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Contribución al estudio de calidad de productos del cerdo Ibérico en el marco de aplicación de la Norma de Calidad

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PHD THESIS

Contribution to the study of the Iberian pork product quality within the framework of the application of the Quality Standard

ALBERTO ORTIZ LLERENA

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The latest Iberian Product Quality Standard, i.e. Royal Decree 4/2014 of 10th of January, which approves the quality standard for Iberian pork meat, ham leg, ham shoulder and loin, sets out the minimum production and industrial requirements the above products must meet in order to be classified under the defined commercial categories with the purpose of guaranteeing the homogeneity and authenticity of the end products within each category. However, there are still new challenges to overcome that can help understand how certain factors can impact on the end quality of Iberian meat and meat products in every link of the value chain.

This doctoral thesis is an attempt to deal with this topic and consists of a number of sequential chapters that are interconnected with the legal framework, and can be of interest from an educational, scientific and technological point of view and of application to the industry.

Chapter I presented a qualitative analysis based on in-depth interviews that aim to understand the perception that the farmers and industrial entrepreneurs involved in the Iberian pig industry have on the application of the Quality Standard (RD 4/2014), with the purpose of identifying the limitations and opportunities of Iberian products in the current environment. The analysis revealed the need for more knowledge about the age at slaughter of Iberian x Duroc crossed animals in order to adapt it to market needs and to the interests of farmers. Chapter II analysed the impact of the age of Iberian x Duroc crossed (50:50) pigs at the beginning of finishing phase (10, 12 and 14 months old) on the production and quality parameters of their carcasses. Animals that were older while at the *Montanera* stage have proven to yield better production parameters, but lesser carcass yields. Subsequently, Chapter III included an evaluation of the impact of animal age at slaughter (12, 14 and 16 months old, respectively) on the quality of fresh loin (m. *Longissimus thoracis et lumborum*) and dry-cured loin, with higher alpha and gamma tocopherol and unsaturated fatty acids being found in both as animals were older. Chapter IV analysed the impact of freezing before the technological process of curing on the main quality parameters of the Iberian dry-cured loin of animals reared in the *Montanera* system. Although this procedure could overcome the system's inherent seasonality, the curing process

would need to be adapted to the raw material, since during the curing process more weight loss and hardness were found in the dry-cured loins deriving from frozen/thawed raw material.

In the subsequent chapters, the focus of study was dry-cured loin in sliced packed format, given the importance that this format has gained against the full-piece selling format in recent years. Chapter V includes a characterisation of the quality parameters of the *Black*, *Red and White* commercial categories compiled in the Quality Standard and their evolution throughout the 12-month refrigeration storage, as well as the influence of the type of packaging (vacuum against modified-atmosphere packaging (MAP) (70% N_2/30% CO_2)) used. *Black* and *Red* categories yielded higher values of alpha and gamma tocopherol and unsaturated fatty acids, whilst quality evolution throughout storage was similar in all categories. The MAP samples revealed a higher lightness (CIE L*) and redness (CIE a*) index but greater lipid oxidation from 8 months onwards in storage in comparison to vacuum-packed products.

Chapter VI includes a technological challenge that analysed the feasibility of using near infrared spectroscopy technology (NIRS) as a tool to monitor the control, traceability and authenticity of the commercial categories of sliced MAP dry-cured loin. Reliable models were obtained for the discrimination amongst the *Black, Red* and *White* commercial categories and amongst the various storage times (0, 4, 8 and 12 months) without the need to open the packaging.

Key words: Quality Standard, *Montanera*, Iberian x Duroc crossed pig, age at slaughter, pre-cure freeing, *Longissimus thoracis et lumborum,* commercial categories, NIRS, authentication.

En la última Norma de Calidad del Ibérico; Real Decreto 4/2014, de 10 de enero, por el que se aprueba la norma de calidad para la carne, el jamón, la paleta y la caña de lomo ibérico, se establecen los requisitos mínimos necesarios que se deben cumplir a nivel productivo e industrial para acogerse a cada una de las categorías comerciales definidas, con el fin de garantizar la homogeneidad y autenticidad de los productos finales dentro de cada una de ellas. Sin embargo, existen aún nuevos retos a abordar que ayuden a entender cómo ciertos factores influyen sobre la calidad final de la carne y productos cárnicos del cerdo Ibérico, en todos los eslabones de la cadena de valor.

En este contexto, se plantea esta tesis doctoral compuesta por una serie de capítulos secuenciales e interconectados por el actual marco legislativo que pueden resultar de interés desde un punto de vista formativa, científico-tecnológica y de aplicación para el sector.

En el capítulo I se utilizó un análisis cualitativo basado en entrevistas en profundidad para conocer la percepción que ganaderos e industriales del sector ibérico tienen sobre la aplicación de la Norma, con el fin de identificar las limitaciones y oportunidades para la comercialización de los productos ibéricos en el entorno actual. El estudio evidenció la necesidad de un mayor conocimiento de la influencia de la edad de sacrificio de los animales en los productos derivados para ajustarla a las necesidades del mercado e intereses de ganaderos. En el capítulo II, se estudió el impacto de la edad de los cerdos Ibéricos cruzados con Duroc (50:50) a la entrada de la fase de acabado en *Montanera* (10, 12 y 14 meses) sobre sus parámetros productivos y calidad de la canal. Los animales de mayor edad obtuvieron mejores parámetros productivos durante el aprovechamiento de la *Montanera,* pero un menor rendimiento de canal. Posteriormente, se evaluó la influencia de la edad de sacrificio de estos animales (12, 14 y 16 meses, respectivamente) en la calidad del lomo fresco (m. *Longissimus thoracis et lumborum*) y curado en el capítulo III, observándose mayores valores de alfa y gamma tocoferol y ácidos grasos insaturados en ambos con la edad de los animales. En el capítulo IV se estudió el impacto de la congelación previa a la curación sobre los principales parámetros de calidad del lomo ibérico curado procedente de animales de *Montanera*. Aunque esta herramienta podría ayudar a mitigar la estacionalidad implícita de este sistema, el proceso de curación debería ser

adaptado a la materia prima de partida, ya que mayores mermas durante la curación y dureza fueron observadas en los lomos curados procedentes de materia prima congelada y descongelada.

En los sucesivos capítulos el objeto de estudio fue el lomo curado en formato loncheado y envasado, dada la importancia ganada con respecto al formato de venta de pieza entera en los últimos años. Así, en el capítulo V se caracterizaron los parámetros de calidad de las categorías comerciales recogidas en la Norma de Calidad *Negra*, *Roja* y *Blanca* y su evolución a lo largo del almacenamiento en refrigeración durante 12 meses, así como la influencia del tipo de envase (vacío frente atmósfera modificada (70% N_2/30% CO_2)) utilizado. Las etiquetas *Negra* y *Roja* mostraron mayores valores de alfa y gamma tocoferol y ácidos grasos insaturados, mientras que la evolución de la calidad a lo largo del almacenamiento fue similar para todas las categorías. Las muestras envasadas en atmósfera modificada mostraron mayor luminosidad (L*) e índice de rojo (a*) pero mayor oxidación lipídica a partir de los 8 meses de almacenamiento con respecto a las envasadas a vacío.

Como reto tecnológico, en el capítulo VI se abordó la viabilidad de la tecnología espectroscópica en el infrarrojo cercano (NIRS) como herramienta para el control, trazabilidad y autenticidad de las categorías comerciales del lomo curado en formato loncheado y envasado en atmósfera modificada. Se obtuvieron modelos fiables para la discriminación entre las categorías comerciales *Negra*, *Roja* y *Blanca* y entre varios tiempos de conservación (0, 4, 8 y 12 meses), sin necesidad de abrir el envase.

Palabras clave: Norma de Calidad, *Montanera*, Cerdo Ibérico cruzado con Duroc, edad al sacrificio, congelación previa a la curación, *Longissimus thoracis et lumborum,* categorías comerciales, NIRS, autentificación.

GENERAL INTRODUCTION

The Iberian pig, its meat and meat products enjoy great social and commercial acceptance. This is probably due to the positive perception there is around this breed and its connection with the preservation of the environment and of animal welfare (Aparicio & Vargas, 2006; Temple, Manteca, Velarde, & Dalmau, 2011), as well as their high organoleptic and nutritional qualities (Pugliese & Sirtori, 2012). Such popularity has turned Iberian products into the object of numerous scientific studies that report their quality attributes and determine the factors associated with their production cycle (Fuentes, Ventanas, Ventanas, & Estévez, 2014; Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012; Ventanas, Tejeda, & Estévez, 2008) and their technological processing (Pérez-Palacios, Ruiz, Martín, Barat, & Antequera, 2011; Ruiz, Ventanas, Cava, Timón, & García, 1998) which have an impact on such attributes.

The high quality of the Iberian meat and meat products, together with the high rusticity of the breed have driven the Iberian breed to become the most relevant autochthonous pig breed in terms of population and economic importance in the Southwest of the Iberian Peninsula. Together with the *dehesa*, this breed forms the basis of a traditional and sustainable livestock farming system that is unique in Europe, the *Montanera* system (extensive management at the final fattening phase of the Iberian pig combined with a feeding strategy based on natural resources, which are basically acorn of the *Quercus* genus and pasture provided from November to March) (López-Bote, 1998) (Figure 1).

Figure 1. Iberian pigs at Valdesequera farm (Regional Council of Extremadura, Badajoz), while grazing in the *Montanera* system (November 2019). Source: own.

The *dehesa* is the largest agroforestry system in Europe (Den Herder et al., 2017), which currently spreads across approximately 3.5 million hectares in Spain (Leco Berrocal, Mateos Rodríguez, & Pérez Díaz, 2011). The Autonomous Community of Extremadura is one of the regions with the largest *dehesa* area in the country, amounting for approximately 1.2 million hectares (López-Rodríguez & Mateos-Rodríguez, 2019) (Figure 2). This explains why the association of the Iberian pig with the *dehesa*, as well as the geographical importance of the products derived from the *Montanera* production system, is mainly linked to Extremadura.

Overall, Extremadura accounts for over 32% of the national population of Iberian pigs with 1,135,451 heads (RIBER, 2021), only behind Castilla y León, which has 36.24%. It is important to point out that, although the number of Iberian pigs that are reared in intensive or extensive systems outside the *dehesa* has gained importance in recent years, this is not the case in Extremadura. Thus, in Extremadura, 21.70% of the registered animals are reared according to the *Montanera* system (246,376 animals) while the national average is 17.57% (MAPA, 2021).

Figure 2. Map of the *dehesa* areas in Spain. Own preparation based on the information of the Spanish Ministry of Transport, Mobility and Urban Agenda (ww.idee.es)

Montanera rearing has been traditionally associated with 100% Iberian breed pigs. However, the balance in the number of animals that on account of their pure genetic breed are put on the *Montanera* system has been changing towards a greater number of Iberian x Duroc crossed pigs rather than the Iberian purebred pigs. This is due to the fact that the crossed pigs provide better production parameters (López-Bote, 1998) and therefore higher profits for the farmer. Specifically, in Extremadura, the highest economic gain in terms of the number of slaughtered heads is provided by 50:50 Iberian x Duroc crossed pigs (MAPA, 2021). These pigs represent nearly half (49.33%) of the total Iberian pigs in the *Montanera* system (MAPA, 2021).

The geographic limitations of the *dehesa* area and the seasonality of its natural resources, i.e. acorn and pasture (from November to March) have led to a diversification of the Iberian pig production system towards the intensification of their management and a feeding strategy based on commercial fodder, in an attempt to meet the growing demand of meat and meat products and as a tool to introduce a differentiation in an increasingly competitive market. This diversity gives rise to differences in the nutritional and sensorial quality of its meat and meat products and, therefore, to differentiated end products. This has led to an interest in a regulatory framework for the production of Iberian pigs that could define and guarantee that each designation met certain quality characteristics in the most uniform possible way. Such framework has been constantly renovated in accordance with the needs and advances of the sector itself, as well as those of the consumer.

The first Spanish Iberian Quality Standard was published in 2001. Its purpose was to define and guarantee the quality characteristics and the control and distribution processes for Iberian dry-cured products (ham leg, ham shoulder and loin) (RD 1083/2001). Its subsequent amendment in 2007 (RD 1469/2007) extended the scope of application to fresh meat. Both standards defined the various Iberian pig production systems; The *Montanera* system, which yields "*bellota*" (acorn-fed animal) designated products and the "*cebo*" (fodder-fed animal) system, which yields "cebo" (fodder-fed animal) products. The middle-of-the-road system between the above two is known as the "*recebo*" system, which includes animals the feeding of which is completed with fodder after they have gained a minimum weight in the free-range *Montanera* system. The latter provided a niche of variability for meat and meat products, and was one of the issues improved in the latest Iberian Quality Standard (RD 4/2014). This standard was approved in 2014 with the purpose of clarifying and providing transparency to the Iberian sector, as well as providing consumers with an easier perception of the products available in the market and their various quality standards. This standard establishes minimum production requirements that can affect the quality of meat and meat products with the purpose of setting out certain quality standards and attaining the homogeneity of the products within each standard. Specifically, the Quality Standard provides four categories, each with a distinctive label and colour for the commercialisation of products deriving from cuttings of the carcass being sold as fresh meat, as well as for ham leg, ham shoulder and dry-cured loin. *Black*, *Red* labels (Iberian purebred animals or Iberian x Duroc crossed bred animals in a proportion of at least 50% Iberian and finished in the

Montanera system), *Green* label (animals with at least 50% Iberian breed, open-air reared and fed on fodder but without detriment that they may also be fed on acorns and pasture in the *Montanera* system) and *White* label (animals with at least 50% Iberian breed that are exclusively fed on fodder and reared in intensive system) (Figure 3).

Figure 3. Quality standard commercial categories according to RD 4/2014.

However, in spite of the huge efforts made by the Iberian sector with the introduction of the aforementioned regulation frameworks, as well as the protected designation of origin (PDO) and the various certification bodies to regulate the production of the Iberian pig and obtain homogeneous products within each commercial designation, there are still new challenges to overcome in order to raise full awareness of all the factors that can have an impact on the quality of meat and meat products and their economic consequences, and therefore in order to cover the needs of consumers, industrial entrepreneurs and farmers. At a production level, the niche of variability found with the products designated as "*recebo*" according to Quality Standard of 2001 (RD 1083/2001) and 2007 (RD 1469/2007) is an issue that has attempted to be solved within the current regulation framework through the creation of the *Green* label. Nevertheless, from our point of view, there is yet a lack of uniformity amongst the products being distributed under this commercial label that derives from the lack of specification of the type of feeding and degree of intensification of the animals, and this causes confusion in the consumer. Another production variation factor is the age of animals at slaughter, due to its economic impact, as it is connected to the production system, but it is clearly dependent on the the growth rate of the animals used. The standard establishes that, for animals finished in the *Montanera* system, the minimum age at which they must be slaughtered is, at least, 14 months old. Given the minimum period of time animals must remain in the *Montanera* system is 60 days (RD 4/2014), animals should be initiated to the final fattening stage in *Montanera* from 12 months old onwards. Nevertheless, it is common knowledge amongst farmers and supported by scientific literature, that production parameters such as the growth rate, feed efficiency and the lean yield of Iberian crossed pigs are better than those of purebred pigs (López-Bote, 1998). This means that crossed bred animals can reach the relevant weight at the beginning of *Montanera* and therefore at age of slaughter at an age below that of the purebred Iberian animals, even at an earlier age than the age established by the Quality Standard, although meeting the weight gain requirements and minimum stay in the *Montanera* system to allow their products to be distributed under the current regulatory framework. Therefore, the difference in age at slaughter could give rise to a disagreement between the requirements of the current regulation framework and the farmers' interests, who broadly use Iberian crossed pigs to increase productivity, reduce the production cycles and their costs (Daza, 2001). Under these assumptions, the age for animals to start in the *Montanera* system and of slaughter for Iberian x Duroc crossed pigs could be different from that of Iberian purebred ones. Additionally, the animal age at slaughter could have an impact on the quality of the meat and meat products, giving rise to a lack of uniformity in products the consumer considers to be homogeneous in principle.

There are many studies dealing with how feeding (especially during the finishing phase of the animal) and the rearing system (Andrés, Cava, Mayoral, Tejada, Morcuende, et al., 2001a; Cava, Ventanas, Florencio, Ruiz, & Antequera, 2000; Tejerina et al., 2012) as well as the genetic line within the Iberian breed (Carrapiso, Tejeda, Noguera, Ibáñez-Escriche, & González, 2020; Muriel, Ruiz, Ventanas, Petrón, & Antequera, 2004) or the Iberian x Duroc cross breed (Andrés, Cava, Mayoral, Tejeda, Morcuende, et al., 2001b; Carrapiso & García, 2005; Ramírez & Cava, 2007) can affect the quality of meat and meat products. Yet, there are very few studies dealing with the animal age at slaughter and its impact on the quality of its products (Daza, López-Bote, Olivares, Menoyo, & Ruiz, 2007; Daza, López-Bote, Rey, & Olivares, 2006; Mayoral, Dorado, Guillén, Robina, Vivo, et al., 1999), and, as far as we are concerned, there are no studies at all on the Iberian x Duroc cross breed, and which therefore integrate the interests of all the stakeholders in the Iberian sector.

In terms of the technological process, the industry challenge of solving the implicit seasonality of the *Montanera* system remains unsolved. This situation derives from the seasonality of its natural resources, i.e. acorns and pastures (from November to March). Such seasonality becomes more accentuated in the case of products with short technological processes such as loin, which takes approximately 70 days to cure (RD 4/2014). This causes for the dry-cured loin to become available to the market in the summer season, while their main commercial season is the Christmas campaign (November to December). Freezing the primal cuts before the curing technological process could balance the time lag between the moment the *Montanera* Iberian dry-cured products become available to the market and their demand by consumers, therefore contributing to overcome their implicit seasonality. It is therefore necessary to create awareness of the pre-cure freezing practice and the changes it may mean to the production yield and the quality of the *Montanera* Iberian dry-cured products in general, and the dry-cured loin in particular, which may affect consumer acceptance.

On the other hand, most studies relating to the impact of the various production and technological factors throughout the entire production cycle of the Iberian pig on its products have been focusing on ham leg, probably because this is the most economically-valued product. However, dry-cured loin is a differentiated product that enjoys wide consumer acceptance due to its particular sensorial characteristics deriving from the composition of the muscle it derives from, i.e. the *Longissimus thoracis et lumborum,* and the physical, chemical and biochemical characteristics deriving from its curing technological process. In fact, the Iberian dry-cured loin represented nearly a third of the Iberian dry-cured products available in the Spanish market under the current Quality Standard (RD 4/2014) in 2020 (MAPA, 2021). It has even a higher ranking in importance within the products from animals produced in the *Montanera* system, reaching 39% of the *Montanera* product units being sold in the national market, with 1,225,382 pieces (RIBER, 2021). Likewise, under the *Montanera* designation, dry-cured loin was the most sold piece in Extremadura with 230,226 pieces (RIBER, 2021). Additionally, as the loin is comprised of a single muscle, it could provide results that are more easily identified than ham leg, which is made of various muscles, in terms of the influence that certain factors could have on the quality parameters of the meat products, and therefore that might impact uniformity and quality standards from a consumer's viewpoint.

The end consumer is an important element in the chain, as they drive and promote the innovation strategies created by the other stakeholders of the Iberian industry. In this sense, the selling and distribution format of Iberian dry-cured products is a factor that has gained importance in recent years. Current sociodemographic changes, consumer lifestyles and purchase habits have led to an increased demand of smaller sliced packed products, in detriment of the whole piece format. The vacuum-packed product was traditionally used because of the benefits it provided in terms of preservation of product characteristics, extension of shelf-life and ease of logistics. Recently, the modified-atmosphere packaging (MAP) has been introduced as an alternative to vacuum packages for these types of products, as an innovation strategy in order to find a product that resembles the freshly-cut product. This could explain the fact that the existing studies on Iberian dry-cured ham tend to praise the superiority of the MAP format over the vacuum-packed format, as it also solves the adherence issues that the vacuum-packed sliced product has (García-Esteban, Ansorena, & Astiasarán, 2004; Parra, Viguera, Sánchez, Peinado, Espárrago, et al., 2010). However, the differences associated to the production costs of each of the quality categories listed in the current Quality Standard (RD 4/2014), as well as the various qualities amongst them, make it necessary to implement a reinforced traceability control process for sliced and packed formats, as these do not carry the label or seal that is present on the whole piece as guarantee assurance of the commercial category. Additionally, the products commercialized in the sliced packed selling formats have a shorter shelf- life in comparison to the whole piece, due to some oxidative processes and alterations that occur in the colour or other nutrients, as they are exposed to a greater number of altering agents. Based on the above, the feasibility of these types of formats to preserve and market the Iberian dry-cured sliced products in general and, of the dry-cured loin, in particular, must be addressed. In the first place, instruments that can guarantee consumers the traceability and commercial category of the products under the current regulatory framework must be devised. In the second place, it would be essential to evaluate the influence of the package itself on the shelf-life of the product, as well as studying the evolution of each of the commercial categories throughout their storage period, since each has various qualities at nutritional and sensorial levels and therefore each is a different product. Such knowledge would help the industry progress in terms of allowing an understanding and decision-making scenario relating to the quality control of the Iberian dry-cured sliced packed products and the control of traceability and shelf-life.

It is therefore necessary to use fast tools as an alternative to conventional analysis methods and allow the involved industries to optimise their time, workforce and economic resources necessary to implement the higher levels of control required for the sliced packed selling formats. In this sense, one of the pig industry's most used and demanded technologies is the near infrared spectroscopic technology (NIRS), which allows to obtain information and make real time decisions in a non-destructive manner, and therefore providing a great advantage given the high value of Iberian pig products.

Previous studies have dealt with the ability of the NIRS technology as a tool for authentication-classification, demonstrating its validity in individual Iberian pig carcasses under the various commercial categories of the former Iberian Quality Standard (RD 1469/2007): i.e. *Acorn-fed*, *free-range fodder-fed* and *fodder-fed* animal categories, and in carcasses, subcutaneous fat, and fresh meat according to the various commercial categories compiled by the current Iberian Quality Standard (RD 4/2014) (Horcada, Valera, Juárez, & Fernández-Cabanás, 2020). According to this, the use of the NIRS technology could also be feasible for the individual control of dry-cured loin in sliced packed format, and therefore protecting and assuring their origin and traceability, making sense of the labelling system and providing additional value to the product. This would also imply an opportunity for industry improvement, since the price differences between the various commercial categories derive from the efforts and resources involved in each production system, especially that of *Montanera,* making it necessary to offer control traceability to the end consumer. On the other hand, the use of fast non-destructive measures in general and, the use of NIRS technology, in particular, could not only be interesting to guarantee the traceability of the commercial category in the sliced packed formats, but also ensure its quality by evaluating its shelf-life throughout storage time. This aspect is particularly interesting for sliced modified-atmosphere packed products, as their quality could be altered to a greater extent than the vacuum-packed formats as stated by Parra et al., (2010) for Iberian dry-cured ham, therefore requiring a more frequent quality control process.

In order to answer all the questions that have been raised, various sequential and interconnected studies were designed under the current Spanish Iberian Quality Standard framework, i.e. RD 4/2014. Details on their development and application have been included in this doctoral thesis with the end purpose of generating scientific knowledge to help clarify such issues and help as a basis for the development of the Iberian sector. Initially, a study was proposed to include the perception of the main stakeholders (farmers and distributors) of the situation of the Iberian sector, with the purpose of identifying the main limitations and opportunities for the distribution of Iberian products within the current regulatory framework. In parallel, other specific studies were designed on relevant production aspects, such as the impact of animal age at the beginning of the finishing phase in *Montanera* and at slaughter on their performance and quality of the carcass, and on quality of meat and meat products, respectively. At industrial level, several issues were discussed with a view to solve two key aspects: i) the seasonality issue associated to the *Montanera* products, ii) the difficulties of setting up a system to control quality and traceability in the production line. This way, the effect of pre-cure freezing was studied as a tool to providing the market with *Montanera* dry-cured products all year round. On the other hand, the feasibility of the use of the NIRS technology was evaluated as a fast non-destructive tool for quality control and categorisation of products in accordance to the categories established by the current Iberian Quality Standard (RD 4/2014).

