEFOOD; contract FOOD-CT-2006-016264), a wide study which looked at different pig production and ham processing systems was simultaneously performed in three countries: France, Slovenia and Spain. The first results of these studies were published by Škrlep et al. (2010) who studied, in the three countries, the association of *PRKAG3* and *CAST* genetic polymorphisms with several raw ham quality parameters (the weight and fat thickness of ham and the colour and pH of muscle)

which are important in the production of dry-cured ham. Significant effects of these two genes on the quality parameters mentioned above were observed, however they differed according to the sample of pigs selected for the ham production in each country, indicating a possible interaction with other genetic or environmental factors. The next step of this project was to evaluate the effect of these polymorphisms on the quality traits of the dry-cured hams produced in each country using specific raw material and processing conditions.

The results of this second step for the case of Spanish dry-cured hams are shown in Chapter II.

Structure of the thesis:

The thesis is structured in three chapters:

Chapter I shows the background, material and methods, results, discussion and conclusions of the pure breeds study, addressing objectives one and two.

Chapter II shows the background, material and methods, results, discussion and conclusions of the *PRKAG3* and *CAST* genotypes study, addressing objectives three and four.

Chapter III compares the breed and genetic markers effects found in previous chapters, addressing objective five.

4. Chapter I

Effect of pure breeds on dry-cured ham

4.1. Background

The quality of dry-cured ham is affected by many factors, the most influential ones being the raw material properties and processing conditions. Raw hams intended for processing are highly heterogeneous. They are from pigs varying in genetic type, age at slaughter, morphology, lean and fat distribution, etc. In addition, there are many factors which have a strong influence on the process, which therefore affect the final quality of the products. These facts have prompted several studies to evaluate the suitability of specific breeds, crossbreds or genetic lines for the production of dry-cured hams with an optimal quality. Nowadays, it is widely accepted that the quality of raw meat, affected by genotype, age, sex, *ante* and *postmortem* treatment, asa well as the processing technology, decisively influence the activity of muscle enzymes, and subsequently, have strong effect on the quality of the final product.

Different methods are usually used in the meat industry to characterize raw materials for the manufacture of dry-cured meat products such as the raw ham weight, pH, temperature, intramuscular fat content, etc. However, it is difficult to interpret these measurements and to establish their impact on the quality of dry-cured meat products. For example, it is generally accepted that an increased level of intramuscular fat has a positive influence on the sensory quality of pig meat and meat products (Steane, 1986), although consumer response is variable (Wood et al., 1988). A careful examination of the literature available reveals contradictory results. Some studies show a positive effect of the intramuscular fat level on the sensory attributes of fresh pork (Barton-Gade & Bejerholm, 1985 and Eikelenboom, Hoving-Bolink, & Van der Wal, 1996), whereas some others do not show any influence (Lentsch, Pruska, Fedler, Meisinger, & Goodwin, 1991; Purchas, Smith, & Pearson, 1990), or even a negative influence (Cameron, 1990; Lan, McKeith, Novakofski, & Carr, 1993). Fat contributes to the technological and sensory quality of dry-cured ham (Antequera et al., 1992; Girard et al., 1986); it influences aroma development due to the lipolytic and oxidative processes which occur during the curing process (López et al., 1992). Knowledge regarding the influence of the fat content and the fatty acid composition on the textural characteristics of the dry-cured ham is scarce and contradictory. For example, Bergonzoni, Rosi, and Fabbri (1985); Parolari, Rivaldi, Leonelli, Bellati, and Bovis (1988) reported a positive relationship between tenderness in dry-cured ham and

the lipid content. To the contrary, Buscailhon, Gandemer and Monin (1994b) found no relationship between textural traits and the lipid fraction. Considerable research has been conducted to assess the role of the fat in the quality of the hams from Iberian pigs, possibly due to its relation to the feeding regimes. The quality of dry-cured meat products is determined by the quality of the raw material and the manufacturing process (Andrés et al., 2001). There are various studies regarding the effect of pig genotype on the quality of dry-cured hams in the literature available (García-Rey, García-Olmo, De Pedro, Quiles-Zafra, & Luque de Castro, 2005; García-Rey, Quiles-Zafra, & Luque de Castro, 2006). In addition, previous research has related specific characteristics on fresh meat to the quality of dry-cured meat products. For instance, the chemical composition has been related to the sensory properties of dry-cured ham (Ruiz-Carrascal, Ventanas, Cava, Andrés, & García, 2000), the *postmortem* pH has been related to the texture parameters and the colour of dry-cured ham (Ruiz-Ramírez, Arnau, Serra, & Gou, 2006; Guerrero, Gou, & Arnau, 1999; García-Rey, García-Garrido, Quiles-Zafra, Tapiador, & Luque de Castro, 2004), the fatty acid profile has been related to some sensory parameters (Cava, Ventanas, Ruiz, Andrés, & Antequera, 2000), and the instrumental colour in fresh meat has been related to the quality of dry-cured ham (Chizzolini et al., 1996). Nevertheless, as far as all these parameters together are concerned, their total impact and their relative importance for dry-cured ham quality have not been evaluated. The Duroc breed was introduced into Europe mainly due to its higher intramuscular fat content in comparison with other breeds (Barton-Gade, 1987). However, the Duroc breed is not homogeneous, since due to its widespread distribution, it has been the object of different selections. Recently, most pig producers have included genetic selection in their production programmes to improve meat quality (Visscher, Pong-Wong, Whittemore, & Haley, 2000). Nonetheless, different studies have reported the dissimilarities between the Duroc lines and the different quality of dry-cured meat products manufactured from them (Soriano, Quiles, Mariscal, & García Ruiz, 2005; Cilla et al., 2006). These authors reported that most of the parameters which affect the overall impression of dry-cured ham such as odour, flavour, and juiciness were affected by the Duroc sire line in crossbreding with industrial genotypes.

Current pig breeding schemes are usually based on three- or four-way crosses. For instance, the most common cross in Spain is a three-way cross where the sow

is an F1 Landrace x Large White (LR x LW) crossbreed. The choice of the terminal sire depends on the profitability obtained per animal and in this sense, slaughterhouses play an important role in selection. The pig carcass price is fixed according to an evaluation consisting of a good score when the backfat is reduced and the conformation provides a high percentage of valuable cuts. Therefore, sows are crossed either with a heavily muscled sire, such as the Piétrain (Pi) or Belgian Landrace (BL), or with a good growth rate and resistance breed like Duroc (DU). Belgian Landrace breed shows a low aptitude of its meat to provide dry-cured hams based on the role of the exopeptidases and free generating flavour precursors (Armero, Baselga, Aristoy, & Toldrá, 1999a). This crossbred, which has a high susceptibility to stress, also showed the largest Pale, Soft and Exudative meat (PSE) incidence, at around 50% of the pigs. On the other hand, the Duroc-sired pigs obtained good scores for meat quality as well as for the presence of intramuscular fat (marbling), which is highly appreciated by the dry-cured ham industry (Armero et al., 1999b). The comparison of the Iberian pig breed with the White crossbred pigs, consisting of (LW×LR) × DU reveals large differences between muscle proteolytic and lipolytic enzymes (Rosell & Toldrá, 1998). In fact, Iberian pigs show higher amounts of cathepsin D, dipeptidylpeptidase III and alanyl aminopeptidase, while the White crossbred pigs show higher amounts of calpain and cathepsins B, B+L and H; dipeptidylpeptidases I1 and IV; aminopeptidase B; leucyl and pyroglutamylaminopeptidase; and acid lipase and neutral esterase (Rosell & Toldrá, 1998).

The Duroc breed was introduced in Spain to improve the growth characteristics of the Iberian pig, which produces the highest quality of processed products in the national market. Nearly 92% of the dry-cured hams produced in Spain are obtained from intensive pig production and the rest from the Iberian breed, either pure or crossed, reared in extensive or semi-extensive conditions. The most common three-way cross used in the intensive pig production in Spain involves well conformed breeds as terminal sires to obtain advantages in carcass yield (Blasco et al., 1993). This strategy has resulted in a high incidence of PSE meat in commercial pig carcasses (Oliver, Gispert, & Diestre, 1990). Furthermore, Arnau, Guerrero, Maneja, and Gou (1992) detected that highly muscled hams present difficulties for dry-cured processing because of their poor meat quality and their low levels of fatness as well as their blocky muscular mass. Therefore, there is a

gap on the Spanish market between the top quality Iberian dry-cured hams and the dry-cured hams from intensive pig production. The Duroc breed does not present the stress syndrome; therefore the crosses with Duroc may be free of the PSE problem and could also produce a better meat quality due to the higher level of intramuscular fat of this breed (Bejerholm & Barton-Gade, 1986; Barton-Gade, 1988; McGlouglin et al., 1988; Oliver, Gispert, & Diestre, 1993).

Several studies show the effect of genetic type on meat quality (Oliver et al., 1994; Guerrero, Gou, Alonso. & Arnau 1996; Armero et al., 1999b). From these studies, it can be concluded that the Duroc breed provides high meat quality with a good intramuscular fat level much appreciated in dry-cured meat products. Additionally, the cross with the Duroc terminal sire grows faster and shows a better food conversion ratio (Blasco, Argente, Haley, & Santacreu, 1994). The Belgian Landrace and Piétrain have a high susceptibility to stress and thus have a high incidence of PSE meats. In these cases, a higher intensity in pastiness, crumbliness, brightness and nonprotein nitrogen and tyrosine content in dry-cured hams has been reported (Guerrero et al., 1996). Belgium Landrace crossbred pigs tend to have a higher percentage of hams discarded during processing and tend to have lower ham flavour scores (Gallo, Montobbio, Carnier, & Bittante, 1994). An intermediate situation, combining reliable conformation and meat quality, can be obtained with the Belgian Landrace × Landrace (BL × LR) cross (Armero et al., 1999b).

Pigs for dry-cured ham production in Spain come principally from female (LR × LW) or (LR × DU) crossbreds. These pigs are highly prolific and show good production parameters and carcass yield, whereas the most commonly used finisher boars are crosses (LW × PI) and, for high-quality production, (LW × DU). The Belgian Landrace breed is also used as terminal (male) line. It is well accepted that highly muscled pig genotypes (i.e. Piétrain and Belgian Landrace) are less appropriate for the production of high-quality dry-cured hams owing to higher processing losses and lower product quality (Buscailhon, Berdagué, Gandemer, Touraille, & Monin, 1994a; Russo & Nanni Costa, 1995). The manufacture of dry-cured hams requires a certain amount of intramuscular fat which regulates water loss and thus ensures sensory quality. Several studies have shown that crossbreds containing DU have higher percentages of intramuscular fat (Barton-Gade, 1987; McGloughlin, Allen, Tarrant, & Joseph, 1988; Wood, Edwards, & Bichard, 1988;

Edward et al., 1992) and higher concentrations of muscle pigments (Meat and Livestock Commission, 1992). The LW breed produces animals that offer meat quality similar to that of the DU breed but with less intramuscular fat, less dry matter and a higher protein content (Barton-Gade, 1987; McGloughlin et al., 1988; Edwards, Wood, Moncrieff, & Porter, 1992; Martel, Minvielle, & Poste, 1988). Guerrero et al. (1996) compared the DU breed with a heavily muscled cross formed from the pure breeds Piétrain and Belgian Landrace and with another cross consisting of DU × LW. They found that after curing for 9 months, hams from DU animals had lower processing weight losses, more marbling and a stronger cured aroma and flavour. Candek-Potokar, Monin and Alender (2002) also found that DU crosses exhibited higher intramuscular fat content and marbling and lower weight losses during the processing of dry-cured ham from Slovenia. Improvements in animal selection by breeding enterprises have had a great effect on production parameters, carcass traits and meat quality in genetic lines. Nowadays, pure breeds do not have homogeneous characteristics owing to selection by enterprises or geographic areas based on different criteria to produce end-products and to satisfy consumer demands.

Many studies have been done about the effect of different breed lines on the quality of dry-cured ham. Significant differences in the percentages of primal cuts (ham, closely trimmed loin, Boston butt, picnic shoulder, and belly) and trimmed ham vs. carcass weight are usually found in differently sired pigs. For instance, the Belgian Landrace sired pigs have the highest proportion of valuable cuts such as ham, shoulder and chops while Duroc sired pigs tend to have a higher proportion of ham and shoulder and a lower proportion of ribs (Blasco el al., 1994; Armero et al., 1999b). On the other hand, the carcass weight also affects the yield of several main cuts. Shoulder, ribs and chops have been observed to increase and bacon trimmings and backfat to decrease with carcass weight (Armero et al., 1999b). However, crossbreding of traditional breeds (Large White, Landrace and Duroc), with different percentages of breeds such as Duroc or Landrace to produce pigs for ham industrial processing, have been found to have, in general, few effects on the curing suitability, processing or chemical and quality properties of dry-cured ham. Berdagué, Bonnaud, Rousset and Touraille (1993) found that crossbreds have a definite effect on the intramuscular fat content but are restricted on the content of volatile compounds and flavour. In studies of Italian crossbreds with different percentages of Duroc and Large White, there were close

relationships between the raw ham weight and the moisture, marbling and muscle firmness in the finished product and between the proteolytic enzyme activity in raw and dry-cured hams (Schivazappa, Virgili, Degni, & Cerati, 1998). Even though the chemical composition of processed meat changed with some breed modification, the observed changes were sometimes insufficient and do not result in perceivable differences of sensory quality of the dry-cured meat products.

Despite the fact that many studies have been done, genetic lines are constantly changing, and new feeding and production systems may modify the expected breed effect on fresh meat quality. Moreover, there is little recent research which throws light upon the effect of pure breeds on Serrano dry-cured ham Most of the previous works were involved with effect of breeds (pure or cross) on the raw meat properties. Several studies have been done on the effect of pure breeds on Parma ham. It is difficult to compare the results of these studies to make clear how and how much influence pure breeds have on the quality of Serrano dry-cured ham. No research on the effect of pure breeds was found on Serrano dry-cured ham produced using the current industrial process. Serrano dry-cured ham accounts for most of the Spanish dry-cured ham and is different from Iberian ham and Parma ham in processing and final quality. Moreover, as an important breed, the Piétrain, which has usually been used as sire line providing heavy muscle characteristics in crossbreds, has not been sufficiently taken into account in previous research.

The aim of the study in this chapter was to estimate the effect of pure breeds (Duroc, Landrace, Large White and Piétrain) on Serrano dry-cured ham quality traits: chemical composition, appearance, texture, flavour and global quality. To better understand the differences between breeds, the relationships between characteristics of raw and dry-cured hams were also studied.

4.2. Material and Methods

4.2.1. Animals

One hundred and twenty two entire male pigs from four pure breeds were studied. Twenty-one Duroc (DU), 44 Landrace (LR), 44 Large White (LW) and 13 Piétrain (PI) pigs were fattened under identical conditions at the Pig Testing Station (IRTA-CAP) in Monells (Girona, Spain). The animals came from different Spanish nucleus herds (4 for DU, 9 for LR, 7 for LW and 3 for PI). At least three animals per herd were sampled. Therefore, the group of animals from each breed can be considered as representative of the genetic lines within each breed currently used in Spain. The four pig breeds (DU, LR, LW, PI) were reared under the same conditions of housing, environment and feeding. The *ante mortem* handling was performed under low stress conditions. The animals were weighed the day before slaughter. The average body weight (kg) was 117.5 ± 9.8 for DU, 116.2 ± 11.2 for LR, 118.5 ± 10.2 for LW, and 103.4 ± 11.9 for PI. The pigs were fasted on-farm for 9 h and then transported for 1.5 h to a commercial slaughterhouse (Vic, Spain). Animals from different pens were not mixed. The animals were slaughtered individually using CO_2 stunning at 90% concentration for 2 min. Animals from different breeds were slaughtered alternatively. The animals were slaughtered in two different days (two batches). In the first batch were 9 DU, 10 LR, 27 LW and 4 PI; in the second batch were 12 DU, 34 LR, 17LW and 9 PI.

4.2.2. Carcass measurements

One day after slaughter the ham was cut off the carcass, trimmed into the prescribed shape of “Jamón Serrano” (skin partially removed) and kept at 1-3ºC. The raw ham weight, length, width, height, and the pH_u value of M. *Semimembranosus* (SM, Fig. 4.1) were recorded. The pH was measured with a puncture electrode and a portable pH meter (Crison 507, Crison Instruments S.A., Barcelona, Spain) by punching the probe into the SM muscle at 24 hours *postmortem* (pH_u).

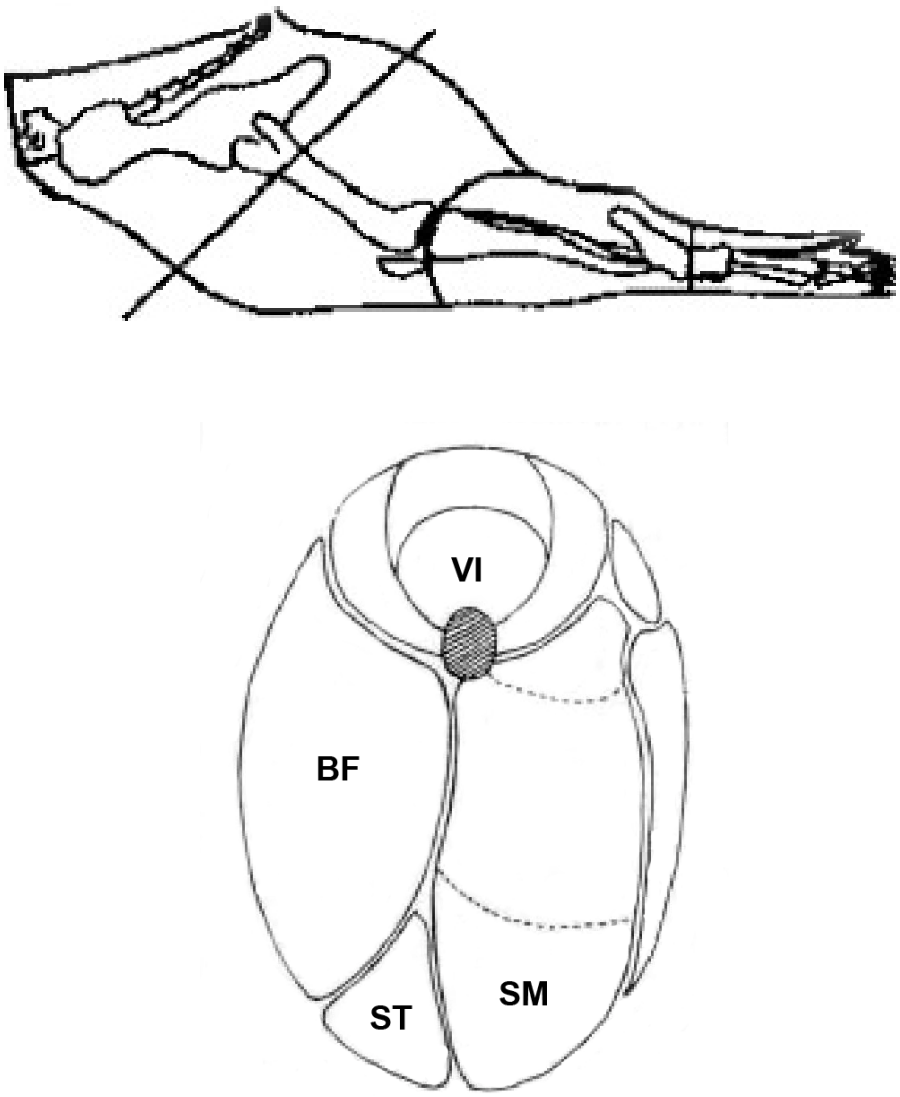

Fig. 4.1. Schematic diagram of a cross section of ham.

BF: *Biceps femoris* muscle; SM: *Semimembranosus* muscle; ST: *Semitendinosus* muscle; VI: *Vastus intermedius* muscle.