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OBJECTIVES

The objectives of this thesis are:

1. To understand the main views of farmers and industry entrepreneurs on the application of the Spanish Iberian Quality Standard (RD 4/2014) in order to identify the limitations and opportunities for the commercialisation of Iberian products in the current environment.
2. To evaluate the impact of animal age and similar body weight at the beginning of the finishing phase in *Montanera,* and of gender on performance, body measurements and carcass quality in Iberian pigs.
3. To assess the impact of animal age at slaughter of *Montanera*-finished Iberian pigs on the quality traits of fresh and dry-cured loins (*Longissimus thoracis et lumborum* muscle).
4. To study the effects of the pre-cure freezing practice on the quality traits of *Montanera* Iberian dry-cured loins.
5. To characterise the main quality traits of pre-sliced Iberian dry-cured loins according to the commercial categories under the Quality Standard (*Black*, *Red* and *White*) and the impact of the packaging type on shelf-life throughout long-term storage.
6. To assess the feasibility of using near infrared spectroscopy technology for pre-sliced modified-atmosphere packaged Iberian dry-cured loin classification according to the commercial categories under the Quality Standard (*Black*, *Red* and *White*) and the various refrigerated storage time.

GENERAL METHODOLOGICAL FRAMEWORK

The structure of this doctoral thesis consists of six sequential and interrelated scientific articles within the framework of the current Spanish Iberian Quality Standard (IQS), i.e. Royal Decree 4/2014 of 10th of January, which approves the quality standard for Iberian pork meat, ham leg, ham shoulder and loin.

Figure 4 describes the methodological framework, including the main objectives and the general experimental design that helped meet them.

The aforementioned objectives are described in the chapters of the doctoral thesis. In Chapter I, a qualitative research process was carried through in-depth interviews with farmers and industrial entrepreneurs from various types of businesses in terms of size (small, medium and large), various product categories being distributed according to the IQS (*Black*, *Red*, *Green* and *White*), various production types (closed cycle, only *Montanera*/fodder-fed animals, other integrations for farmers), and various market structures (local, national or international), in order to identify their main views on the limitations and opportunities of Iberian product commercialisation under the framework of the QS.

Chapter II addressed the influence of two production factors, i.e. gender (surgically castrated males vs. immunologically-castrated females) and animal age at the beginning of the finishing phase in the *Montanera* system when animals are initiated at similar body weights, on performance, body measurements and carcass quality in Iberian x Duroc crossed bred pigs (50:50). Chapter III used the experimental design and the animals mentioned in the previous chapter to evaluate the impact of animal age at slaughter on the quality traits of fresh and dry-cured loin. Thus, the effects of the pre-curing freezing practice were evaluated on the quality traits of *Montanera* Iberian dry-cured loins. Chapters V and VI are based on the same experimental design to address another industrial issue, i.e. the challenge of establishing a quality control and traceability system for dry-cured products when they are presented as pre-sliced packed selling formats. In the first place, the differences in the physico-chemical and sensorial qualities and their evolution throughout long-term refrigeration storage according to the various commercial categories under the Quality Standard (*Black, Red* and *White*) and the packaging type (vacuum vs. modified-atmosphere) on pre-sliced Iberian dry-cured loin were evaluated (Chapter V). Lastly, Chapter VI assessed the feasibility of use of near infrared spectrocopy

(NIRS) technology in combination with various chemometric approaches for pre-sliced Iberian dry-cured loin in modified-atmosphere packaging classification according to the aforementioned commercial categories under the Quality Standard and various refrigeration storage time (0, 4, 8 and 12 months) without opening the package.

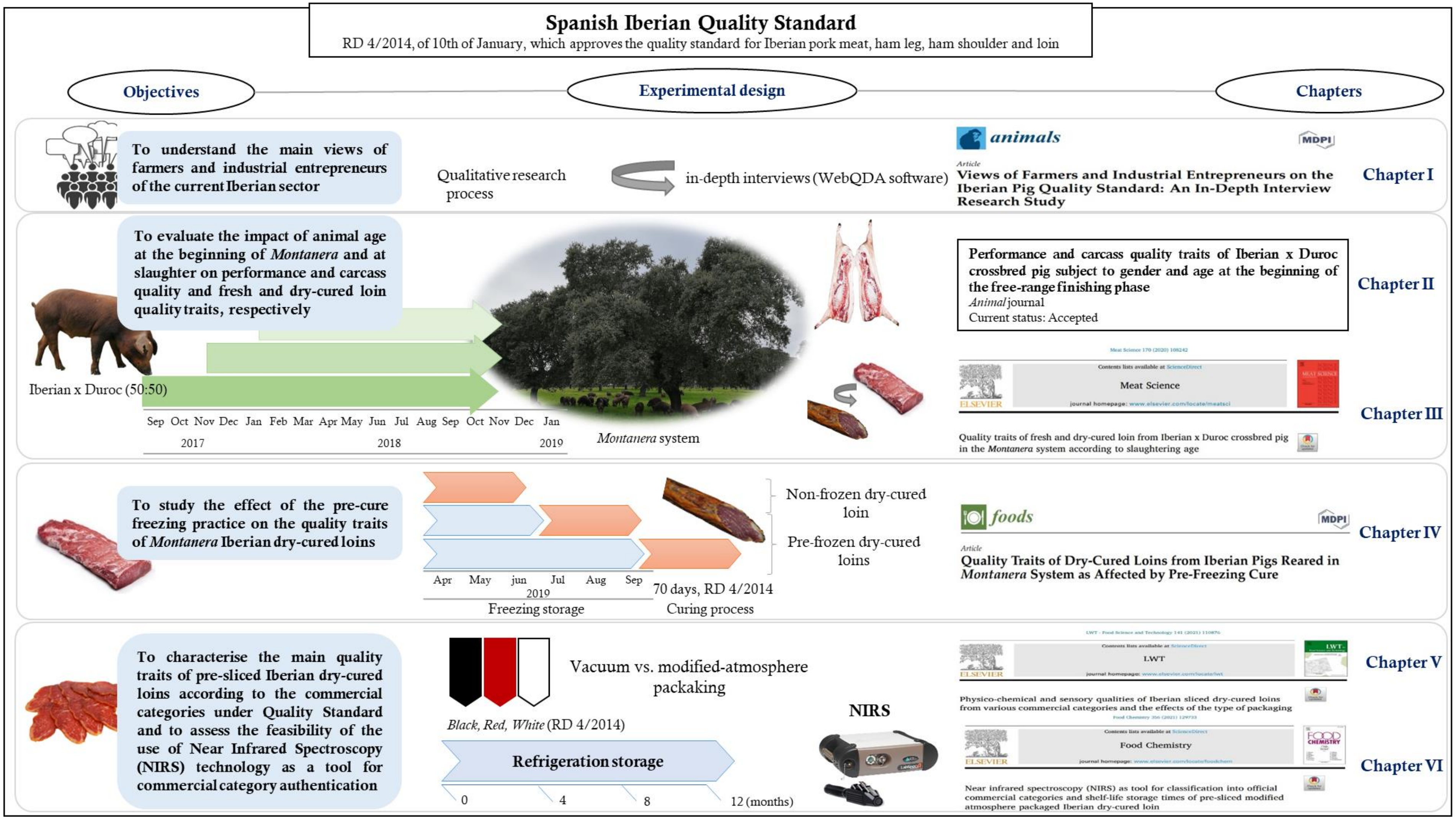

Figure 4. Objectives, experimental design and chapters.

Chapter I. Views of farmers and industrial entrepreneurs on the Iberian pig Quality Standard: An in-depth interview research study

Ortiz, A., Carrillo, N., Elghannam, A., Escribano, M., Gaspar, P., 2020. Views of Farmers and Industrial Entrepreneurs on the Iberian Pig Quality Standard: An In-Depth Interview Research Study. *Animals, 10.* https://doi.org/10.3390/ani10101772

Views of Farmers and Industrial Entrepreneurs on the Iberian Pig Quality Standard: An In-Depth Interview Research Study

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Simple Summary

This paper aims to assess the main opinions of farmers and industrial entrepreneurs on the implementation of the current Spanish Iberian Quality Standard regulation as well as on the processing technologies of Iberian dry-cured products. The study is based on a qualitative research process through in-depth interviews, and has allowed the identification of aspects that can be improved both at the level of the Iberian meat industry and in the administrative processes in the view of the main actors of the Iberian pork sector in Spain. The aspects of the Quality Standard related to the protection of the base of the Iberian breed, the conditions of production in the traditional system (*Montanera*), as well as the ripening time of the products were mostly supported by the farmers and industrial entrepreneurs. However, they showed certain inconformity with the requirements established by the Quality Standard for other production systems such as the fodder-fed and free-range fodder-fed, therefore they demanded changes in these aspects.

Abstract

Since 2014, the Quality Standard for Iberian meat, ham leg, ham shoulder ham and loin has regulated production factors and processes involved in the raw material and manufactured products from Iberian pigs, the most important pig breed in both population size and economic importance of the Southwest Iberian Peninsula. Regarding the changes to the Quality Standard that industrial entrepreneurs and farmers are currently demanding, a qualitative research study has been developed through 14 in-depth interviews with the purpose of understanding the perception of Iberian pig farmers and industrial entrepreneurs of the requirements of the currently-effective Quality Standard, as well as the conditions under which this is being applied. The results showed a consensus amongst the majority of the participants in aspects such as the maintenance of the breed base as 100% Iberian for reproductive females, weight and age requirements at the time of slaughter for the *Montanera* category and the manufacturing lengths for dry-cured products. On the other hand, there were discrepancies between the requirements defined by the Quality Standard and those requested by the respondents for the fodder-fed and free-range fodder-fed categories, with the industrial entrepreneurs and farmers being inclined towards the reduction in the age of slaughter of the former and the distinction in the production conditions of the latter.

Key words: Iberian pork, Quality Standard, qualitative analysis, in-depth interviews

1. Introduction

Pork meat consumption represents a major part of the diet of the European countries, with pork being the most consumed and preferred meat, before chicken and beef (European Commission, 2012). In recent years there has been an increasing demand of meat products deriving from autochthonous breeds that are reared in extensive systems, which is potentially due to a positive perception of society as to their contribution in the preservation of the environment (Trícia, Monteiro, Wilfart, Utzeri, Lucka et al., 2019), animal welfare (Temple, Manteca, Velarde, & Dalmau, 2011), as well as the perceived high quality of the derivative products (Pugliese & Sirtori, 2012).

This is the case of the Iberian pork, the most important pig breed in both population size and economic importance of the Southwest Iberian Peninsula (Serra, Gil, Pérez-Enciso, Oliver, Vázquez et al., 1998). High acceptance and demand of Iberian products have enabled the development of the industry involved, which still has major problems to deal with, such as the great variability of factors associated with the various stages of the production chain, giving rise to a diversity of production models and therefore differences in the final quality of the products. These factors include the genetic background of animal (Ramírez & Cava, 2007), the production system, the feed provided to the animal, especially during the final finishing phase (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012). Additionally, animal age and weight at the beginning of the finishing phase (Daza, López-Bote, Olivares, Menoyo, & Ruiz,, 2007) and at the time of slaughter (Bahelka, Hanusová, Peškovičová, & Demo, 2007; Candek-Potokar, Ilender, Lefaucheur, & Bonneauc, 1998) are factors to consider in the quality of the products derived. Further, this variability to which the Iberian pork products are subjected makes it difficult for the detection of any fraudulent activity that may take place within the industry (Espárrago, Cabeza de Vaca, & Molina, 2001).

The first Spanish Iberian Quality Standard (RD 1083/2001) emerged in 2001 within this context with the main purpose of guaranteeing and defining the quality traits and control process, regulating Iberian pig production factors and the commercialization of their derived dry-cured products. Its subsequent amendment in 2007 (RD 1469/2007) extended its scope of application to pork cuts that are commercialized as fresh meat. The current Spanish Iberian Quality Standard, known as the Quality Standard for Iberian pork meat, ham leg, ham shoulder and loin, came into force in 2014 (RD 4/2014) (hereinafter, QS) as an attempt to clarify and provide transparency to the industry, as well as providing a simpler perception of the market products and their various agreed quality standards grouping them under a new labeling system. Thus, four commercial categories (labels) were defined: the *Black* label (100% Iberian breed acorn-fed pigs), *Red* label (at least 50% Iberian breed acorn-fed pigs), *Green* label (at least 50% Iberian breed pigs, reared in pastures (*dehesas*) and fed on fodder but without detriment that they may also be fed on acorns and pasture) and *White* label (at least 50% Iberian breed pigs, reared in confinement and fed only on fodder (Table I.1).

Table I. 1. Requirements for the production aspects of the various categories of Iberian products and manufacture minimum times according to the current Quality Standard (QS).

Production aspects	**Commercial Label**			
	Black	***Red***	***Green***	***White***
Breed (100%, 75% and 50% Iberian) provided that the female is 100% Iberian breed and the male is Duroc breed, both registered in a genealogic tree	100 %	75%, 50%	100%, 75%, 50%	100%, 75%, 50%
Management system (the animals can be reared under various levels of intensiveness)	Extensive		Semi-intensive	Intensive
	(0.25-1.25 animals/ ha) subject to the wooded area available and the availability of acorns		At least 100 square metres/ animal when the body weight exceeds 110 kg	At least 2 square metres/ animal when the body weight exceeds 110 kg
Weight and minimum weight gain during the finishing stage	46 kg for over 60 days		At least 60 days prior to slaughter	
Minimum age at slaughter	14 months		12 months	10 months
Feed allowed during the finishing stage for each category	Feed based only on acorn, grass and other natural resources found during *Montanera* in the *dehesa*		Feed based on fodder made of cereal and legumes with the possibility for the animals to either fully or partially rearing in *Montanera*	Fodder made of cereal and legumes
Carcass minimum weight	115 kg, except for 100% Iberian animals, which will be 108 kg			
Product	**Ham leg**		**Ham shoulder**	**Loin**
Minimum time of manufacture of the products	W < 7 kg 600 days, W ≥ 7 kg 730 días		365 days (regardless of weight)	70 days (regardless of weight)

Own source based on the current QS (RD 4/2014).

Thus, the commercialization of the products under the aforementioned labels requires an effort by farmers, industrial entrepreneurs and traceability and control systems. This in turn means an increase in the production costs, which at times is not compensated by the selling price, given that all this effort to improve the industry is not always perceived and translated in the consumer purchasing decisions (Díaz-Caro, García-Torres, Elghannam, Tejerina, Mesías, et al., 2019).

Several years since the implementation of the current QS, there is certain degree of disagreement amongst the involved stakeholders, i.e., farmers and industrial entrepreneurs, with regards to some of the requirements set out in the QS for the various categories, specifically in relation to production factor in the farm, certification process and technological processing of the products. Amongst others, one of the factors raising most of the interest and controversy within the production aspects is the age of slaughter of animals, which is determined according to production system (*Montanera*, free-range fodder-fed and fodder-fed) and, disregarding the animal Iberian breed percentage. Although traditionally, a long production cycle has been the preferred option for the Iberian breed in order to obtain high-quality products (Daza et al., 2007), the improvement in the production parameters deriving from the use of the Duroc breed (Ramírez & Cava, 2007) -authorized by the current QS- could generate a misalignment between the QS's required age of slaughter and the farmers' interests, who broadly use this breed in order to increase productivity, reduce production cycles and therefore costs. Nevertheless, in spite of the relevance of the age of slaughter (Bonneau & Lebret, 2010), as far as we are concerned, there are no studies that explore the recommended age in association to genetics, and there are only few studies that assess its influence on the quality of the meat derivatives (Daza et al., 2007; Mayoral, Dorado, Guillén, Robina, Vivo et al., 1999), and therefore that may combine the demands and interests of farmers and consumers alike. On the other hand, there is a clear lack of definition of the feeding and rearing system used for pigs under the *Green* label category (free-range fodder-fed animals), a fact that has even led to this category being excluded from sensorial studies on account of the lack of uniformity of its production aspects (Ortiz, Tejerina, Díaz-Caro, Elghannam, García-Torres et al., 2020).

Additionally, the current QS does not contemplate any measures to help overcome the seasonality to which Iberian products are subjected, especially *Montanera* Iberian dry-cured loins, which are launched to the market in the summer months with lesser demand, and considering that the period of greatest consumption of this type of products is from November to December (because of Christmas season), there is a gap between industry and consumer demand. In order to overcome this situation, industrial entrepreneurs use practices such as freezing the raw material prior to its technological process of curing, but the lack of European Regulations for the freezing of animal products (European Regulation 16/2012), together with scarce scientific literature (Abellán, Salazar, Vázquez, Cayuela, & Tejada, 2018; Lorido, Ventanas, Akcan, & Estévez, 2016), has led to a situation of uncertainty regarding how this practice may affect the quality of the end product, which in turn has made it difficult to regulate and control it at the industrial level.

Given the above concerns and the over four years since the implementation of the QS, it is vital to assess the current QS, as well as any improvement proposals made by the stakeholders, and thus contribute to form a bases for a decision tool that may focus on these specific industry and administrative issues.

In this context our purpose was to understand the main views of farmers and industrial entrepreneurs on the application of the current QS for the Iberian product through an in-depth interview qualitative research study, in order to identify the limitations and opportunities for the commercialization of Iberian products in the current environment.

2. Material and methods

This research study has been based on a qualitative method throughout in-depth interviews on account of its exploratory nature and because of the high level of controversy amongst the various stakeholders involved in this industry. The research team selected a widely-recognized semi-structured model that is largely used in these types of research studies (McEachern & Seaman, 2005). Two versions of the interview script were designed and adapted to the purposes of the study, one per type of stakeholder (farmers and industrial entrepreneurs). Figure I.1 presents the full methodological process followed for the research study.

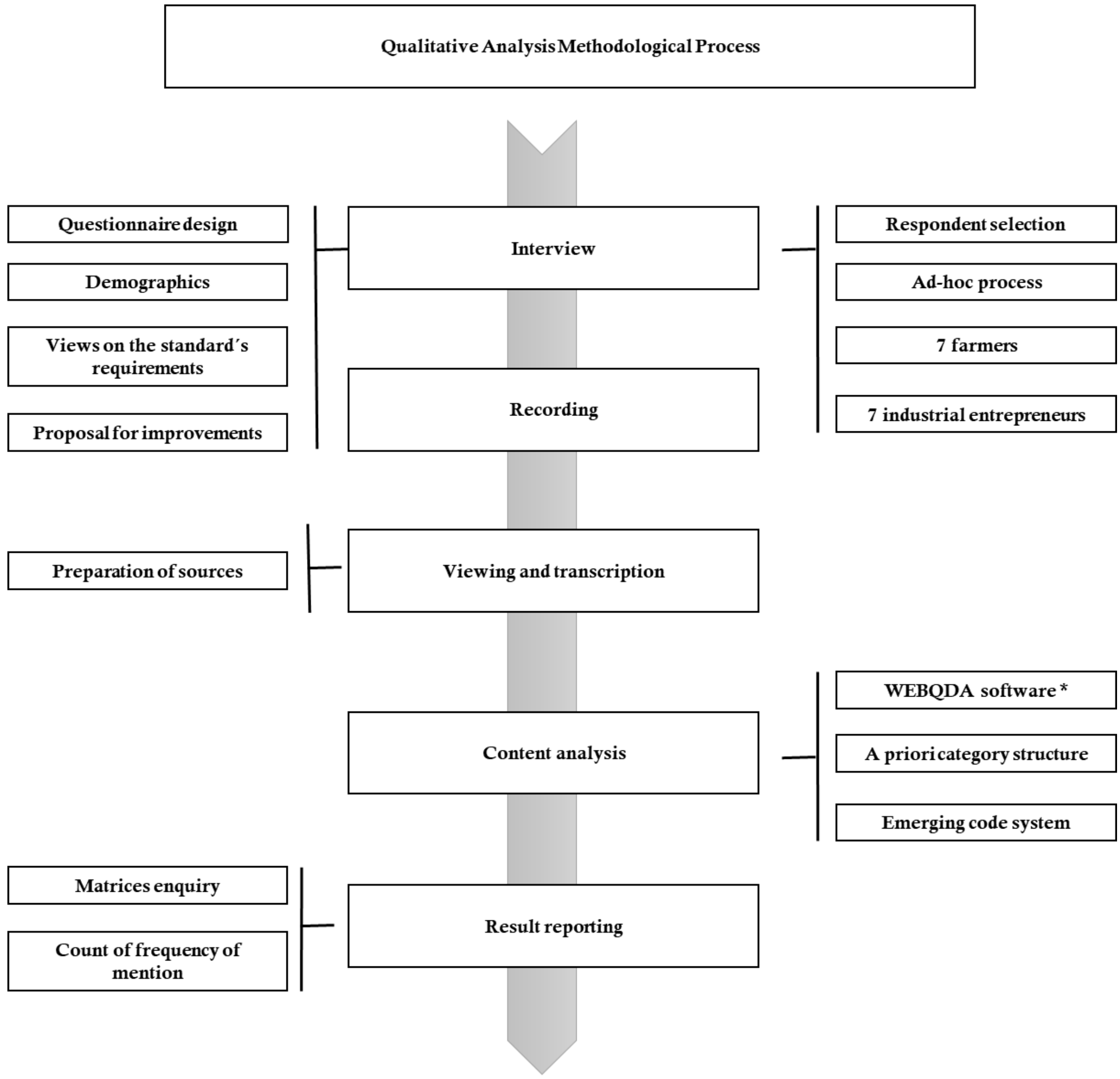

Figure I. 1. Methodological process followed in order to carry out the in-depth interviews. * WebQDA Software V 3.1. is a qualitative data software analysis.

2.1. Data collection

For the purposes of this research study, all the interviews were face to face interviews carried out at the workplace of the respondents. The final sample was selected following a convenience sampling process, which is a non-probability type of sampling that is broadly used in qualitative research (Kinnear & Taylor, 1993; Mesias, Pulido, Escribano, Gaspar, Pulido, et al., 2013). For this piece of research, the respondent selection process was progressive, as interviews were conducted at the same time as the respondents were classified by characteristics. Subsequently, from the information obtained, new farmers and industrial entrepreneurs with different characteristics from the ones that had already been interviewed were sought. This particular selection process was adopted with the purpose of covering the various types of businesses in terms of size (small, medium and large), categories (or labels) of products sold (*Black*, *Red*, *Green* and *White* labels) and production type; both in the case of farmers (closed cycle, only *Montanera*/fodder, other integrations) and industrial entrepreneurs (dry-cured products, fresh products, both) and finally, the various market plans for the products (local, national or international).

Subsequently, a telephone conversation was held with the selected respondents, where the purpose of the research study was explained, and they were asked cooperation in the conduction of the semi-structured interviews. Additionally, they were assured that the data provided would be confidential in compliance with the Spanish Data Protection Act. Fourteen in-depth interviews were conducted in total, seven of which were conducted with farmers and the other seven with industrial entrepreneurs. This number of interviews was in line with that of other qualitative research studies carried out using semi-structured interviews (Gatley, Caraher, & Lang, 2014). All the participants were asked for their consent to be audio recorded during the interviews and they all accepted. The average length of each interview was approximately 120 minutes.

2.2. Interview script design

The interviews were structured in five sections designed to meet the purposes of the research study. As Table I. 2 shows, the interviews included similar questions for farmers and industrial entrepreneurs, although in some cases there were specific questions for each group.

Table I. 2. Script of the interviews conducted with farmers and industrial entrepreneurs.

Interview Design	Farmers	Industrial Entrepreneurs
Demographics	Information on the age, sex, education level, job title and experience in the position	
Type of farm/ industry	Business activity (farming, industrial or both), number of employees and their distribution in the various departments, production type under the QS, commercialisation channels and sales	
	Nº of pigs sold per category	Main product type sold (fresh, cured)
	Animal breed base	Brands under which the products are commercialised
		Countries for export, if any
Views on the various aspects of the Quality Standard (RD 4/2014)	Views on the requirements of the QS in terms of breed base, feeding type, weight gain at the finishing stage and weight/ age at time of slaughter	
	Views on the certification process for farms and industrial businesses	
		Manufacturing time for products according to the QS
Views on production seasonality and Iberian product demand	-	Strategies used to correct discontinuity of demand of Iberian products
	-	Freezing of the fine cuts prior to the curing process. Impact on the final quality of the product and production costs. Need to specify such practice on the label
Proposals for improvement	Applicable measures aimed at improving the identified deficiencies or others not referred to previously. Individuals responsible for their implementation.	

2.3. Data analysis

Once the interviews had been conducted, the recordings were transcribed and the information collected was analyzed by means of a Content Analysis methodology (Stewart, Shamdasani, & Rook, 2007). Content analysis is a method attempting to obtain valid and applicable inferences from the texts with the purpose of reducing the original material (Flick, 2009). The webQDA software (v. 3.1, Ludomedia, Oliveira de Azeméis, Portugal) was used for the purpose of this analysis, establishing a tree structure with a priori categories and an "emerging" code system linked to each category. Building a priori categories consists of establishing a preliminary hierarchical system prior to reading the documents, which for the purposes of this research study was predetermined by the items included in the semi-structured interview. Coding is a systematic way of developing and refining the data interpretation. The coding process includes the collection and analysis of all the data relating to subjects, ideas, concepts, interpretations and propositions (Taylor &

Bogdan, 1986). Specifically, emerging coding is characterized by being inductive or open with codes being generated as the information is processed, in a way that as the data are being read or interpreted, new amendments emerge. Subsequently, the frequency of mention of each opinion was obtained from the views of each respondent that were coherent with the ideas or concepts contained in each code with respect to the total of responses, converted to a percentage (Neri de Souza, Costa, & Moreira, 2011).