4.2.3. Dry-cured ham processing

The hams were processed according to the specifications of the quality system of Serrano Ham (European Commission, 1998), as a Traditional Speciality Guaranteed (European Commission, 2006). The salting of the hams was carried out 48 h *postmortem*. The hams were purged of blood residues and then pre-salted with 36.5 g of a mixture of dextrose (5 g), sodium nitrite (0.5 g), potassium nitrate (0.5 g), sodium ascorbate (0.5 g), fine salt (15 g), and coarse salt (15 g) per kg of ham. After 4 days, the hams were manually salted with 20 g fine salt and 16.5 g coarse salt per kg of ham and allowed to rest for 9 days at 3 ±2 ºC. After washing with cold water, the hams were hung at 3 ±2 ºC and at a relative humidity of 75-80% for 2 months (post-salting period). Subsequently (drying period), the temperature and the relative humidity were gradually increased up to 25 ºC and decreased to 60%, respectively. After four months of drying, the lean surface of the hams was covered with melted fat to control the excessive drying of the lean surface (crusting). The hams were weighed periodically, until 35% of weight losses were obtained. The processing time, final weight and weight losses were recorded after processing.

4.2.4. Sampling

Sampling for instrumental texture and physicochemical analysis

At the end of the process, transversal cuts were made on the hams at A (level of coxofemoral joint) and B (Fig. 4.2) to obtain the slice AB (20 mm thick). The parts of *Semimembranosus* and *Biceps femoris* muscles (Fig. 4.1) from each slice were removed. Five specimens per sample (BF and SM muscles) were accurately carved with a scalpel into parallelepipeds of 20 mm x 20 mm x 15 mm. The specimens were wrapped in plastic film to avoid drying and stored for 24 h at 4 ºC for the Stress relaxation test. The rest of the BF and SM muscle was minced, vacuum packaged and stored at 2–4 °C for further physicochemical analyses (Sánchez-Molinero & Arnau, 2010).

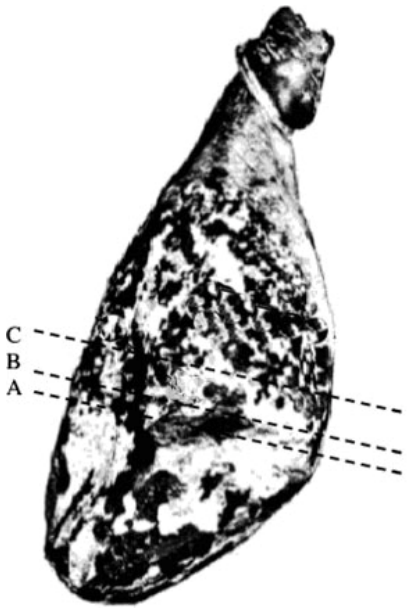

Fig. 4.2. Sampling zones.
A, B, C: zones where transversal cuts were made.

Sampling for instrumental colour analysis

The transversal surface B (Fig. 4.2) was the sample for instrumental colour measurement.

Sampling for sensory analysis

The dry-cured hams were boned, cut at the head of the femur level BC (Fig. 4.2) and then sliced (1.5 ± 0.2 mm-thick) perpendicularly to the femur axis in the distal part direction with a vertical slicer Kolossal 350 BVK (Marconi, Italy).

4.2.5. Analysis of processed ham

Physicochemical measurements

The pH (final pH), a_w and water and NaCl contents were measured on the minced SM and BF muscles; collagen, fat and protein contents and proteolysis index were measured only on the BF muscle. The final pH was measured with a pH penetration electrode (Crison 52-32) on a portable pH-meter (Crison pH 25, Crison Instruments, SA, Alella, Spain). The water activity (a_w) measurement was

carried out at 25 ºC with a Novasina AW SPRINT – TH 500 instrument (Axair Ltd., Pfäffikon, Switzerland) which allows temperature control during the a_w measurement. Collagen, fat and protein contents were measured by near infrared transmittance spectroscopy FoodScan®, FOSS Electric A/S, Denmark); water content by drying the samples at 103 ± 2ºC until a constant weight was achieved (AOAC, 1990); chloride content with a potentiometric titrator (785 P Titrino, Metrohm Ltd., Herisau, Switzerland) by using a standard silver nitrate titrant (0.1 M) following (ISO, 1996). A proteolysis index (PI) was calculated by the formula PI=100 x NPN x TN^{-1}. Non-protein nitrogen content (NPN) was assessed by precipitation of proteins with trichloroacetic acid (Kerese & Chalmers, 1984), followed by determination of the nitrogen in the extract using the Kjeldahl method.

Rheological analysis

Texture was assessed on the *Biceps femoris* and *Semimembranosus* muscles using the Stress Relaxation (SR) test. The SR test was performed using a RT/5 Universal MTS Alliance Texture Analyser (SEM, Barcelona, Spain) with a 25 kg load cell and a 50 mm diameter compression plate. The specimens were compressed to 25 % of original height, perpendicular to the fibre bundle direction at a crosshead speed of 1 mm/s. The force versus time after the compression was recorded at a speed of 50 points per second for 90 s (relaxation time). The relaxation curves obtained for each specimen were normalized, i.e., the force decay Y(t) was calculated as follows:

$$Y(t)=\frac{F0-F(t)}{F(t)}$$

where F0 (kg) is the initial force and F(t) is the force recorded after t seconds of relaxation. The force decay at 2 s (Y2) and 90 s (Y90) were calculated (Morales, Guerrero, Serra & Gou, 2007a). The average of the five specimens per sample was used for statistical analysis.

Instrumental colour analysis

Colour measurements were carried out with a colourimeter Minolta Chroma Meter CR-200 (illuminant D65, 2˚ standard observer and the specular component included) in the CIELAB space: lightness (L*), redness (a*) and yellowness (b*).

Colour measurements of *Semimembranosus* (SM), *Biceps femoris* (BF), *Vastus intermedius* (VI), *Semitendinosus* (ST) (Fig. 4.1) muscles were carried out on the slice surface, and averaged over five zones.

4.2.6. Sensory analysis

Quantitative Descriptive Analysis (QDA)

Seven selected and trained assessors (ASTM, 1981; ISO 8586-1, 1993; ISO 8586-2, 1994) took part in the sensory analysis on 1.5 mm-thick slices of dry-cured ham obtained from a transverse cut at the femur level which included the BF and the SM muscles (Fig. 4.1, Fig. 4.2).

The generation of the descriptors had been carried out in open discussion in two previous sessions. The descriptors retained are shown in Table 4.1. The references used to illustrate the maximum intensity of hardness, crumbliness and pastiness were those described by Guerrero et al. (1999). A non-structured scoring scale (Amerine, Pangborn, & Roessler, 1965) was used, where 0 meant absence of perception and 10 meant high intensity of the descriptor.

Sensory evaluation was undertaken in 25 sessions. Five samples per session were analysed in 20 sessions and 4 samples in the other 5 sessions. Within each session, samples came from at least three different breeds and a maximum of two samples per breed were analysed. The samples were coded with three-random numbers and were presented to the assessors balancing the first-order and the carry-over effects as much as possible according to Macfie, Bratchell, Greenhoff and Vallis (1989). The means of the seven panellists for each muscle and ham was used for data analysis.

Table 4.1. Definitions of sensory descriptors of dry-cured ham

Descriptor	Definition
Sensory texture of Biceps femoris muscle	
Adhesiveness	Textural property rated by the degree to which the surface of the ham slice adheres to the palate when compressed with the tongue
Hardness	Amount of pressure required to completely compress the sample
Crumbliness	Textural property characterized by the ease of which a sample can be separated into smaller particles during chewing
Pastiness	Textural property characterized by the feeling of paste detected in hams with a high proteolytic index
Fibrousness	Textural property characterized by the perception of the amount of muscle fibres detected during chewing
Sensory appearance	
Marbling	Visual evaluation of the fat tissue infiltrations within muscle tissue on the surface of the slice evaluated in the *Semimembranosus*, *Biceps femoris* and *semitendinosus* muscles
Red ring	Visual assessment of the presence of colour rings due to a lack of nitrite in the core of the ham
Tyrosine crystals	Number of small white crystal spots appearing on the surface of the slice
White film	Intensity of white colour that appears on the cut surface of a vacuum packaged product evaluated on 8 cm slices that had previously been vacuum packaged and stored for 15 days at 2–4 °C (Arnau, Gou, & Guerrero, 1994).
Sensory flavour of Biceps femoris muscle	
Metallic taste	Flavour similar to a solution of $FeSO_4 \cdot 7H_2O$
Sweetness	Basic taste sensation elicited by sugar
Saltiness	Basic taste sensation elicited by NaCl
Piquantness	Stinging sensation in the mouth and throat
Bitterness	Basic taste sensation elicited by caffeine and L-tryptophan
Umami taste	Basic taste sensation elicited by sodium glutamate
Matured flavour	Set of complex nuances characteristic of dry-cured meat products, not described by other flavour attributes
Global sensory quality of dry-cured hams	
Global sensory quality	Global quality assessed by panelists

4.2.7. Statistical analysis

The effects of breeds and batch were analyzed by the GLM procedure of SAS (SAS Inst., Inc., Cary, NC). A two-way analysis of the variance (breeds and batch) was carried out. For sensory traits, the model also included session as a random effect. Least square means (LSMEAN) were used to compare differences. Tukey's test was applied to compare the LSMEAN values of the breeds. LSMEAN values and root mean square error (RMSE) are reported. The relationships between traits (raw ham vs final product; raw ham and final product VS. rheological analysis, instrumental colour, sensory texture, appearance, flavour and global sensory quality) were analyzed by calculation of Pearson's correlation coefficient using the CORR procedure of SAS. Principal component analysis (PCA) with the variables of texture, appearance and flavour, along with the global sensory quality studied in this experiment were performed with the FACTOR procedure of the statistical package SAS (SAS Inst., Inc., Cary, NC).

4.3. Results

4.3.1. Correlation coefficients between traits

4.3.1.1. Correlation coefficients between traits of raw hams and dry-cured hams

Table 4.2 shows the correlation coefficients between the raw ham properties and the physicochemical parameters of *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured hams.

Table 4.2. Correlation coefficients between the raw ham properties and the physicochemical parameters of *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured hams

	Raw ham properties				
	Weight	Length	Width	Thickness	pH_u (SM)
Physicochemical parameters of BF *muscle*					
Final pH	0.130	0.108	0.046	0.110	**0.352*** **
Water content	**0.329*** **	-0.004	**0.200***	**0.259*** **	0.002
NaCl content	**-0.427*** **	-0.105	**-0.354*** **	**-0.269*** **	0.107
a_w	**0.428*** **	0.132	**0.308*** **	**0.325*** **	0.106
Collagen content	**0.245*** **	**0.179***	0.105	-0.025	**-0.226***
Fat content	0.128	0.092	0.064	0.083	0.096
Protein content	**-0.451*** **	0.043	**-0.224***	**-0.331*** **	0.131
Proteolysis Index	**0.441*** **	0.06	**0.218***	**0.205***	**-0.262*** **
Physicochemical parameters of SM muscle					
Final pH content	-0.026	0.003	0	-0.019	**0.211***
Water content	0.129	-0.068	0.036	**0.191***	0.097
NaCl content	**-0.421*** **	-0.162	**-0.352*** **	**-0.206***	0.048
a_w	**0.354*** **	0.123	**0.254*** **	**0.257*** **	0.131

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

Physicochemical parameters of BF muscle

The final pH had positive relationship with pH_u. The water content and a_w were positively related to the weight, width and thickness of the raw hams, but not with the length or pH_u measured. There were negative correlations between the salt content and weight, width and thickness of the raw hams. The collagen content was positively correlated with weight and length, but negatively related to pH_u. The fat content of the dry-cured ham was not significantly correlated with any measured traits of the raw hams. The total protein content was negatively correlated with weight, width and thickness. The proteolysis index was positively correlated with weight, width and thickness and negatively correlated with pH_u.

Physicochemical parameters of SM muscle

The final pH was positively related to pH_u. The water content was not significantly correlated with traits of the raw hams except for a slight correlation with thickness. The salt content had negative correlations with the weight, width and thickness. The a_w was shown to positively correlate with the weight, width and thickness of the raw hams.

4.3.1.2. Correlation coefficients between rheological values and traits of raw hams and dry-cured hams

Rheological traits of BF muscle

There were significant correlations between the rheological variables, namely F0, Y2 and Y90, with the weight and thickness of the raw hams (Table 4.3). As to final composition, the rheological variable of BF was significantly correlated with the total protein content and proteolysis index. Relationships were also observed between the salt content of BF and F0, Y2 and Y90. Moreover, F0 and Y90 were significantly related to a_w of BF. Y2 and Y90 were correlated with the collagen content.

There were also significant correlations between the rheological attributes of BF and some attributes of SM, i.e., between Y2 and pH_u and final pH of SM, between F0 and a_w of SM, and between F0, Y2 and Y90 and the salt content of SM.

Table 4.3. Correlation coefficients between the rheological parameters (F0, Y2, Y90) of the *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured ham and the traits of the raw hams and those of the BF and SM muscles of dry-cured hams

	Rheological parameters of BF muscle			*Rheological parameters of SM muscle*		
	F0 (BF)	Y2 (BF)	Y90 (BF)	F0 (SM)	Y2 (SM)	Y90 (SM)
Attributes of raw hams						
Weight	**-0.389*** **	**0.386*** **	**0.403*** **	0.020	-0.042	-0.029
Length	-0.029	0.028	0.066	0.174	**-0.254*** **	**-0.251** **
Width	-0.126	0.134	0.158	0.064	-0.103	-0.110
Thickness	**-0.280*** **	**0.244** **	**0.267*** **	**-0.243** **	**0.194***	**0.181***
pH_u (SM)	0.020	**-0.240** **	-0.135	0.021	**-0.232***	**-0.269*** **
Physicochemical parameters of BF muscle						
Final pH	-0.016	-0.124	-0.077	0.083	**-0.258*** **	**-0.301*** **
Water content	-0.154	0.052	0.117	-0.162	0.057	0.082
NaCl content	**0.251** **	**-0.212***	**-0.250** **	**0.187***	-0.138	-0.153
a_w	**-0.321*** **	0.136	**0.253*** **	**-0.314*** **	0.146	0.149
Collagen content	-0.178	**0.467*** **	**0.378*** **	0.136	0.053	0.082
Fat content	-0.025	0.140	0.165	**0.253** **	**-0.198***	**-0.198***
Protein content	**0.402*** **	**-0.385*** **	**-0.374*** **	0.094	-0.055	-0.071
Proteolysis Index	**-0.378*** **	**0.606*** **	**0.452*** **	0.008	0.157	0.153
Physicochemical parameters of SM muscle						
Final pH	-0.002	**-0.197***	-0.163	0.082	**-0.200***	**-0.255** **
Water content	-0.132	-0.003	0.107	**-0.573*** **	**0.193***	**0.294*** **
NaCl content	**0.246** **	**-0.253*** **	**-0.262*** **	-0.074	-0.005	0.038
a_w	**-0.274*** **	0.093	**0.215***	**-0.333*** **	0.144	0.140

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

Rheological traits of SM muscle

Y2 and Y90 were negatively related to length and pH_u (Table 4.3). Correlations were also found between thickness and F0, Y2 and Y90. With regards to the final composition, F0 was significantly and negatively correlated with the a_w and water content of SM. Y2 was related to the water content and final pH of SM. Y90 was related to the water content and final pH of SM.

There were also relationships between the rheological attributes of SM and some attributes of BF, i.e., between F0 of SM and both salt content and a_w of BF, between both Y2 and Y90 of SM and final pH of BF, and between F0, Y2 and Y90 of SM and fat content of BF.

4.3.1.3. Correlation coefficients between instrumental colour and traits of raw hams and dry-cured hams

Correlation coefficients between the instrumental colour in muscles of the dry-cured ham and traits of raw hams and dry-cured hams are shown in Table 4.4.

Colour of BF muscle

Lightness (L*) was positively correlated with weight, a_w and fat content, and negatively correlated with total protein content. A negative correlation between redness (a*) and a_w was found. Yellowness (b*) was also shown to positively relate to both pH_u and fat content.

Correlation was also found between lightness of BF and a_w of SM.

Colour of SM muscle

The lightness and yellowness of SM were both positively correlated with a_w and water content of SM.

Negative correlations were discovered between the lightness of SM and the collagen content and proteolysis index of BF, while a positive one was discovered between the lightness and a_w of BF. The yellowness of SM was positively correlated to a_w of BF.

Table 4.4. Correlation coefficients between the instrumental colour (Lightness, L*; redness, a*; yellowness, b*) of *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles and the traits of raw and dry-cured hams

	Instrumental colour of BF muscle			*Instrumental colour of SM muscle*		
	L*	a*	b*	L*	a*	b*
Attributes of raw hams						
Weight	**0.200***	-0.011	0.143	-0.083	0.030	-0.023
Length	0.090	0.112	0.134	-0.168	0.097	0.082
Width	0.077	-0.048	0.151	-0.051	-0.031	0.088
Thickness	-0.002	-0.111	0.056	0.041	**-0.212***	-0.129
pH_u (SM)	0.129	0.049	**0.187***	0.052	0.127	0.176
Physicochemical parameters of BF muscle						
Final pH	-0.058	-0.011	0.176	0.075	-0.146	0.051
Water content	0.108	-0.118	-0.072	0.008	0.009	0.009
NaCl content	-0.139	0.161	-0.156	-0.034	0.038	-0.143
a_w	**0.246****	**-0.223***	0.123	**0.224***	-0.013	**0.259*****
Collagen content	0.033	0.062	0.087	**-0.310*****	0.126	-0.073
Fat content	**0.309*****	-0.108	**0.189***	0.087	0.050	0.059
Protein content	**-0.347*****	0.208	-0.074	-0.128	0.124	0.090
Proteolysis Index	-0.053	0.169	0.096	**-0.354*****	0.175	-0.167
Physicochemical parameters of SM muscle						
Final pH	0.069	-0.037	0.101	0.082	-0.050	0.080
Water content	0.152	**-0.202***	-0.007	**0.232***	0.013	**0.208***
NaCl content	-0.030	0.000	-0.219	0.038	-0.001	-0.132
a_w	**0.257*****	**-0.223***	0.075	**0.267*****	0.006	**0.261*****

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$.

4.3.1.4. Correlation coefficients between sensory traits of dry-cured ham and attributes of raw and dry-cured hams

Sensory texture

Table 4.5 shows the correlation coefficients between the sensory texture (Hardness, Fibrousness, Crumbliness, Pastiness, Adhesiveness) of *Biceps femoris* (BF) muscle and traits of raw and dry-cured hams.

Table 4.5. Correlation coefficients between the sensory texture of *Biceps femoris* (BF) muscle and traits of the raw and dry-cured hams.

	Sensory texture of BF muscle				
	Hardness	Fibrousness	Crumbliness	Pastiness	Adhesiveness
Attributes of raw hams					
Weight	**-0.368*** **	**-0.425*** **	-0.116	**0.364*** **	**0.455*** **
Length	-0.154	-0.105	-0.083	0.084	0.172
Width	-0.136	**-0.183***	0.047	0.055	0.126
Thickness	-0.163	**-0.266*** **	0.049	**0.180***	**0.246****
pH_u (SM)	-0.003	-0.047	0.080	**-0.217***	**-0.182***
Physicochemical parameters of BF muscle					
Final pH	0.068	0.009	-0.012	-0.150	-0.154
Water content	-0.048	-0.057	0.072	0.059	0.045
NaCl content	0.129	0.141	-0.040	**-0.193***	**-0.233****
a_w	-0.071	-0.096	0.090	0.101	0.170
Collagen content	**-0.219***	**-0.248****	-0.165	**0.343*** **	**0.345*** **
Fat content	-0.052	-0.121	-0.122	0.009	0.053
Protein content	**0.229***	**0.291*** **	0.081	**-0.338*** **	**-0.293*** **
Proteolysis Index	**-0.398*** **	**-0.503*** **	-0.109	**0.590*** **	**0.554*** **
Physicochemical parameters of SM muscle					
Final pH	0.076	0.017	0.081	-0.168	-0.165
Water content	0.116	0.131	-0.067	0.001	-0.009
NaCl content	**0.251****	**0.281*** **	-0.137	**-0.195***	**-0.264*** **
a_w	-0.008	-0.022	0.091	0.059	0.092

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

Hardness showed negative relationship with the weight of the raw ham, and the collagen content and proteolysis index of BF. On the other hand hardness was positively related to the total protein content of BF and salt content of SM. Fibrousness was negatively related to the weight, width and thickness of the raw ham, and the collagen content and proteolysis index of BF, and positively related to the total protein content of BF and the salt content of SM. No significant correlation was found between crumbliness and any of the raw ham traits or final composition. Pastiness was positively correlated with the weight and thickness of the raw ham, and the collagen content and proteolysis index of BF; and negatively correlated with pH_u, salt content and total protein content of BF and salt content of SM. Adhesiveness of BF was positively correlated with the weight and thickness of the raw ham, and the collagen content and proteolysis index of BF: and negatively correlated with pH_u, salt content and total protein content of BF and salt content of SM.