3. Results

The results are presented according to the a priori categories established in the methodological process. These are a total of eight categories of which the first four are categories related to the requirements of the QS at the farms, the next two have to do with the administrative aspects that the QS also establishes, such as certification and inspection processes as well as product labeling. The last two categories refer to aspects related to the processing of the products by the manufacturers.

3.1. Farmers and industrial entrepreneur views on the requirements of the QS for farms

3.1.1. Requirements in terms of the breed base of the reproductive animals

With the purpose of protecting the genetic value of the Iberian breed, the QS establishes that all females must be 100% Iberian breed. These can be used for the Iberian female x Duroc male cross-breeding, whereas the Duroc breed is reserved for the male line, provided always that both are registered in the herd book. In Figure I. 2 we can see how in their majority, both farmers and industrial entrepreneurs who are asked about this, are inclined towards the protection and preservation of a 100% Iberian female, with 63.6% and 56.3% in frequency of mention, respectively.

Category	Code	Frequency of mention INDUSTRIAL ENTREPRENEURS	Frequency of mention FARMERS
Breed base	Maintain 100% Iberian sows	56.3%	63.6%
	100% Duroc registered boars	18.8%	18.2%
	More flexible genetic requirements	25.0%	18.2%

Figure I. 2. Industrial entrepreneur/ farmer views on the requiremts of the QS in terms of breed base. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

The following were some of the literal comments made by the respondents:

> *"This has been one of the major contributions of the QS to the industry because it has maintained production at the same time as preserving the pure Iberian female".*

> *"It is great for the Standard to protect the Iberian female because, otherwise, this would all get out of hand".*

Another idea that was contributed during the interviews by industrial entrepreneurs and farmers was the need to guarantee the breed purity of Duroc boars in the farms where they decide to have cross-bred animal products, with over 18% in the frequency of mention both by farmers and industrial entrepreneurs.

Lastly, some views were in favor of making the genetic requirements more flexible (Figure I. 2), mainly amongst the industrial entrepreneurs (25.0% of frequency of mention), in a way that the QS would allow animals whose characteristics were compatible with the breed standards, even when they were not registered in the herd books. This goes against the currently effective regulations which are supported by Iberian Breed Association (RD 4/2014).

3.1.2. Requirements in terms of minimum weight gain at the finishing phase and minimum carcass weight

According to the current QS, the average weight of the animal lot at the beginning of the *Montanera* stage (*Black* and *Red* labels) must be between 92 and 115 kg, gaining a minimum of 46 kg during at least 60 days. In the case of animals produced under the free-range fodder-fed and non-free-range fodder-fed categories (*Green* and *White* labels, respectively), the QS does not establish the weight gain applicable during the finishing phase. As a common feature for all categories, the carcass weight must be greater than 115 kg in cross-breeds (Iberian x Duroc), and 108 kg in pure Iberian animals.

Almost half of respondents when asked for this requirement, both from the farm and the industrial backgrounds, were of the opinion that the weight gain and weight at slaughter were adequate (in order to achieve the minimum carcass weight) in all categories (42.9% and 44.4% in frequency of mention, respectively) (Figure I. 3). Examples of comments in this line are:

> *"With regards to weight, I think it is important to maintain the limits established by the QS, because a pig that does not reach the adequate weight will not prove an adequate carcass later on".*

Nevertheless, some interviewed farmers would agree to not establishing minimum-weight gains for animals reared under the *Montanera* system (*Black* and *Red* labels) (21.4% of frequency of mention).

On the other hand, a large proportion of farmers, and especially industrial entrepreneurs, (35.7% and 44.4%, respectively) pointed out that the issue might not be so much in the minimum carcass weights required by the QS (108 to 115 kg) but in the minimum age at slaughter, which was inferred from the interviews through comments such as the following:

> "*I think it is OK, although the industry complains about it being a bit high and with a little less weight they would have better selling hams, especially because the new Duroc hams have caused the ham meat yield to increase and, back then, when the Standard was published in 2014, it was assumed that a 115 kg carcass would yield 7–8 kg hams, which are easy to sell. They are now finding this is not the case, the average weight per ham is 8.5 to 8.6 kg which is way higher than the expected average".*

Category	Code	Frequency of mention INDUSTRIAL ENTREPRENEURS	Frequency of mention FARMERS
Minimum weight gain at the finishing phase and weight at slaughter	Weight gain and weight at slaughter are adequate in all the categories	44.4%	42.9%
	No established minimum weight gain for the *Montanera* stage	11.1%	21.4%
	The issue is not the minimum weight at slaughter, but age	44.4%	35.7%

Figure I. 3. Industrial entrepreneur/farmer views on the requirements of the QS in terms of weight gain at the finishing stage and minimum weight at slaughter. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

3.1.3. Requirements in terms of feeding at the finishing phase

The QS establishes that pigs reared under the *Montanera* system (*Black* and *Red* labels) must be only fed on natural resources (acorns and grass); free-range fodder-fed pigs (*Green* label) must feed on fodder made of cereal and legumes, without prejudice to the use they may make of the natural resources, and that fodder-fed animals (*White* label) are fed only on fodder. Figure I. 4 collects the respondents' main views on the aspects set out by the QS in terms of the feeding at the finishing phase.

Categoria	Code	Frequency of mention INDUSTRIAL ENTREPRENEURS	Frequency of mention FARMERS
Feeding at the finishing phase	There are fraudulent practices in the *Montanera* category	8.3%	7.7%
	The *acorn + fodder-fed* category should be included as in the previous QS	16.7%	7.7%
	The free-range fodder-fed category requires improvement	33.3%	23.1%
	The feeding requirements are adequate for *fodder-fed* animals	25.0%	23.1%
	There is fraudulent activity everywhere	8.3%	7.7%
	All the feeding requirements of the QS are adequate	8.3%	30.8%

Figure I. 4. Industrial entrepreneur/farmer views on the QS feeding requirements. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

The perception that "there are fraudulent practices with animals reared in the *Montanera* production system" represented 8.3% and 7.7%, in frequency of mention by industrial entrepreneurs and farmers, respectively. On the other hand, there were some which were in favor of "including again the free-range acorn + fodder-fed animal category that was contemplated in the previous QS" (16.7% and 7.7% of frequency of mention by industrial entrepreneurs and farmers, respectively).

With regards to the animal feeding requirements for free-range fodder-fed animals (*Green* label), most of the views of the industrial entrepreneurs and farmers, with 33.3% and 23.1% in frequency of mention, respectively, concluded that it was necessary to improve the current QS requirements.

Participants generally showed a consensus with the requirements of the QS in terms of feeding in the fodder-fed category (*White* label).

Lastly, in spite of not being a majoritarian view, some participants expressed the idea that there are generally fraudulent practices in the industry in terms of the feeding at the finishing phase (coded as "There is fraudulent activity everywhere"; Figure I. 4), which attempts to classify animals fed on fodder as *Montanera* animals.

3.1.4. Requirements in terms of the minimum age at slaughter

For each of the production systems set out in the QS, a series of requirements is established for minimum age at slaughter: for pigs reared under the *Montanera* system (*Black* and *Red* labels), the minimum age for slaughter is 14 months and for free-range fodder-fed (*Green* label) and fodder-fed (*White* label) the age is 12 and 10 months, respectively.

A percentage of industrial entrepreneurs and farmers (30.0% and 45.5% in frequency of mention, respectively) were supportive of a reduction in the age at slaughter by two months for fodder-fed pigs (*White* label), going from 10 to 8 months (Figure I. 5). Some examples of these comments were:

> *"The fair thing to do would be to adjust the age of the animal in order to obtain a product that is more adapted to the market and able to make it stable».*

> *"Thanks to the existing technological advances, pigs cannot be slaughtered at the age of 10 months, which would not comply with the requirements of the market; this is where by trying to improve the pig or other parameters,*

we may forget that the purpose is to sell it in a market demanding an 8-month-old pig, not because it is 8 months old, but because this is the age at which it can be sold into the market; the consumer does not want a ham that weights more than 7 kg".

"The age at slaughter should be earlier for fodder-fed animals at least by two months".

"The genetics of pigs have been improved and currently 8-month-old pigs or younger would be ready for slaughter".

Category	Code	Frequency of mention INDUSTRIAL ENTREPRENEURS	Frequency of mention FARMERS
Minimum age for slaughter	Reduction of the minimum age for *fodder-fed* category by two months	30.0%	45.5%
	Adequate only for the *Montanera* category	25.0%	18.2%
	Adequate for the *Montanera* and the *free-range fodder-fed* categories	15.0%	18.2%
	The minimum age at slaughter is adequate for all categories	25.0%	18.2%

Figure I. 5. Industrial entrepreneur/ farmer views on the QS requirements for age of slaughter (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

In the case of animals that are fed under the *Montanera* system, industry entrepreneurs and farmers thought (with 25.0% and 18.2% of frequency of mention, respectively) that the currently-established age at slaughter is adequate. Amongst their statements, the following can be highlighted:

"Animals like those my father used to rear, Montanera all his life. If I tell him that the current minimum is 14 months old, he will laugh at me, because that is so little time, if you think of the time an animal with good qualities and traits needs to fully rear".

Finally, other farmers and industrial entrepreneurs also expressed their views on keeping the age at slaughter proposed by the QS for the various production systems as they thought they were adequate (*Montanera*, free-range fodder-fed and fodder-fed), with 18.2% and 25% in frequency of mention, respectively.

3.2. Farmer and industrial entrepreneur views on the certification processes and the product labeling

3.2.1. Views on the certification/ inspection process

The certification and inspection processes are key for Iberian pigs and their meat products, as they are the tool that guarantees they comply with the QS both at the production stage in the fields and the manufacturing and commercialization stages. In this regard, various matters were dealt with during the interviews, with the results being shown in Figure I. 6.

Category	Code	Frequency of mention INDUSTRIAL ENTREPRENEURS	Frequency of mention FARMERS
Certification/ inspection process	The certification process is too complicated	13.6%	52.6%
	There are fraudulent practices involved in the certification process	22.7%	26.3%
	Industry unrelated inspectors should be involved	9.1%	5.3%
	Farms are strictly controlled whereas industrial businesses are not	31.8%	0.0%
	The certification process is adequate	22.7%	15.8%

Figure I. 6. Industrial entrepreneur/ farmer views on the requirements of the QS in terms of certification/ inspection. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

Notably, the participating farmers (52.6% in frequency of mention) complained about the excessive control measures they are required to submit their practices, especially at production level, with the purpose of ensuring the quality of the inspections, as well as the number of documents that they need to provide in order to prove their situation.

3.2.2. Views on the labeling process

In terms of the labeling of the Iberian pork products, the majority of the industrial entrepreneurs mentioned the excessive importance that the QS places on the font size and type to be used, which was considered irrelevant by the above in contrast with more important aspects, such as the information that is provided to the consumer.

The participants thought it was much more important to mention the animal's breed purity (whether it is pure or a cross-breed with Duroc), the production system (extensive or in confinement) and even indicating the age at which it was slaughtered.

On the other hand, they also felt that it would be important to select the key words to be used on each label depending on the type of animal (words such as Iberian, black leg, free-range fodder-fed, etc.).

3.3. Industrial entrepreneur views on the processing of the products by the manufacturers

3.3.1. Views on the freezing process of raw materials

Part of industrial entrepreneurs saw freezing as a feasible solution to manage the excess product in the industry and to adapt it to market demand, with 19.2% mentions. On the other hand, with the same frequency of mention, industrial entrepreneurs pointed that freezing was a solution for importing and exporting products since the useful life of fresh meat is quite limited. However, the participants pointed out that consideration must be given to the alterations the product might suffer in terms of quality. Thus, the same proportion of respondents indicated that freezing affects the quality of the products as those stating that it does not (15.4%).

On the other hand, the seasonality to which the Iberian products are subject, especially those deriving from pigs reared under the *Montanera* system (*Black* and *Red* labels), makes it necessary for this industry to innovate in order to adapt production to consumer demand. A potential solution would be freezing the hams raw material prior to their curing process as mentioned by the industrial entrepreneurs in this research study with 11.5% frequency of mention (Figure I. 7).

Category	**Code**	**Frequency of mention INDUSTRIAL ENTREPRENEURS**
Freezing of raw meats	It is a good solution to help manage the excess product in the industry	19.2%
	It is a good solution for imports/ exports	19.2%
	Freezing reduces the product quality	15.4%
	Freezing does not affect product quality	15.4%
	It is a good solution to prevent seasonality issues	11.5%
	Freezing increases cost too much	19.2%

Figure I. 7. Industrial entrepreneur views on the requirements of the QS in terms of product freezing. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

On the other hand, other opinions were related with the idea that freezing the product increases the costs.

Some contributed the above opinions with statements such as:

> *"The freezing process clearly increases the cost of the product, which does necessarily translate into an increase in the final product".*
>
> *"Freezing the hams is a solution that would help optimize the facilities of this industry".*
>
> *"Slaughtering and acorn-fed animals have a seasonal component to them, and Christmas is when most of the acorn-fed product is sold. For example, loin is always frozen in preparation for the Christmas demand".*

3.3.2. Views on the minimum obligatory maturity time

An aspect that is defined by the Quality Standard is the minimum manufacturing times, which range from 600 to 730 days for ham legs, 365 days for ham shoulders and 70 for loins. It is important to point out that these timings are minimums, and the final maturity period can be much longer, depending on the manufacturing method used in the industry. In this regard, Figure I. 8 portrays the views of respondents on these maturity times.

Category	**Code**	**Frequency of mention INDUSTRIAL ENTREPRENEURS**
Obligatory minimum manufacturing times	Adequate	85.7%
	Non-adequate	14.3%

Figure I. 8. Industrial entrepreneurs' views on the requirements of the QS in terms of the maturity times. (Scale: frequency of mention of each opinion. Percentage is based on responses in the given code out of all responses to equal 100%).

As Figure I. 8 shows, with over 85% in frequency of mention, industrial entrepreneurs stated that the maturity times for Iberian products were adequate.

> *"The acorn-fed product fully complies with it under the traditional system and in the case of the free-range fodder-fed product, it must comply too, as it is the case of a greased animal. If anyone were able to put it out earlier in the market by artificial means, they will do it, but… to what extent should we tell them: put it out now! One thing is clear: quality takes time and the standard is the reference stating the times in order to prevent us from going the fastest way possible".*

4. Discussion

The regulation of the breed base of reproductive animals was certainly the main motivating aspect for the implementation of the first QS in 2001 (RD 1083/2001) and this has been maintained through to the current QS (RD 4/2014). Both sectors, farmers and industrial entrepreneurs, were of the opinion that this requirement has given rise to a positive increase in the number of Iberian animals (MAPA, 2020), thus preventing the loss of purity of the Iberian breed against the cross with Duroc, as was the case before the application of the first Iberian QS (Espárrago et al., 2001; Vargas Giraldo, & Aparicio Tovar, 2001). On the other hand, the main reasons for the need to guarantee the breed purity of Duroc boars may be associated with characteristics of the Duroc breed itself, such as an improvement in the production parameters, prolificacy, higher energy efficiency, lean meat yield and growth ratio of the cross-bred animals, which are consistent with the scientific literature (Ramírez & Cava, 2007). In spite of this requirement being effective since the QS was published in 2001, it became more demanding with the application of a new inspection protocol in 2017 (MAPAMA, 2017). This gave rise to an increase in the demand of registered pure Duroc boars and a price increase (Vinagre, 2020).

With respect to the minimum weight gain at the finishing phase and minimum carcass weight, the fact that some responders, mainly farmers, considered not to establish a minimum weight gain during *Montanera* could be explained due to years of bad weather resulting in lack of natural resources (acorns and grass), and therefore inability to reach the minimum weight gain threshold required -46 kg- on only natural resources. On the other hand, the large proportion of respondents that indicated that the minimum age of slaughter was the main issue to solve, instead of the minimum weight gain during the finishing phase, could be attributed to financial terms. Thus, most of the animals currently being slaughtered in Spain are those yielding *White* and *Red* label products, being 80% and 14%, respectively, of the total slaughtered animals (Higuera, 2018), which are therefore Iberian x Duroc crossed animals. Since the QS establishes the age at slaughter for each category, regardless of the breed purity, the greater growth speed of Iberian × Duroc crossed animals (Ramírez & Cava, 2007) could lead to animals reaching the age at slaughter required by the QS with heavier weights to those the farmer deems optimum to render the most profit (Muñoz, 2001). On the other hand, industrial entrepreneurs are faced with the difficulty of processing hams from animals which are overweight, especially leg hams above 8 kg, which forces the increase of curing and stocking times, as well as the commercializing of products that are not in line with the current trends and habits of consumption (Parra, Viguera, Sánchez, Peinado, Espárrago, 2012).

Regarding to the requirements in terms of feeding at the finishing phase it should be highlighted that respondents think it is not well defined for free-range fodder-fed animals (*Green* label). This may be due to the lack of specific criteria to differentiate the degree of intensiveness in which the animals are reared (animals per hectare), as well as the percentage of feed the animal has eaten from natural resources in the *dehesa* and from fodder. This situation gives rise to a lack of an adequate distinction within the free-range fodder-fed category that is translatable into a differentiated type and therefore into the price of the derived products, thus acting in detriment of the profitability threshold of the extensive producer. In this sense, the scientific literature we find on the relevance of the production factors (Díaz-Caro et al., 2019) or the influence of the various QS Iberian packaged products (Ortiz et al., 2020) on consumer preference have omitted the *Green* label as a commercial category due to the lack of uniformity of the farming conditions and therefore the high degree of heterogeneity of the end product.

With respect to the requirements in terms of the minimum age at slaughter, the relative high portion of respondents who agreed with the adequacy of the slaughtering age established by the QS for *Montanera* animals could be explained by the greater presence of 100% Iberian animals in the *Montanera* production system (*Black* label), which, according to the existing scientific literature, grow and mature slowly and have low meat yield (Bonneau & Lebret, 2010), which indicates the requirement for them to have a longer production cycle in order to obtain quality products. In this line, there are studies which analyzed the traits of the carcass and the meat quality of pure Iberian pigs by age, concluding that the animals being introduced to the *Montanera* rearing system at a younger age might not be mature enough to make the most of it (Mayoral et al., 1999), and could led to poorer growth rate, as well as carcass quality and fat traits (Daza et al., 2007). As far as we are aware, there are no studies on the impact of the age at slaughter on cross-breed pigs reared under the *Montanera* system (*Red* label). The free-range fodder-fed animal (*Green* label), however, has a more complex context, due to the great variability of the production factors. On the one hand, extensive farmers state these animals are very similar to the pigs reared under the *Montanera* system and therefore, the age at slaughter is adequate. On the other hand, intensive farmers state that the age at slaughter should be reduced or maintained due to its similarities with the non-free-range fodder-fed animals (*White* label). Contrarily, most of the industrial entrepreneurs and farmers agreed with a reduction in the age of slaughter by two months for fodder-fed pigs (*White* label), maybe because animals reared under this category come from Iberian × Duroc crossed animals and therefore, they grow faster and yield more meat (Ramírez & Cava, 2007) in comparison to the pure Iberian breed, and they could reach the optimum weight for slaughter at a younger age.

Moving to certification processes and the product labeling, the high frequency of mentions reporting the too complicated certification process by farmers could be explained by overlap between the various institutions carrying out inspections and the certifying companies in the first place, and then the Autonomous Communities Authorities and Interprofessional Iberian Pig Association (ASICI, in its Spanish abbreviation), which leads to a general sense of irritation in the industry (Resano, Sanjuán, & Albisu, 2007). To overcome this situation, farmers and industrial entrepreneurs suggested that inspections could be conducted by independent experts unrelated to the farming industry, but with sufficient knowledge and skills to perform the job, since the main purpose of this is to prevent industry fraud.

On the other hand, the importance given by respondents to mention the animal's breed purity in product´s labeling may be due to the fact that participants understood that breed is a quality indicator for the consumer, in spite of the fact that various studies may not support the same position, demonstrating that consumer places much more importance on the type of feed than the breed (Hallenstvedt, Øverland, Rehnberg, Kjos, & Thomassen, 2012) and concluding, additionally, that consumers cannot distinguish, from the sensorial point of view, between dry-cured products coming from pure Iberian pigs or crossed with Duroc, when the feed is the same (Díaz-Caro et al., 2019).

In regard to the views on the freezing process of raw materials, there was no consensus about its impact on quality. In this line, no detriment in quality has been reported in Iberian pork meat after a year and a half frozen (Martín Mateos, 2013). On the other hand, freezing raw material from pigs under *Montanera* system could help to overcome the seasonality to which these products are subjected. However, this practice is not contemplated by the current QS (RD 4/2014) nor by the European standards dealing with freezing animal meats (European Regulation Nº 16/2012). As far as we are concerned, there are few research studies relating to the freezing of meats prior to their curing process and mostly carried out on ham from commercial pigs (Bañón, Cayuela, Granados, & Garrido, 1999; Flores, Soler, Aristoy, & Toldrá, 2006). Few studies deal with such topic in hams from Iberian pigs (Pérez-Palacios, Ruiz, Martín, Barat, & Antequera, 2011; Pérez-Palacios, Ruiz, Barat, Aristoy, & Antequera, 2010, Pérez-Palacios, Ruiz, Grau, Flores, & Antequera, 2009), and that analyze its effects on Iberian loins (Lorido et al., 2016), so further studies being required in order to assess the effects of freezing on the final product as well as consumer acceptance.

Lastly, where a consensus was observed was in the fact that freezing the product increases the costs. In the first place, because a frozen product translates into money that is not circulating and, in the second place, because the maintenance of the freezing process implies a relevant cost for any industry.

With respect to the views on the minimum obligatory maturity time, the general agreement about the manufacturing length follows the lines of the scientific literature. Research studies concluded that consumers prefer hams that have a long process to mature, as they positively associate this fact with an improvement in texture, flavor and aroma (Flores, Ingram, Bett, Toldrá, & Spanier, 1997). We can conclude that maturity time is a parameter that does not give rise to much dispute, as the nature itself of the production process defines minimum times that must be observed.

5. Conclusion

A qualitative research study involving the use of in-depth interviews allowed the stakeholders to identify key aspects for future potential modifications in the current Iberian QS. Our findings showed industrial entrepreneurs and farmers were of the same opinion in aspects of the QS that have a significant impact on the profitability, the production yield and the quality of the end product. There was general consensus in terms of the preservation of the Iberian breed for sows, the elimination of the minimum weigh gains in animals under the *Montanera* system as well as establishing an additional difference within the free-range fodder-fed category. Additionally, the participants shared the view that the age at slaughter established by the QS for fodder-fed animals (*White* category) is too high, which leads to a detriment in the commercial value of derivatives due to excessive weight.

On the other hand, this research study highlighted the dissatisfaction of the participants with the excessive bureaucracy required for the commercialization of products under the current QS. This aspect could potentially pose a risk for the industry, as farmers may be inclined to abandon their activities given the highly atomized environment with a lack of qualified personnel that characterizes the Iberian sector.

With regards to technological processing, the participants thought freezing was an adequate solution in order to manage the balance between production and demand, which was particularly relevant for the animals reared under the *Montanera* system. They believe that any future amendments to the QS should take the regulation of such practice into account.

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Chapter II. Performance and carcass quality traits of Iberian x Duroc crossbred pig subject to gender and age at the beginning of the free-range finishing phase

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Performance and carcass quality traits of Iberian x Duroc crossbred pig subject to gender and age at the beginning of the free-range finishing phase

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Abstract

The traditional production of the Iberian breed pig involves a long production cycle. It might be shortened by using Iberian x Duroc crossed bred pigs and by reducing the growing phase, but the age-related changes on productive performance and carcass quality should be addressed. Thus, productive performance, live measurements and carcass and primal cuts traits were evaluated on Iberian x Duroc 50:50 crossed bred pigs according to animal age at the beginning of the free-range finishing phase (*Montanera*): 10, 12 and 14 months old (IBxD10 (n = 15), IBxD12 (n = 17) and IBxD14 (n = 18) animal batches, respectively) and gender (immunologically-castrated (IC) female -consisted of the Improvac® vaccination- and surgically castrated (SC) males). During the growing period, animals were fed with restrictions; 1.49, 1.29 and 1.20 ± 0.023 (mean ± standard error of the mean; SEM) kg/day of commercial feeds to start *Montanera* with similar body weight (BW); 103.9, 102.9 and 102.1 ± 0.22 kg, for IBxD10, IBxD12 and IBxD14, respectively. IBxD14 animals yielded the highest average daily gain (ADG) and BW after *Montanera*, as well as larger rump height and croup width. In contrast, these animals had the lowest carcass yield. Although animals from IBxD10 yielded hams of inferior size, this could be of interest to the sector, as there is a certain segment of the market that demands hams of smaller size and, generally, this is difficult to obtain with the traditional *Montanera* production system.