Sensory appearance

Table 4.6 shows the correlation coefficients between the sensory appearance (Marbling of BF, SM and ST, Red ring, Crystals, White film) of *Biceps femoris* (BF), *Semimembranosus* (SM) and *Semitendinosus* (ST) muscles and traits of the raw and dry-cured hams.

The marbling of the muscles studied (SM, BF and ST) showed positive correlations with the weight and length of the raw ham. Positive correlation was found between the marbling of BF and width.

With respect to the final composition, the marbling of BF was positively correlated with the collagen content, fat content, and proteolysis index of BF, and negatively correlated with the total protein content of BF as well as the water content of SM.

The marbling of SM was negatively correlated with the water content of SM as well as with the total protein content of BF while it showed positive relationship with some other traits of BF, namely collagen content, fat content, and proteolysis index.

Positive correlations were observed between the marbling of ST and the collagen content, fat content and proteolysis index of BF, and negative correlation were found between the marbling of ST and the water content and a_w of SM.

Red ring score was negatively correlated with the length, width and pH_u of the raw hams and the final pH and fat content of BF. Presence of tyrosine crystals was positively correlated with the weight of the raw ham, collagen content and proteolysis index of BF, and negatively correlated with the pH_u, salt content of BF and final pH and salt content of SM. White film was positively correlated with the weight and width of raw ham, and the water content and fat content of BF; and negatively correlated with the salt content of both BF and SM.

Table 4.6. Correlation coefficients between the sensory appearance (Marbling of BF, SM and ST, Red ring, Tyrosine crystals, White film) of *Biceps femoris* (BF), *Semimembranosus* (SM) and *Semitendinosus* (ST) muscles and traits of the raw and dry-cured hams.

	Sensory appearance of BF, SM and ST *muscles*					
	Marbling(BF)	Marbling(SM)	Marbling(ST)	Red ring	Tyrosine Crystals	White film
Attributes of raw hams						
Weight	**0.325*** **	**0.332*** **	**0.270*** **	-0.024	**0.255*** **	**0.218***
Length	**0.235** **	**0.220***	**0.200***	**-0.256*** **	0.049	0.012
Width	**0.292*** **	0.161	0.151	**-0.183***	0.085	**0.299*** **
Thickness	0.010	0.106	-0.001	-0.009	0.135	0.142
pH_u (SM)	0.073	-0.024	0.051	**-0.302*** **	**-0.212***	-0.120
Physicochemical parameters of BF muscle						
Final pH	-0.050	-0.024	-0.014	**-0.291*** **	-0.157	0.002
Water content	-0.174	-0.132	-0.176	0.066	-0.065	**0.198***
NaCl content	-0.046	-0.099	0.075	0.008	**-0.218***	**-0.274*** **
a_w	-0.130	-0.013	-0.152	-0.048	0.056	0.175
Collagen content	**0.375*** **	**0.313*** **	**0.294*** **	0.041	**0.439*** **	0.169
Fat content	**0.494*** **	**0.578*** **	**0.547*** **	**-0.213***	-0.062	**0.211***
Protein content	**-0.255** **	**-0.242** **	-0.181	-0.072	-0.120	-0.224
Proteolysis Index	**0.345*** **	**0.234***	**0.237***	0.074	**0.460*** **	0.060
Physicochemical parameters of SM muscle						
Final pH	-0.020	-0.097	0.056	-0.150	**-0.199***	0.141
Water content	**-0.349*** **	**-0.196***	**-0.360*** **	0.094	-0.080	-0.025
NaCl content	-0.173	-0.150	-0.092	0.085	**-0.233** **	**-0.279*** **
a_w	**-0.183***	-0.061	**-0.192***	-0.031	0.004	0.150

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

Sensory flavour

Table 4.7 shows the correlation coefficients between the sensory flavour (Saltiness, Bitterness, Sweetness, Umami, Metallic taste, Piquantness, Matureness) of *Biceps femoris* (BF) muscle and traits of the raw and dry-cured hams.

Sensorial assessment of BF saltiness was positively correlated with the chemically determined salt content of BF and SM, and negatively correlated with the a_w and collagen content of BF, and the final pH and a_w of SM. Bitterness was positively correlated with the weight and length of the raw ham, the collagen content and proteolysis index of BF, and negatively correlated with the total protein content of BF and the final pH of SM. Sweetness was positively related to pH_u and to a_w of BF, and negatively related to the proteolysis index of BF. There were also positive correlations between sweetness and the a_w and water content of SM, and negative correlation between sweetness and the length of the raw ham. Umami taste showed positive correlations with the weight of the raw ham, the collagen content and proteolysis index of BF and negative correlations with the total protein content of BF and pH_u. Perception of metallic taste was positively correlated with the collagen content and proteolysis index of BF, and had negative relationship with pH_u. Negative correlations were observed between piquantness and the length of the raw ham. Matured flavour was positively related to the salt content, final pH and total protein content of BF and the pH_u while it was negatively related to the a_w of BF, the thickness of the raw ham and the a_w and water content of SM.

Global sensory quality

Table 4.8 shows the correlation coefficients between the global sensory quality of the dry-cured hams and all recorded traits of the raw and dry-cured hams. There were positive correlations between the global sensory quality and pH_u of the raw ham, final pH, the total protein content of BF, F0 of BF and SM, hardness, fibrousness, crumbliness and matured flavour of BF and b* of SM. There were negative correlations between the global sensory quality and the weight and thickness of the raw ham, the collagen content and proteolysis index of BF, Y2 and Y90 of BF and SM, the pastiness and adhesiveness of BF, the marbling of BF and SM, red ring, tyrosine crystals, bitterness, umami, metallic taste and piquantness.

Table 4.7. Correlation coefficients between the sensory flavour of *Biceps femoris* (BF) muscle and traits of the raw and dry-cured hams

	Sensory flavour of BF muscle						
	Saltiness	Bitterness	Sweetness	Umami	Metallic	Piquantness	Matured
Attributes of raw hams							
Weight	-0.079	**0.331*** **	-0.174	**0.278*** **	0.130	-0.067	-0.148
Length	-0.033	**0.182***	**-0.261*** **	0.048	0.081	**-0.217***	0.008
Width	-0.106	0.101	-0.061	0.066	0.037	0.001	-0.037
Thickness	-0.126	0.143	0.103	0.076	-0.099	0.121	**-0.198***
pH_u (SM)	0.034	**-0.215***	**0.226***	**-0.258*** **	**-0.221***	-0.119	**0.206***
Physicochemical parameters of BF muscle							
Final pH	-0.111	-0.122	-0.012	-0.099	-0.155	-0.143	**0.187***
Water content	-0.086	0.061	0.135	0.061	-0.052	-0.045	-0.134
NaCl content	**0.438*** **	-0.139	-0.138	-0.128	0.036	0.046	**0.242****
a_w	**-0.408*** **	0.029	**0.255*** **	0.050	-0.127	0.017	**-0.318*** **
Collagen content	**-0.201***	**0.367*** **	-0.125	**0.328*** **	**0.271*** **	-0.082	-0.147
Fat content	-0.050	0.016	0.095	0.006	-0.068	-0.051	0.077
Protein content	0.039	**-0.218***	-0.093	**-0.248****	-0.038	-0.172	**0.225***
Proteolysis Index	-0.084	**0.468*** **	**-0.208***	**0.473*** **	**0.290*** **	-0.091	-0.179
Physicochemical parameters of SM muscle							
Final pH	**-0.206***	**-0.188***	0.146	-0.174	-0.110	-0.044	0.174
Water content	-0.140	-0.055	**0.305*** **	-0.059	-0.147	0.042	**-0.318*** **
NaCl content	**0.417*** **	-0.171	-0.023	-0.141	-0.045	0.088	0.146
a_w	**-0.382*** **	-0.006	**0.304*** **	-0.011	-0.156	0.017	**-0.291*** **

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

Table 4.8. Correlation coefficients between the global sensory quality of dry-cured hams and all the recorded traits in the raw and dry-cured hams.

	Global sensory quality		Global sensory quality
Attributes of raw hams		*Sensory texture of BF muscle*	
Weight	**-0.3256*** **	Hardness	**0.4173*** **
Length	-0.079	Fibrousness	**0.4149*** **
Width	0.0022	Crumbliness	**0.3938*** **
Thickness	**-0.1749***	Pastiness	**-0.9162*** **
pH_u (SM)	**0.2563*** **	Adhesiveness	**-0.8567*** **
Physicochemical parameters of BF muscle		*Instrumental colour of BF muscle*	
Final pH	**0.1819***	L*	-0.147
Water	-0.0168	a*	-0.0393
NaCl	0.1581	b*	**0.1911***
a_w	-0.0841	*Instrumental colour of SM muscle*	
Collagen	**-0.3166*** **	L*	0.065
Fat	-0.0043	a*	-0.096
Protein	**0.313*** **	b*	**0.2509****
Proteolysis Index	**-0.5033*** **	*Sensory appearance of BF, SM and* ST *muscles*	
Physicochemical parameters of SM muscle		Marbling(BF)	**-0.2371****
Final pH	0.1565	Marbling(SM)	**-0.1969***
Water	-0.0369	Marbling(ST)	-0.1296
NaCl	0.1453	Red ring	**-0.3077*** **
a_w	-0.0295	Tyrosine Crystals	**-0.5194*** **
Rheological parameters of BF muscle		White film	0.0633
F0	**0.5991*** **	*Sensory flavour of BF muscle*	
Y2	**-0.7846*** **	Saltiness	0.0479
Y90	**-0.6742*** **	Bitterness	**-0.6788*** **
Rheological parameters of SM muscle		Sweetness	**0.1815***
F0	**0.2706*** **	Umami	**-0.5994*** **
Y2	**-0.416*** **	Metallic	**-0.3354*** **
Y90	**-0.403*** **	Piquantness	**-0.298*** **
		Matured	**0.7207*** **

*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.005$

4.3.2. Differences between breeds

4.3.2.1. Traits of raw hams

The morphometrical traits and pH_u of the raw ham were measured. Table 4.9 shows the differences in recorded attributes of the raw hams between breeds. Significant difference was found between the Piétrain and the other three breeds (Duroc, Landrace and Large White) in ham length and thickness, Piétrain hams being shorter and thicker than those of the other three breeds. No significant differences were found in weight or width or in the pH_u of the raw hams. No breed effect was detected for ham weight and pH_u.

Table 4.9. Differences in the raw ham properties (n=122) between breeds. Least squares means, root mean standard error (RMSE) and p-value.

	Breeds									
	DU		LR		LW		Pi		RMSE	P-value
Number of animals	21		44		44		13			
Weight (kg)	11.724		11.255		11.257		11.553		0.997	0.243
Length (cm)	57.586	**a**	56.766	**a**	57.402	**a**	53.577	**b**	2.170	**<.0001**
Width (cm)	29.943		29.411		29.707		30.446		1.888	0.339
Thickness (cm)	15.338	**b**	14.925	**b**	14.907	**b**	16.654	**a**	1.250	**0.0001**
pH_u (SM)	5.602		5.554		5.563		5.555		0.081	0.154

ab: means within a row without a common letter are significantly different (P<0.05).

4.3.2.2. Physicochemical parameters analysis of dry-cured hams

The effect of breed on dry-cured ham propertiesrecorded on *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles is presented in Table 4.10. Pi differed from LR and LW in water content of SM and water activity of both BF and SM muscles, while DU was intermediate, not differing from the other three breeds. The salt content of BF was found to be lower lower in Piétrain than in the other three breeds. There was no breed effect detected for salt content of SM, however it showed the same trend than in BF: Piétrain also gave the lowest value. No

significant differences were found in the pH of BF and SM between the four breeds. Duroc had higher fat content in BF than the other breeds. Duroc had the lowest total protein content. No significant breed effect was found in proteolysis index, but Piétrain had the highest value.

Table 4.10. Differences in recorded attributes of the *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured hams (n=122) between breeds. Least squares means, root mean standard error (RMSE) and p-value.

	Breeds									
	DU		LR		LW		Pi		RMSE	P-value
Number of animals	21		44		44		13			
Physicochemical parameters of BF muscle										
Final pH	5.811		5.818		5.823		5.827		0.059	0.869
Water content (%)	59.160		59.518		59.067		60.267		1.463	0.070
NaCl content (%)	6.083	**a**	6.109	**a**	6.047	**a**	5.666	**b**	0.384	**0.001**
a_w	0.913	**ab**	0.911	**b**	0.911	**b**	0.918	**a**	0.006	**0.001**
Collagen content (%)	0.567		0.530		0.511		0.445		0.161	0.234
Fat content (%)	2.577	**a**	1.253	**b**	1.182	**b**	1.035	**b**	0.674	**<.0001**
Protein content (%)	29.121	**b**	29.533	**ab**	30.324	**a**	29.486	**ab**	1.020	**0.011**
Proteolysis Index (%)	27.318		28.413		27.806		29.195		3.485	0.575
Physicochemical parameters of SM muscle										
Final pH	5.786		5.794		5.771		5.806		0.110	0.739
Water content (%)	53.475	**ab**	52.953	**b**	53.240	**b**	55.000	**a**	2.021	**0.017**
NaCl content (%)	5.259		5.339		5.288		5.056		0.410	0.188
a_w	0.912	**ab**	0.910	**b**	0.911	**b**	0.917	**a**	0.006	**0.002**

ab: means within a row without a common letter are significantly different (P<0.05).

4.3.2.3. Rheological analysis

Table 4.11 shows the differences in rheological values (F0, Y2, Y90 value of stress relaxation test) of the *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured hams between breeds.

In BF, Large White showed a higher F0 than Landrace and Piétrain. Duroc was not significantly different from the other breeds. No breed effect was found in Y2 and Y90.

In SM, Piétrain showed lower F0 than Landrace and Large White, higher Y2 than other three breeds, higher Y90 than Landrace and Large White. F0 and Y90 of Duroc were not significantly different from other three breeds.

Table 4.11. Differences in rheological values (F0, Y2, Y90 value of stress relaxation test) of the *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles of the dry-cured hams (n=122) between breeds. Least squares means, root mean standard error (RMSE) and p-value.

	Breeds									
	DU		LR		LW		Pi		RMSE	P-value
Number of animals	21		44		44		13			
Rheological parameters of BF muscle										
F_0 (kg)	1.488	**ab**	1.505	**b**	1.961	**a**	1.256	**b**	0.699	**0.003**
Y_2	0.404		0.397		0.391		0.409		0.028	0.159
Y_{90}	0.702		0.697		0.690		0.701		0.022	0.164
Rheological parameters of SM muscle										
F_0 (kg)	5.017	**ab**	5.337	**a**	5.301	**a**	3.592	**b**	1.589	**0.005**
Y_2	0.359	**b**	0.355	**b**	0.356	**b**	0.381	**a**	0.022	**0.003**
Y_{90}	0.628	**ab**	0.625	**b**	0.626	**b**	0.649	**a**	0.024	**0.017**

ab: means within a row without a common letter are significantly different (P<0.05).

4.3.2.4. Instrumental colour analysis

Table 4.12 shows the differences in instrumental colour (Lightness, L*; redness, a*; yellowness, b*) of the *Biceps femoris* (BF), *Semimembranosus* (SM), *Vastus intermedius* (VI), and *Semitendinosus* (ST) muscles of the dry-cured hams between breeds.

Table 4.12. Differences in instrumental colour (Lightness, L*; redness, a*; yellowness, b*) of the *Biceps femoris* (BF), *Semimembranosus* (SM), *Vastus intermedius* (VI), and *Semitendinosus* (ST) muscles of the dry-cured hams (n=122) between breeds. Least squares means, root mean standard error (RMSE) and p-value.

	Breeds									
	DU		LR		LW		Pi		RMSE	P-value
Number of animals	21		44		44		13			
Instrumental colour parameters of BF muscle										
L*	41.86	**a**	39.96	**b**	39.61	**b**	39.46	**b**	1.64	**<.001**
a*	14.58	**b**	15.53	**a**	15.71	**a**	15.03	**ab**	1.35	**0.01**
b*	5.97	**a**	5.75	**ab**	5.58	**ab**	5.05	**b**	0.96	**0.05**
Instrumental colour parameters of SM muscle										
L*	37.66	**a**	36.43	**ab**	36.20	**b**	37.08	**ab**	1.98	**0.036**
a*	14.33		14.62		14.69		13.90		1.46	0.335
b*	6.04		6.09		5.81		5.46		1.03	0.226
Instrumental colour parameters of VI muscle										
L*	40.97	**a**	37.90	**b**	37.79	**b**	37.59	**b**	2.12	**<.001**
a*	12.22		13.09		13.13		12.94		1.35	0.07
b*	6.40		5.88		5.62		5.08		1.55	0.09
Instrumental colour parameters of ST muscle										
L*	42.59	**a**	41.13	**b**	41.26	**b**	41.09	**b**	2.12	**0.05**
a*	14.00		14.04		13.99		13.01		1.63	0.23
b*	5.88		5.89		5.71		5.65		1.05	0.79

ab: means within a row without a common letter are significantly different (P<0.05).

In BF, Duroc had higher L* than other three breeds, lower a* than Landrace and Large White and higher b* than Piétrain.

In SM, difference in L* was found between Duroc and Large White, L* of Duroc being higher. No significant difference was found in a* or b* of SM between the four breeds.

In VI and ST, difference in L* was found between Duroc and the other three breeds. Duroc showed higher L*. No significant difference was found in a* or b* between the four breeds.

4.3.2.5. Sensory analysis

Table 4.13 shows the differences in the sensory texture, sensory appearance, sensory flavour, and global sensory quality of the dry-cured hams between breeds.

Of all the sensory texture traits, only fibrousness was affected by breed. Higher fibrousness of BF was observed in dry-cured ham of LW than in DU or PI pigs, LR being intermediate. Duroc showed the highest marbling in BF, SM and ST of the four breeds. In the rest three breeds, Landrace was higher in marbling than Piétrain, neither of which were different from Large White. No breed effect was found on red ring pale core, tyrosine crystals or white film.

No significant differences were found between the four breeds regarding the sensory flavour items (metallic taste, sweetness, saltiness, piquantness, bitterness, umami, matured). The panellists did not find any significant differences between breeds in the global sensory quality.

Table 4.13. Differences in sensory texture of the *Biceps femoris* (BF) muscle, sensory appearance of BF, *Semimembranosus* (SM) and *Semitendinosus* (ST) muscles, sensory flavour of BF, and global sensory quality of dry-cured hams (n=122) between breeds. Least squares means, root mean standard error (RMSE) and p-value.

	Breeds									
	DU		LR		LW		Pi		RMSE	P-value
Number of animals	21		44		44		13			
Sensory texture of BF muscle										
Adhesiveness	3.091		2.492		2.460		3.353		1.294	0.132
Hardness	3.113		3.236		3.412		3.071		0.522	0.154
Crumbliness	4.057		4.180		4.050		4.307		0.473	0.332
Pastiness	2.869		2.428		2.271		3.333		1.541	0.230
Fibrousness	2.521	**b**	2.707	**ab**	2.985	**a**	2.477	**b**	0.534	**0.010**
Sensory appearance of BF, SM and ST *muscles*										
Marbling (BF)	3.708	**a**	3.030	**b**	2.613	**bc**	1.967	**c**	0.906	**<.0001**
Marbling (SM)	2.479	**a**	1.689	**b**	1.544	**bc**	1.114	**c**	0.674	**<.0001**
Marbling (ST)	4.312	**a**	3.332	**b**	2.889	**bc**	2.006	**c**	1.117	**<.0001**
Red ring	1.769		1.551		1.493		2.608		1.503	0.119
Tyrosine crystals	6.731		6.838		8.511		10.405		7.631	0.404
White film	4.674		3.951		3.513		3.936		1.759	0.114
Sensory flavour of BF muscle										
Metallic	2.578		2.620		2.493		2.538		0.532	0.780
Sweetness	0.946		0.871		0.872		0.970		0.208	0.395
Saltiness	3.068		3.135		3.087		3.041		0.289	0.748
Piquantness	1.425		1.338		1.382		1.409		0.272	0.699
Bitterness	1.423		1.339		1.312		1.580		0.338	0.152
Umami	2.169		2.152		2.184		2.304		0.413	0.758
Matured	2.638		2.879		2.823		2.597		0.531	0.306
Global sensory quality	3.876		4.131		4.213		3.734		0.769	0.257

abc: means within a row without a common letter are significantly different (P<0.05).

All attributes except Tyrosine crystals: 0-10 points non-structured scales (0: absence; 10: maximum intensity).

Tyrosine crystals: Number of small white crystal spots appearing on the surface of the slice.

4.3.3. Principal Component Analysis (PCA)

4.3.3.1. Instrumental and sensory texture

Fig. 4.3 shows the principal component loadings and principal component scores for the four breeds in the PCA of the rheological parameters and sensory texture attributes of BF muscle and the global sensory quality of the dry-cured hams. The first component, which explained 60.76 % of the total variation, contrasts instrumental texture parameters and sensory texture attributes related with softer texture (Y2, Y90, pastiness and adhesiveness) with those related with harder texture (F0, fibrousness and hardness), as well as the the global sensory quality. The second component, which explains 18.05 % of the total variation, contrasts fibrousness and hardness with crumbliness. The F0 of BF positively correlated with the global sensory quality while Y2, Y90, pastiness and adhesiveness were negatively related to the global sensory quality. Hardness and fibrousness were positively correlated. The four breeds were discriminated in the first component with the order of Piétrain, Duroc, Landrace and Large White, according to the direction of global sensory quality.

4.3.3.2. Instrumental colour and sensory appearance

Fig. 4.4 shows the principal component loadings and principal component scores for the four breeds in the PCA of instrumental colour (Lightness, L*; redness, a*; yellowness, b*) of *Biceps femoris* (BF) and the sensory appearance and global sensory quality of the dry-cured hams. The first component which explains 25.89 % of the total variantion, contrasts b*, L* and white film with a*, red ring and tyrosine crystals. The second component contrasts the global sensory quality with marbling, L* and tyrosine crystals. Tyrosine crystals, red ring, marbling and L* of BF are negatively correlated with the global sensory quality. Mean values of four breeds shows Duroc is far from other three breeds and it is positively correlated with L*, b*, marbling and white film.

4.3.3.3. Sensory flavour

Fig. 4.5 shows the principal component loadings and principal component scores for the four breeds in thePCA of sensory flavour of *Biceps femoris* (BF) muscle of the dry-cured hams. The first component, which explains 34 % of total variation, contrasts the global sensory quality and matured flavour with umami, bitterness and metallic flavour. The second component, which explains 22.75 % of total variation, contrasts sweetness and piquantness with saltiness, metallic and matured flavour. Global sensory quality is positively related to matured flavour while negatively related to piquantness, metallic taste, umami and bitterness. The four breeds were well discriminated on the second component. Large White is characterized by matured flavour and saltiness. Landrace is characterized by saltiness, matured flavour and metallic taste. Duroc and Piétrain are characterized by sweetness and piquantness. Large white and Landrace are positively characterized by global sensory quality while Duroc and, especially Piétrain are characterized by negative global sensory quality.

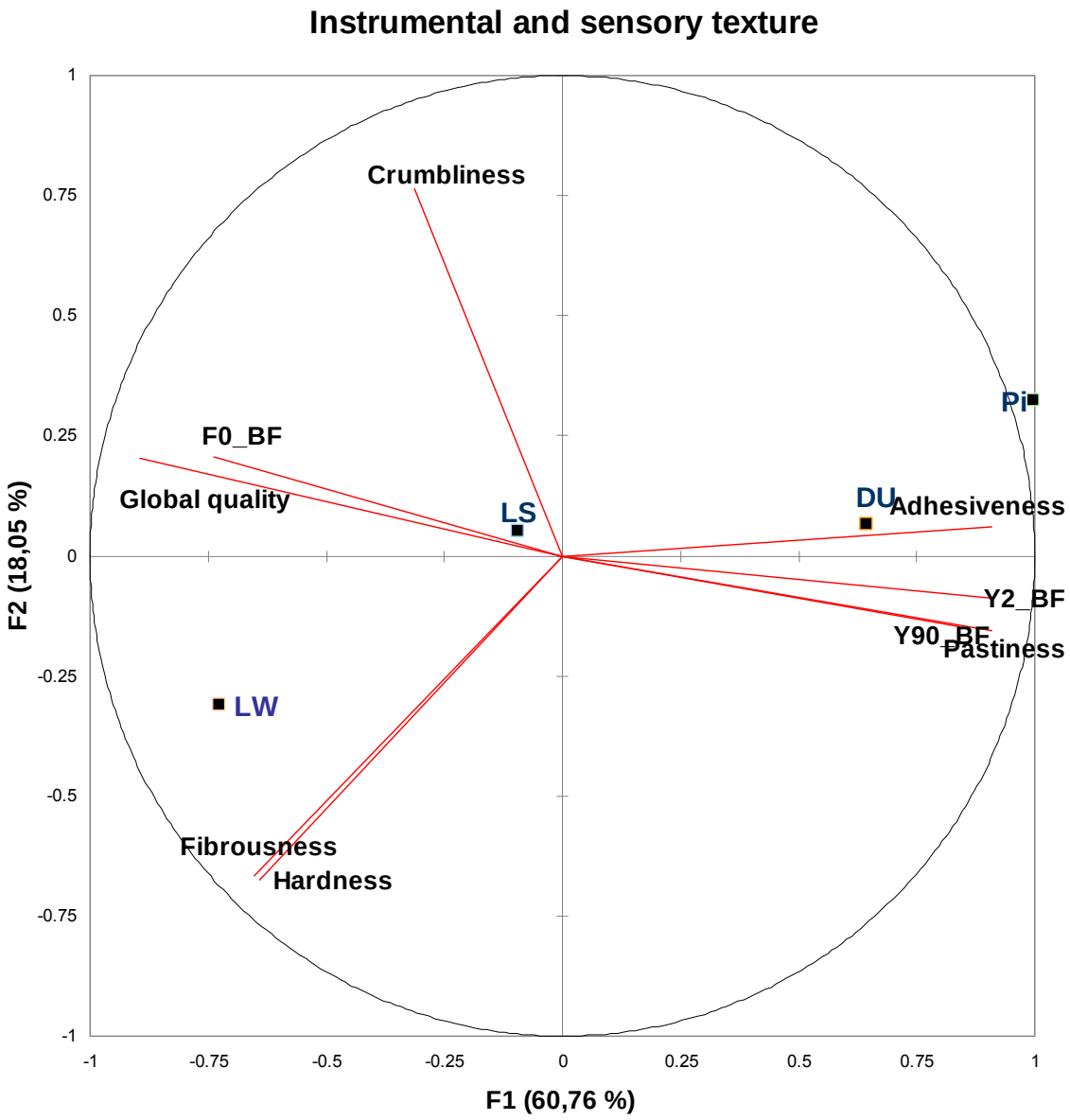

Fig. 4.3. Principal component analysis (PCA) of rheological parameters (F0, Y2, Y90) and sensory texture attributes of *Biceps femoris* (BF) muscle and global sensory quality of dry-cured hams (n=122). Principal component loadings and principal component scores for four pure breeds of Duroc (DU), Landrace (LR), Large White (LW) and Piétrain (PI).

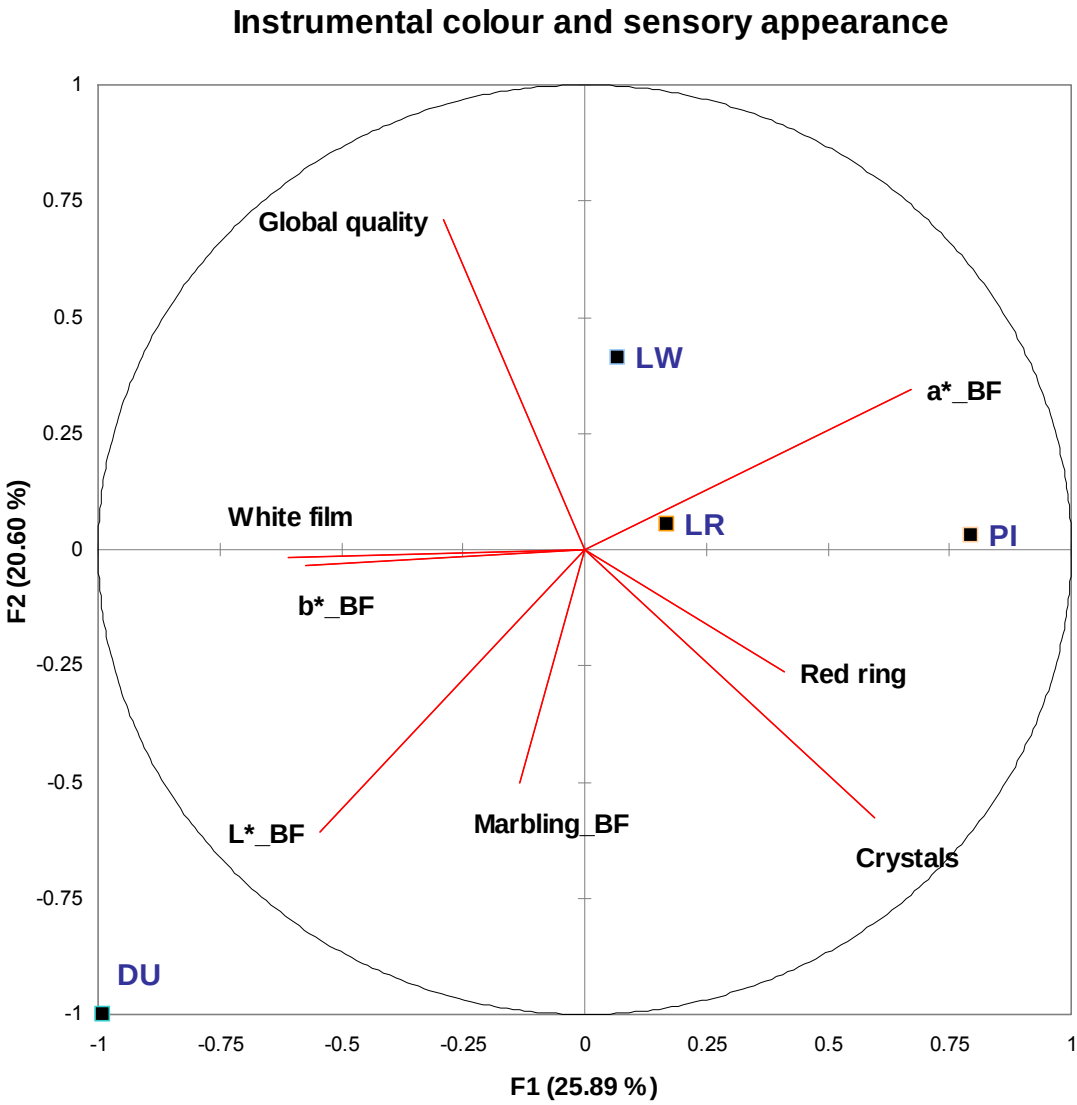

Fig. 4.4. Principal component analysis (PCA) of Instrumental colour (Lightness, L*; redness, a*; yellowness, b*) of *Biceps femoris* (BF), sensory appearance and global sensory quality of dry-cured hams (n=122). Principal component loadings and principal component scores for four pure breeds of Duroc (DU), Landrace (LR), Large White (LW) and Piétrain (PI).

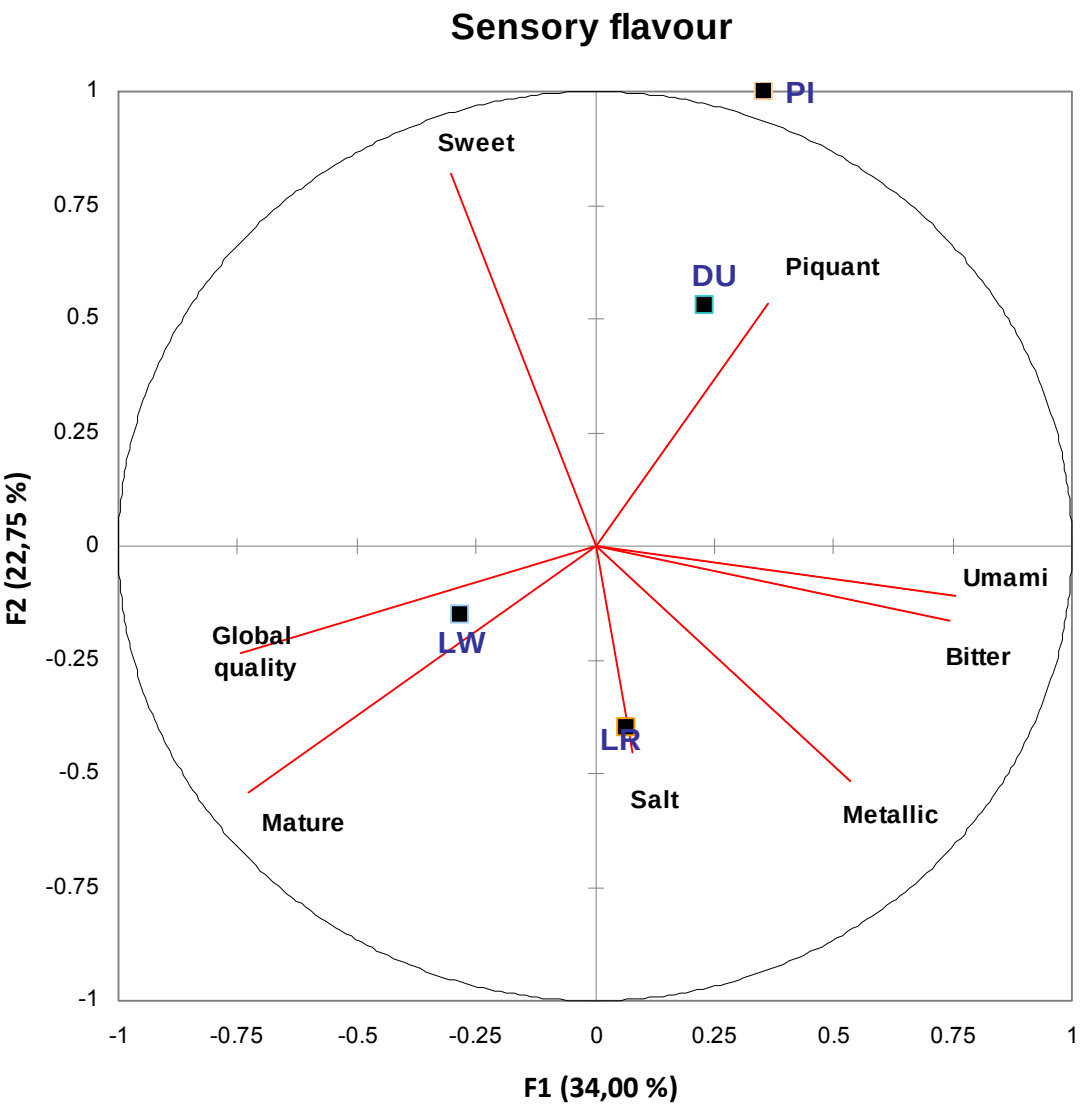

Fig. 4.5. Principal component analysis (PCA) of sensory flavour (Saltiness, Bitterness, Sweetness, Umami, Metallic taste, Piquantness, Matured) of *Biceps femoris* muscle of dry-cured hams (n=122). Principal component loadings and principal component scores for four pure breeds of Duroc (DU), Landrace (LR), Large White (LW) and Piétrain (PI).

4.4. Discussion

4.4.1. Traits of raw hams

In the present study, raw hams of PI were significantly shorter and thicker, which agrees with the results of Fisher, Green, Whittemore, Wood, & Schofield (2003). In general the literature dealing with dry-cured ham does not provide many data on morphometrical traits of hams, although they are important for salt intake and diffusion. The differences in carcasses and primal cuts between breeds have been mentioned before (Raj et al., 2010; Edwards, Bates, & Osburn, 2003; Ellis et al., 1996; Kanis et al., 1990; García-Macías et al., 1996; Affentranger, Gerwig, Seewer, Schwörer, & Künzi, 1996; Peloso, Lopes, Gomide, Guimarães, & Carneiro, 2010; Fisher et al., 2003) and show that the Piétrain breed grows more slowly than other breeds and that they have shorter carcass. Wagner, Schinckel, Chen, Forrest and Coe (1999) demonstrated that the effect of genetic population was significant for carcass measurements as well as for ham primal cut weight. Edwards et al. (2003) reported that Piétrain progeny had a higher percentage of ham within the carcass than Duroc progeny. However, with a heavier carcass weight, Duroc progeny had greater primal cut weights as a function of age, similar to that which Affentranger et al. (1996) reported. However, the weights of the raw ham from the four breeds were not significantly different in this study, probably due to the fact that the weight here was measured from a trimmed thigh instead of from a primal cut. This was also referred to by Lo Fiego, Santoro, Macchioni and De Leonibus (2005), who established that Landrace × Large White pigs produced thighs with significantly higher weights compared with the Cotswold commercial hybrid line, but the differences disappeared after trimming because of higher trimming losses, arising from the presence of a thicker fat covering. García-Macías et al. (1996) demonstrated no ham percentage difference between Duroc and Piétrain progeny. Morphometrical differences of dissected ham have barely been discussed in previous research. Fisher et al. (2003) used the logarithmic transformation of data in the allometric model to predict the dimension of carcass components between the breeds of Piétrain, Landrace and Meishan, and the results showed that Piétrain had a wider ham than Landrace. No significant difference in the length of the pelvic limb (similar to the length of the

ham) were found between the two breeds. This result is partly in accordance with what was found in this study when a comparison was made between four breeds, i.e. that raw hams from Piétrain were significantly shorter and thicker than those from the other three breeds, including Landrace. Slightly different methods of measurement could account for the dissimilarity between the two results.

No significant breed effect on pH_u was found in the present study although some previous research reported significant breed differences (Schivazappa et al., 2002; Edwards et al., 2003; Affentranger et al., 1996; García-Macías et al., 1996; Soriano et al., 2005). Similarly, Peloso et al. (2010) found no significant difference for pH_u between six crossbreeds (crossed by Duroc, Landrace and Large White) in two weight groups of 130kg and 160kg.

4.4.2. Physicochemical analysis of dry-cured hams

Water content, water activity and salt content were significantly correlated and were also significantly affected by breed. Piétrain showed the highest water content and water activity, and the lowest salt content. Salt diffusion and water movement are two main and strongly connected processes taking place in the initial stages of dry-cured ham processing. Salt must diffuse in the muscle through a liquid phase while water moves from the inner to the outer part of the muscle in the opposite direction. Both movements follow Fick's law. Salt diffusion, caused by salt gradient, is much more important since its De, the diffusive coefficient, is twenty times higher than the De of water. Some previous research has revealed a difference in the moisture of raw meat between breeds (Sellier & Monin, 1994; Schivazappa et al., 2002; Soriano et al., 2005). On the other hand, some studies have shown that difference in the moisture of raw meat between breeds could be eliminated after curing (Franci et al., 2007).

The final pH of dry-cured ham rises slightly from pH_u, which has been proved to be due to the generation of amino acids in proteolysis. In this study, the final pH of BF was correlated with the pH_u of SM. The pH_u showed no significant difference between breeds, neither did the proteolysis index. This could explain why significant differences on the final pH between the breeds were not detected.

On the other hand, pH affects the activities of muscle enzymes. It has been demonstrated that the proteolysis is more intense in low pH hams (Arnau,

Guerrero, Casademont，& Gou, 1995 & 1998), particularly at the end of processing (Buscailhon et al., 1994). No difference was found in proteolysis between the breeds in this study probably because of the similar pH_u of the raw hams used. The difference in the total protein content may be due in part to the different raw meat composition of breeds.

Similarly, different fat content in the raw hams could also account for the differences in fat content of the dry-cured hams between breeds. The Duroc breed has been demonstrated to be rich in fat content, especially intramuscular fat content (Schivazappa et al., 2002; Peloso et al.,2010; Edwards et al, 1992).