The gender had no major effects on performance and carcass and primal cuts traits, so both IC female and SC males are suitable for finishing in *Montanera.*

Keywords: slaughter age, immunological castration, body measurements, carcass yield, primal cuts

Implications

A more efficient management and maximisation of natural resources of the production system of Iberian pigs (the most important European autochthonous breed pig in terms of population and economic importance) finished under free-range could be possible in reduced animal age at the beginning of free-range finishing phase and by using Iberian x Duroc crossed bred pigs, given they have better production parameters compared to purebred Iberian pigs. The results from this study suggest that animals that are initiated in free-range finishing phase at a younger age reveal worse productive performance, and primal cuts of inferior size, but higher carcass yield, without differences between immunologically castrated females or surgically castrated males.

1. Introduction

The Iberian is an autochthonous breed pig traditionally produced in the Southwest of Iberian Peninsula, but from which products are obtained recognised and appreciated by the European market (Pugliese & Sirtori, 2012). The traditional cycle for Iberian breed pigs involves a long production time, given the slow-growth and poor food efficiency of the Iberian breed (Bonneau & Lebret, 2010), which involves a free-range finishing phase taking place between the late-fall and early winter in *dehesas* -rangelands with evergreen oaks and pastures found in the Southwest of the Iberian Peninsula, (*Montanera*)-. During this phase, feed is based exclusively on *ad libitum* consumption of acorns mainly from *Quercus ilex* and grass. Subsequently, pigs are slaughtered at 150-160 kg body weight (BW) (Rodríguez-Estévez, García, Peña, & Gómez, 2009).

In order to take full advantage of the availability of acorns and grass, animals need to begin the free-range finishing phase at a weight range between 90 and 120 kg of BW, at which they are assumed to be at least 10-12 months old (López-Bote, 1998), thus guaranteeing adequate development and a sufficient level of maturity in their locomotive and digestive system. In fact, the current Spanish Iberian pig production regulation –the Iberian Quality

Standard (QS) (RD 4/2014)- does not allow pigs to commence the *Montanera* phase before they are 12 months old, with 14 months being the minimum age at slaughter. Additionally, pigs need to gain at least 46 kg while being fed exclusively on natural resources to be ingested during at least 2 months during the *Montanera* in order to ensure that they meet the requirements of the above-mentioned Iberian QS (RD 4/2014).

Iberian x Duroc crossed bred pigs (Iberian x Duroc) has been proven to have better production parameters such as growth rate, feed efficiency and leaner performance (López-Bote, 1998). Therefore, it would be possible for crossed pigs to be initiated in the *Montanera* phase at an earlier age, provided that their growth is adequate during the growing period mainly by the encouragement of feed intake. Additionally, these crossed animals should reach the minimum weight gain during the *Montanera* phase in order to meet the requirements of the current QS (RD 4/2014) as well as the common BW at slaughter in a shorter period of time than purebred Iberian pigs. This would result in a more efficient use of the natural resources with the consequential profitability for the farm. An efficient management and the maximisation of the resources becomes especially relevant in the *Montanera* production system, since the main constraint to further increasing the output of *Montanera* meat and meat products is the limited range of the breed's traditional habitat –*dehesa-,* whereas there is a demand for the products (Rodríguez-Estévez, Sánchez-Rodríguez, García, & Gómez-Castro, 2011).

Previous studies have evaluated the age-related changes in carcass traits (Mayoral, Dorado, Guillén, Robina, Vivo, et al., 1999) or the effect of animal age prior to the fattening phase on growth performance, productive parameters and meat quality traits in Iberian purebred pigs (Daza, López-Bote, Rey, & Olivares, 2006; Daza, López-Bote, Olivares, Menoyo, & Ruiz, 2007). Recently, the effects of animal age on subcutaneous back fat layers (Ortiz, García-Torres, González, Contador, & Tejerina, 2020a), meat and meat product quality traits (Ortiz, García-Torres, González, De Pedro-Sanz, Gaspar, et al., 2020b) of the Iberian x Duroc crossed bred pigs have been addressed, but to our knowledge there is no literature dealing with the effects of animal age at the beginning of the *Montanera* phase of Iberian x Duroc crossed bred pigs on growth performance, carcass quality traits and primal cuts, despite the fact that the meat and meat products derived from them are of high commercial value (MAPA, 2019).

On the other hand, given that during the *Montanera* phase, animals are reared outdoors for several months after puberty, castration of both males and females is required. In males, castration aims to avoid the boar taint in meat and meat products, whereas in females, the main reason is to prevent undesirable mounts by wild boars. Although castration is allowed in males, this is not the case in females (Council Regulation Nº 2008/120). So, a potential solution could involve immunological castration, consisting of a vaccination being delivered against the gonadotrophin-releasing factor (GnRF). The vaccination against GnRF may impact on feed intake (Poulsen, Van, & Mah, 2020) and growth performance (Sánchez-Esquiliche, Arce, García-Martínez, Sánchez-Rodríguez, & Rodríguez-Estévez, 2011). However, there is scarce literature dealing with the subject of vaccination against GnRF in free-range Iberian females (Martinez-Macipe, Rodríguez, Izquierdo, Gispert, Manteca, et al., 2016), and to our knowledge, its effect on productive performance, carcass traits and primal cuts yield Iberian x Duroc female have not yet been addressed.

Within this framework, this research study evaluates the effects of various ages of Iberian x Duroc crossed bred pigs at the beginning of the free-range finishing phase in the *Montanera* system (10, 12 and 14 months) when slaughtered with similar BW and the effects of gender (surgically castrated (SC) males and immunologically castrated (IC) females) on performance, body measurements and carcass quality after the *Montanera* phase.

2. Material and methods

2.1. Animals, experimental design and growth performance

A total of 50 Iberian *Retinto* (Valdesequera line, Junta de Extremadura, Badajoz, Spain) crossed with Duroc animals (50:50) were used. The animals were divided into three batches (IBxD14, IBxD12 and IBxD10) according to their age at the beginning of the free-range finishing phase in the *Montanera* system. The animal's date of birth for each batch was successive and spaced 2 months from each other, in order to start the *Montanera* phase simultaneously but with different ages for each batch of animals;14, 12 and 10 months old for IBxD14, IBxD12 and IBxD10, respectively. The gender distribution was as follows: 9 males and 6 females for the IBxD14 batch, 11 males and 6 females for the IBxD12 batch and 12 males and 6 females for the IBxD10 batch.

The males were surgically castrated in the first week of life following Spanish regulations (RD 1221/2009), whereas the females were immunologically castrated through vaccination against GnRF, consisting of the application of three subcutaneous doses of 2 mL of Improvac® (Zoetis) at the 20th, 24th and 40th week of life, together with a fourth (remainder) dose at approximately 120 kg of weight (three weeks after the beginning of the *Montanera* phase).

During the growing phase -from weaning to the beginning of the *Montanera* phase (with a BW of 102.1 kg, 102.9 kg and 103.9 kg ± 0.22 for batches IBxD14, IBxD12 and IBxD10, respectively)- the control of their consumption of feed was performed every three weeks by means of weight control with the purpose of fitting the weight increments to a previously-defined theoretical growth curve, so that all the animals could attain a similar weight at the beginning of the *Montanera* phase, regardless of their age (Figure II. 1). Thus, from weaning to 40 kg approx., (42.7 kg, 40.2 kg and 41.6 ± 0.86 kg for batches IBxD14, IBxD12 and IBxD10, respectively) feed was provided per batch on common feeders. From that weight on, the animals were provided their daily feed individually, by placing each pig in an individual pen, where they received the amount of feed according to their needs.

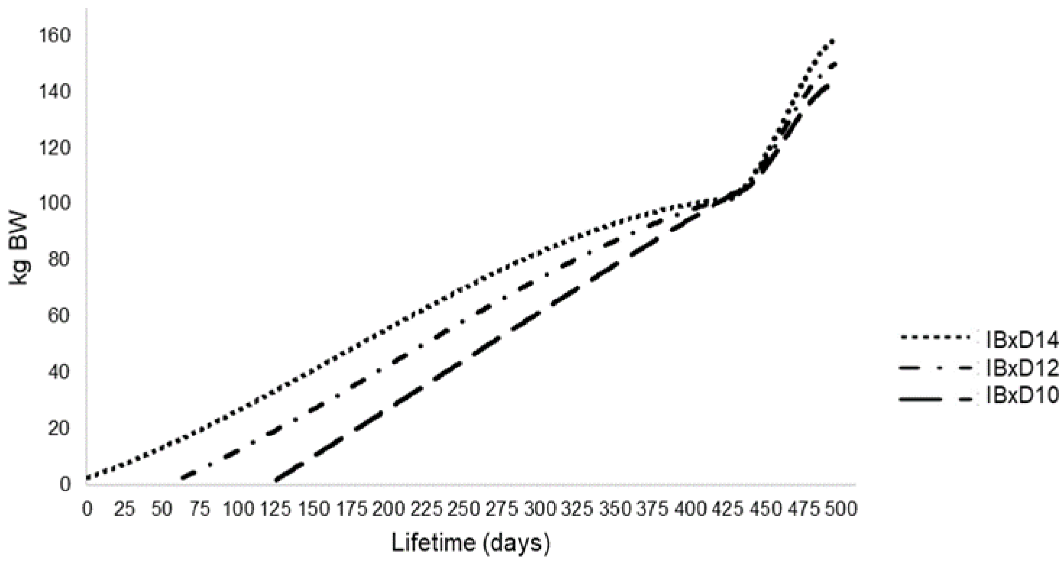

Figure II. 1. Growth curve during growing and fattening period of the Iberian x Duroc pigs. The average values of the weights of each batch were used in each weighing, representing the adjustment to a third-degree polynomial curve. IBxD14, IBxD12 and IBxD10: Iberian x Duroc crossed animal batches according to the age at the beginning of the fattening period under *Montanera*, with 14, 12 and 10 months old, respectively. BW, body weight.

The relevant weight controls were performed early in the morning, before the consumption of the day's feed, in order to calculate the ADG. The feed conversion ratio (FCR) was calculated by dividing the weight of the feed consumed by the ADG. The animals were housed in open-air pigsties according to animal batches. The nutritional characteristics of the feeds supplied (commercial feeds) according to BW are shown in Table II. 1, and the production performance during the growing period is showed in Table II. 2.

Table II. 1. Analytical composition of the feed used during the growing and the free-range fattening phase of the *Montanera* system.

	Grower diet					*Montanera* diet	
	From weaning to12 kg BW	12-25 kg BW	25-40 kg BW	40-70 kg BW	70-100 kg BW	Acorn (kernel) [1]	Grass [1]
DM	912.6	911.8	913.5	910.5	914.7		
Crude protein	197.2	190.4	182.4	176.6	164.2	64.5	395.6
Ether extract	60.3	54.0	30.0	22.3	43.1	72.4	4.97
Crude fibre	34.0	39.6	28.8	33.8	58.5	66.0	187.9
Lys (g/kg) [a]	14.6	13.4	12.3	11.9	10.3	-	-
Met (g/kg) [a]	5.7	4.9	3.9	3.4	2.8	-	-
ME [a]	15.04	14.68	15.16	14.39	14.00	-	-

Values were presented as mean values. DM, dry matter; Lys; lysine, Met; methionine; ME, Metabolisable Energy, MJ/kg. Proximate composition data: DM, crude protein, ether extract and crude fibre were expressed as g/kg DM. [a] calculated by FEDNA 2017 tables. [1] data of acorns and grass shown is the average for each parameter taken from 3 samplings (at the beginning, middle and end) during the *Montanera* season.

The pigs were reared during the finishing period of the *Montanera* phase in the Valdesequera farm, Junta de Extremadura, Badajoz, Spain. During the *Montanera* stage all the animal batches (IBxD14, IBxD12 and IBxD10) were grouped into a single batch in order to manage them collectively. The stocking rate was approximately 0.60 pigs per hectare. The length of the *Montanera* phase was between 63 and 71 days from November 2018 to January 2019. Feeding was based on *ad libitum* consumption of acorns mainly from the species *Quercus ilex* and grass (Table II. 1). Specifically, the animals were fed on these natural resources in plots, through the use of rotational grazing in order to ensure that their supply was continually renewed. Once a day, the oaks were shaken to help the acorn fall so acorns were fully available to all pigs. The shift to another plot took place when acorns were detected to begin to be scarce after visual inspection. The animals had free access to water. At the beginning and at the end of the *Montanera* phase, the animals were weighted.

Additionally, the weight was also taken twice during this time with an interval of three weeks, in order to calculate their ADG.

2.2. Animal measurements

At the beginning and at the end of the *Montanera* phase (the latter corresponding to the day before slaughtering), subcutaneous back fat thickness, *Longissimus thoracis et lumborum* (LTL) area, and length, height and width (at the level of the rump and croup, respectively) of the animals were measured on live animals.

Thus, for the subcutaneous back fat thickness and LTL muscle area, animals were ultrasonically scanned using a hand-held ultrasonic device (Aquila vet, Esaote Pie Medical, Genoa, Italy) equipped with an ultrasonic linear probe (3.5 Mhz and 18 cm long) with a silicone acoustic loin adapter, by placing the probe perpendicularly to the LTL muscle at the level of the last rib. The captured images were processed using AutoCAD® academic 2017 (AutoDESK®, AutoDESK, Inc. San Rafael, USA) software. Subsequently, a series of photographs were taken per animal, with a Sony video camera located 2.5-meter height from the animal. Specifically, three photographs were taken at three levels: caudal, central and cranial area of animals in order to avoid the effect of parallelism. A label was placed on the surface of the animal's skin for each photo as a reference for future measurements. Then, measurements were taken through image processing with AutoCAD® academic 2017, for which the image was scaled taking the reference of the label. Thus, animal width -width of pelvic limb at its widest point- was obtained from the caudal photograph, whereas the length was obtained by measuring from the atlanto-occipital insertion of the cranial photograph up to the insertion of the tail of the caudal photograph. A lateral photograph taken with a Canon EOS 550D camera was also captured by putting the camera at 3-meter distance from the animal and at a height of 0.5 meters from the ground, referenced in the rear area of the animal in order to obtain animal height -measured from the highest point of the rump to the ground-. Thus, using imageJ (U. S. National Institutes of Health, Bethesda, Maryland, USA) the height was measured, after scaling the image. For ultrasound measurement and image collection, pigs were restrained in a pen in order to restrict their movements and maintain a standing posture.

2.3. Slaughtering and carcass quality

At the end of the *Montanera* phase, the animals had reached their slaughtering age at 16, 14 and 12 months (496, 433 and 370 ± 7 days) for batches IBxD14, IBxD12 and IBxD10, respectively. They were slaughtered on three different days (with a difference of 7 days between the first and the last one) with the number of pigs ranging from 15 to 17 per slaughter batch. Each slaughter batch was balanced in age and gender, with the presence of animals from all batches (IBxD14, IBxD12 and IBxD10) as well as SC males and IC females.

The day before slaughter, after weighing the animals (early in the morning without previous fasting), they were transported (Council Regulation Nº 1/2005) to a local slaughterhouse (Mafrivisa, Castuera (Badajoz), 06420, Spain), located approximately 150 km from the Valdesequera farm, in a vehicle pen that exposed them to air flow during transport, but without food or water. The animals were offloaded after approximately a 2-hour journey. During lairage (less than 24 hours), the animals had access to water but not feed. Successively, animals were stunned using a carbon dioxide stunning system and then randomly exsanguinated. The slaughtering practices used in the slaughterhouse were performed in compliance with European Regulations (Council Regulation Nº 1099/2009) for the protection of animals during operations at the time of slaughter. After scalding, skinning, evisceration and splitting down the midline according to standard commercial procedures (20 minutes after slaughter) were carried out. Carcasses were weighted including perirenal fat and kidneys. Subsequently, 45 min *post mortem*, carcass length (from the rear edge of the pubic symphysis to the front edge of the first rib) and subcutaneous back fat thickness at the level of the last rib (including skin) were measured -with a tape measure- on the left carcass side.

After the quartering stage (4 h after slaughtering) following the Iberian pig standard methods, the ham length (from the front edge of the pubic symphysis to the hock joint), ham perimeter (widest diameter), and weight of the ham, shoulder and loin (from the left half of the carcass) were measured, with the latter being free of fat, which meant that primal cut yields were obtained.

2.4. Statistical analysis

The data were analysed using the statistical SPSS package (SPSS for Windows Ver. 19.0; SPSS Inc., Chicago, IL, 2004). The model included animal age at the beginning of the *Montanera* finishing period (14, 12, and 10 months of age for batches IBxD14, IBxD12 and IBxD10, respectively), gender (SC males and IC females) and age x gender interaction as the main factors for the two-way-ANOVA analysis of variance of the variables related to productive performance, lineal body measurements in live animal and carcass and primal cut quality traits. The carcass weight was considered as covariate for ham length, ham perimeter, ham weight, shoulder weight and LTL weight. Each animal was considered as one unit. The data were presented as mean values (calculated through least mean squares) ± standard error of the mean (SEM) for each group of age and sex. The statistical significance was assessed using Tukey's HSD test, and the level of signification was set at $P = 0.05$. The significant differences post-hoc test was used to compare groups.

The average weight for each batch was adjusted to a third-degree polynomial curve to represent the growth curve during both the growing and the finishing period.

3. Results

3.1. Influence of age and gender at the beginning of the free-range fattening phase on the productive performance of Iberian x Duroc crossed bred pigs

As far as productive performance during the growing phase is concerned (Table II. 2), the ADFI during the growing period yielded 1.20, 1.29 and 1.49 ± 0.023 kg /day for animals from batches IBxD14, IBxD12 and IBxD10, respectively. These values allowed all animals to attain a similar weight (102.1, 102.9 and 103.9 ± 0.22 kg) at the beginning of the free-range fattening phase (Figure II. 1). The significant differences between BW from batches IBxD10 and IBxD14 ($P \leq 0.01$), were negligible in practical terms, and they could be explained by the slight SEM identified in BW among animals.

Table II. 2. Productive performance of the pigs during the growth and the free-range fattening phases.

	Age (1)			Gender (2)		SEM	Sig.	
	IBxD14	IBxD12	IBxD10	IC females	SC males		1	2
Growing period: restricted feeding								
Initial weight (kg)	42.7	40.2	41.6	42.9	40.2	0.86	ns	ns
Final weight (kg)	102.1b	102.9Ab	103.9a	103	103	0.22	**	ns
ADFI (kg/day)	1.20b	1.29b	1.49a	1.3	1.3	0.023	***	ns
ADG (g/day)	214c	256b	321a	258	269	7.3	***	ns
FCR (kg/kg)	5.69a	5.09b	4.68c	5.2	5.1	0.071	***	ns
Finishing period: free-range feeding								
Final weight (kg)	158.8a	148.4b	142.5b	148.2	151.5	1.59	***	ns
ADG (g/day)	842a	673b	569c	675	714	23.4	***	ns
Weight gain (kg)	57.0a	45.4b	39.0c	45.1	49.0	1.60	***	ns

Values were presented as mean values. IBxD14, IBxD12 and IBxD10: Iberian x Duroc crossed animal batches according to the age at the beginning of the fattening period under *Montanera*, with 14, 12 and 10 months old, respectively. IC, immunologically-castrated female -consisted of the Improvac® vaccination; SC, surgically castrated males; ADFI, average daily feed intake (expressed as fed basis); AGD, average daily gain; FCR, feed conversion ratio; SEM, standard error of the mean; Sig, significance; a, b, c: different letters in the same row indicate significant differences due to animal age at the beginning of *Montanera* for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Regarding ADG, the lowest value (214 g/day) was obtained for animals from batch IBxD14, whereas the highest value was obtained from batch IBxD10 (321 g/day), with batch IBxD12 yielding an intermediate value: 256 g/day (SEM: 7.3) ($P \leq 0.001$). FCR during the growing period reached the following values: 5.69, 5.09 and 4.68 ± 0.071 kg/kg for batches IBxD14, IBxD12 and IBxD10, respectively, all differing from each other statistically ($P \leq 0.001$).

With reference to the productive performance at the free-range finishing phase under the *Montanera* system (Table II. 2), an increase in ADG was identified in association with an increase in animal age, with significant differences among the three batches ($P \leq 0.001$) and values of 842, 673 and 569 g/day ± 23.4 for batches IBxD14, IBxD12 and IBxD10, respectively. These results can be also graphically inferred in Figure II. 1 which shows how the growth curve of the animals from batch IBxD14 was more pronounced during the finishing phase than in the other batches. This is the opposite case in the growing period. As a result, the weight gain followed the same pattern identified for ADG, with animals from batch IBxD14 yielding the highest value ($P \leq$ 0.001). Thus, despite to the similarity in weight at the beginning of the *Montanera* phase, there were clear differences after that for BW deriving from animal age, with animals from batch IBxD14 yielding the highest value compared to those from both batches IBxD12 and IBxD10 ($P \leq 0.001$).

No differences were identified in the productive performance results during the growing or finishing phases that could be accounted for gender or age x gender interaction ($P > 0.05$).

3.2. Influence of age and gender at the beginning of the free-range fattening phase on the live measurements of Iberian x Duroc crossed bred pigs

Animals from batch IBxD14 began the free-range fattening phase with the lowest back fat thickness ($P \leq 0.001$) (Table II. 3). Nevertheless, these animals had the highest back fat thickness increment during this phase, which was higher than animals in batch IBxD10 ($P \leq 0.001$), but showed no differences against animals from batch IBxD12 ($P > 0.05$). In contrast, no differences on account of animal age were identified for the LTL area at the beginning or at the end of the finishing phase (Table II. 3). The values for the LTL area ranged from 2038 for batch IBxD10 to 2216 ± 35.27 mm^2 for batch IBxD12 after the *Montanera* phase (Table II. 3).

Table II. 3. Lineal live measurements of Iberian x Duroc crossed bred pigs at the beginning and at the end of the free-range fattening phase of the *Montanera* system.

	Age (1)			Gender (2)		SEM	Sig.	
	IBxD14	IBxD12	IBxD10	IC females	SC males		1	2
				Ultrasound measurements				
Back fat thickness (cm)								
Beginning	2.3b	2.7a	3.0a	2.7	2.7	0.06	***	ns
End	5.1a	5.1a	4.6b	4.9	5.0	0.07	**	ns
Increment	2.8a	2.4a	1.7b	2.3	2.3	0.09	***	ns
Loin area (mm^2)								
Beginning	1833	1963	1864	1938	1835	23.95	ns	*
End	2110	2216	2038	2159	2084	35.27	ns	ns
Increment	277	253	173	220	249	28.53	ns	ns
				Photographic measurements				
Rump height (cm)								
Beginning	77.2	76.5	75.5	76.5	76.3	0.32	ns	ns
End	84.9a	82.7a	80.4b	82.4	82.9	0.46	***	ns
Increment	6.9a	5.7ab	4.6b	5.2	6.2	0.37	*	ns
Body length (cm)								
Beginning	108	107.3	108.4	108.5	107.3	0.42	ns	ns
End	116	114.6	115.3	116.2	114.4	0.62	ns	ns
Increment	8.0	7.4	6.9	7.7	7.2	0.55	ns	ns
Croup width (cm)								
Beginning	25.3	26.0	26.1	25.6	26	0.15	ns	ns
End	30.5a	29.7ab	29.3a	29.8	29.9	0.18	*	ns
Increment	5.1a	3.7b	3.2b	4.2	3.9	0.21	**	ns

Values were presented as mean values. IBxD14, IBxD12 and IBxD10: Iberian x Duroc crossed animal batches according to the age at the beginning of the fattening period under *Montanera*, with 14, 12 and 10 months old, respectively. IC, immunologically-castrated female -consisted of the Improvac® vaccination; SC, surgically castrated males; SEM, standard error of the mean; Sig, significance; a, b: different letters in the same row indicate significant differences due to animal age at the beginning of *Montanera* for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

As far as live measurements are concerned (Table II. 3), animal age had a significant impact on rump height and croup width following the free-range fattening phase, as well as on the increments of both parameters during this period ($P \leq 0.05$), with animals from batch IBxD10 yielding the lowest values for both ($P \leq 0.05$). In contrast, no differences in body length on account on animal age were identified either at the beginning or after the free-range fattening phase ($P > 0.05$). On the other hand, no differences on lineal live measurements were identified accounting for gender or age x gender interaction ($P > 0.05$).

3.3. Influence of age and gender at the beginning of the free-range fattening phase on carcass quality traits of Iberian x Duroc crossed bred pigs

An increase in carcass weight was associated with an increase in animal age, with animals from batch IBxD14 yielding the highest value ($P \leq 0.001$). No differences were found between carcasses from batches IBxD12 and IBxD10 ($P > 0.05$) (Table II. 4). On the contrary, a progressive decrease in carcass yield was associated with an increase in animal age ($P \leq 0.05$), with animals from batch IBxD14 yielding the lowest value compared to those in batch IBxD10 ($P \leq 0.05$), whilst an intermediate carcass yield was seen in animals from batch IBxD12. No differences were reported for carcass length due to animal age at the beginning of the free-range fattening phase under the *Montanera* system. Neither gender nor age x gender interaction resulted in differences on carcass traits ($P > 0.05$). With the exception of ham length, for which higher values were observed with animal age, yielding the pieces from IBxD10 the lowest value ($P \leq 0.05$) (Table II. 4), the ham perimeter and weight of ham, shoulder and loin were not influenced by animal age at the beginning of the *Montanera* phase ($P > 0.05$) (Table II. 4). The ham, shoulder or loin yield did not vary either ($P > 0.05$). As far as the impact of gender on primal cuts is concerned, a significant effect on weight and yield of loin was identified, with IC females yielding higher values for both parameters compared with SC males ($P \leq 0.01$).

Table II. 4. Carcass traits and primal cuts from Iberian x Duroc crossed bred pigs.

	Age (1)			Gender (2)		SEM	Sig.	
	IBxD14	IBxD12	IBxD10	IC females	SC males		1	2
Carcass weight (kg)	129.4b	123.2a	120.6a	124	125	0.90	***	ns
Carcass yield (%)	81.5b	83.2ab	84.9a	84.0	82.4	0.48	*	ns
Carcass length (cm)	84.8	83.8	83.7	84.7	83.5	0.44	ns	ns
Back fat thickness (cm)	5.2a	4.9a	4.3b	4.7	4.9	0.11	**	ns
Ham length (cm) [a]	71.0a	69.6a	67.8b	69.5	69.4	0.31	**	ns
Ham perimeter (cm) [a]	74.4	72.9	72.3	73.8	72.6	0.36	ns	ns
Ham weight (kg) [a]	15.0	14.4	13.9	14.5	14.4	0.14	ns	ns
Shoulder weight (kg) [a]	9.3	9.1	8.6	9.0	9.0	0.09	ns	ns
LTL weight (kg) [a]	1.73	1.73	1.68	1.78	1.65	0.024	ns	**
Ham yield (%)	23.2	23.3	22.9	23.2	23.1	0.13	ns	ns
Shoulder yield (%)	16.8	16.2	15.4	16.2	16.1	0.32	ns	ns
LTL yield (%)	2.67	2.81	2.78	2.87	2.65	0.035	ns	**

Values were presented as mean values. IBxD14, IBxD12 and IBxD10: Iberian x Duroc crossed animal batches according to the age at the beginning of the fattening period under *Montanera*, with 14, 12 and 10 months old, respectively. IC, immunologically-castrated female -consisted of the Improvac® vaccination; SC, surgically castrated males. LTL = *Longissimus thoracis et lumborum* muscle. [a] The carcass weight was considered as covariate in the model; SEM, standard error of the mean; Sig, significance; a, b: different letters in the same row indicate significant differences due to animal age at the beginning of *Montanera* for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

4. Discussion

In terms of the productive performance data for the growing phase, our results showed that the lengthening of the growing period with feed restriction led to an increase in FCR due to the increase in feed used for animal maintenance. Despite the fact that the lowest FCR was attained by animals within batch IBxD10, which were those under the less strict feeding restrictions, the FCR values obtained from the current research study were in general slightly higher than those reported by Serrano, Valencia, Nieto, Lázaro, & Mateos, (2008) in Iberian crossed with various Duroc lines of pigs from 80 to 110 kg BW subjected to *ad libitum* access to feed and by Oliveira, González, Asmar, Batllori, Vera, et al. (2020) for Iberian x Duroc crossed bred pigs subjected to *ad libitum* or restricted feeding (10% restriction with respect to *ad libitum* feed) during the growing period (50 to 100 kg BW).

With regards to the free-range fattening phase of the *Montanera* system, the ADG values of animals from batch IBxD14 were in agreement with those reported by Rodríguez-Estévez et al. (2011) for purebred Iberian pigs that started the free-range fattening phase at an older age than 12 months. In contrast, the ADG values identified in batches IBxD12 and IBxD10 were lower than the values reported by the latter author for animals that started the free-range fattening phase younger than 12 months old. The increment in ADG value as animal age increased during the *Montanera* phase reported in the current study would agree with the results reported by Rodríguez-Estévez et al. (2011) and by Daza et al. (2006) in which greater ADG values were identified for purebred Iberian pigs that started the *Montanera* phase at over one year of age with respect to those that started younger, both of them with similar BW as the animals used in the current study. This could be explained by compensatory growth, which takes place as a consequence of restricted feeding practice (Daza, Mateos, Rey, & López-Bote, 2005a; Daza et al., 2007; Almeida, Bressan, Amaral, Bettencourt, Santos-Silva, et al., 2019). Thus, although feed intake during the *Montanera* phase was not measured in this study, it is likely that animals from batch IBxD14 ate more acorns and grass during the *Montanera* phase, as they were offered *ad libitum,* as a consequence of on the one hand, their increased age at the beginning of that stage (López-Bote, Rey, & Isabel, 2001), and, on the other hand, the compensatory feed intake as a result of feed restriction during growing phase (Font-i-Furnols, Luo, Brun, Lizardo, Esteve-García et al., 2020) which probably led to a higher ADG. Indeed, Font-i-Furnols et al., (2020) reported higher ADG and ADFI for Pietrain x (Large White x Landrace) gilts feed restricted during growing phase (from 30 to 70 kg) compared with those *ad libitum* fed during the finishing phase (from 70 to 120 kg of BW). The higher ADG of IBxD14 would explain their higher weight gain during the free-range fattening phase and BW compared to animals from IBxD12 and IBxD10. Additionally, a higher weight gain in animals from batch IBxD14 in the free-range finishing phase could also be a consequence of the increase in their digestive contents deriving from a greater filling of the digestive system, which would have been probably caused by the aforementioned higher intake of acorns and grass and the higher development of the large intestine (Rodríguez-Sánchez, Ripoll, & Latorre, 2010).

The lack of differences in the productive performance parameters results on account of gender suggests that early castration in males and vaccination against GNRF in females can lead to similar productive behaviours in both genders of Iberian x Duroc crossed bred animals. The results obtained in the current study are in line with the results obtained byPeinado, Serrano, Nieto, Sánchez, Medel, et al., (2011), in which no differences were identified in ADG, ADFI or FCR between Landrace x large whites x Duroc male and female castrated pigs of approximately 28 to 130 kg in BW, although female castration was carried out by ovariectomy. At a later stage, Martinez-Macipe et al. (2016) reported there was no difference in BW between SC males and IC females, although this referred to Iberian purebred pigs reared in the *Montanera* system. Nevertheless, the latter study did not report results from other productive parameters such as ADG, weight gain during the free-range finishing phase or ADFI during the previous growing phase.

The finding that there is a greater increase of back fat thickness as animals grow older is in agreement with previous studies carried out in Iberian purebred pigs using a broader age range and a longer free-range fattening phase in the *Montanera* system, e.g. 8 to 14 months old animals and 117 days, respectively, in the study carried out by Daza et al. (2007), and 12 to 18 months old and 107 days of *Montanera* length for the study carried out by Rodríguez-Sánchez et al., (2010). The increase in back fat thickness in older animals might be attributed to their probably higher fed consumption as a result of their advanced age and compensatory feed intake (Font-i-Furnols, et al., 2020), as their higher ADG proves.

With respect to live measurements, in general, rump height and body length yielded slightly higher values than those reported by Almeida et al. (2019) for purebred Iberian pigs in the free-range fattening phase of the *Montanera* system, that were slaughtered at a similar BW as the animals used in the current study, whilst croup width was slightly lower. The higher values of rump height and croup width after the free-range fattening phase for batch IBxD14 could support the idea of a higher growth rate associated with animal age during the *Montanera* phase, given that the animals from the three batches started the free-range finishing phase ($P > 0.05$) at similar values. On the other hand, the lack of difference in lineal live measurements due to gender would derive from the similarity in the productive performance pattern between the IC females and SC males above described.

The mean values obtained in the present study for carcass traits (weight, yield and length) were in line with those previously reported for Iberian purebred pigs fattened under the *Montanera* system (Daza, Mateos, Rey, & López-Bote 2005b; Daza et al., 2007). As far as the effect that animal age at the beginning of the *Montanera* phase had on these, the increase of carcass weight as animals are older was not unexpected, given the productive performance data and live body measurements previously registered. The opposite trend in carcass yield could be attributed to the following aspects: in the first place, the age of the animals and therefore, their stage of maturity while they are at the fattening stage of the *Montanera* system could have led to a different ratio of distribution of the energy they produce, which goes to growth of muscle tissue -lean- in younger animals, to growth of adipose tissue, more common in older animals. Back fat thickness yielded higher values in older animals rather than younger. In this line, Daza et al. (2007) also found higher total back fat thickness in animals that started the finishing period of the *Montanera* system at a more advanced age. Secondly, the potential higher development of the large intestine as a consequence of the likely higher intake of crude fibre from grass by animals from batch IBxD14 (Hawe, Walker, & Moss, 1992) may have contributed to a lower carcass yield. The results referring to carcass quality traits found in the current study are in agreement with those reported by Daza et al. (2006) when comparing animals that started the finishing period under the *Montanera* system at 12 months old compared to those starting at 8 months old for Iberian purebred pigs with a longer *Montanera* period of time than the period under consideration in the current research. On the other hand, these authors also reported differences in carcass length on account on animal age, whereas this was not the case in the current study. The lack of difference in carcass length among animal batches (IBxD10, IBxD12 and IBxD14) was in correlation with the lack of difference in body length among those previously observed. The lack of difference on account of gender in carcass traits was in agreement with the the findings of Martinez-Macipe et al. (2016), who had previously described the lack of difference in carcass weight and length between GnRF-vaccinated female and SC male Iberian purebred pigs reared in *Montanera*.

On the matter of primal cuts quality traits, despite the higher values observed for length, perimeter and weight of ham, and weight of shoulder from oldest animals in comparison with those from youngest ones, these differences only became significant for ham length. This could be explained by the influence of carcass weight on the conformation and weight of primal cuts, which is corroborated by the lack of differences in the yields of ham, shoulder and loin. In contrast, higher perimeter in hams and higher weight in hams and shoulders were found in Iberian pigs that started the *Montanera* phase at 12 months old compared to those that started at 8 months old by Daza et al. (2006). Later, Daza et al. (2007) also obtained the same pattern for ham and shoulder of older animals (between 8 and 14 months old) at the beginning of the free-range fattening phase in the *Montanera* system; however, in both studies, animals were Iberian purebred pigs and the length of time they remained in *Montanera* was higher than the length in this research study (117 days). Discrepancies between the results might also derive from the non-consideration of carcass weight or body weight as a covariate to study the effect of age on the conformation and weight of primal cuts in previous studies, since in terms of primal cuts yield, a lack of differences deriving from animal age was observed (Daza et al. 2006). The lack of difference in loin weight on account of animal age was in disagreement with Daza et al., (2006), who reported an increase when the animals were older; whilst was in line with Daza et al., (2007).

With respect to how primal cuts were affected by gender, the pattern obtained followed the same trend reported by Martinez-Macipe et al. (2016) with a higher loin area and yield in IC Iberian purebred females compared to SC males. Nevertheless, in the latter study, these differences did not become statistically significant. Unfortunately, to our knowledge, there is no scientific literature dealing with the effects of vaccination of Iberian x Duroc females against GnRF on their productive performance, carcass traits and primal cuts yield to enable a comparison of results.

5. Conclusion

Our results suggest that Iberian x Duroc crossed bred animals that start the free-range finishing phase at an older age (14 months old) would have yielded lower back fat thickness at the beginning of the *Montanera* phase. During the fattening phase, the production performance indicators were better as the age of the animals was higher, but also higher increments in back fat thickness were observed with age. No differences in body length during both the growing and the finishing period were found in association with animal age.

Animals that started the *Montanera* phase at 10 months old had the highest carcass yield, but their ham were inferior in length compared with those of older animals. Rearing immunologically-castrated females or surgically-castrated males proved to yield no differences in productive performance, body measurements and carcass traits with the exception of the higher loin yield of the former.

Ethics approval

The consideration of ethical and welfare aspects by the Animal Care & Ethics Committee (ACEC) was not required for the development of the current study, because animals were subjected to standard production practices during both the growing and the finishing stages, in compliance with the Council Regulation Nº 2008/120 regarding the minimum standards for the protection of pigs, which was transposed into national legislation for the care and handling of animals by RD 1392/2012, and to pigs reared on extensive systems by RD 1221/2009. No additional measures were required.

Data and model availability statement

None of the data were deposited in an official repository. The data that support the study findings are available to reviewers.

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Declaration of interest

The authors declare no conflicts of interest.

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Chapter III. Quality traits of fresh and dry-cured loin from Iberian x Duroc crossbred pig in the *Montanera* system according to slaughtering age

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Quality traits of fresh and dry-cured loin from Iberian x Duroc crossbred pig in the *Montanera* system according to slaughtering age

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Abstract

Iberian x Duroc crossed bred grower pigs of similar weight, but 12, 10 and 8 months of age, were finished using the *Montanera* system to study effects on fresh and dry-cured loin quality. Pigs were slaughtered simultaneously at 16, 14 and 12 months of age (IBxD16, IBxD14 and IBxD12) and *Longissimus thoracis et lumborum* were removed and processed into dry-cured loin. Fresh and dry cured loins from the oldest animals (IBxD16) had higher tocopherols, C18:2 n-6 and polyunsaturated fatty acids as well as higher insoluble collagen, which led to greater hardness. Fresh loin from the youngest animals (IBxD12) had the lowest myoglobin content, redness (a*), chroma and hue angle. No differences in proximate composition or instrumental colour were found in dry-cured loins.

Keywords: *Longissimus thoracis et lumborum*, Iberian x Duroc crossbred, slaughter age, meat quality, dry-cured loin

1. Introduction

The Iberian pig is an autochthonous breed that is traditionally produced in the Southwest of the Iberian Peninsula. The economic importance of the Iberian pig production is based on the quality of both its dry-cured and its fresh meat products (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012a), which are appreciated not only by Spanish consumers but also in the European market (Pugliese & Sirtori, 2012). The quality of these products results from a combination of factors that are associated with their genetic background, the production system used (Daza, Rey, Ruiz, & López-Bote, 2005) and the feeding provided –especially during the last fattening phase– (Rey, Daza, López-Carrasco, & López-Bote, 2006).

The most widely-renowned production system is the free-range breeding system from November to January, with a diet based on acorns and grass, and referred to as *Montanera* (Tejerina et al., 2012a). Products from *Montanera* Iberian pigs achieve the highest sensory quality, being the most-highly appreciated by consumers (Díaz-Caro, García-Torres, Elghannam, Tejerina, Mesías et al., 2019). Quality is guaranteed by compliance with the Spanish Iberian Quality Standard (QS) (RD 4/2014), which defines the production factors and conditions necessary (age and slaughtering weight, breed percentage, feeding, or length of last fattening stage, among others) to guarantee the various agreed upon quality standards are met.

Traditionally, the Iberian breed has been described as late-maturing, slow-growing and of poor feed efficiency (Bonneau & Lebret, 2010). In this sense, Daza, López-Bote, Olivares, Menoyo, & Ruiz (2007) argued that an older age should be aimed for before introduction of the animal to the *Montanera* system. Nevertheless, the increase in slaughter age of animals, and consequently length of productive cycle imply an increase in the farmers' production costs (Daza, 2001). Additionally, proof has been shown that the growth rate of Iberian x Duroc crossed bred pigs is higher than Iberian purebred pigs. Thus, the Duroc breed is more widely used in order to reduce the costs, based on its higher growth rate, meat yield (Ramírez & Cava, 2007a) and final meat quality properties (Bonneau & Lebret, 2010).

Currently, the minimum age at slaughter of Iberian pigs under *Montanera* system is established at 14 months, regardless of whether they are purebred or crossbreed with

Duroc animals. There are only a few studies that have analysed the recommended slaughter age as dependant on the genetic background. Some authors have described its influence on the pure Iberian breed (Daza et al., 2007; Mayoral, Dorado, Guillén, Robina, Vivo, et al., 1999), pure Duroc breed (Bosch, Tor, Reixach, & Estany, 2012), as well as on the crossing of Duroc with rustic breeds, such as the Celtic (Lorenzo, Fernández, Iglesias, Carril, Rodríguez et al., 2014) and other commercial crossbreeds (Candek-Potokar, Ilender, Lefaucheur, & Bonneauc, 1998). Nevertheless, to our knowledge, literature on the effects of slaughter age on the meat quality of Iberian x Duroc crossed bred pigs, which integrates the demands and interests of farmers, manufacturers and consumers, does not exist.

In addition, most of studies on meat quality of Iberian pigs have focused on ham, the most valued dry-cured product. However, the loin (m. *Longissimus thoracis et lumborum*) is a different product, which has high consumer acceptance (Cava, Tárrega, Ramírez, & Carrasco, 2009). In fact, according to MAPA, (2019) commercialisation of dry-cured loins represented over 30% of commercialised Iberian products under the current QS (RD 4/2014), only second to Iberian dry-cured shoulder. Although there are studies dealing with the influence of production system (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012b), feeding regime (Daza, Rey, et al., 2005) or genetic background (Ramírez & Cava, 2007a) on the quality traits of loins, as far as we know, there are no studies on the effects of age at slaughter on loins from Iberian x Duroc crossed bred pigs.

Thus, the purpose of this paper was to study the influence of age at slaughter (16, 14 and 12 months old) with similar live weight at the beginning of the final fattening phase, *Montanera*, on the quality traits of fresh and dry-cured loin from 50:50 Iberian x Duroc crossed bred pigs.

2. Materials and Methods

2.1. Animal rearing and batches

A total of 49 (male and female) Iberian *Retinto* breed (Valdesequera line, Junta de Extremadura, Badajoz, Spain) × Duroc bred pigs up to 50% according to the current QS (RD 4/2014) were used. Three batches (IBxD16, IBxD14 and IBxD12) of 15, 17 and 17 grower pigs with equal weights at 12, 10 and 8 months (± 10 days) were produced and finished using the *Montanera* system for simultaneous slaughter at 16, 14 and 12 months age, respectively. The gender distribution was as follows; 10 males and 5 females for the IBxD16, and 11 males and 6 females for the IBxD14 and IBxD12. The males were surgically castrated following Spanish regulations (RD 1221/2009) and the females were inmunocastrated through vaccination against GnRF consisted on the application of three doses of 2 mL of IMPROVAC® (Zoetis) subcutaneously.

The rearing period (from weaning to slaughtering) was divided into two phases: growing and finishing (*Montanera*) phases (Figure III. 1).

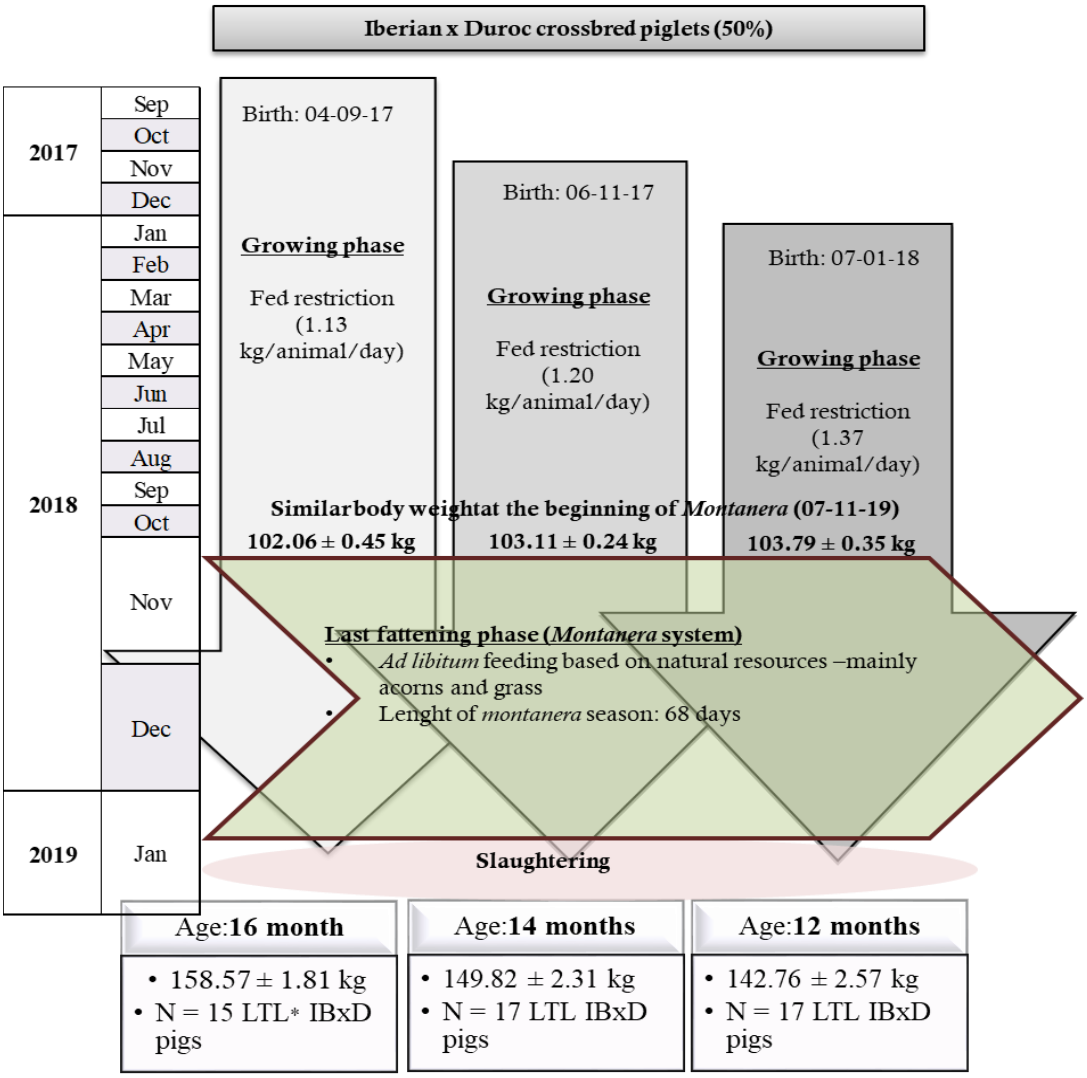

Figure III. 1. Experimental design of animal rearing and batches. Growing phase; from weaning (September 2017–January 2018) to start of *Montanera* (7th of November). Slaughtering (14th January) of batches IBxD16, IBxD14 and IBxD12 with 16, 14 and 12 months and 158.6 ± 1.8, 149.8 ± 2.3 and 142.8 ± 2.6 kg of body weight, respectively.

During the grower phase -from weaning to start of *Montanera*- each batch followed a feed restriction program with commercial feeds according to their needs, as they had different average daily gains (ADG), resulting in weights of 102.1 ± 0.5, 103.1 ± 0.2, 103.8 ± 0.4 kg for the IBxD16, IBxD14 and IBxD12, respectively. A theoretical growth curve was established for each batch, which was verified every 3 weeks, and diets were adjusted accordingly. The ADG during growing phase were 228.2 ± 1.5, 244.4 ± 2.1 and 314.8 ± 1.5 g/day for the IBxD16, IBxD14 and IBxD12, batches, respectively. The composition of the last commercial fed from 70 to 103 kg live weight is showed in Table III. 1, and would have had the largest influence on the quality of the final product.

During the fattening phase under the *Montanera* system (7th of November to 14th of January at the Valdesequera farm, Junta de Extremadura, Badajoz, Spain), pigs had *ad libitum* access to acorn and grass (Table III. 1) for 68 days in a single pasture. Each pig had access to approximately 2.5 ha, and the average temperature was 8ºC. When animals reached the established slaughtering ages (IBxD16 = 16, IBxD14 = 14 and IBxD12 = 12 months old), they achieved average body weight of 158.6 ± 1.8, 149.8 ± 2.3 and 142.8 ± 2.6 kg (Figure III. 1) and an ADG of 836.8 ± 26.3, 690.9 ± 31.2, 575.1 ± 34.8 g/day, respectively. The experimental procedures animals were subjected to during the rearing and fattening phases were in compliance with the Council regulation 2008/120, regarding to minimum standards for the protection of pigs, transposed into national legislation for the care and handling animals by RD 1392/2012, and to pigs reared extensively (RD 1221/2009), but exempted from consideration for ethical and welfare aspects by Animal Care & Ethics Committee (ACEC), since they were considered standard husbandry practices.