4.4.3. Rheological analysis of dry-cured ham

The texture of dry-cured ham is a characteristic directly related to the muscle structure, and especially related to the degradation of myofibrillar protein and collagen as well as to the intramuscular fat content and drying rate (Toldrá, 1998). Morales, Serra, Guerrero and Gou (2007b) indicated that a lower F0 value and higher F2 and F90 values were related to a higher softness. The correlation coefficients in this study showed that the weight and thickness of the raw ham were positively related to softness of BF in the dry-cured ham. The characteristics of fresh ham (conformation, fat thickness, and fat content) from different genetic origins have also been reported to be responsible for the differences found in dry-cured ham texture (Gou, Guerrero, & Arnau, 1995; Guerrero et al., 1996). Heavier and thicker hams tend to give a higher a_w and a lower salt content in BF because water movement and salt diffusion are slow in such hams. Heavier hams, which are usually from heavy pigs, have also been related to lower calpain and cathepsin (B, L and D) levels in raw ham as well as in dry-cured ham, (Sárraga et al., 1993) which could cause lower proteolysis. Softness positively correlated with a_w whereas negatively correlated with salt content. Parolari et al. (1988) reported that softness in dry-cured hams is increased by low salt to moisture ratios. Virgili, Parolari, Schivazappa, Bordini and Borri (1995) also reported that hardness was enhanced by salt concentration. Softness is associated with increased proteolysis (Parolari, 1994; Virgili et al., 1995), high cathepsin activity (Parolari, Virgili, & Schivazappa, 1994; Parreño, Cussó, Gil, & Sárraga, 1994; Arnau, Guerrero, & Sárraga, 1998), in agreement with the positive correlation found between

proteolysis index and softness, and the negative correlation between protein content and softness.

In this study the moisture content showed a significantly positive effect on softness only in SM, not in BF, probably due the influence of other factors such as fat content and perhaps because of the small difference in water content between the samples. Ruiz-Carrascal et al. (2000) also found no significant relationship between the moisture (water content) and the sensory textural characteristics (hardness, dryness and juiciness) in Iberian dry-cured hams.

Ruiz-Ramírez et al. (2006) observed that dry-cured hams with higher proteolysis show lower hardness and higher cohesiveness and springiness, especially at low water content, which is in agreement with the different effects of water content on texture in this study.

Rheological differences were found between the breeds in F0 of BF and F0, Y2 and Y90 of SM. Piétrain showed the lowest F0 and the highest Y2 and Y90, which means that Piétrian had the softest texture. Landrace and Large White presented the highest F0 and the lowest Y2 and Y90 in SM. Duroc gave an intermediate value without significant differences from the other breeds. This is in accordance with the difference found in a_w and water content of SM, where Piétrain showed higher value than Landrace and Large White. Duroc showed no significant difference fromthe other three breeds. Moreover, the texture of SM changed partly in agreement with the morphometrical properties of the raw hams, Piétrain being shorter and thicker than the other three breeds. The SM muscle is located at the surface of the ham and is one of the hardest muscles in the ham, therefore its texture is affected by morphometrical properties, trimming and internal water diffusion much more than the texture of BF. The BF is more complicated. A difference was only found inn F0, where Large White was higher and Landrace and Piétrain were lower, Duroc showed no significant difference from the other three breeds. This could be explained by the different protein and fat content in the dry-cured hams, as the Large White gave a higher protein content and a lower fat content. Ruiz-Carrascal et al. (2000) found that the intramuscular fat content had a strong influence on the texture of ham.

4.4.4. Instrumental colour of dry-cured ham

Breed effects on instrumental colour (CIELAB space) were found both in the inner muscle (BF) and in the outer muscles (SM, VI, ST). Significant differences of L*, a* and b* between breeds were found, with DU being lighter, less red and yellower; LR and LW being less light, redder and intermediate yellow; PI being less light, intermediate red and less yellow.

The colour of meat and meat products is influenced by its moisture and fat content as well as the content of hemoprotein, particularly myoglobin, and its existing form. The colour of the dry-cured meat products mainly depends on the concentration of pigment (myoglobin), the percentage of conversion to the nitrosyl pigment. The myoglobin concentration depends on the type of muscle (Aristoy & Toldrá, 1998), being higher in muscles with oxidative patterns than in muscles with glycolytic patterns like M. *Semimembranosus*. Most of the muscles in ham exhibit glycolytic or intermediate metabolism (Laborde, D., Talmeant, A., & Monin, 1985). The age of the animal also has an important contribution to the increase in the concentration of myoglobin. However, age was not discussed since the animals in this study had the same age at slaughter.

It has been shown that heme pigments contents, especially that of myoglobin (Mb) and its metmyoglobin (MetMb) and oxymyoglobin (MbO) forms represent the parameters that most contribute to colour variations in pig muscles (Warriss, Brown, & Adams, 1990). Lindahl, Lundström and Tornberg (2001) demonstrated that the pork muscles became darker at higher pigment content and more myoglobin oxidation (higher fractions of MetMb), while more blooming (higher fractions of MbO) resulted in a lighter colour. The pork muscles became redder at higher pigment content and more myoglobin oxidation (higher fractions of MetMb), while less blooming (higher fractions of Mb) resulted in less redness. The pork muscles became more yellow at more blooming (higher ratio of MbO/Mb) and higher fibre optic probe (FOP) value.

Water activity was found to be positively related to lightness in this study in agreement with previous studies (Pérez-Alvarez, 1996; Pérez-Alvarez, et al., 1997; 1999). Hunt (1980) demonstrated that lightness is related to the thin aqueous layer on the muscle's surface. It has beensuggested that lightness in the muscles depends on the free water content and water movement towards the surface

(Pérez-Alvarez et al., 1997). More free water is also beneficial to oxygen solubilization, and is therefore beneficial to myoglobin oxidation, which contributes to high redness, according to the report of Lindahl et al. (2001). This could also explain the positive relationship between water content and lightness in SM. Furthermore, a higher water content leads to a higher internal reflectance, which can result in a higher yellow colour in SM (Lindahl et al., 2001). The positive relationship between water content and colour of lightness and yellowness in SM was also found by Pérez-Alvarez et al. (1999) and Ramos, Serenius, Stalder and Rothschild (2007). Pérez-Alvarez et al. (1997) also reported that in other dry-cured meat products, adding water during processing increased L* value.

It has previously been estimated that salt content plays an important role in the change of L* (decrease) (Pérez-Alvarez et al., 1997), a* (increase) (Femández-López, 1998) and b* (decrease) (Pérez-Alvarez et al., 1997; Pérez-Alvarez et al., 1999). Ramos et al. (2007) demonstrated that salt content was not correlated with instrumental colour of dry-cured ham. The salt content showed no correlation with any value of the instrumental colour in this study, which was in agreement with the result of Carrapiso and García (2005), in BF of Iberian ham.

In this study, positive correlations were found between pH_u (SM) and b* of BF and SM, which means that the ham with higher pH is yellower and lighter. The pH effects on meat colour, both the fresh and dry-cured, have been reported. Offer and Knight (1988), as well as Lindahl et al. (2001), reported that the pH_u was a main determinant of fresh meat colour. The meat surface may be more or less translucent, depending on the rate of the post mortem pH drop, the ultimate pH and the extent of protein denaturation (Bendall & Swatland, 1988; Feldhusen, 1994). Ramos et al. (2007) did not find a significant correlation between the pH_u and the lightness of dry-cured ham. These results are not in agreement with previous research (Pérez-Alvarez et al., 1997; Brewer, Zhu, Bidner, Meisinger, & McKeith, 2001; Huff-Lonergan et al., 2002; Fernández-López, Sayas-Barberá, Pérez-Alvarez, & Aranda-Catalá, 2004; Gjerlaug-Enger, Aass, Ødegård, & Vangen, 2010), which demonstrates that pH_u does not affect meat colour and proved that pH45 is negatively related to lightness. More MbO, resulting in a higher ratio of MbO/Mb, is formed at low temperatures and at low pH values,

conditions that increase oxygen solubility and inhibit oxygen consumption enzyme activity (Ledward, Johnston, & Knight, 1992).

Visible intramuscular fat depot denoted as marbling in dry-cued ham, which is derived from marbling of fresh meat, can affect (increase) the L value (Van Der Wal, Olsman, Garssen and Engel,1992; Jones, Tong, Campbell and Dyck, 1994; Hermesch, Luxford and Graser, 2000; Huff-Lonergan et al., 2002; Suzuki et al., 2005), which could explain a high fat content of BF yielded higher lightness or L* values of BF and ST in the present study. On the contrary, Hovenier, Kanis, van Asseldonk and Westerink (1992) reported a negative genetic correlation between lightness and IMF in fresh meat. The disagreement probably came from the difference between breeds. Gjerlaug-Enger et al. (2010) demonstrated that there was a negative genetic correlation between L* value and IMF of Landrace, whereas this correlation was positive in Duroc. Muscles with higher levels of IMF are usually darker (Essén-Gustavsson & Fjelkner-Modig, 1985). Muscles with high levels of IMF are usually more oxidative than muscles with low levels, since oxidative muscles use more fat during metabolism (Essén-Gustavsson & Fjelkner-Modig, 1985). Essén-Gustavsson, Karlsson, Lundstrom and Enfalt (1994) showed that lipids are stored mainly in type I fibres but alsoin some type IIA fibres. Oxidative muscle contains more mitochondria, has a higher myoglobin content and a higher pH *post mortem*. All these factors give oxidative muscles a darker colour than glycolytic muscles. The positive genetic correlation between L* value and IMF in this study probably did not come from the meat colour, but from visible fat cells detected when the colour was measured. There was almost no marbling in Landrace, and the darker meat (lower L* value) also had more IMF in this breed. Peloso et al. (2010) indicated that a high IMF probably affected the lightness, as measured by Göfo readings, thus revealing a lighter colour of the *Semimembranosus muscle* surface. Carrapiso and García (2005) observed that L* was significantly correlated to visible intramuscular fat (marbling) but not to total intramuscular fat in Iberian dry-cured ham. Marbling was also positively correlated with L* in this study ($r=0.26$, $p<0.005$). However, the results gave very proximate value, to those from this study, of correlation coefficient between L* and IMF as well as between L* and marbling. That was probably due to the smaller number of samples of thirty-four dry-cured Iberian hams, whereas one hundred twenty two hams were sampled in this study.

The protein content in BF was found to be negatively correlated with the lightness of BF, probably because a lower protein content comes with a higher fat content, which causes higher lightness.

The colour of Serrano ham is a typical bright-red colour from the action of nitrite with myoglobin that will depend on the percentage of myoglobin transformed into nitrosylmyoglobin. Nitrates and nitrites constitute the curing agents added in the formulation of some kinds of dry-cured ham. The former is reduced to nitrite through the action of microbial nitrate reductase activity, typically present in Gram (+) catalase (+) *cocci*. Most of the formed nitrite is reduced to nitric oxide either by meat reductants or by additives such as sodium ascorbate/erythorbate. The curing colour in dry-cured ham is formed through several steps (Lücke, 1985). First, oxygenated myoglobin (red) reacts with nitrous acid to give metmyoglobin (brown) and nitrate. Second, nitrous acid is reduced to nitric oxide, favored by the presence of ascorbate, and metmyoglobin to myoglobin. Then, myoglobin and nitric oxide interact forming the red coloured nitric oxide myoglobin. This reaction is speed up at low pH. During cooking, the protein moiety of nitric oxide myoglobin is denatured giving the formation of the pink nitric oxide myochromogen.

4.4.5. Sensory analysis of dry-cured ham

The marbling (BF, SM and ST) was positively correlated with the initial weight, length and width (only with marbling of BF) of the raw hams. The relationship indicated that bigger raw hams, generally with more intramuscular fat, tend to result in more marbling, which is in line with many previous authors (Daszkiewicz, Denaburski, & Sáiz-Cidoncha, 2004; Asenjo, Miguel, Ciria, & Calvo, 2005; Galián, Poto, & Peinado, 2009) all of whom showed the relationship between IMF levels and factors such as age and weight at slaughter. As was ecpected, marbling, i.e. visible intramuscular fat depot, is correlated with intramuscular fat, which was also demonstrated by (Ruiz-Carrascal et al., 2000; Van der Wal et al., 1992). Therefore, different fat contents between the breeds can account for differences in marbling between breeds in the present study. Du, which has the highest fat content in BF of the dry-cured ham, presented the highest marbling score among four breeds. Similar results have been reported in studies with raw meat (Barton-Gade, 1987; Wood et al.,1988; McGloughlin et al., 1988; Edwards et al., 1992; Oliver et al., 1994; Ellis, Webb, Avery, & Brown, 1996) and with dry-cured ham (Guerrero et al.,

1996). In the later study, DU breed exhibited more marbling than pure breeds Piétrain and Belgian Landrace and another cross consisting of DU × LW.

A significant correlation was found between the collagen content of dry-cured ham and the weight of raw ham. A similar relationship was also found in the study of Zeng, Wang and Wei (2008), which demonstrated that an increased weight of pigs was associated with an increase in the total collagen content and insoluble collagen content of muscle, but tended to be related to a decrease in the soluble collagen content, collagen solubility and tenderness. Meanwhile, both the collagen content of the dry-cured ham and the weight of the raw ham were positively correlated with the marbling and the intramuscular fat content. This could be explained by the fact that pork with a higher collagen content usually exhibits less glycolytic and more oxidative metabolism, potentially using more lipids as an energetic substrate and is prone to higher intramuscular lipid deposit (Hu, Wang, Zhu, Guo, & Wu, 2008).

A breed effect on the water content was found in SM in the present study. The water content in the SM of the dry-cured ham was significantly correlated with marbling. Higher intramuscular fat content means a decrease in water content The different fat contents rather than the differences in dehydration were suggested to account for it (Schivazappa et al., 2002). Differences in moisture between muscles, as a consequence of their position, are showed by Monin et al (1997) and Ruiz-Ramírez et al (2006). It has previously byee reported that SM had a significantly lower moisture content between three muscles (Pérez-Alvarez et al., 1997) and that BF had the highest fat content and the lowest protein content (Franci et al., 2007).

No significative correlations between salt content and marbling in both the BF and the SM were found, although the correlation coefficients were negative, in agreement with the negative relationship between salt content and marbling found by Arnau (1991). deducing that water between the myofibrillar helps diffusion of sodium chloride in pork (Offer & Knight, 1988), and more intramuscular fat content would be an unfavorable factor (Schivazappa et al., 2002).

Although no significant relationship was found, marbling, namely intramuscular fat, was presumed to reduce diffusion of sodium chloride to a certain degree in pork (Offer & Knight, 1988; Schivazappa et al., 2002), which consequently, favoured proteolysis.

The presence of white precipitates is a problem in dry-cured ham and is closely related to the changes in its proteins. The formation of tyrosine crystals has been proved to be developed by strong proteolysis, which benefits from a low pH 24 h *post mortem* or a low salt content (Guerrero et al., 1996; Virgili et al., 1995; Virgili, Schivazappa, Parolari, Soresi Bordini, & Degni, 1998; Schivazappa et al., 2002). The same relationships were found in this study. The proteolysis index showed a positive correlation with the initial weight of the raw ham. A positive correlation between tyrosine crystals and the initial weight of the raw ham, as well as the collagen content, could be due to the fact that the initial weight of raw ham and the collagen content of BF were positively correlated with proteolysis.

The release of tyrosine during the curing process, as in the case of other aminoacids, has an enzymatic origin (Toldrá & Etherington, 1988; Sárraga, Gil, & García-Regueiro, 1993). It depends on the factors which regulate the cathepsin and calpain activity, although tyrosine is less soluble in water than the other amino acids found in meat, having a special tendency to form precipitates when the moisture content of the ham falls during the ageing process.

White film (WF) is similar to tyrosine crystals in composition and formation. Butz, Blumer, Christian and Swaisgood (1974) identified white film on cut surfaces of dry-cured ham as crystalline L-tyrosine. It was also found that free L-tyrosine averaged 287.3 μmol/g (5.2% by weight) in the film and 6.1 μmol/g in the ham tissue from which the film had been removed. It is concluded that the film forms on the cut surface because the concentration of free L-tyrosine and certain other free amino acids in ham, is greater than the solubility levels. Salt content and pH_u were negatively correlated with white film, which agrees with previous studies (Virgili et al., 1995; Arnau et al., 1994; Arnau, Guerrero, & Pere, 1997). The positive relationship between white film and the width of the raw ham as well as a less significant relationship between white film and the weight of raw ham could be due to the fact that heavier and wider hams are prone to having a low salt content.

Regardng PCA for texture parameters, hardness was positively correlated with fibrousness, both of which showed positive relationship with the global sensory quality. Pastiness and adhesiveness were positively related to each other and both negatively related to the global sensory quality. The most correlating factors with global sensory quality were instrumental texture (F0, Y2 and Y90), adhesiveness and pastiness, all of which can be considered as predictors of final

product quality. Large White showed the highest hardness and fibrousness and the lowest adhesiveness and pastiness, therefore resulting in the best global sensory quality, while Piétrain was the worst. Serra, Ruiz-Ramírez, Arnau, & Gou (2005) reported a negative non-linear relationship between hardness and both water content and water activity. Hardness was positively related to the total protein content and negatively to the proteolysis index. Adhesiveness and pastiness were negatively correlated to the proteolysis index. Large White had a lower water content and a higher total protein, which resulted in the highest positive effect on the global sensory quality.

The PCA for the instrumental colour and sensory appearance showed that no factors seemed to have strongly positive effect on the global sensory quality, whereas tyrosine crystals, red ring and marbling of BF negatively affected the global sensory quality. Red ring was produced because nitrite did not reach the central part of the ham and was negatively correlated with the pH of raw ham, which agrees with Arnau, Gou and Comaposada (2003). The red ring was also affected by the width and fat content, which could have reduce the percentage of absorbed nitrite. Marbling is usually thought to be a positive visual trait, but the high intramuscular content of the pure breed Duroc might have turned it into an adverse one. In this study, Duroc was far different from the other three breeds in that it showed the most negative position. Large White was the best one according to the global sensory quality, and was differentiated from Landrace and Piétrain on the second principal component. Landrace and Piétrain were similar on the second principal component although being well differentiated on the first one. Piétrain tended to have more red ring and tyrosine crystals, which decreased the sensory quality of the product.

The PCA for sensory flavour attributes showed that matured flavour positively affected the global sensory quality. Umami and bitterness were closely related to each other and along with piquantness, gave a strongly negative effect on the global sensory quality. Metallic taste had a slightly negative effect on the global sensory quality while sweetness and saltiness hardly showed any. All four breeds were well differentiated on the first principal component, which mainly consisted of sweetness, piquantness, saltiness, matured flavour and metallic taste. Large White showed the best sensory flavour according to the global sensory quality while Piétrain showed the worst.

4.5. Conclusion

Most of the differences in the raw material, final composition and rheology found in this study were between Piétrain and the other three breeds (Large White, Landrace and Duroc).

Differences in the instrumental colour and sensory appearance were mainly between Duroc and the other three breeds, which were probably caused by the high intramuscular fat content of the Duroc breed.

No important differences between breeds were found in the sensory texture, flavour or the global sensory quality. The sensory differences between the four breeds were too small to discriminate them on single traits.

The Instrumental texture test was related more closely to adhesiveness and pastiness than to hardness, fibrousness and crumbliness. The former three also had a bigger effect on the global sensory quality than the latter three.

Large White showed a tendency to be the most appropriate according to the appreciated sensory characteristics for dry-cured ham of the four pure breeds under the processing conditions used in this study regarding the principal component analyses for texture, colour and flavour attributes, while Piétrain was the least appropriate.

5. Chapter II

Effect of *PRKAG3* and *CAST* genetic polymorphisms on dry-cured ham

5.1. Introduction

Mediterranean countries use traditional procedure for ham preservation consisting of dry salting (usually also curing), without smoking and long drying and ageing periods. During processing, the characteristics of raw hams such as weight, amount of subcutaneous fat and the properties of muscle, such as the pH at 45 min *post mortem* and the pH at 24 hours *post mortem*, affect the salt uptake (Costa-Corredor, Muñoz, Arnau, & Gou, 2010; Sánchez, Albarracin, Grau, Ricolfe, & Barat, 2008) and drying (Gou, Comaposada, & Arnau, 2002). The role of salting and drying in dry-cured hams is not only to provide a bacteriostatic effect but also to modulate biochemical modifications (such as proteolysis and lipolysis) which are responsible for the final texture and flavour of the product (Antequera et al., 1992; Toldrá, 1998). Therefore, the selection of raw hams with similar behaviour during processing results in more homogeneous products, which in turn allows optimizing the process in terms of quality.