Table III. 1. Proximate composition, antioxidant and fatty acid profile of concentrate and *Montanera* feed.

	Grower diet	*Montanera* diet	
	Concentrate feed	Acorn (kernel) [1]	Grass [1]
	Proximate composition (g/kg DM)		
DM	915 ± 0.1	565 ± 0.5	138 ± 0.1
Crude protein	164 ± 0.2	64.5 ± 0.43	395 ± 1.4
Crude fat	43.1 ± 0.10	72.4 ± 0.21	4.97 ± 0.03
Crude fibre	58.5 ± 0.07	66.0 ± 0.15	187.90 ± 0.92
Ash	52.7 ± 0.03	20.5 ± 0.11	126.3 ± 0.50
NFE	680 ± 0.2	777 ± 1.9	286 ± 1.6
	Antioxidant Composition (mg/kg DM)		
α-Tocopherol	16.1 ± 0.23	35.8 ± 0.32	36.1 ± 0.24
γ-Tocopherol	tr	87.2 ± 0.40	2.80 ± 0.02
	Fatty acids composition (g/100 g FAMEs)		
C16:0	21.3 ± 0.24	16.1 ± 0.97	15.8 ± 0.34
C16:1	1.91 ± 0.02	0.62 ± 0.01	1.53 ± 0.01
C18:0	7.41 ± 0.13	3.84 ± 0.01	4.11 ± 0.03
C18:1 n-9	34.5 ± 0.04	58.3 ± 0.54	4.62 ± 0.29
C18:2 n-6	32.4 ± 0.35	20.5 ± 0.36	11.9 ± 0.29
C18:3 n-3	2.51 ± 0.02	0.74 ± 0.01	62.1 ± 0.20

Values were presented as mean values ± standard error. DM, dry matter; tr, traces; NFE, nitrogen-free extract; FAME, fatty acids methyl esters C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid, C18:3 n-3, linolenic acid. [1] data of acorns and grass shown is the average for each parameter taken from 3 samplings (at the beginning, middle and end) during the *Montanera* season.

After finishing, animals were transported to a commercial slaughterhouse located 160 km from the farm in a single trip, thereby reducing the length of transport between the farm and slaughterhouse. The animals were not fed or watered during transport and exposed to air flow during the transport. The animal transport was in compliance with the Council Regulation Nº 1/2005. Animals were unloaded after the 2 h journey, provided water but no feed, and spent less than 24 h in lairage. Subsequently, animals were randomly slaughtered by exsanguination after stunning using carbon dioxide. The slaughtering practices used in the slaughterhouse were performed in compliance with the European Rules (Council Regulation 1099/2009) for the protection of animals during operations at the time of slaughter.

2.2. Meat sampling and curing

2.2.1. *Longissimus thoracis et lumborum* (LTL)

Carcasses were quartered 4 h after slaughter according to standard commercial procedures. Subsequently, LTL muscles were removed from the left half of the carcasses and refrigerated to 4ºC. After 24 h, the first 20 cm of sample belonged to *Longissimus lumborum* muscle (LL) was taken for raw meat analytical determinations (fresh loin), (n = 15 from IBxD16 (LL16), n = 17 from IBxD14 (LL14) and n = 17 from IBxD12 (LL12)) and the remaining sample (belonging to the *Longissimus thoracis* muscle (LT)) was immediately subjected to dry-curing.

2.2.2. Dry-curing

The curing process was carried out according to common practice used in the meat processing industry, but adapted to the weight of the loins in this study. The conditions were the same for the three sets of loins manufactured (n = 10 from IBxD16 (DCL16), n = 10 from IBxD14 (DCL14) and n = 10 from IBxD12 (DCL12)). Loins were seasoned in a mixing bowl with nitrified salt (3%) and nitrites (0.8% of the total salt content), percentages refer to the total meat content. Garlic, paprika and olive oil, which are commonly used ingredients in the seasoning of dry-cured loins, were not used in order to avoid interferences with oxidative reactions. The loins were kept at 4ºC t for 48 h –in darkness– to allow the seasoning mixture to penetrate. Subsequently, the loins were stuffed into 10 cm-diameter collagen casings and kept at 4ºC in 75-80% relative humidity for 30 days. Finally, the loins were ripened at 15-18ºC and in 65-70% relative humidity up to 70 days, according to the current QS (RD 4/2014). Once the technological process was completed, the dry-cured loins were vacuum-packed and refrigerated until quality analyses were performed (less than one week).

2.3. Fresh and dry-cured loin's quality measurements

2.3.1. Proximate analysis

Dry matter (DM) was tested following the AOAC method (AOAC, 2003). The results were expressed in g/100 g of fresh (Table III. 2) or dry-cured loin (Table III. 5) as mean values. Intramuscular fat (IMF) was analysed according to Folch, Lees, & Sloane-Stanley (1957). The results were expressed as g/100 g moisture-free tissue of fresh (Table III. 2) or dry-cured loin (Table III. 5) as mean values. In fresh loins, total collagen content was determined by quantifying hydroxyproline content following the method described by Hill, (1966). Soluble collagen was determined following the same method and insoluble collagen content was determined by

calculating the difference between the previous two. Results were expressed as g/100 g moisture-free tissue of fresh loin (Table III. 2) as mean values.
Chloride content (NaCl) was tested in dry-cured loins using the Volhard method (AOAC, 2000). NaCl values were expressed as g/100 g moisture-free tissue of dry-cured loin (Table III. 5) as mean values.

2.3.2. Water holding capacity (WHC) and cooking loss (CL) (only fresh loin)

WHC was determined as a percentage of the free water released after the application of a centrifugal force, following the method proposed by Irie & Swatland (1992) expressed as g of water released /100 g of fresh loin (Table III. 2).
For the determination of CL, samples from fresh loin were vacuum-packed in nylon/polyethylene bags and cooked by immersion at 80ºC for 45 min in a water bath until an internal temperature of 75ºC was reached (Combes, Lepetit, Darche, & Lebas, 2003). The difference in weight before and after cooking was used to calculate CL. The results are presented as g of water/100 g of fresh loin (Table III. 2).

2.3.3. Instrumental colour

For both, fresh (Table III. 2) and dry-cured loins (Table III. 7), the following colour coordinates were determined: lightness (L*), redness (a*, red ± green) and yellowness (b*, yellow ± blue) using a Minolta CR-400 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0º standard observer and a 2.5 cm port/viewing area. The colorimeter was standardised before use with a white tile. Additionally, the saturation index or chroma (C*), defined as ($C = (a^{*2} + b^{*2})*0.5$) and hue angle (Hº) as arctangent (b*/a*) were determined. The measurements were repeated at five randomly selected sites on each sample and averaged.

2.3.4. Myoglobin content (only fresh loin)

The chemical determination of the meat myoglobin was performed according to the method proposed by Hornsey, (1956) by which the heme pigment content (myoglobin) was calculated using the following formula;
Mg of myoglobin/g = OD*(12.5 ml*652*1 kg) *0.026/($9.52*10^{3}$*2.5 g*10^{-3} g)
Where: OD = Optical density (absorbance of the myoglobin molecule at 512 nm).

2.3.5. Determination of α and γ-tocopherol

Samples were homogenised using an IKA homogeniser. α- and γ-tocopherol contents in fresh and dry-cured loins were measured using the method proposed by Liu, Scheller, & Tornberg (1996) with some modifications proposed by Cayuela, Garrido, Sancho Bañón, & Ros (2003). For the purpose of extracting the tocopherols, 250 mg of ascorbic acid, 7.5 mL of saponifying solution (KOH 11.5% in EtOH/H_2O 55:45) and 4 mL of 0.01% BHA in isooctane were added to 1 g of sample. Samples were heated at 80°C for 15 minutes. Once cooled, samples were centrifuged at 1500 rpm for 5 minutes and the upper layer was collected for HPLC analysis. Tocopherol determination was performed on an Agilent Technologies HPLC Series 1100 instrument (Agilent Technologies, Santa Clara, CA, USA), equipped with a Kromasil Silica column (5 µm particle size, 150 x 4.6 cm) (Symta, Madrid, Spain) and a Kromasil Silica Guard Column (10 µm) (Symta, Madrid, Spain). The mobile phase was hexane:isopropanol:etanol (98.5:1:0.5 v/v), at a flow rate of 1 mL/min and the fluorescence detector (Agilent Technologies Series 1200) was fixed at λ-excitation: 295 nm and λ-emission: 330 nmn. Identification and quantification of the peaks were performed by comparison with α-tocopherol and γ-tocopherol standards (0.2-14 µg/mL). The results were expressed as µg of α- or γ-tocopherol/g fresh (Table III. 3) or dry-cured (Table III. 6) loin, as mean values.

2.3.6. Determination of the fatty acid profile

For the determination of the fatty acid profile, the intramuscular fat extracted according to Folch et al., (1957), was dissolved in 2 ml of hexane. From this, 210 µl were taken and mixed with 4 ml of hexane and 200 µl of KOH (85% in MetOH). It was vortexed and after centrifugation (10 minutes at 3000 rpm) the organic phase was collected in vials. One microlitre was injected into a gas chromatograph (model 4890 Series II; Hewlett-Packard, Palo Alto, CA, USA) equipped with a split/split-less injector and a flame ionisation detector. Fatty acid methyl esters (FAMEs) were separated on a Carbowax™ fused silica capillary column (30m×0.25 mm id; 0.25 µm film thickness; (Ohio Valley, Marietta, OH, USA). The oven temperature was held at 200°C. The injector and detector were set at 250°C. The carrier gas was nitrogen at 1.8 mL min^{-1}. The identification of individual FAME was based on a standard mixture of 37 Component FAME Mix (Sigma–Aldrich, Supelco 37 Component

FAME Mix- CRM47885, St. Louis, MO, USA). The amount of each fatty acid and of the different fatty acid groups were calculated on the total of fatty acids detected, and expressed as g/100 g of FAMEs in both fresh (Table III. 3) and dry-cured loins (Table III. 6), as mean values.

2.3.7. Lipid and protein oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid (TBA) method of Salih, Smith, Price, & Dawson (1987). TBA-RS values were calculated from the standard (1,1,1,3- tetraethoxypropane, TEP) curve and expressed as mg malondialdehyde (MDA)/kg DM in both fresh (Table III. 3) or dry-cured loin (Table III. 6) as mean values.
Protein oxidation was estimated by measuring the content of free thiol groups, following the method proposed by Batifoulier, Mercier, Gatellier, & Renerre (2002). The concentration of free thiols was measured spectrophotometrically at 412 nm and was calculated using an absorption coefficient of 13.6 $mM^{-1}cm^{-1}$. Protein concentration was determined by spectrophotometry at 280 nm using bovine serum albumin (BSA) as standard. The results were expressed as nmol of free thiols per mg of protein in both fresh (Table III. 3) and dry-cured loin (Table III. 6).

2.3.8. Weight loss (WL) of dry-cured loins

For the purpose of establishing the weight loss WL during the curing process, the loins were weighed at the beginning and end of the curing process (70 days), and losses were calculated using the following equation:

% weight loss = $((W_0 - W_f)/W_0) * 100$, were W_0 is the weigh at the beginning of the process and W_f the weigh at the end of the process. The results are presented as g of water/100 g of dry-cured loin (Table III. 5) as mean values.

2.3.9. Texture analysis: Texture Profile Analysis (TPA) and Warner-Bratzler shear force test (WBSF)

- **Fresh loins tests** (Table III. 4): Fresh loin tests were performed on cooked meat (Combes et al., 2003). The texture profile analysis (TPA) was determined at two different deformation percentages: (i) TPA I (20%

deformation) (ii) TPA II (80% deformation) in order to determine the contribution of myofibrillar structures with and without the intervention of the connective tissue on textural properties (Lepetit & Culioli, 1994). The texture analysis was performed using a TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, U.K.). For the determination of TPA, uniform portions of muscle were cut into 1 cm^3 cubes. Samples were axially compressed to 20% (TPA I) and 80% (TPA II) of the original height with a 20 mm diameter (P/20) flat plunger using a 25-kN load cell applied at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from force–deformation curves (Bourne, 1978): hardness (N/cm^2), springiness (cm), cohesiveness (dimensionless), gumminess (N cm s^2), chewiness (N cm s^2) and resilience (dimensionless), as mean values. For the purpose of performing a Warner–Bratzler test, samples were prepared as 10 x 30 x 10 mm^3 slices (width x length x thickness). Samples were cut with a Warner–Bratzler blade (HDP/BS) in perpendicular direction to the muscle fibres. The maximum shear force (N/cm^2) was measured to cut samples. Instrumental determinations were repeated 8 times per sample and results were averaged.

- **Dry-cured loin tests** (Table III. 7): A TPA with 50% deformation (Ramírez & Cava, 2007b) was performed following the above-described method. The WBSF test was similar to that performed on fresh loins.

2.4. Statistical analysis

Statistical analysis was performed using SPSS version 20.0. Proximate composition, instrumental colour, antioxidant and fatty acid profile and texture analysis were the variables subjected to one-way-ANOVA for both, fresh (*Longissimus lumborum*) and dry-cured loin (*Longissimus thoracis*) separately, with age at slaughter (with three groups for both -fresh and dry-cured loins- from IBxD16, IBxD14 and IBxD12 animal batches) as the fixed factor. In addition, water losses and myoglobin were assessed for fresh loin, whereas WL was evaluated only for dry-cured loins. Each loin (fresh or dry-cured) was considered as the experimental unit. Data are presented as mean values ± standard error for each age group. Statistical significance was assessed by Tukey's HSD test, and the level of signification was set at $P = 0.05$. The least significant differences post-hoc test was used to compare groups.

3. Results and discussion

3.1. Effect of age at slaughter on the quality traits of fresh loin

3.1.1. Proximate composition and water losses

The influence of slaughter age on proximate composition, water losses and instrumental colour of fresh loin (LL) are shown in Table III. 2.

Table III. 2. Effect of age at slaughter on proximate composition, water losses, instrumental colour and myoglobin content of LLs from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	LL16	LL14	LL12	
	Proximate composition			
DM [a]	33.2a ± 0.8	29.1b ± 0.4	30.7b ± 0.3	***
IMF [b]	15.6 ± 1.1	15 ± 1.0	15.9 ± 0.9	ns
Total collagen [b]	7.78 ± 0.18	7.88 ± 0.10	7.62 ± 0.10	ns
Insoluble collagen [b]	7.62a ± 0.17	7.08b ± 0.10	7.01b ± 0.11	***
	Water losses (g/100 g)			
WHC [c]	28.4 ± 0.3	28.9 ± 0.3	28.5 ± 0.3	ns
CL [c]	23.0 ± 0.8	22.8 ± 0.6	21.2 ± 0.7	ns
	Instrumental colour coordinates			
L*	47.5 ± 0.4	47.7 ± 0.8	48.1 ± 0.5	ns
a*	14.6a ± 0.3	14.3a ± 0.4	13.1b ± 0.4	**
b*	8.22 ± 0.25	8.20 ± 0.30	8.14 ± 0.21	ns
C*	16.7a ± 0.4	16.6a ± 0.4	15.0b ± 0.4	*
H°	29.5b ± 0.5	29.2b ± 0.5	31.3a ± 0.4	***
Myoglobin (mg/g)	2.10a ± 0.07	1.89a ± 0.08	1.62b ± 0.06	***

Values were presented as mean values ± standard error. LL16, LL14 and LL12 = *Longissimus lumborum* muscle from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). [a] expressed as g/100 g muscle. [b] expressed g/100 g moisture-free tissue. [c] expressed as g water released per 100 g muscle. DM, dry matter; IMF, intramuscular fat; WHC, water holding capacity; CL, cooking loss; L*, lightness; a*, redness; b*, yellowness; C*, Chrome; H°, hue angle (dimensionless instrumental colour coordinates measured in CIELab space). Sig, significance; a, b: different letters in the same row indicate significant differences due to animal slaughtering age for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

The data reveals that differences were found in DM ($P \leq 0.001$). LL16 yielded the highest value in DM. These results were in line with Lorenzo et al., (2014), who studied the effect of age at slaughter on meat traits of Celtic x Duroc crossed bred pigs, but at a lower age range. The DM values obtained in this study were the same in general terms as those obtained by Tejerina et al., (2012a) for fresh loin from Iberian purebred pigs under the *Montanera* system.

Regarding IMF, mean values obtained were lower than those reported by Tejerina et al., (2012a) for *Longissimus dorsi* muscle but from Iberian purebred pigs. No differences were found on account of slaughtering age ($P > 0.05$). Our findings were in line with those of Mayoral et al., (1999) and Daza et al., (2007) for the same muscle but from Iberian purebred pigs that were slaughtered at a different ages. Lorenzo et al., (2014) also reported how IMF remained constant with age at slaughter. Therefore, the different growth rates, given by the different ADGs, during the growing (228.2 ± 1.5, 244.4 ± 2.1 and 314.8 ± 1.5 g/day) and finishing (836.8 ± 26.3, 690.9 ± 31.2, 575.1 ± 34.8 g/day) phases from IBxD16, IBxD14 and IBxD12, respectively, did not have an impact on fat deposition in the case of LLs. Contrarily, Bosch et al., (2012) found a positive correlation between IMF and age in muscle *Longissimus dorsi* from Duroc pigs, but at a lower age range. The lack of differences on IMF content could be explained by the narrow age range under study, together with others factors, such as genetic background (Fuentes, Ventanas, Ventanas, & Estévez, 2014), management and feeding (Tejerina et al., 2012a) which remained constant in this study.

No differences were found in total collagen on account of slaughtering age ($P > 0.05$). In contrast, the amount of insoluble collagen increased with animal age, so LL16 attained the highest value (7.62 ± 0.17 from LL16 vs. 7.08 ± 0.10 and 7.01 ± 0.11 from LL14 and LL12, respectively; $P \leq 0.001$). This was not an unexpected trend, since previous studies such as carried out by Mayoral et al., (1999) have proven an increase in insoluble collagen in m. *Longissimus dorsi* throughout the animal lifetime.

No differences were found in WHC and CL on account of slaughtering age ($P > 0.05$), which could be due to the fact that WHC together with CL mainly depend on factors such as the production system (Tejerina et al., 2012a) and the composition of the muscle –especially the IMF– (Gandemer, Bonnot, Vedrenne, Caritez, Berge, Briant et al., 1990), which remained constant ($P > 0.05$) in all batches. Both WHC and CL revealed higher values than those found by Tejerina, García-Torres, & Cava, (2012c) in the *Longissimus dorsi* obtained from Iberian purebred pigs raised using the *Montanera* system. Few authors have reported findings on the effect of slaughtering age effect on water loss in pig muscles. Mayoral et al., (1999) found a progressive decrease in WHC with age in *Longissimus dorsi* and *Biceps femoris* obtained from 100% purebred Iberian pigs.

3.1.2. Instrumental colour and myoglobin content

Instrumental colour coordinates were reported in Table III. 2. Slaughtering age had a significant effect ($P \leq 0.05$) on redness (a*), intensity of colour (C*) and Hº. In contrast, lightness (L*) and yellowness (b*) were not affected ($P > 0.05$). The lower values of a* and C* and higher values of Hº observed in LL12 ($P \leq 0.05$) pointed to a decrease in the intensity of the red colour. This could be explained by the close relationship between heme pigment concentration and redness, which was observed previously by Lindalh, Lundstrom, & Tornberg (2001) and reported for the purebred Iberian by Tejerina et al., (2012a). In fact, a lower myoglobin content was observed in LL12 ($P \leq 0.001$) (Table III. 2).

In terms of the L* and b* coordinates, previous studies in the Iberian breed reported their relationship with the fatty acid profile (Carrapiso & García, 2005). However, this association was not observed in this study. The differences in the fatty acid profiles identified in this study (Table III. 3) ($P \leq 0.05$) were probably not enough to allow changes in the colour coordinates.

In general, the CIELab colour coordinates were in agreement with the ranges reported by other authors for Iberian meat (Tejerina et al., 2012a).

Myoglobin was lowest in LL12 (Table III. 2). The differences identified could be caused by age factor, as other factors which have been shown to have an impact on heme pigments, such as exercise (Pearson, 1990), which is related to the productive system, were similar for the three batches of animals. Our results were in agreement with those obtained by Mayoral et al., (1999), who identified an increase in pigment content associated with animal age for *Longissimus dorsi* obtained from Iberian purebred pigs. The myoglobin content values reported in this study are similar to those found previously by Mayoral et al., (1999).

3.1.3. Antioxidant and fatty acids profile and oxidative status

The effect of slaughter age on the antioxidant composition and fatty acids profile and oxidative status of LLs obtained from IBxD crossed breed pigs was shown in Table III. 3. Differences in both α- and γ- tocopherol were identified related to age at slaughter ($P \leq 0.05$). In terms of α-tocopherol content, LL16 yielded higher values in comparison with LL14 (5.31 ± 0.25 and 4.29 ± 0.22, respectively; $P \leq 0.001$), and similar to LL12 (4.58 ± 0.21; $P > 0.05$). However, for γ-tocopherol, only LL16 yielded values that were significantly higher ($P \leq 0.001$). The scientific literature has widely reported the influence of feeding on the tocopherol content of muscle. Specifically, the α-tocopherol content of muscles obtained from free-range pigs is attributed to the high α-tocopherol content of grass; whereas the presence of γ-tocopherol in the muscles is due to their high content of acorns (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2011). Henceforth, the higher values of γ-tocopherols found in LLs obtained from IBxD16 animals ($P \leq 0.001$) could be explained by a higher intake of acorns of animals in the *Montanera* system as a consequence of their advanced age (López-Bote, Rey, & Isabel, 2001), evidenced by its largest ADG (836.8 ± 26.3 g/day) with respect to IBxD14 (690.9 ± 31.2 g/day) and IBxD12 (575.1 ± 34.8 g/day). On the other hand, reasons for differences in α-tocopherol are not clear, and could be derived from variability in grass intake as reported previously by Rey et al., (2006). Both α- and γ-tocopherol values revealed in this study were similar to those reported previously for the same muscle obtained from Iberian purebred pigs in Tejerina et al., (2012a).

With regards to the fatty acid profile (Table III. 3), the values identified in this study were similar to those reported previously for *Montanera Longissimus dorsi* obtained from Iberian purebred pigs (Muriel, Ruiz, Ventanas, Petrón, & Antequera, 2004). The most predominant fatty acids were oleic acid (C18:1 n-9), which reached values ranging from 50.5 ± 0.7 for LL14 to 52.4 ± 0.6 g/100 g FAMEs for LL12, as well as palmitic acid (C16:0), with values ranging between 22.8 ± 0.6 for LL16 and 23.5 ± 0.5 g/100 g FAMEs for LL14.

No differences were observed for the SFA and MUFA fatty acid groups, neither for any of the individual fatty acids which comprise them ($P > 0.05$). On the contrary, lower PUFA values were obtained for LL12 ($P \leq 0.01$), mainly due to their lower C18:2 n-6 and C18:3 n-3 values ($P \leq 0.01$). This led to variations in n-6/n-3 ratio ($P \leq 0.05$), attaining the IMF from LL16 the highest value with 12.3 ± 0.2 ($P \leq 0.01$). Anyway, n-6/n-3 ratios were higher than nutritional recommendations established by Wood, Richardson, Nute, Fisher, Campo, Kasapidou et al. (2003).