Inconsistent muscle quality may lead to variation in yield and muscle colour of dry-cured hams. Pork quality was demonstrated to be different between pure breeds and composite lines from different breeding companies (Goodwin & Burroughs, 1995). It has been indicated that some breeds possess muscle quality traits that affect the characteristics of dry-cured Spanish Serrano hams (Oliver et al., 1993). Some other breeds or lines, which have a high frequency of the gene marker of *HAL 1843*™ or rendement *napole (RN-)* will produce pigs with undesirable muscle quality variation. This variation may increase economic losses to the dry-cured ham industry with excessive water loss, poor processing characteristics and possibly increased spoilage. Moreover, these factors may affect consumers' acceptance of dry-cured ham, their eating experience, and therefore, their decision to buy dry-cured ham again. Previous work has demonstrated that there are other genetic factors that play a role in meat quality. Ciobanu et al. (2004) reported a gene region on chromosome 2 which affected pig meat tenderness in the pig. The causative gene for these effects is called *CAST*. *CAST* affects calpastatin - an inhibitor of calpains which affect meat tenderization (Koohmarie, 1992). Another genetic region on chromosome 15, associated with the *PRKAG3* gene marker *RN199*, has been reported to explain 4-6% of ultimate pH variation in Berkshire x Yorkshire F2 pigs (Ciobanu et al. 2001).

DNA polymorphisms identified in the porcine calpastatin (*CAST*) and the protein kinase AMP-activated (*PRKAG3*) genes have been associated with certain quality traits of fresh meat (Garnier, Klont, & Plastow, 2003; Plastow et al., 2005; Stefanon et al., 2004). The calpastatin is the physiological inhibitor of calpain enzymes (Goll, Thompson, Li, Wei, & Cong, 2003) responsible for early *post mortem* muscle proteolysis (Huff-Lonergan et al., 1996; Koohmaraie & Geesink, 2006). Ciobanu et al. (2004) associated two *CAST* polymorphisms (*Arg249Lys* and *Ser638Arg*) to the texture of pork. Stalder, Rothschild and Lonergan (2005) further demonstrated the effect of *CAST Ser638Arg* polymorphism on water content, ham weight, salt content and colour in country-style dry-cured hams. The effect of several other *CAST* polymorphisms on backfat thickness, meat colour, leanness and pH value has also been proven (Emnett et al., 2000; Kocwin-Podsiadla, Kuryl, Krzecio, Zybert, & Przybylski, 2003; Krzecio et al., 2008; Krzecio, Kocwin-Podsiala, & Monin, 2005; Kuryl, Kapelanski, Pierzchala, Grajewska, & Bocian, 2003).

The subunit gamma-3 of the protein kinase AMP-activated is involved in regulating energy homeostasis in eukaryotes (Hardie, Carling, & Carlson, 1997). The *Ile199Val* polymorphism has been proven to affect ultimate pH in muscle (Ciobanu et al., 2001), which has been reported to affect proteolysis in dry-cured ham (Arnau et al., 1998; Buscailhon et al., 1994a; Tabilo, Flores, Fiszman, & Toldrá, 1999). However, Stalder, Knauer, Baas, Rothschild and Mabry (2004) reported that the *PRKAG3* gene marker had no affect on dry-cured ham processing characteristics. The *CAST Ser638Arg* gene marker was a significant source ($P<0.05$) variation for dry-cured ham moisture content and tended to be a significant source ($P<0.10$) of variation for yield, ham weight loss, salt and Minolta colour change. The *CAST Arg638/Arg638* genotype appears to have beneficial effects for processing yield, which would be preferred by processors as they would have more saleable ham when compared to hams having *CAST* genotypes *Arg638/Ser638* or *Ser638/Ser638*. However, consumer is likely to prefer *CAST* genotype *Ser638/Ser638* for a dried ham with a more traditional flavour. This demonstrates that the benefit of a particular *CAST* genotype that is most favorable can be dependent on which portion of the pork chain being discussed.

The relationship between proteolysis and texture has been previously reported in Italian dry-cured hams (Parolari et al., 1994; Virgili et al., 1995) and in Spanish

dry-cured hams (Ruiz-Ramírez et al., 2006). Sensorial characteristics of dry-cured ham such as those related to an abnormal texture (pastiness and softness) can be considered as a consequence of increased proteolysis (Careri et al., 1993; Gou, Morales, Serra, Guàrdia, & Arnau, 2008; Parolari et al., 1988).

Although some research on the effect of the above mentioned genes on pork quality has been conducted, there is still a lack of information especially regarding the traits of interest in dry-cured ham. Recently, within the frame of an Integrated Project funded by the European Commission under the 6th Framework Programme for RTD (Project TRUEFOOD - "Traditional United Europe Food", contract n. FOOD-CT-2006-016264), a wide study which looked at different pig production and ham processing systems was simultaneously performed in three countries: France, Slovenia and Spain. The first results of these studies were published by Škrlep et al. (2010) who studied, in the three countries, the association of *PRKAG3* and *CAST* genetic polymorphisms with several raw ham quality parameters (weight and fat thickness of ham and colour and pH of muscle) which are important for dry-cured ham production. Significant effects of these two genes on the quality parameters mentioned above were observed, however they differed according to the sample of pigs selected for ham production in each country, indicating a possible interaction with other genetic or environmental factors. The next step of this project was to evaluate the effect of these polymorphisms on the quality traits of the dry-cured hams produced in each country using specific raw material and processing conditions.

The section aims to show the effect of previously identified *PRKAG3* and *CAST* genetic polymorphisms on the quality traits of the Spanish dry-cured ham Jamón Serrano.

5.2. Materials and methods

5.2.1. Animal and raw ham evaluation

Six hundred and sixty five left hams were selected from a total of 1128, from seven batches of slaughter pigs coming from several grow-out units (Turolense Ganadera S.A., Spain) and processed at a commercial plant (Agroalimentaria de Teruel S.A., Spain). The crossbred pigs were sired from the PIC Duroc Terminal Sire (Line 16) on the PIC Camborough 23 sow (50 % Duroc; 25 % Large White and 25 % Landrace dam lines). The day after slaughter, fat thickness was measured on the carcass mid-line over the thickest depth of the exposed area of M. *gluteus medius* (GM). The pH of M. *Semimembranosus* (SM) (pH_u) was recorded with a portable pH meter (Crison 507, Crison Instruments S.A.). The left hams were cut off the carcass, trimmed to the "Jamón Serrano" type and weighed. Marbling on the exposed area of the SM was assessed after trimming, using a five point scale (1 = not visible; 2 = slightly visible; 3 = visible; 4 = very visible and 5 = highly visible).

The rejected hams did not achieve the minimum 2.5 marbling score required by the industrial partner (Jamones Segovia S.A., Spain), responsible for the dry-cured ham processing. The 71 % of rejected hams also had a weight out of the range accepted by industrial specifications. The selection of hams by weight and visible marbling for dry-cured ham elaboration is common practice in Spain. Therefore, the hams chosen can be considered a representative sample of hams which are being used in the Spanish dry-cured ham industry for long ageing processes.

5.2.2. Genotype determination

A tissue sample from the selected hams was taken at the abattoir and frozen in liquid nitrogen until genotyping. Samples were genotyped using PCR-RFLP method according to Ciobanu et al (2001, 2004) for *PRKAG3 Ile199Val*, *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms.

5.2.3. Ham processing

Prior to salting, the hams were trimmed into a prescribed shape (rind was partially removed forming the typical V shape) and kept at 1-3ºC. Hams were pre-salted with sodium nitrite (0.150 g/Kg), potassium nitrate (0.150 g/Kg) and sodium ascorbate (0.500 g/Kg) and then put into a salting pile for 0.7 days/Kg at 0-4ºC. Subsequently, the hams were washed with water at 15 ºC ± 2 ºC and left to rest in a chamber at 5 ºC and 75-85% relative humidity for approximately ten weeks. Following the resting period the hams were dried for six months by progressively increasing the temperature up to 12-15ºC and reducing the relative humidity to 60-70%. Thereafter, the open surface of the hams was coated with a layer of rendered fat to prevent any excessive superficial drying and hollow defect (Fulladosa et al., 2010). Finally, the hams were aged for ten months in a natural dryer-chamber to develop an optimal maturation, until a weight loss around 35% was reached.

5.2.4. Analysis of processed ham

At the end of processing there were 652 hams correctly processed and genotyped. A subsample of 120 hams was selected to perform physico-chemical, rheological, instrumental colour and sensory analyses on the M. *Biceps femoris* (BF). The selection was done to obtain the maximum homozygous allele in the three gene polymorphisms. In this way, the power of the comparison between homozygous was optimized, although the sample did not represent the occurrence of the polymorphism in the population. Moreover, in order to facilitate the haplotype analysis of *CAST*, no ham heterozygote on both *CAST* codons was sampled.

Physico-chemical analysis

Water and NaCl contents were evaluated according to AFNOR NF V04-401 method (AFNOR, 2001) and AFNOR NF V04-405 method (AFNOR, 1972a) respectively; the a_w with a Novasina AW-SPRINT-TH 500 instrument (Axair Ltd., Pfäffikon, Switzerland) at 25 ºC ± 0.3 ºC; fat content (%) by Soxhlet method (ISO-1443, 1973); total nitrogen (TN) according to AFNOR NF V04-407 method (AFNOR, 1972b) and expressed as protein content percentage (TN x 6.25); non-protein nitrogen content (NPN) by precipitation of proteins with trichloroacetic

acid (Kerese & Chalmers, 1984), followed by determination of the nitrogen in the extract by the Kjeldahl method; the lipid oxidation by Thiobarbituric acid-reactive substances (TBARs) assay (mg of malonaldehide / kg of muscle) assessed according to the method of Shahidi, Rubin, Diosady and Wood (1985). A proteolysis index (PI) was calculated by the formula PI=100 x NPN x TN^{-1}.

Rheological analysis

The Stress Relaxation (SR) test was performed in triplicate, using the Texture Analyser TA.TX2 (Stable Micro Systems Ltd., Surrey, UK) with a 25 kg load cell and a 50 mm diameter compression plate, on BF muscles following the procedure described by Morales et al. (2007a). The samples were compressed to 25% of their original height at a crosshead speed of 5 mm/s. Force (kg) versus time after the compression was recorded and the relaxation curves were normalized, i.e., the level of force decay Y(t) was calculated as follows:

$$Y(t)=\frac{F_0 - F(t)}{F(t)}$$

where F0 (kg) is the initial force and F(t) is the force recorded after t seconds of relaxation.

Initial force (F0) and the level of force decay after 2 seconds of relaxation (Y2) and after 90 seconds of relaxation (Y90) were recorded.

Instrumental colour analysis

Colour measurements were taken with a Minolta Chroma Meter CR-410 (Minolta, Co., Ltd., Japan) using a 50 mm port size, illuminant D65 and a 2º standard observer on BF muscles. CIELAB lightness L*, redness a* and yellowness b* values were determined as indicators of lightness, redness and yellowness, respectively. The data presented are means of five measurements.

Sensory analysis

Quantitative descriptive analyses (Stone & Sidel, 1993) were carried out to assess texture and flavour on BF muscle of the dry-cured hams by six trained panellists (ASTM/Committee-E-18, 1981; ISO-8586-1, 1993; ISO-8586-2, 1994), using 0-10 point non-structured scale i.e. 0 for absence of the descriptor and 10 for maximum intensity (Amerine et al., 1965). The samples were coded with three-random numbers and were presented to the panellists. Four samples with different

genotypes were assessed in each session. The saltiness, bitterness, metallic taste, matured and aged flavours, hardness, fibrousness, crumbliness, pastiness and adhesiveness were evaluated using the definitions proposed by Sánchez-Molinero and Arnau (2008). The generation of the descriptors had been carried out in open discussions in four previous sessions. For each descriptor, the average score of the six panellists was recorded.

5.2.5. Statistical analysis

An analysis of variance was performed using the MIXED procedure of the SAS statistical package (SAS Inst., Inc., Cary, NC). The linear model included the gene polymorphisms and sex as fixed effects, batch as a random effect and fresh ham weight as a covariate. For sensory traits, the model also included session as a random effect. When a significant effect of gene polymorphism was encountered, least squares means were compared using LSMEANS with Tukey's test option.

Additionally, the haplotypes defined by the *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms were compared. Four haplotypes were inferred from *CAST249* and *CAST638*: *249Lys-638Arg*, *249Arg-638Arg*, 249Lys-638Ser and *249Arg-638Ser*. For haplotypes analysis, equivalent models to those applied for single gene polymorphisms were used, including the combined *CAST249-CAST638* genotype instead of the single *CAST* gene polymorphisms. When the combined *CAST249-CAST638* genotype effect was significant, the haplotype differences were tested with statistical contrasts of *CAST249-CAST638* genotype, considering animals having 0, 1 or 2 copies of each haplotype.

A principal component analysis (PCA) with all the variables studied in this experiment was performed with the FACTOR procedure of the statistical package SAS (SAS Inst., Inc., Cary, NC).

5.3. Results

5.3.1. Allele and genotype frequencies

Table 5.1 shows the allele and genotype frequencies by gene markers in our population. The Hardy-Weinberg-equilibrium (HWE) distribution of genotypes considering the allele frequencies is also shown.

Table 5.1. Allele and genotype frequencies by gene markers in the genotyped population (n=652). The expected Hardy-Weinberg-equilibrium (HWE) distribution of genotypes considering the allele frequencies is also shown.

	Allele frequencies		
PRKAG3 Ile199Val	*Ile*		*Val*
	0.475		0.525
CAST Arg249Lys	*Arg*		*Lys*
	0.513		0.487
CAST Ser638Arg	*Ser*		*Arg*
	0.360		0.640
	Genotype frequencies		
PRKAG3 Ile199Val	*Ile/Ile*	*Ile/Val*	*Val/Val*
	0.126	0.698	0.176
CAST Arg249Lys	*Arg/Arg*	*Arg/Lys*	*Lys/Lys*
	0.249	0.529	0.222
CAST Ser638Arg	*Ser/Ser*	*Ser/Arg*	*Arg/Arg*
	0.133	0.453	0.414
	Expected HWE distribution		
PRKAG3 Ile199Val	*Ile/Ile*	*Ile/Val*	*Val/Val*
	0.226	0.498	0.276
CAST Arg249Lys	*Arg/Arg*	*Arg/Lys*	*Lys/Lys*
	0.263	0.500	0.237
CAST Ser638Arg	*Ser/Ser*	*Ser/Arg*	*Arg/Arg*
	0.130	0.460	0.410

The genotype distribution of a crossed population, such as our sample, is in HWE if both the parental lines have this genotype distribution. Table 5.2 shows the association between codons 249 and 638 of the *CAST* gene polymorphisms.

The allele frequencies of *PRKAG3* were approximately 0.50, but its genotype distribution did not follow a HWE, being the frequency of the heterozygote higher than expected. The allele frequencies at *CAST Arg249Lys* were around 0.50 and its genotype distribution was the same as in the HWE. On the contrary, the allele frequency of *Arg638* at *CAST Ser638Arg* polymorphic site was 0.64.

There was significant linkage disequilibrium between the *CAST* polymorphisms (Table 5.2). Genotypes *249Lys/249Lys-638Ser/638Ser* and *249Lys/249Lys-638Arg/638Ser* were not observed, and only two animals were *249Lys/249Arg-638Ser/638Ser* and 10 animals *249Arg/249Arg-638Arg/638Arg*.

Table 5.2. Combined distribution of the two *CAST* polymorphisms analysed (652 genotyped animals).

CAST Arg249Lys	*CAST Ser638Arg*		
	Ser/Ser	*Arg/Ser*	*Arg/Arg*
Arg/Arg	0.130	0.103	0.015
Arg/Lys	0.003	0.350	0.176
Lys/Lys	0	0	0.222

5.3.2. Descriptive analysis of the recorded attributes in the selected subsample of hams

Mean and standard deviation (SD) of pH of *Semimembranosus* muscle (SM) in raw hams and recorded attributes of *Biceps femoris* muscle (BF) in dry-cured hams in the selected subsample of hams (n=120) are shown in Table 5.3.

Table 5.3. Mean and standard deviation (SD) of pH of *Semimembranosus* muscle (SM) in raw hams and recorded attributes of *Biceps femoris* muscle (BF) in dry-cured hams in the selected subsample of hams (n=120).

	Mean	SD
Raw ham properties		
pH_u	5.64	0.17
Physico-chemical parameters of BF muscle		
Water content (%)	56.33	1.58
NaCl content (%)	5.82	0.62
a_w	0.899	0.009
Fat content (%)	6.67	1.44
Protein content (%)	31.20	1.40
Fat/protein (%)	21.43	4.90
Proteolysis index (%)	30.55	3.11
TBA[1]	0.430	0.154
Rheological parameters of BF muscle		
F0 (kg)	2.840	0.774
Y2	0.338	0.020
Y90	0.618	0.021
Instrumental colour parameters of BF muscle		
L*	40.72	1.79
a*	16.20	1.43
b*	5.85	1.14
Sensory attributes of BF muscle[2]		
Saltiness	3.13	0.46
Bitterness	0.80	0.36
Metallic	0.52	0.53
Matured flavour	4.56	0.72
Aged flavour	1.49	0.69
Hardness	4.26	0.40
Crumbliness	5.88	0.38
Fibrousness	2.95	0.42
Pastiness	0.39	0.77
Adhesiveness	0.71	0.69

[1] mg of malonaldehide / kg of muscle
[2] 0-10 points non-structured scale (0: absence; 10: maximum intensity)

The mean pH_u of raw ham was 5.64, and 25.8% of the hams had pH below 5.55.

The chemical analysis of BF of dry-cured hams showed mean contents for water, NaCl, fat and protein of 56.33%, 5.82%, 6.67% and 31.20% respectively. The average ratios Proteolysis index and fat/protein were 30.55% and 31.20% respectively.

The rheological parameters F0, Y2 and Y90 of the relaxation test were 2.840 kg, 0.338 and 0.618 respectively. The averages of L*, a* and b* were 40.72, 16.20 and 5.85 respectively.

The mean values for saltiness, bitterness, matured flavour, hardness, crumbliness and fibrousness were 3.13, 0.80, 4.56, 4.26, 5.88 and 2.95 respectively. In addition, many samples scored lower than 1 for metallic taste (87), pastiness (94) and adhesiveness (82). The panellists agreed that scores lower than 1 for these attributes could be considered unimportant.

5.3.3. *PRKAG3* associations with recorded attributes of dry-cured hams

There were significant ($p<0.05$) and suggestive associations ($p<0.10$) of the *PRKAG3 Ile199Val* genotype with the pH of the fresh ham (measured on SM muscle) and with the physicochemical composition, instrumental colour and sensory attributes related to the flavour and texture of the dry-cured ham (Table 5.4).

Differences in pH_u of raw ham were found between the three *PRKAG3* genotypes, being higher in *Ile199Ile* genotype of the selected subsample of fresh hams.

Water content was higher in *Ile/Val* genotype. Fat content, and fat/protein as well, was higher in *Ile/Ile* than in *Ile/Val* genotype. Proteolysis index was higer in *Val/Val* than in *Ile/Ile* genotype.

Differeces of instrumental colour parameter (b*) of BF muscle were found, being higher in *Ile/Ile* than in *Ile/Val* genotype.

With regard to sensory attributes of BF muscle, matured flavor and aged flavor were both lower in Ile/Val than in other two genotypes. Crumbliness was higher in Ile/Ile than in Ile/Val genotype. Fibrousness was lower in Val/Val than in other two genotypes. Pastiness was higher in Val/Val than in Ile/Ile genotype

Table 5.4. Association results between *PRKAG3 Ile199Val* genotypes and pH of *Semimembranosus* muscle (SM) in raw hams and recorded attributes of *Biceps femoris* muscle (BF) in dry-cured hams in the selected subsample of hams (n=120). Least square means ± standard error.

	PRKAG3 genotypes			
	Ile/Ile	*Ile/Val*	*Val/Val*	P-value
Number of animals	44	26	50	
Raw ham properties				
pH_u	$5.73^{a} \pm 0.04$	$5.64^{ab} \pm 0.05$	$5.63^{b} \pm 0.05$	0.021
Physico-chemical parameters of BF muscle				
Water content (%)	$56.18^{b} \pm 0.38$	$57.31^{a} \pm 0.45$	$56.20^{b} \pm 0.43$	0.014
NaCl content (%)	5.74 ± 0.17	5.46 ± 0.20	5.55 ± 0.19	0.240
a_w	0.900 ± 0.003	0.906 ± 0.003	0.900 ± 0.003	0.137
Fat content (%)	$7.30^{a} \pm 0.42$	$6.14^{b} \pm 0.48$	$6.88^{ab} \pm 0.45$	0.036
Protein content (%)	31.04 ± 0.47	31.07 ± 0.51	31.20 ± 0.50	0.890
Fat/protein (%)	$23.65^{a} \pm 1.49$	$19.88^{b} \pm 1.66$	$21.95^{ab} \pm 1.60$	0.030
Proteolysis index (%)	$31.42^{b} \pm 1.01$	$31.47^{ab} \pm 1.12$	$33.01^{a} \pm 1.08$	0.096
TBA[1]	0.433 ± 0.038	0.428 ± 0.044	0.425 ± 0.043	0.976
Rheological parameters of BF muscle				
F0 (kg)	2.963 ± 0.237	2.620 ± 0.262	2.723 ± 0.254	0.173
Y2	0.348 ± 0.006	0.346 ± 0.006	0.352 ± 0.006	0.416
Y90	0.629 ± 0.007	0.632 ± 0.007	0.635 ± 0.007	0.369
Instrumental colour parameters of BF muscle				
L	41.30 ± 0.53	40.31± 0.64	41.37 ± 0.57	0.114
a*	15.22 ± 0.41	14.52 ± 0.53	14.60 ± 0.45	0.160
b*	$4.72^{a} \pm 0.35$	$3.81^{b} \pm 0.44$	$4.16^{ab} \pm 0.38$	0.039
Sensory attributes of BF muscle[2]				
Saltiness	3.08 ± 0.12	2.92 ± 0.14	3.03 ± 0.13	0.428
Bitterness	0.79 ± 0.09	0.91 ± 0.11	0.84 ± 0.10	0.439
Metallic	0.55 ± 0.13	0.49 ± 0.16	0.64 ± 0.15	0.581
Matured flavour	$4.46^{a} \pm 0.16$	$4.11^{b} \pm 0.19$	$4.53^{a} \pm 0.18$	0.061
Aged flavour	$1.30^{a} \pm 0.15$	$0.96^{b} \pm 0.19$	$1.47^{a} \pm 0.17$	0.019
Hardness	4.26 ± 0.10	4.18 ± 0.12	4.13 ± 0.11	0.409
Crumbliness	$5.90^{a} \pm 0.09$	$5.71^{b} \pm 0.11$	$5.89^{ab} \pm 0.10$	0.093
Fibrousness	$3.05^{a} \pm 0.10$	$3.01^{a} \pm 0.12$	$2.76^{b} \pm 0.11$	0.006
Pastiness	$0.31^{b} \pm 0.19$	$0.59^{ab} \pm 0.22$	$0.65^{a} \pm 0.21$	0.070
Adhesiveness	0.79 ± 0.19	0.99 ± 0.21	1.05± 0.20	0.126

[1] mg of malonaldehide / kg of muscle
[2] 0-10 points non-structured scale (0: absence; 10: maximum intensity)
[a,b] : means within a row without a common letter are significantly different (P<0.05).

5.3.4. *CAST* associations with recorded attributes of dry-cured hams

There were significant ($p<0.05$) and suggestive associations ($p<0.10$) of *CAST249* (Table 5.5) and *CAST638* polymorphisms (Table 5.6) with NaCl content, rheological parameters, instrumental colour and saltiness. *CAST249* also showed associations to sensory attributes of texture (pastiness and adhesiveness).

With respect to *CAST249* genotypes, NaCl content was higher in *Arg/Arg* than in *Lys/Lys* and *Lys/Arg* genotypes. Rheological parameters, Y2 and Y90, of BF muscle were both lower in *Arg/Arg* than in other two genotypes. Instrumental colour parameters of BF muscle, a* and b*, were both higher in *Arg/Arg* than in other two genotypes. In sensory attributes of BF muscle, saltiness was higher in *Arg/Arg* than in other two genotypes. Pastiness and Adhesiveness were both higher in *Lys/Arg* than in other two genotypes. No differences were found in proteolysis index of BF muscle between three *CAST249* genotypes.

With respect to *CAST638* genotypes, NaCl content was higher in *Arg/Arg* than in *Arg/Ser* and *Ser/Ser* genotypes. Rheological parameters, Y2 and Y90, of BF muscle were both lower in *Arg/Arg* than in *Ser/Ser* genotype. Regarding to instrumental colour parameters of BF muscle, a* was higher in *Arg/Arg* than in *Ser/Ser* genotype, and b* was higher in *Arg/Arg* than in other two genotypes. In sensory attributes of BF muscle, only saltiness was found higher in *Arg/Arg* than in other two genotypes. No differences were found in proteolysis index of BF muscle between three *CAST249* genotypes.

Table 5.5. Association results between *CAST Arg249Lys* genotypes and pH of *Semimembranosus* muscle (SM) in raw hams and recorded attributes of *Biceps femoris* muscle (BF) in dry-cured hams in the selected subsample of hams (n=120). Least squares means ± standard error.

	CAST249 genotypes			
	Lys/Lys	*Lys/Arg*	*Arg/Arg*	P-value
Number of animals	47	21	52	
Raw ham properties				
pH_u	5.68 ± 0.06	5.70 ± 0.07	5.63 ± 0.04	0.722
Physico-chemical parameters of BF muscle				
Water content (%)	56.44 ± 0.58	56.91 ± 0.65	56.35 ± 0.34	0.543
NaCl content (%)	5.30^{b} ± 0.26	5.22^{b} ± 0.28	6.23^{a} ± 0.16	0.018
a_w	0.905 ± 0.004	0.907 ± 0.005	0.895 ± 0.002	0.119
Fat content (%)	6.92 ± 0.59	7.03 ± 0.64	6.37 ± 0.37	0.705
Protein content (%)	30.91 ± 0.60	31.25 ± 0.64	31.15 ± 0.44	0.698
Fat/protein (%)	22.43 ± 2.01	22.38 ± 2.15	20.66 ± 1.37	0.768
Proteolysis index (%)	33.06 ± 1.34	32.85 ± 1.43	29.99 ± 0.94	0.160
TBA[1]	0.403 ± 0.056	0.459 ± 0.063	0.423 ± 0.034	0.415
Rheological parameters of BF muscle				
F0 (kg)	2.817 ± 0.320	2.675 ± 0.350	2.813 ± 0.222	0.799
Y2	0.354^{a} ± 0.008	0.358^{a} ± 0.008	0.334^{b} ± 0.006	0.037
Y90	0.640^{a} ± 0.008	0.642^{a} ± 0.009	0.614^{b} ± 0.006	0.010
Instrumental colour parameters of BF muscle				
L	41.28 ± 0.74	41.02 ± 0.82	40.68 ± 0.51	0.751
a*	14.19^{b} ± 0.62	13.87^{b} ± 0.69	16.27^{a} ± 0.38	0.027
b*	3.74^{b} ± 0.52	3.27^{b} ± 0.57	5.67^{a} ± 0.33	0.006
Sensory attributes of BF muscle[2]				
Saltiness	2.83^{b} ± 0.18	2.84^{b} ± 0.20	3.37^{a} ± 0.11	0.074
Bitterness	0.77 ± 0.13	0.84 ± 0.15	0.93 ± 0.08	0.574
Metallic	0.52 ± 0.20	0.54 ± 0.22	0.63 ± 0.12	0.913
Matured flavour	4.37 ± 0.24	4.19 ± 0.27	4.55 ± 0.14	0.487
Aged flavour	1.26 ± 0.24	0.97 ± 0.27	1.50 ± 0.14	0.159
Hardness	4.21 ± 0.15	4.24 ± 0.17	4.12 ± 0.09	0.861
Crumbliness	5.81 ± 0.13	5.71 ± 0.15	5.98 ± 0.08	0.352
Fibrousness	3.00 ± 0.15	2.91 ± 0.17	2.90 ± 0.09	0.674
Pastiness	0.36^{b} ± 0.27	0.85^{a} ± 0.30	0.33^{b} ± 0.18	0.037
Adhesiveness	0.89^{b} ± 0.25	1.24^{a} ± 0.28	0.71^{b} ± 0.19	0.069

[1] mg of malonaldehide / kg of muscle
[2] 0-10 points non-structured scale (0: absence; 10: maximum intensity)
[a,b] : means within a row without a common letter are significantly different (P<0.05).

Table 5.6. Association results between *CAST Ser638Arg* genotypes and pH of *Semimembranosus* muscle (SM) in raw hams and recorded attributes of *Biceps femoris* muscle (BF) in dry-cured hams in the selected subsample of hams (n=120). Least squares means ± standard error.

	CAST638 genotypes			
	Arg/Arg	*Arg/Ser*	*Ser/Ser*	P-value
Number of animals	73	8	39	
Raw ham properties				
pH_u	5.62 ± 0.04	5.75 ± 0.08	5.64 ± 0.06	0.253
Physico-chemical parameters of BF muscle				
Water content (%)	56.43 ± 0.30	56.81 ± 0.76	56.46 ± 0.58	0.859
NaCl content (%)	6.06^{a} ± 0.14	5.53^{ab} ± 0.33	5.16^{b} ± 0.26	0.030
a_w	0.896 ± 0.002	0.904 ± 0.005	0.906 ± 0.004	0.228
Fat content (%)	6.34 ± 0.35	6.86 ± 0.72	7.12 ± 0.58	0.588
Protein content (%)	31.07 ± 0.42	31.09 ± 0.70	31.14 ± 0.60	0.992
Fat/protein (%)	20.54 ± 1.29	22.13 ± 2.41	22.81 ± 1.99	0.654
Proteolysis index (%)	30.32 ± 0.89	33.19 ± 1.59	32.39 ± 1.33	0.267
TBA	0.462 ± 0.031	0.415 ± 0.072	0.408 ± 0.057	0.777
Rheological parameters of BF muscle				
F0 (kg)	2.763 ± 0.209	2.932 ± 0.402	2.611 ± 0.322	0.596
Y2	0.333^{b} ± 0.005	0.357^{ab} ± 0.009	0.356^{a} ± 0.008	0.030
Y90	0.612^{b} ± 0.006	0.643^{ab} ± 0.010	0.640^{a} ± 0.008	0.007
Instrumental colour parameters of BF muscle				
L	40.58 ± 0.46	41.20 ± 1.00	41.20 ± 0.74	0.808
a*	16.16^{a} ± 0.33	14.23^{ab} ± 0.87	13.94^{b} ± 0.61	0.039
b*	5.55^{a} ± 0.29	3.41^{b} ± 0.72	3.72^{b} ± 0.51	0.023
Sensory attributes of BF muscle[2]				
Saltiness	3.37^{a} ± 0.10	3.07^{a} ± 0.23	2.59^{b} ± 0.18	0.002
Bitterness	0.83 ± 0.07	0.90 ± 0.18	0.81 ± 0.14	0.832
Metallic	0.47 ± 0.11	0.62 ± 0.26	0.60 ± 0.20	0.872
Matured flavour	4.68 ± 0.13	4.25 ± 0.32	4.18 ± 0.25	0.347
Aged flavour	1.53 ± 0.12	1.04 ± 0.31	1.17 ± 0.24	0.416
Hardness	4.24 ± 0.08	4.02 ± 0.20	4.31 ± 0.15	0.246
Crumbliness	5.93 ± 0.07	5.83 ± 0.17	5.73 ± 0.14	0.498
Fibrousness	2.95 ± 0.08	2.94 ± 0.20	2.94 ± 0.16	0.999
Pastiness	0.35 ± 0.16	0.44 ± 0.34	0.76 ± 0.27	0.354
Adhesiveness	0.65 ± 0.17	1.09 ± 0.30	1.10 ± 0.26	0.335

[1] mg of malonaldehide / kg of muscle
[2] 0-10 points non-structured scale (0: absence; 10: maximum intensity)
[a,b] : means within a row without a common letter are significantly different (P<0.05).

5.3.5. *CAST* haplotype associations with recorded attributes of dry-cured hams

In the subsample studied there were five different combined *CAST249-CAST638* genotypes: *249Lys/249Lys-638Arg/638Arg* (C1), *249Lys/249Arg-638Arg/638Arg* (C2), 249Arg/*249Arg-638Arg*/638Arg (C3), *249Arg/249Arg-638Arg/638Ser* (C4) and *249Arg/249Arg-638Ser/638Ser* (C5). These genotypes allowed the estimation of three *CAST* haplotype differences: *249Lys-638Arg* vs *249Arg-638Arg*, *249Lys-638Arg* vs *249Arg-638Ser* and *249Arg-638Arg* vs *249Arg-638Ser* (Table 5.7).

To estimate each haplotype difference, different individual contrasts were performed considering animals having 0, 1 or 2 copies of each haplotype (Table 5.7). The arithmetic mean value of the individual contrasts does not take into account that the number of animals was different for each *CAST249-CAST638* genotype, which implies that each individual contrast has a different error.

If we assume that the variability in the population was the same in all groups (σ_o), the square error for each individual contrast can be estimated as:

$$se^2 = \frac{n_i + n_j}{n_i \cdot n_j} \cdot \sigma_o^2$$

where n_i and n_j are the number of animals of the combined *CAST249-CAST638* genotype contributing to the contrast.

A weighted mean contrast was calculated for each haplotype difference using $(n_i \cdot n_j)/(n_i + n_j)$ as the weight for each contrast contributing to the mean contrast (Table 5.7). In this weighted mean contrast, the genotype with more animals contributes to the final average more than those with fewer animals.

Table 5.7. Contrasts constructed with *CAST249-CAST638* genotype estimates to estimate *CAST* haplotype differences.

Haplotype difference	*CAST249-CAST638* genotypes*				
	C1	C2	C3	C4	C5
Number of animals	47	21	5	8	39
249Lys-638Arg vs 249Arg-638Arg					
Contrast 1	1	-1	0	0	0
Contrast 2	0.5	0	-0.5	0	0
Contrast 3	0	1	-1	0	0
Weighted mean contrast	0.727	-0.454	-0.273	0	0
249Lys-638Arg vs 249Arg-638Ser					
Contrast 1	0.5	0	0	0	-0.5
Contrast 2	0	1	0	-1	0
Weighted mean contrast	0.393	0.213	0	-0.213	-0.393
249Arg-638Arg vs 249Arg-638Ser					
Contrast 1	0	0	1	-1	0
Contrast 2	0	0	0.5	0	-0.5
Contrast 3	0	0	0	1	-1
Weighted mean contrast	0	0	0.374	0.252	-0.626

*C1: 249Lys/249Lys-638Arg/638Arg; C2: 249Lys/249Arg-638Arg/638Arg; C3: 249Arg/249Arg-638Arg/638Arg; C4: 249Arg/249Arg-638Arg/638Ser; C5: 249Arg/249Arg-638Ser/638Ser.

Table 5.8 shows the differences between the three haplotypes compared in this study (*249Lys-638Arg*, *249Arg-638Arg* and *249Arg-638Ser*) for those attributes which were significantly affected by the *CAST249-CAST638* genotype effect and where there were significant differences between haplotypes. There were significant ($p<0.05$) and suggestive associations ($p<0.10$) of *CAST249-CAST638* genotype with NaCl content, rheological parameters (Y2 and Y90), instrumental colour (b*) and saltiness. NaCl content was found higher in *249Arg-638Arg* than in *249Arg-638Ser* genotype. Rheological parameters of Y2 and Y90 were lower in *249Arg-638Arg* than in *249Arg-638Ser* genotype. Y90 was also found lower ($p<0.10$) in *249Arg-638Arg* than in *249Lys-638Arg* genotype. Instrumental colour parameter of b* was found higher ($p<0.10$) in *249Arg-638Arg* than in

249Arg-638Ser genotype. Saltiness was found higher in in *249Arg-638Arg* than in *249Arg-638Ser* genotype.

Table 5.8. Differences between *CAST* haplotypes compared in this study (H1: *249Lys-638Arg*, H2: *249Arg-638Arg*, H3: *249Arg-638Ser*) for those attributes with significant (P<0.05) or suggestive (P<0.10) association with *CAST249-CAST638* genotype. SE: standard error.

		CAST haplotypes differences		
		H1 – H2	H1 – H3	H2 – H3
NaCl (%)	Estimate	-0.21	-0.11	0.43
(P=0.046)	SE	0.13	0.08	0.16
	p-value	0.113	0.189	0.009
Y2	Estimate	0.003	-0.001	-0.008
(P=0.099)	SE	0.003	0.002	0.004
	p-value	0.320	0.483	0.047
Y90	Estimate	0.006	-0.001	-0.010
(P=0.036)	SE	0.004	0.002	0.004
	p-value	0.085	0.589	0.027
b*	Estimate	-0.31	-0.09	0.60
(P=0.026)	SE	0.27	0.18	0.33
	p-value	0.259	0.606	0.076
Saltiness	Estimate	-0.15	0.05	0.41
(P=0.012)	SE	0.09	0.06	0.11
	p-value	0.109	0.457	0.001

5.3.6. Principal component analysis (PCA) for all the parameters

The PCA for all the parameters (Figure 5.1) shows the main relationships between them. Regarding the first axis, texture parameters related to softness (Y2, Y90, adhesiveness and pastiness) were positively correlated with PI and aw and negatively correlated with the NaCl content. F0 and hardness were negatively correlated with softness indicators. Bitterness and metallic taste also showed a negative correlation with aged and matured flavours. Regarding the second axis, the fat content or fat/protein ratio were negatively correlated with the water content,

due to the lower water content of fatty tissue in comparison to lean tissue. The fat content was also slightly correlated with TBA, lightness (L*), crumbliness and aged and matured flavours.