Table III. 3. Effect of age at slaughter on the antioxidant and fatty acids profile and oxidative status of LLs obtained from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	LL16	LL14	LL12	
	Antioxidant Composition (µg/g)			
α-Tocopherol	5.31a ± 0.25	4.29b ± 0.22	4.58ab ± 0.21	***
γ-Tocopherol	0.91a ± 0.08	0.66b ± 0.05	0.65b ± 0.03	***
	Fatty acid composition (g/100g FAMEs)			
C16:0	22.8 ± 0.6	23.5 ± 0.5	23.2 ± 0.5	ns
C16:1	3.75 ± 0.18	3.74 ± 0.14	4.04 ± 0.13	ns
C18:0	11.0 ± 0.4	11.4 ± 0.4	10.6 ± 0.3	ns
C18:1 n-9	52.2 ± 0.7	50.5 ± 0.7	52.4 ± 0.6	ns
C18:2 n-6	6.20a ± 0.16	6.39a ± 0.17	5.53b ± 0.15	***
C18:3 n-3	0.47b ± 0.02	0.53a ± 0.01	0.43b ± 0.01	**
SFA	35.4 ± 1.0	36.6 ± 0.9	35.5 ± 0.8	ns
MUFA	56.9 ± 0.9	55.2 ± 0.8	57.5 ± 0.7	ns
PUFA	7.71ab ± 0.20	8.12a ± 0.22	7.05b ± 0.21	**
n-6/n-3	12.3a ± 0.2	11.1b ± 0.2	11.7ab ± 0.3	**
	Oxidative status			
Lipid oxidation [a]	0.22 ± 0.01	0.26 ± 0.02	0.24 ± 0.02	ns
Protein oxidation [b]	167 ± 4.7	168 ± 5.2	164 ± 6.4	ns

Values were presented as mean values ± standard error. LL16, LL14 and LL12 = *Longissimus lumborum* muscle from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). FAMEs: fatty acid methyl esters; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid, C18:3 n-3, linolenic acid; SFA: sum of all saturated fatty acids detected (C14:0, C16:0, C18:0, C20:0); MUFA: sum of all monounsaturated fatty acids detected (C16:1, C17:1, C18:1, C20:1); PUFA: sum of all polyunsaturated fatty acids detected (C18:2 n-6, C18:3 n-3; C20:2 n-9; C20:3 n-6; C20:4 n-6; C20:3 n-3); n-6/n-3: PUFA n-6/PUFA n-3 ratio. [a] expressed as mg malondialdehyde/kg DM. [b] expressed as nmol thiol/mg protein. Sig, significance; a, b: different letters in the same row indicate significant differences due to animal slaughtering age for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Fatty acid profile of tissues results from a combination of both direct deposition and endogenous synthesis, with the latter being of particular relevance to IMF due to its eminently adipogenic nature. This fact leads to a more stable composition and lower influence of the diet with respect to other tissues such as the subcutaneous back fat (D'Souza, Mullan, & Pethick, 2012). A further factor to be taken into account is the amount and composition of IMF at the beginning of the *Montanera*, which despite not being studied, can be inferred to have lower amounts in older animals (IBxD16) with respect to IBxD14 and specially IBxD12 because of the restrictive feeding of the former (Candek-Potokar et al., 1998).

Henceforth, the higher PUFA content in fresh loin from IBxD16 could be partly explained by a higher grass consumption of these animals during *Montanera,* as a consequence of a more advanced age (López-Bote et al., 2001) as demonstrated the higher ADG (836.8 ± 26.3, 690.9 ± 31.2, 575.1 ± 34.8 g/day) and weight increase (56.5 ± 1.8, 45.7 ± 2.3, 39.0 ± 2.6 kg) for IBxD16, IBxD14 and IBxD12, respectively, together with its higher synthesis of fat during this phase. On the other hand, the lowest content of PUFA, as well as of the main fatty acids that compose this group (C18:2 n-6 and C18:3 n-3) in IMF from LL12 could be linked to the likely higher IMF at the beginning of *Montanera* and the lower fat deposition during this phase of animals from IBxD12. Within this context, Benítez, Núñez, Fernández, Isabel, Fernández, Rodríguez et al., (2015) concluded the most noticeable changes due to the feeding in the C18:2 n-6 and PUFA group of the neutral lipid fraction of IMF in the same muscle from Iberian pigs. Also, Oliveira, González, Asmar, Batllori, Vera, Valencia et al., (2020) found lower C18:2 n-6 in Iberian pigs fed *ad-libitum* with respect to restricted-fed ones, but in subcutaneous back fat.

Regarding to oxidative status, values of mg MDA/kg DM ranged from 0.22 ± 0.01 ((LL16) to 0.26 ± 0.02 (LL14), which were in line with those reported by Lorido, Ventanas, Akcan, & Estévez (2016) in *Longissimus dorsi* from Iberian pigs, without differences on account on slaughter age ($P > 0.05$). In the same way, the slaughter age did not exert any effect on protein oxidation on fresh loin ($P > 0.05$).

3.1.4. Textural properties

The effect of slaughter age on TPA (20% and 80% deformation) and the results from the WBSF test on LLs were shown in Table III. 4. Test I and II from the TPA tests yielded values that were similar to those reported by Tejerina et al., (2012c) for the same muscle obtained from Iberian purebred pigs reared in the *Montanera* system, except for hardness in Test II, where the values identified in this study were higher.

No difference was found for Test I ($P > 0.05$), which indicates that the myofibrillar structure (Poste, Butler, Mackie, Agar, Thompson, Cliplef et al., 1993) of the LLs remained constant in the age range under study. This could be attributed to the fact that factors associated with the production system, such as feeding and exercise were similar for the three groups of animals (IBxD16, IBxD14 and IBxD12), as well as the lack of differences in physical-chemical factors such as WHC and specially IMF content ($P > 0.05$) among the three groups (LL16, LL14 and LL12) (Table III. 2) (Tejerina et al., 2012c).

In contrast, LL12 showed the lowest values in hardness, gumminess and chewiness ($P \leq 0.05$) in Test II. TPA tests with high percentages of deformation have been used previously to determine the strength of connective tissue (Lepetit & Culioli, 1994). Subsequently, the higher value of hardness in LL16 could be explained by the close relationship between the age of the animals and the collagen content. In this sense, Wilson, Bray, & Phillips (1954) suggested that total collagen was not always adequate to explain differences in meat textural properties, especially on account of the age of animals, since as McCormick, (1994) reported at a later stage, there was a little variation in the total collagen of skeletal muscle with animal growth, revealing that the age of animals had a direct correlation with insoluble collagen fraction and, consequently, with meat toughness. Thus, the higher amount of insoluble collagen in LL16 (Table III. 2) ($P \leq 0.05$) could explain the higher hardness values.

Regarding the WBSF, the values obtained were higher than those identified by Tejerina et al., (2012c) in *Longissimus dorsi* from Iberian purebred pigs, maybe due to the lower IMF values of muscles used in the current study. Slaughtering age did not affect WBSF ($P > 0.05$), which could be due to the lack of differences between the physical-chemical parameters, such as WHC (Table III. 2) ($P > 0.05$) (Rosenvold &

Andersen, 2003) and the properties evaluated in Test I (P > 0.05) (Koohmaraie, 1994).

Table III. 4. Effect of age at slaughter on the textural properties of LLs obtained from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	LL16	LL14	LL12	
Compression test I (TPA-20% compression)				
Hardness (N/cm^2)	2.06 ± 0.15	1.76 ± 0.09	1.76 ± 0.12	ns
Springiness (cm)	0.82 ± 0.01	0.84 ± 0.01	0.82 ± 0.01	ns
Cohesiveness	0.72 ± 0.01	0.71 ± 0.01	0.71 ± 0.01	ns
Gumminess (N cm s^2)	1.27 ± 0.07	1.18 ± 0.05	1.18 ± 0.06	ns
Chewiness (N cm s^2)	1.08 ± 0.08	0.98 ± 0.05	0.98 ± 0.06	ns
Resilience	0.47 ± 0.01	0.46 ± 0.01	0.47 ± 0.01	ns
Compression test II (TPA-80% compression)				
Hardness (N/cm^2)	110a ± 4.2	107ab ± 2.6	92.1b ± 2.6	**
Springiness (cm)	0.45 ± 0.01	0.45 ± 0.01	0.44 ± 0.01	ns
Cohesiveness	0.40 ± 0.01	0.41 ± 0.01	0.39 ± 0.01	ns
Gumminess (N cm s^2)	41.5ab ± 2.4	46.1a ± 1.8	36.8b ± 1.0	**
Chewiness (N cm s^2)	17.4b ± 1.1	20.6a ± 0.9	15.4c ± 0.5	***
Resilience	0.24 ± 0.01	0.24 ± 0.01	0.23 ± 0.01	ns
Warner-Braztler shear force test (WBSF)				
Shear force (N/cm^2)	73.7 ± 5.5	71.3 ± 5.8	71.3 ± 5.0	ns

Values were presented as mean values ± standard error. LL16, LL14 and LL12 = *Longissimus lumborum* muscle from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). TPA: textural profile analysis. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to animal slaughtering age for P = 0.05 according to Tukey´s HSD test. ns (P > 0.05); ** ($P \leq$ 0.01); *** ($P \leq$ 0.001).

3.2. Effect of age at slaughter on the quality traits of dry-cured loins

3.2.1. Proximate composition and water losses

Table III. 5 shows the proximate composition and WL of dry-cured loins. No differences were found in DM, IMF, NaCl and WL (P > 0.05) on account of age at slaughter. The DM values are higher than those reported by Ramírez & Cava, (2007b). Also, the IMF values identified in this study were higher than the values reported by Fuentes et al., (2014) for dry-cured loins obtained from pigs raised in *Montanera* system.

Table III. 5. Effect of age at slaughter on the proximate composition, WL and instrumental colour of dry-cured loins from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	DCL16	DCL14	DCL12	
		Proximate composition		
DM [a]	55.4 ± 0.6	55.0 ± 0.6	57.8 ± 0.4	ns
IMF [b]	17.5 ± 0.6	17.1 ± 1.3	16.7 ± 1.1	ns
NaCl [b]	9.54 ± 0.68	10.3 ± 0.5	10.4 ± 0.3	ns
		Weight loss (g/100g)		
WL	36.8 ± 0.6	39.5 ± 0.6	37.2 ± 0.6	ns
		Instrumental Colour coordinates		
L*	35.2 ± 0.8	35.7 ± 0.7	35.2 ± 0.7	ns
a*	13.2 ± 0.3	13.2 ± 0.3	12.1 ± 0.4	ns
b*	6.32 ± 0.14	6.88 ± 0.26	6.51 ± 0.27	ns
C*	14.6 ± 0.3	14.9 ± 0.3	13.8 ± 0.4	ns
H°	25.6 ± 0.3	27.5 ± 0.5	28.2 ± 1.1	ns

Values were presented as mean values ± standard error. DCL16, DCL14 and DCL12 = dry-cured loins from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). DM, dry matter; IMF, intramuscular fat; NaCl, salt content; WL, weight loss. L*, lightness; a*, redness; b*, yellowness; C*, Chrome; H°, hue angle (dimensionless instrumental colour coordinates measured in CIELab space). [a] expressed g/100 g muscle. [b] expressed g/100 g moisture-free tissue. Sig, significance; ns (P > 0.05) according to Tukey´s HSD test.

Regarding the curing process, the WL values found in this study were slightly lower than those found by Muriel et al., (2004). The lack of differences in WL could be explained by the fact that the loins were manufactured using similar times and conditions for the curing process, together with the lack of differences on salt and IMF content (Table III. 5) (P > 0.05); as the main factors that have an effect on WL are the presence of salt during the rubbing of the pieces (due to osmotic dehydration) and the constant increase in temperature, as well as the reduction in relative humidity (which causes water to continuously evaporate from the surface of the loins) (Ventanas & Cava, 2001).

3.2.2. Instrumental colour

Instrumental colour coordinates on dry-cured loins are shown in Table III. 5. Values were similar to those reported previously for dry-cured loins obtained from Iberian x

Duroc crossed bred pigs by Ramírez & Cava, (2007b). No differences were identified in instrumental colour coordinates for dry-cured loins on account of the age at slaughter (Table III. 5) ($P > 0.05$). Therefore, differences in a* and C* observed in fresh loins were not reproduced in dry-cured loins. This was not unexpected, since previous studies have reported a decrease in the variability of instrumental colour after processing Iberian pig products (Carrapiso & García, 2005). Whereas the colour in raw meat is determined by muscle composition, in dry-cured products, the drying degree and salt content –for which no differences were found (Table III. 5) ($P > 0.05$)– have a direct influence on colour (Pérez-Álvarez, Sayas-Barberá, Fernández-López, Gago-Gago, Pagán-Moreno et al., 1998).

3.2.3. Antioxidant and fatty acids profile and oxidative status

Table III. 6 shows the effect of age at slaughter on the antioxidant and fatty acid profile and oxidative status of dry-cured loins. The α-tocopherol values were higher than those found by Ventanas, Estevez, Tejeda, & Ruiz (2006) for dry-cured loins obtained from crossed bred Iberian pigs under *Montanera* system, whereas the γ-tocopherol values were similar. A similar trend can be noted for α- and γ-tocopherol in dry-cured loins with respect to fresh loin (Table III. 3), being the DCL16 which reported the highest values for α- and γ-tocopherol ($P \leq 0.05$). Within this context, previous studies have reported that such compounds are highly stable throughout the technological processing, and therefore, they do not show significant changes in comparison to the initial values (Isabel, López-Bote, Rey, & Sanz Arias, 1999; Rey, López-Bote, Daza, & Lauridsen, 2010). Therefore, the age at slaughter did reveal a direct correlation with the antioxidant profile of dry-cured loins, which could improve oxidative stability (Daza et al., 2005; Rey et al., 2006) and therefore could have an impact on the sensory features.

The fatty acid profile of dry-cured loins was affected by the age at slaughter, with differences being identified ($P \leq 0.05$) in the main fatty acids (C16:0, C18:0, C18:1 n-9, C18:2 n-6) and therefore in SFA and MUFA fatty acids groups. When the correlation (R^2) among the main fatty acid groups between fresh and dry-cured loin was explored, higher values were obtained; 0.973, 0.964 and 0.860 for SFA, MUFA and PUFA, respectively. The most remarkable differences with respect to the fresh fatty acid profile were related to unsaturation, especially in polyunsaturated fatty acids and PUFA group, which decreased during the curing process in all dry-cured loins sets (DCL16, DCL14 and DCL12) (Ventanas et al., 2006) and to a lesser extent in the C18:1 n-9 and MUFA group (in DCL14 and DCL12 with respect to LL14 and LL12, respectively), leading to an increase in the percentage of SFA after curing process in loins from IBxD14 and IBxD12 with respect to those from IBxD16. Also, an increase in the n-6/n-3 quotient was observed in dry-cured loins with respect to fresh ones.

The lack of differences in the oxidative status among groups accounting on age at slaughter remained after curing ($P > 0.05$), but, an increase in mg MDA/ kg DM (Table III. 6) was observed in all groups with respect to fresh loin (Table III. 3). Henceforth, the decrease of unsaturation after curing could be explained by the degradation during processing through lipolysis and oxidation, which affect PUFA preferentially (Gandemer, 2002).

Table III. 6. Effect of age at slaughter on the antioxidant and fatty acids profile and oxidative status of dry-cured loins obtained from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	DCL16	DCL14	DCL12	
	Antioxidant Composition (µg/g)			
α-Tocopherol	6.71a ± 0.39	6.06b ± 0.12	5.65b ± 0.24	*
γ-Tocopherol	1.12a ± 0.08	0.93b ± 0.03	0.89b ± 0.04	*
	Fatty acid composition (g/100g FAMEs)			
C16:0	22.1b ± 0.7	25.2a ± 0.8	24.1ab ± 0.8	*
C16:1	4.04 ± 0.34	3.70 ± 0.37	4.08 ± 0.28	ns
C18:0	10.6b ± 0.7	13.2a ± 0.7	11.0ab ± 0.5	*
C18:1 n-9	53.3a ± 1.2	48.3b ± 1.1	52.0ab ± 1.0	*
C18:2 n-6	5.90a ± 0.33	5.48ab ± 0.30	4.75b ± 0.10	*
C18:3 n-3	0.32 ± 0.04	0.30 ± 0.02	0.24 ± 0.01	ns
SFA	34.2b ± 1.5	40.0a ± 1.5	36.7ab ± 1.3	*
MUFA	58.4a ± 1.5	52.9b ± 1.5	57.0ab ± 1.3	*
PUFA	7.48 ± 0.43	7.08 ± 0.33	6.32 ± 0.15	ns
n-6/n-3	17.3 ± 1.0	16.9 ± 0.4	17.0 ± 0.8	ns
	Oxidative status			
Lipid oxidation [a]	1.78 ± 0.16	1.79 ± 0.11	1.75 ± 0.15	ns
Protein oxidation [a]	216 ± 10.0	210 ± 6.8	205 ± 8.4	ns

Values were presented as mean values ± standard error. DCL16, DCL14 and DCL12 = dry-cured loins from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). FAMEs: fatty acid methyl esters; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid, C18:3 n-3, linolenic acid; SFA: sum of all saturated fatty acids detected (C14:0, C16:0, C18:0, C20:0); MUFA: sum of all monounsaturated fatty acids detected (C16:1, C17:1, C18:1, C20:1); PUFA: sum of all polyunsaturated fatty acids detected (C18:2 n-6, C18:3 n-3; C20:2 n-9; C20:3 n-6; C20:4 n-6; C20:3 n-3); n-6/n-3: PUFA n-6/PUFA n-3 ratio. [a] expressed as mg malondialdehyde/kg DM. [2] expressed as nmol thiol/mg protein. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to animal slaughtering age for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$).

3.2.4. Textural properties

TPA –with 50% deformation– and WBSF tests on the dry-cured loins were shown in Table III. 7. No differences were found in the majority of the texture parameters under analysis, which indicates that the slaughtering age range used did not have any effect in texture traits of dry-cured loins. Thus, the curing process appeared to unify the differences found in hardness, gumminess and chewiness observed in fresh loins due to animal slaughter age ($P > 0.05$). This could be explained by the greater influence of moisture (Ruiz-Ramírez, Arnau, Serra, & Gou, 2005) and IMF (Ruiz, García, Muriel, Andrés, & Ventanas, 2002) on the textural properties of dry-cured Iberian products over collagen content.

With regards to WBSF, the lack of differences reported for fresh loins (Table III. 4) remained after the curing process (Table III. 7) ($P > 0.05$), which may be explained by the lack of differences in weight losses (WL) and IMF (Table III. 5) ($P > 0.05$). These findings were in line with previous studies on dry-cured loins (Ramírez & Cava, 2007b) and dry-cured hams (Ruiz-Ramírez et al., 2005).

Table III. 7. Effect of age at slaughter on the textural properties of dry-cured loins obtained from Iberian x Duroc crossed bred pigs reared in the *Montanera* system.

	Slaughtering age			Sig.
	DCL16	DCL14	DCL12	
Compression test (TPA-50% compression)				
Hardness (N/cm^2)	29.1 ± 1.2	32.3 ± 1.7	32.4 ± 2.7	ns
Springiness (cm)	0.61a ± 0.01	0.54b ± 0.01	0.55b ± 0.01	*
Cohesiveness	0.62 ± 0.01	0.59 ± 0.01	0.60 ± 0.01	ns
Gumminess (N cm s^2)	17.2 ± 0.5	19.3 ± 0.9	19.9 ± 1.8	ns
Chewiness (N cm s^2)	10.5 ± 0.2	10.6 ± 0.6	10.8 ± 1.2	ns
Resilience	2.18 ± 0.06	2.02 ± 0.08	2.09 ± 0.09	ns
Warner-Braztler shear force test (WBSF)				
Shear force (N/cm^2)	34.6 ± 3.1	53.8 ± 3.5	39.4 ± 2.8	ns

Values were presented as mean values ± standard error. DCL16, DCL14 and DCL12 = dry-cured loins from IBxD16, IBxD14 and IBxD12 animal batches (slaughtered with 16, 14 and 12 months, respectively). TPA: textural profile analysis. Sig, significance; a, b: different letters in the same row indicate significant differences due to animal slaughtering age for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$).

4. Conclusion

Our findings suggest that the age at slaughter at constant weight of Iberian x Duroc crossed bred pigs reared using the *Montanera* system effects certain quality traits (mainly textural properties, colour intensity, and the antioxidant and fatty acids profile) in fresh loins (LL) and these were partially reflected in dry-cured loins. Further research must now be carried out to assess whether differences would be perceived by consumers and influence their preferences.

Declaration of Competing Interest

All authors state no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work.

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Chapter IV: Quality traits of dry-cured loins from Iberian pigs reared in *Montanera* system as affected by pre-cure freezing

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Quality traits of dry-cured loins from Iberian pigs reared in *Montanera* system as affected by pre-cure freezing

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Abstract

Iberian dry-cured loins from pigs under *Montanera* system are usually subjected to seasonal production, which could be overcome through freezing the raw material prior to curing. Hence, three homogeneous batches (n = 15 per batch) were established to evaluate the effect of different pre-cure freezing (-20 ± 2°C) times (3 and 6 months) of raw material on main quality traits of dry-cured loins in comparison to those elaborated from unfrozen meat. All samples were subjected to similar seasoning and same curing length to obtain dry-cured loins. Results showed a decrease in lightness, redness, chrome and hue values, polyunsaturated fatty acids (PUFA), higher oxidative phenomena, and changes in textural properties in fresh loins on account on freezing ($P \leq 0.05$). Some quality traits of dry-cured loins were affected by pre-cure freezing, weight loss and hardness being higher as well as salt content being lower. PUFA and Warner-Braztler Shear Force ($P \leq 0.05$) also showed higher values in dry-cured loins from frozen/thawed meat than those elaborated from unfrozen counterparts.

Keywords: pre-cure freezing, pre-frozen dry-cured loin, raw material; *Longissimus thoracis et lumborum*; *Montanera* seasonality

1. Introduction

Iberian products enjoy great prestige worldwide due to their quality attributes, especially those which derive from pigs reared under *Montanera* system (outdoor rearing and final fattening phase based on acorns and grass) (Díaz-Caro, García-Torres, Elghannam, Tejerina, Mesías, et al., 2019). Nevertheless, considering the seasonality of *Montanera* system, dry-cured loins reach the market in summer, as a result of its short technological process (about 70 days) (RD 4/2014). The greatest consumption of this type of products takes place from November to December (because of Christmas season), this fact resulting in the existing time gap between industry production and consumer demand.

A growing practice among manufactures involves freezing raw material before its technological process of curing. It would allow producers to balance production and demand, avoiding the fluctuations in the market price and subsequently, the seasonality could be overcome. On the contrary, the use of frozen/thawed counterparts as raw material could be detrimental to the inherent quality of the final product. Physical (water losses or changes on textural properties) and chemical (colour or oxidative phenomena) changes in raw meat could have an impact on technological and nutritional attributes of dry-cured products (Pérez-Palacios, Ruiz, Martín, Barat, & Antequera, 2011). Moreover, the use of frozen/thawed material could be a factor to be considered during seasoning and curing. Oxidation phenomena are of particular interest in this sense, since these are not completely inhibited during frozen storage. In fact, both protein (Utrera, Parra, & Estévez, 2014) and lipid oxidation (Pérez-Palacios, Ruiz, Grau, Flores, & Antequera, 2009) are defined by (Soyer & Hultin, 2000) as the major forms of the deterioration in foods from muscle origin.

To date, scientific literature concerning to pre-cure freezing is scarce, and mostly carried out in ham from commercial breed pigs (Bañón, Cayuela, Granados, & Garrido, 1999; Flores, Soler, Aristoy, & Toldrá, 2006). Few studies deal with such a topic in hams from Iberian pigs (Pérez-Palacios et al., 2011; Pérez-Palacios, Ruiz, Barat, Aristoy, & Antequera, 2010; Pérez-Palacios et al., 2009), and only Lorido, Ventanas, Akcan, & Estévez, (2016) reported substantial contributions about the potential effect of pre-cure freezing on oxidative stability of the proteins of Iberian loins. Nevertheless, the evaluation of different freezing times has never been approached to the best of our knowledge. In addition, the aforementioned studies were carried out in meat from Iberian pigs fattened in confinement and commercially feeding system, even though the *Montanera* production system is the one which seasonality is an important issue, as has been previously explained. Abellán Salazar, Vázquez, Cayuela & Tejada, (2018) focused on the effect of pre-cure freezing on proteolysis changes and sensory acceptance of loins but not from Iberian breed pigs.

On the other hand, some Specific Designations of Origin exclude the pre-cure freezing, whereas the current European regulation on freezing of food of animal origin (European Regulation 16/2012) and the current Iberian Quality Standard (QS) (RD 4/2014) make no mention to this practice. Thus, the effect of this practice on the quality of the final product –dry-cured loin- remains unknown.

In order to generate scientific knowledge to help solving the seasonality of the production of dry-cured loin from Iberian pigs under the *Montanera* system, the objective of this study was to evaluate the effect of different freezing times (3 and 6 months) on the quality characteristics of the raw material (fresh loins) and final quality characteristics of the dry-cured loins from Iberian x Duroc crossed bred pigs under the *Montanera* system with respect to counterparts elaborated from fresh (unfrozen) ones.

2. Materials and methods

2.1. Meat sampling

A total of 45 *Longissimus thoracis et lumborum* muscles (LTL) were obtained from Retinto Iberian (Valdesequera line, Junta de Extremadura, Badajoz, Spain) crossed with Duroc (50%) pigs under the *Montanera* system. The rearing system conditions to which animals were subjected were as follows:

During growing period -from weaning to the beginning of *Montanera* (with a body weight (BW) of 102.9 ± 0.85 kg (mean ± standard deviation)) animals were housed in open-air corrals and fed based on commercial feeds. The average daily feed intake (ADFI) was 1.29 ± 0.08 kg/day, and the average daily gain (ADG) 256 ± 28.3 g/day. Afterwards, the finishing period in *Montanera*, was carried out in the Valdesequera farm, Junta de Extremadura, Badajoz, Spain, with a stocking rate of approximately 0.60 pigs per hectare. The length of the *Montanera* was of 67 days; from November 2018 to January 2019. *Montanera* feeding was based on *ad libitum* consumption of acorns mainly from *Quercus rotundifolia* and grass with free access to water. At the end of *Montanera*, animals had reached a BW of 148.4 ± 6.15 kg, with an ADG of 673 ± 34.9 g/day. The day previous to the slaughter animals were transported to a local slaughterhouse located 150 km approx. from the Valdesequera farm in a vehicle pen, where they were exposed to air flow during the transport, but they were not fed or watered. Animals were unloaded after 2-hours journey approx. During lairage (less than 24 hours) animals were provided of water whilst feed was withdrawn. Successively, animals were randomly slaughtered by exsanguination and previously stunned using a carbon dioxide stunning system.