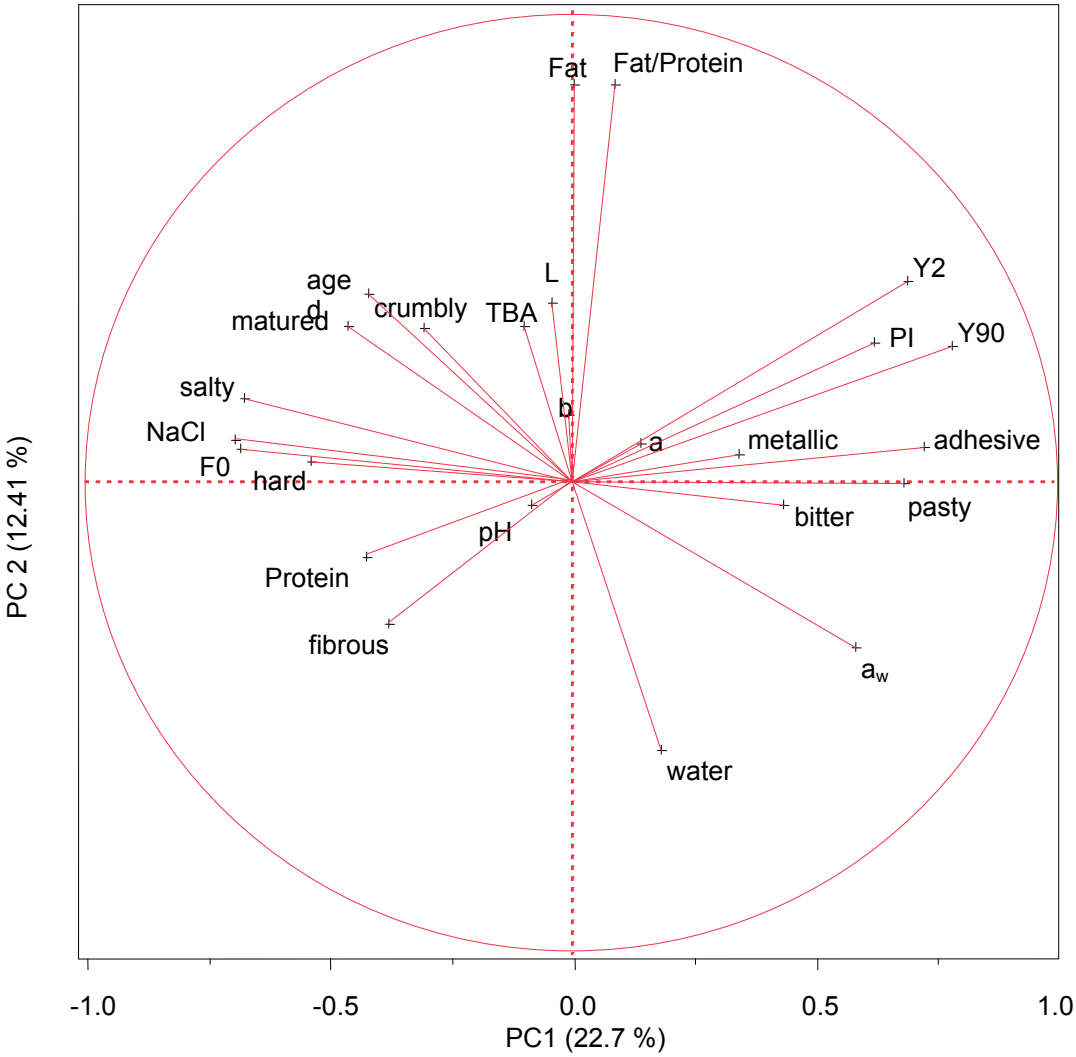

Fig. 5.1. Principal component analysis (PCA) of all factors including raw ham properties (pHu), physico-chemical parameters of BF muscle (water content, NaCl content, aw, fat content, protein content, fat/protein, proteolysis index, TBA), rheological parameters (F0, Y2, Y90), instrumental colour parameters of BF muscle (L*, a*, b*), sensory texture attributes of BF muscle (saltiness, bitterness, metallic taste, matured flavour, aged flavour, hardness, crumbliness, fibrousness, pastiness, adhesiveness).

5.4. Discussion

5.4.1. Allele and genotype frequencies

The allele frequency of the heterozygote of *PRKAG3* was higher than expected in Hardy-Weinberg-equilibrium (HWE). This could be due to the fact that the genotype distribution is different in the parental lines, i.e., breeding criteria in each line has modified this distribution in a different way, or that marbling (the criteria used for hams selection) is higher in *Ile/Val* heterozygote. The lower fat content in *Biceps femoris* muscle found in the *Ile/Val* heterozygote (Table 5.4) invalidates the hypothesis that marbling is higher in heterozygote *Ile/Val*, reinforcing the hypothesis of different distributions in the parental lines.

The allele frequencies at *CAST Arg249Lys* were around 0.50 and its genotype distribution was the same as in the HWE. On the contrary, the allele frequency of *Arg638* at *CAST Ser638Arg* polymorphic site was higher than *Ser638*, which underlines that selection has occurred in the population against the *Ser638* allele. This selection could have been done at breeding level (in one or both parental lines) or in the crossbred population. Including attributes dependent on *CAST638*, both in the selection criteria for breeding and in the criteria for hams selection, would result in indirect selection for the *CAST638* gene marker. However, the genotype distribution of *CAST638* is similar to the expected HWE. This reinforces the hypothesis that the selection against the *Ser638* allele must have been done in the parental lines.

The significant linkage disequilibrium between the *CAST* polymorphisms was also found in previous study. Ciobanu et al. (2004) found a linkage disequilibrium between these *CAST* polymorphisms. Due to the significant linkage disequilibrium between the *CAST* polymorphisms, the haplotypes were tested. In the subsample of hams used in this study, three haplotypes were compared: *249Lys-638Arg*, *249Arg-638Arg* and *249Arg-638Ser*.

5.4.2. Descriptive analysis of the recorded attributes in the selected subsample of hams.

The low pH of SM in raw hams (25.8% of the hams had pH below 5.55), indicates that they were more prone to showing texture defects (García-Rey et al., 2004). Water content was slightly lower than that found by García-Rey et al. (2004) in dry-cured hams subjected to a similar process (57.91% for Low pH hams and 59.29% for Normal pH hams), while the average NaCl content on dry matter (13.3%) was slightly higher. This would explain, in part, the lower PI in our experiment compared with those obtained by García-Rey et al. (2004) where the results were 40.9% in Low pH hams and 38.9% in Normal pH hams. The average fat content was similar to that found in Iberian hams (Carrapiso & García, 2008) due to the criteria used for ham selection at the slaughterhouse. TBARs values were also similar to those usually found in dry-cured hams with long ageing periods (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004).

The means of rheological parameters of the relaxation test were similar to those found by Gou et al. (2008). Instrumental colour parameters in dry-cured hams are affected by water content (Pérez-Alvarez et al., 1999). Consequently, a wide range of values can be found in literature depending on the drying level which has been applied during processing. The averages of L*, a* and b* are similar to those found by Pérez-Palacios, Ruiz, Martín, Barat, and Antequera (2011) in M. *Biceps femoris* of Iberian hams with a water content of 52%.

The mean values for saltiness, bitterness, matured flavour, hardness, crumbliness and fibrousness were considered standard for Spanish dry-cured Serrano ham by the panel of experts (Guàrdia, Aguiar, Claret, Arnau, & Guerrero, 2010).

5.4.3. *PRKAG3* associations with recorded attributes of dry-cured hams

The differences in pH of the fresh SM muscle found between *PRKAG3* genotypes were similar to those observed by Škrlep et al. (2010) in the whole population. This is in accordance with (Ciobanu et al., 2001) and (Otto et al., 2007) who reported higher pH in *Ile199Ile* genotype. Lindahl et al. (2004a) also reported

higher pH in *Ile* carriers and Stalder et al. (2005) and Škrlep, Kavar, Santé-Lhoutellier and Čandek-Potokar (2009) found a similar tendency, though not statistically significant. In concordance with (Arnau et al., 1994), who showed lower proteolytic activity in hams with higher pH, the *Ile/Ile* hams had the lowest proteolysis index ($p<0.10$).

Lower proteolysis resulted in lower pastiness and higher fibrousness in *Ile/Ile* hams, but, although softer texture has previously been related to higher Y2 and Y90 values (Morales et al., 2007b), no significant difference in the rheological parameters was detected. Nevertheless, *199Val* allele can be considered to have negative effects on hams selected for dry-cured hams processing, due to their higher proteolysis during processing, which increases pastiness, especially if salt content is reduced (Arnau et al., 1998; Buscailhon et al., 1994a; Tabilo et al., 1999).

The fat content was lower in the *Ile/Val* than in *Ile/Ile* genotype ($p<0.05$). The fat/protein ratio, which could also be considered a raw ham property, was also lower in the heterozygotes. No effect of *PRKAG3* on fat content has previously been reported in hams (Ramos, Glenn, Serenius, Stalder, & Rothschild, 2008; Stalder et al., 2005) or loins (Josell et al., 2003; Lindahl, Enfält et al., 2004a; Otto et al., 2007; Skrlep et al., 2010).

Yellowness (b*) of dry-cured ham subcutaneous fat is increased by fat oxidation (Sánchez-Molinero, García-Regueiro, & Arnau, 2010). Although there was no significant differences in TBA values between genotypes, the lower fat content of heterozygotes could have been responsible for their lower yellowness ($p<0.05$) in this study. Lindahl et al. (2004b) also found higher b* values in fresh loins from *Val/Val* animals than in a pooled sample of loins from *Ile/Val* (81%) and *Ile/Ile* (19%) animals, which was explained by differences in internal reflectance and distribution of myoglobin derivatives. Although the cured meat pigment (nitrosyl myoglobine) is different from those of fresh meat (e.g. myoglobin, oxymyoglobin), their distribution could also be affected by the *PRKAG3* genotype, therefore contributing to the differences observed in b* value in dry-cured hams.

Heterozygotes showed the lowest score for matured and aged flavour, which might be related to their lower fat content (Guàrdia et al., 2010). Therefore, *Ile/Val* hams are expected to have a lower preference by consumers who are aware of

the matured and aged flavours, which are considered quality characteristics of the Serrano ham (Morales et al., 2008).

It can be concluded, taking into account texture and flavour properties, that the most favourable genotype of the *PRKAG3 Ile199Val* polymorphism for the production of the Spanish dry-cured ham *Jamón Serrano* is *Ile/Ile*.

5.4.4. *CAST* associations with recorded attributes of dry-cured hams

The gene *CAST* codes for calpastatine, which is the physiological inhibitor of calpain enzymes (Goll et al., 2003), responsible for early *post mortem* muscle proteolysis (Huff-Lonergan et al., 1996; Koohmaraie & Geesink, 2006). It is expected that different *CAST* genotypes may produce different proteolysis patterns. However, no significant differences in the proteolysis index between genotypes of both individual *CAST* polymorphisms were detected at the end of the process. Nevertheless, higher salt contents were found in *Arg/Arg* genotype for *CAST249* and in *Arg/Arg* genotype for *CAST638*. These differences in salt content could have affected the proteolysis at the initial stages of the process, masking the potential effect of *CAST* polymorphisms on proteolysis. The effect of the calpain activity on the muscle structure during the early stages of curing time could modify some textural traits and perhaps the colour of dry-cured hams. In fact, lower Y2 and Y90 and higher a* and b* values were found in *Arg/Arg* genotype for *CAST249* and in *Arg/Arg* genotype for *CAST638*. Although pastiness and adhesiveness tended to be lower in these genotypes, only *Arg/Arg* genotype for *CAST249* showed significantly lower pastiness and adhesiveness than *Lys/Arg* genotype. The salty perception was in concordance with the salt content.

It was not possible from these results to conclude if the effect on texture and colour was due only to the different salt content shown by each *CAST* genotype or whether there was also an effect caused by their different proteolysis pattern. To test the effect of *CAST* genotype independently from the NaCl content, a new statistical analysis was performed for Y2, Y90, a*, b*, pastiness and adhesiveness, adding as a covariate the NaCl content to the linear model. NaCl content as the covariate was statistically significant for Y2, Y90, pastiness and adhesiveness, but

not for colour parameters. When NaCl covariate was included in the model the differences between genotypes were lower than when NaCl was not considered. Only differences in Y90 between *CAST638* genotypes and differences in pastiness between *CAST249* genotypes were significant. Differences in Y2 were mainly due to differences in NaCl content.

5.4.5. *CAST* haplotype associations with recorded attributes of dry-cured hams

Haplotype *249Arg-638Arg* had the highest salt content and accordingly, the highest salty taste. Additionally, it had slightly lower Y2 and Y90 values and tended to show a slight higher b* value. When salt content was added as a covariate to the linear model, there were no significant differences in Y2 and Y90 between haplotypes. Therefore, the differences in texture between haplotypes could be explained mainly by their differences in NaCl content.

5.4.6. Principal component analysis (PCA) for all the parameters

The negative correlation between the salt content and proteolysis has been extensively reported in dry-cured hams (Schivazappa et al., 2002). A higher proteolysis has been reported for hams with a lower pH (Gou et al., 2008), but only a slight negative correlation between the salt content and the proteolysis index was found in the present study due to the narrow range of pH used. This indicated that other factors apart from pH had more extensively affected the PI. The relationship between proteolysis and softness is also well known (Ruiz-Ramírez et al., 2006). As expected, NaCl was negatively correlated with a_w and positively correlated with saltiness. It was also negatively correlated with bitterness and metallic taste, which could have been produced by proteolysis products (Virgili et al., 1995). Bitterness and metallic taste also showed a negative correlation with aged and matured flavours, which might have been due to a masking effect of the former on the ‘standard’ dry-cured ham flavours.

To sum up, soft texture, bitterness and metallic taste were correlated with proteolysis index, which was mainly correlated with NaCl content and to a lesser extent, with the fresh ham pH. Moreover, TBA, aged and matured flavours and lightness are slightly correlated with fat content.

5.5. Conclusions

Significant linkage disequilibrium between the *CAST* polymorphisms was observed in our population, which reduces the potential number of *CAST* haplotypes. There were significant ($p<0.05$) and suggestive associations ($p<0.10$) between *PRKAG3*, *CAST249*, *CAST638* genotypes or *CAST* haplotypes and several quality traits of Spanish dry-cured ham Jamón Serrano, mainly related to flavour and texture.

The *PRKAG3 Ile/Ile* genotype, the *CAST249 Arg/Arg* genotype, the *CAST638 Arg/Arg* genotype and the haplotype *CAST 249Arg-638Arg* are the most favourable for the production of the Spanish dry-cured ham Jamón Serrano.

6. Chapter III

General discussion

The quality of dry-cured ham is, to some extent, defined by the sensory texture, taste and flavour, which are the fundamental attributes influencing overall consumer acceptability of dry-cured ham (Hersleth, Lengard, Verbeke, Guerrero, & Næs, 2011; Issanchou, 1996; Pham et al., 2008; Resano, Sanjuán, Cilla, Roncalés, & Albisu, 2010; Resano et al., 2011). Some authors have highlighted proteolysis and endogenous proteolytic activity of ham as major factors affecting the flavour and texture (Arnau et al., 1998; Parolari et al., 1994; Rosell & Toldrá, 1998; Sandrolini, 2000; Virgili et al., 1998) of long aged dry-cured ham, through the release of free amino acids and peptides in appropriate amounts and ratios (Careri et al., 1993; Rosell & Toldrá, 1998; Toscani, Virgili, Corbari, & Calzolari, 2000; Virgili, Parolari, Bordini, Schivazappa, Cornet, & Monin, 1999). The breakdown of myofibrillar proteins accounts for texture. The generation of peptides and free amino acids directly influences the taste. Products of proteolysis, such as amino acids, are flavour precursors contributing to further reactions. The endogenous muscle enzyme systems are those mainly responsible for proteolysis because there is a low microbial enzyme activity (Virgili et al., 1995; Virgili et al., 1998) because the microbial growth is limited by unfavorable conditions within muscle such as a high salt content and low water content and water activity (Molina & Toldrá, 1992). The pH at 24 h *postmortem* (Arnau et al., 1994; Buscailhon et al., 1994a; Gigli, Pacchioli & Barchi, 1993), salt content and water activity (Flores, Aristoy, Spanier, & Toldrá, 1997; Toldrá, Rico, & Flores, 1992) have been reported to be some of the major conditions affecting proteolysis by modifying the muscle enzyme activity.

The differences between breeds were mainly observed on physiochemical items such as the morphometrical properties of the raw hams (length and thickness), the components of the dry-cured ham (a_w and contents of water, salt, fat and protein), rheological traits (stress relaxation test) and the instrumental colour (L*, a* and b*). However, significant sensory differences were only found in the marbling and fibrousness. Moreover, the proteolysis index of the four breeds were not significantly different.

No significant difference in the pH_u of the raw ham between the breeds was found in this study. It is supposed that the pH_u measurement in chilled pig meat is largely determined by the effects of the harvest day (Hambrecht et al., 2003), the interaction between muscle energy stores at the time of the harvest and the *RYR1*

and *PRKAG3* genes (Hocquette, Ortigues-Marty, Pethick, Herpin, & Fernandez, 1998) as well as the predominant fibre type present in the muscle (Ryu & Kim, 2005). In this study, the NaCl content in BF at the end of the process was found to be significantly different between the breeds, which could be due to the differences in the water content and does not necessarily mean that these differences existed during the first stages of the processing, when a higher proteolysis is expected. This could explain why no differences between the breeds were found on proteolysis and therefore, on the most important sensory traits defining quality of dry-cured ham. The panel's global sensory quality test did not find significant differences between the breeds either. This result implied that the effect of the four pure breeds studied was not strong enough to produce significant difference between the dry-cured hams.

On the other hand, *PRKAG3* and *CAST* genetic polymorphisms were found to significantly affect the pH of SM of the raw ham and the physicochemical traits of the dry-cured ham such as water content, fat content, stress relaxation value, instrumental colour and proteolysis index. The sensory texture and flavour attributes such as adhesiveness, crumbliness, fibrousness, pastiness, saltiness, matured flavour and aged flavour were also significantly affected.

Recently published studies have demonstrated a certain connection between genetic polymorphisms and meat quality characteristics such as fatness, ham weight, pH and water-holding capacity (Ibeagha-Awemu, Kgwatalala, & Zhao, 2008). With regard to the genetic polymorphisms (*PRKAG3* and *CAST*) investigated in this study, *PRKAG3* gene encodes a specific isoform of the γ subunit of the adenosine monophosphate-dependant protein kinase (AMPK), an enzyme with a key role in cell energy metabolism regulation. Among five non-synonymous substitutions on the gene, the *Ile199Val* substitution/polymorphism which was found in several different pig breeds, was proven to affect carcass leanness, muscle glycogen content, colour, ultimate muscle pH and meat water holding capacity (Ciobanu et al. 2001; Enfält et al. 2006; Milan et al., 2000; Otto et al. 2007). *CAST* gene encodes calpastatin, a physiological inhibitor of calpains which are responsible for early post mortem muscle proteolysis (Koohmaraie & Geesink, 2006). The two *CAST* polymorphisms (*Arg249Lys* and *Ser638Arg*) in this study were highlighted and associated to pork texture (Ciobanu et al., 2004). In the only study carried out up to the present time,

made on US country-style dry-cured hams, Stalder et al. (2005) demonstrated the effect of *CASTSer638Arg* polymorphism on water content, ham weight, salt content and colour.

In this study, significant differences in the pH of the raw ham were found between genotypes of *PRKAG3* genetic polymorphism. This is in accordance with previous studies that showed higher pH in *Ile199Ile* genotype (Ciobanu et al., 2001; Otto et al., 2007) or higher pH in *Ile* carriers (Lindahl et al., 2004a). Stalder et al. (2005) and Škrlep et al. (2009) found a similar tendency, though not statistically significant. In accordance with (Arnau et al., 1994), who showed lower proteolytic activity in hams with a higher pH, the *Ile/Ile* hams had the lowest proteolysis index ($p<0.10$).

Due to the reported associations of *PRKAG3* and *CAST* polymorphisms with meat quality and carcass traits, it can be hypothesized that proteolysis would be affected either through the dynamics of dehydration and salt penetration or through the inhibiting action of calpastatin on the activity of calpains.

The differences between the genotypes of *PRKAG3* and *CAST* polymorphisms in the pH of the raw ham and in the salt content, water activity and proteolysis of the dry-cured ham can be compelling reasons for differences in sensory traits, which indicate that *PRKAG3* and *CAST* polymorphisms could induce perceivable quality and sensory differences in dry-cured hams. Therefore, these genetic polymorphisms could be better indicators for raw material screening in the dry-cured ham industry than breeds.

7. Conclusions

The majority of the differences in raw material, final composition and rheology of dry-cured ham between the breeds were found between Piétrain and the other three breeds (Duroc, Large White and Landrace).

Differences in the instrumental colour and sensory appearance were mainly between Duroc and the other three breeds, caused by the high intramuscular fat content of the Duroc breed.

No important differences between breeds were found in sensory texture, flavour or global sensory quality. The sensory differences between the four breeds were too small to discriminate them on single traits.

The multivariate analysis (PCA) highlighted that Large White breed showed the most appropriate sensory characteristics for dry-cured ham, while Piétrain showed the least appropriate ones.

There were significant associations between *PRKAG3*, *CAST249*, *CAST638* genotypes or *CAST* haplotypes and several quality traits of the dry-cured ham, mainly related to flavour and texture.

The *PRKAG3 Ile/Ile* genotype, the *CAST249 Arg/Arg* genotype, the *CAST638 Arg/Arg* genotype and the haplotype *CAST 249Arg-638Arg* are the most favourable for the production of the Spanish dry-cured ham “Jamón Serrano”.

The differences between the genotypes of *PRKAG3* and *CAST* polymorphisms in proteolysis and sensory traits were more important than differences caused by the pure breed used, indicating that *PRKAG3* and *CAST* polymorphisms could induce higher perceivable quality differences in dry-cured hams than breeds. Accordingly, genetic polymorphisms could be better indicators for raw material screening in the dry-cured ham industry than breeds.

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