After 4 hours post-mortem, the LTL muscles were removed from the left side of the carcass and freed from intermuscular fat and connective tissue. Then, they were allowed to cool for 24 h at 4 ± 1ºC. Subsequently and previously to curing, the loins were randomly divided into three sets: unfrozen (n = 15) and pre-frozen dry-cured loin during 3 (n = 15) and 6 months (n = 15). The average weight of the LTL muscles was 1.72 ± 0.15, 1.66 ± 0.08 and 1.64 ± 0.12 kg, respectively. Each whole muscle (LTL) was divided into two parts: (i) the first portion corresponding to *Longissimus lumborum* muscle (LL) was used for fresh loin analyses (including those resulting from the freezing/thawing process) and (ii) the rest of the loin corresponding to the thoracic part -*Longissimus thoracis*- (LT), was used for dry cured loin's analyses (Figure IV. 1).

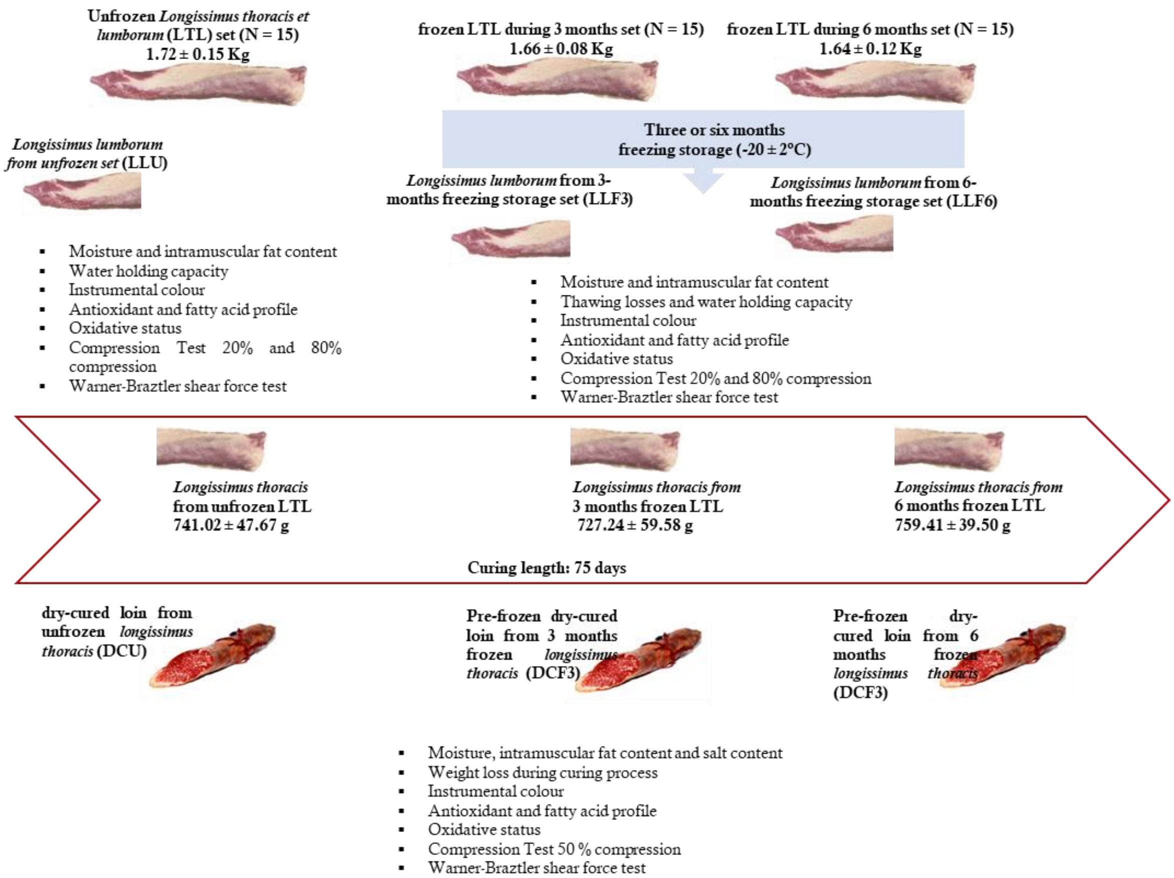

Figure IV. 1. Experimental design.

2.2. Freezing and thawing process

The muscle *Longissimus Lumborum* assigned to unfrozen loins (LLU) was subjected to analysis after cooling for 24 hours. The remaining muscle samples (belonging to LT muscle) were cured, in order to obtain dry-cured loins (dry-cured loins from unfrozen pieces).

The rest of LTL muscles from pre-frozen dry-cured loin sets were individually packaged in nylon/polyethylene bags (O_2 permeability, 9.3 ml $O_2/m^2/24$ h at 0°C) and frozen during 3 or 6 months in a freezing room through cold air (-20 ± 2°C) at 20 km/h. The freezing rate ranged between 1 and 5 cm/h, according to the common practice in meat industry. Thawing was carried out by storing loins at cooling temperature (4 ± 1°C) for 48 hours.

After thawing, a section of sample belonging to LL muscle was taken to evaluate the effect of freezing during three (LLF3) or six months (LLF6) on fresh loin. The remaining muscle samples (belonging to LT muscle) were immediately subjected to curing, in order to obtain dry-cured loins (pre-frozen dry-cured loins).

2.3. Technological process of curing

Curing was carried out according to common practice in the meat processing industry though it was adapted to the weight of the loins (741.02 ± 47.67, 727.24 ± 59.58 and 759.41 ± 39.50 g for unfrozen and frozen during three- and six-months *Longissimus thoracis*, respectively). The processing conditions were homogenous in the three sets of manufactured dry-cured loins, (n = 15 unfrozen dry-cured loin (DCU) from unfrozen LT; n = 15 pre-frozen dry cured loin (DCF3) and n = 15 DCF6 from frozen during 3 and 6 months, respectively, and thawed LT muscle). Therefore, all loins were seasoned in the same way within a mixing bowl with added nitrified salt (3%) and nitrites (0.8% of the total salt content) (percentages referred to total meat content). Garlic, paprika and olive oil, commonly used ingredients for seasoning of dry-cured loins, were not used to avoid interferences with oxidative reactions. They were kept at a temperature of 4°C for 48 h -in darkness- to allow the seasoning mixture to distribute into the meat. Subsequently, loins were stuffed into 10 cm diameter collagen casings and held at 4°C and 75-80% relative humidity (RH) for 30 days. Then, loins were ripened at temperatures which ranged from 10 to 16ºC whereas RH decreased from 75 to 65% (45 days). Thus, the total curing length was 75 days (RD 4/2014). Processing, seasoning and curing length were similar in the current study, in order to evaluate the single effect of pre-cure freezing on quality traits of raw material and dry-cured loins.

Finally, once the technological process was completed, dry-cured loins were vacuum-packed and kept in refrigeration until the corresponding analyses were carried out (less than one week). The average weight of the dry-cured loins was 462.77 ± 25.59, 442.79 ± 19.16 and 448.05 ± 19.15 g for DCU, DCF3 and DCF6 sets, respectively).

2.4. Methods

2.4.1. Proximate composition

Moisture was assayed following the AOAC method (AOAC, 2003). Intramuscular fat (IMF) was extracted with chloroform/methanol (2:1, v/v), following the Folch, Lees, & Sloane-Stanley, (1957) method. In dry-cured loins chloride content (NaCl) was assayed, using Volhard method (AOAC, 2000).

2.4.2. Thawing loss (TL) and water holding capacity (WHC)

The difference between the weight of the sample before freezing and after thawing was expressed as TL through the following equation:

$$\% \text{ TL} = ((W_0\text{-}W_f)/W_0) * 100,$$

were W_0 is the weight of sample (the whole muscle, LTL) before freezing and W_f the weigh after freezing and thawing process.

WHC (only in LL) was evaluated by measuring the water released of the sample after the application of a centrifugal force (3000 rpm during 3 min) following the method proposed by Tejerina García-Torres & Cava, (2012).

2.4.3. Weight loss (WL) in dry-cured loins

The WL after curing was determined by gravimetric differences using the equation:

$$\% \text{ WL} = ((W_0 - W_f)/W_0) * 100,$$

were W_0 is the sample weigh (belonged to LT muscle) before seasoning and W_f the weigh at the end of the process (75 days).

2.4.4. Colour measurement

For both fresh and dry-cured loins, using a Minolta CR-400 colorimeter (Minolta Camera, Osaka, Japan) with illuminant D65, a 0° standard observer and a 2.5 cm port/viewing area using colour system CIE (L*a*b*) for determinate lightness (L*), redness (a*) and yellowness (b*) and the saturation index or chroma (C*), defined as ($C = (a^{*2} + b^{*2}) * 0.5$) and Hue angle (H°) as arctg (b*/a*) were determined. The measurements were repeated at five randomly selected sites on each sample and averaged.

2.4.5. Determination of α and γ-tocopherol

Antioxidants (α- and γ-tocopherol) content were assayed by the method proposed by Liu, Scheller, & Schaeffer (1996). Thus, tocopherol extraction was carried out with a saponifying solution (KOH 11.5% in EtOH/H_2O 55:45), and determination was performed on an Agilent Technologies HPLC Series 1100 instrument (Agilent Technologies, Santa Clara, CA, USA) equipped with a Kromasil Silica column (5 µm particle size, 150 × 4.6 cm) (Symta, Madrid, Spain) and a Kromasil Silica Guard Column (10 µm) (Symta, Madrid, Spain). The mobile phase was hexane:isopropanol:etanol (98.5:1:0.5 v/v), at a flow rate of 1 mL/min and the fluorescence detector (Agilent Technologies Series 1200) was fixed at λ-excitation: 295 nm and λ-emission: 330 nm. Individual identification and quantification of the peaks were done by comparing with those of α- and γ-tocopherol authenticated commercial standards from Sigma-Aldrich in the 0.2–14 µg/mL range (Sigma-Aldrich, St. Louis, MO, USA).

2.4.6. Lipid and protein oxidation

Lipid oxidation was assayed by the 2-thiobarbituric acid (TBA) method of Salih, Smith, Price, & Dawson (1987). TBA-RS values were calculated from the standard (1,1,1,3-tetraethoxypropane, TEP) curve.

Protein oxidation was estimated by measuring the content of free thiol groups, spectrophotometrically at 412 nm and was calculated using an absorption coefficient of 13.6 $mM^{-1}cm^{-1}$. Protein concentration was determined by spectrophotometry at 280 nm using bovine serum albumin (BSA) as standard according to Batifoulier, Mercier, Gatellier, & Renerre (2002).

2.4.7. Determination of fatty acid profile

Fatty acid methyl esters (FAME) were obtained from intramuscular fat (previously extracted) by methylation using. KOH (85% in MetOH). Separation of FAMEs was carried out using a gas chromatograph (model 4890 Series II; Hewlett-Packard, Palo Alto, CA, USA) fitted a Carbowax™ fused silica capillary column (30 m × 0.25 mm id; 0.25 µm film thickness; (Ohio Valley, Marietta, OH, USA). For the identification of individual FAME was used a standard mixture of 37 Component FAME Mix (Sigma–Aldrich, Supelco 37 Component FAME Mix- CRM47885, St. Louis, MO, USA).

2.4.8. Texture analysis

Prior to fresh loin texture assays, samples were vacuum-packing in nylon/polyethylene bags and cooking by immersion at 80°C for 45 min in a water bath preheated at until an internal temperature of 75°C was reached. Texture analysis was performed in a texturometer TA XT-2i Texture Analyser (Stable Micro Systems Ltd., Surrey, UK).

Two texture analysis were carried out: Texture Profile Analysis (TPA) and Warner-Bratzler shear force test (WBSF).

TPA was determined at two deformation percentages; (i) TPA I (20%) in order to evaluate the contribution of myofibrillar structures and (ii) TPA II (80%) to analyze the intervention of the connective tissue on textural properties (Lepetit & Culioli, 1994). For the determination of TPA, each sample was cut into uniform cubes of 1 cm^3 and which were compressed in a parallel direction to the muscle fibers to 20% (TPA I) and 80% (TPA II) of the original height with a flat plunger of 20 mm diameter (P/20) using a 25-kN load cell applied at a crosshead speed of 2 mm/s through a 2-cycle sequence. The following texture parameters were measured from force–deformation curves (Bourne, 1978); hardness (N/cm^2), springiness (cm), cohesiveness (dimensionless), gumminess (N cm s^2), chewiness (N cm s^2), and resilience (dimensionless), as mean values. TPA analysis in dry-cured loin was performing with a 50% deformation (Ramírez & Cava, 2007).

Both cooked and dry-cured loins were prepared as 10 x 30 x 10 mm^3 (width x length x thickness) slices. Afterwards, samples were cut with a Warner–Bratzler device (HDP/BS) in perpendicular direction to the muscle fibers. The maximum shear force to cut the sample was measured. Results were expressed as WBSF (N/cm^2).

Instrumental determinations were repeated 8 times per sample and results were data averaged.

2.4.9. Statistical analysis

The effects of freezing and pre-cure freezing time (0, 3, and 6 months) on fresh and dry-cured loin, respectively, were evaluated on proximate composition, water losses, instrumental colour, antioxidant, oxidative status, fatty acid profile, and textural properties by one-way analysis of variance (ANOVA) using SPSS version 20.0 (SPSS Inc. Chicago, IL, USA). Each loin (raw, frozen/thawed, or dry-cured) was considered as the experimental unit. Data are presented as mean values ± standard error for each group (LLU, LLF3, and LLF6 for raw and frozen/thawed loins and DCU, DCF3, and DCF6 for dry-cured and pre-frozen dry-cured loins, respectively). Statistical significance was assessed by Tukey's HSD test, and the level of signification was set at $P = 0.05$. Post-hoc test was used to compare groups.

3. Results and discussion

3.1. Effect of pre-cure freezing time on proximate composition, water losses and instrumental colour

Freezing did not significantly affect proximate composition of fresh loins (Table IV. 1) ($P > 0.05$). With regard to water losses (Table IV. 1), freezing storage time did not influence this parameter -on fresh loin- ($P > 0.05$), but differences in the capacity of the muscle to retain water were observed ($P \leq 0.001$). In this respect, there is a general agreement regarding the formation of relatively long and irregular ice crystals in conventional freezing (-20°C). This could have led to damaging the structure of the LLF3 and LLF6, resulting in a loss of water after freezing and thawing process (Estévez, Ventanas, Heinonen, & Puolanne, 2011). This phenomenon might have been the reason of the lower volume of water available to be released when an external force was exerted in LLF3 and LLF6 with respect to LLU (Table IV. 1) ($P \leq 0.001$). Our results are in line with Kim, Jung, Lim, Yang, Joo, et al. (2013) who observed less released water after freezing/thawing of m. *Longissimus thoracis et lumborum* from pigs when measuring cooking losses.

Table IV. 1. Effect of freezing and pre-cure freezing on proximate composition, water losses and instrumental colour.

	Fresh loin			Sig.	Dry-cured loin			Sig.
	LLU	LLF3	LLF6		DCU	DCF3	DCF6	
				Proximate composition (g/100g)				
Moisture	70.1 ± 1.28	70.7 ± 0.70	70.1 ± 0.74	ns	44.2a ± 2.09	41.9b ± 3.21	42.4b ± 2.28	*
IMF [a]	12.1 ± 1.08	13.5 ± 1.55	12.7 ± 2.75	ns	16.4 ± 1.08	17.6 ± 0.97	17.3 ± 2.44	ns
NaCl	-	-	-		5.6a ± 0.66	4.1b ± 0.43	4.1b ± 0.50	***
				Water losses (g water/100 g muscle)				
TL	-	2.5 ± 0.54	2.7 ± 0.62	ns	-	-	-	
WL	-	-	-		37.6c ± 1.78	39.2b ± 1.47	41.3a ± 1.97	***
WHC [b]	29.0a ± 0.50	26.4c ± 1.39	28.0b ± 0.46	***	-	-	-	-
				Instrumental colour coordinates				
L*	47.9a ± 2.48	44.9b ± 2.51	45.4b ± 2.67	***	35.3 ± 2.17	36.7 ± 3.87	36.3 ± 3.29	ns
a*	14.0a ± 1.51	12.5b ± 1.20	12.0b ± 1.24	***	12.6a ±1.04	11.0b ± 0.50	12.6a ± 0.85	ns
b*	7.5 ± 1.28	7.4 ± 0.70	7.5 ± 0.54	ns	6.3a ± 0.54	4.5b ± 0.19	3.5c ± 0.39	***
C*	16.2a ± 1.70	14.3b ± 1.47	13.8b ± 1.66	***	14.2 ± 1.12	12.0 ± 0.93	12.8 ± 0.85	ns
H°	20.0b ± 1.32	30.0a ± 1.55	30.4a ± 1.20	***	26.3a ± 1.47	22.0a ± 2.05	15.4b ± 1.08	***

Values were presented as mean values ± standard error. LLU, LLF3 and LLF6 = unfrozen, and frozen during three- or six-months loins (*Longissimus lumborum* muscle), respectively and DCU, DCF3 and DCFF6 = dry-cured loins from *Longissimus thoracis* muscle unfrozen and frozen during three and six months, respectively. IMF, intramuscular fat; NaCl, salt content; TL, thawing loss; WL, weight los; WHC, water holding capacity. L*, lightness; a*, redness; b*, yellowness; C*, Chrome; H°, hue angle (dimensionless instrumental colour coordinates measured in CIELab space). [a] expressed as g /100 g moisture-free tissue. [b] expressed as g water released per 100 g muscle. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to freezing storage time and pre-freezing cure time on fresh and dry-cured loin, respectively, for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); *** ($P \leq 0.001$).

The higher WL observed in pre-frozen dry-cured loins (DCF3 and DCF6) ($P \leq 0.001$) (Table IV. 1) could be therefore explained by the easier release of water from the meat piece during curing because of a breakdown of the muscle fibers and subsequent loss of structural integrity caused by ice crystal formation. Nonetheless, similar studies carried out on Iberian dry-cured hams observed no differences in this parameter accounting on pre-cure freezing (Grau, Albarracín, Toldrá, Antequera, & Barat, 2008; Pérez-Palacios et al., 2011). Discrepancies between studies may be related with proximate composition –especially the amount of IMF, higher in hams than loins, which could have overcome the possible differences exerted by pre-cure freezing practice-. Also, Iberian dry-cured hams are subjected to a longer curing process, and therefore the dynamics of water may differ with respect to dry-cured loins. Therefore, the influence of pre-cure freezing could affect differently to dry-cured ham and loin. The higher values of WL in pre-frozen dry-cured loins (DCF3 and DCF6) possibly led to their lower moisture content ($P \leq 0.001$) (Table IV. 1). Therefore, pre-cure freezing could allow the production of *Montanera* Iberian loin to be adjusted to consumer demand. However, this practice might cause a lower performance of the pieces, which would lead to greater economic losses as a result of the cost of the freezing process and the lower final weight of the pre-frozen dry-cured loins.

The lower values found in NaCl content in DCF3 and DCF6 with respect to DCU ($P \leq 0.001$) (Table IV. 1) could be closely connected with the fact that pre-frozen dry-cured loins showed a lower moisture content ($P \leq 0.05$). This fact could have led to a lower diffusion of salt within the loin. This hypothesis is in line with the results found by Pérez-Álvarez, Sayas-Barberá, Fernández-López, Gago-Gago, Pagán-Moreno et al. (1998) in dry-cured hams. Conversely Abellán et al. (2018) and Pérez-Palacios et al. (2011) reported an opposite pattern for this parameter, attaining higher salt content in pre-frozen dry-cured loins and hams respectively, when they were compared to those from unfrozen counterparts. The penetration of salt also depends on other factors such as muscle structure. Differences in this latter because of the different conditions of the process of freezing/thawing (Abellán et al., 2018) may be responsible of the discrepancies in results of salt content among studies. It is therefore important to identify the characteristics of the process of freezing/thawing of raw material previous to the technological process of curing, as this could have an impact during salting/seasoning. Unfortunately, references on the effect that the frozen storage of the raw muscle has on salt content in dry-cured loin is scarce (Abellán et al., 2018) and inexistent when it comes to Iberian dry-cured loin.

Regarding instrumental colour coordinates, freezing and thawing showed a significant effect on lightness (L*), redness (a*), chroma (C*) and hue angle values (H°) ($P \leq 0.001$) of fresh loins, regardless of storage time ($P > 0.05$). Contrarily, no effect was observed for yellowness values (b*) ($P > 0.05$).

The lower L* values in LLF3 and LLF6 are in line with results by Kim et al. (2013), and could be ascribed to their higher water losses as compared to LLU, as a result of freezing/thawing (Table IV. 1). Freezing and thawing also promoted a decrease in a* and C* values, as well as an increase in H° values leading to a decrease in the intensity of the red colour in LLF3 and LLF6. Our results were in concordance with those reported by Martín Mateos (2013), who found a decrease in the red colour of frozen and thawed m. *Serratus ventralis* from Iberian pigs after 18 months of freezing storage. Similarly, Bañón et al. (1999) observed a slight decrease in a* in frozen/thawed commercial hams. On the contrary, Pérez-Palacios et al. (2011) found higher values of a* and C* in frozen/thawed Iberian hams, when they were compared to unfrozen ones, which was attributed to a higher level of desiccation.

No differences were observed for b* on fresh loin after freezing. There is a wide range of results within the existing scientific literature. Whereas Bañón et al. (1999) described a reduction of b* values as a result of freezing in commercial hams, Pérez-Palacios et al. (2011) did not observed any difference in Iberian hams pieces. Similarly, Kim et al. (2013) did not observe changes in b* coordinates in m. *Longissimus thoracis et lumborum* from commercial breed as a result of freezing/thawing process.

The discrepancies noted for some of the coordinates studied among results from the current study and those dealing with pre-cure freezing in hams could be attributed not only to differences in chemical composition between products, but also to the influence of the component distribution on instrumental colour, as concluded Carrapiso & García (2005).

Despite the differences in instrumental colour observed in the raw material due to freezing, these differences are not evident for L*, a* and C* values after curing process (P > 0.05) (Table IV. 1). This is consistent with previous studies in Iberian products, in which the instrumental colour differences found in cured meat were not exactly alike in the fresh meat (Carrapiso & García, 2005).

On the opposite, differences in b* were found in dry-cured loin because of the pre-cure freezing, with DCU yielding the highest value ($P \leq 0.001$), which led to a decrease in H° ($P \leq 0.001$) (Table IV. 1). This could be attributed to their higher moisture and salt content (Pérez-Álvarez et al., 1998).

3.2. Effect of pre-cure freezing time on antioxidant content, oxidative status, and fatty acids profile

As far as antioxidants content in fresh loin is concerned, no differences were observed due to freezing/thawing neither to freezing storage time (P > 0.05) (Table IV. 2). The values of α- and γ-tocopherol are influenced by the feeding of the animals. In particular, in the meat from Iberian pigs fattened in *Montanera*, the- and γ- tocopherol content is attributed to the intake of grass and acorns (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2011). The lack of differences in tocopherol content between LLU and LLF3 and LLF6 could be explained by the stability of these compounds. In fact, previous studies have reported a low α-tocopherol degradation in Iberian meat after 18 months of freezing storage (Martín Mateos, 2013). Contrariwise, this latter author reported a degradation of γ-tocopherol in the muscle *Serratus ventralis* after 12 months of freezing storage. In any case, the time of freezing storage studied in our research was shorter, which could explain the lack of degradation of γ-tocopherol in both LLF3 and LLF6.

Table IV. 2. Effect of freezing and pre-cure freezing on antioxidant content, oxidative status, and fatty acids profile.

	Fresh loin			Sig.	Dry-cured loin			Sig.
	LLU	LLF3	LLF6		DCU	DCF3	DCF6	
				Antioxidant composition and oxidative status				
α-Tocopherol [a]	16.4 ± 1.43	16.6 ± 2.28	16.3 ± 2.21	ns	10.7 ± 0.89	11.1 ± 2.67	11.7 ± 0.97	ns
γ-Tocopherol [a]	2.6 ± 0.43	2.9 ± 0.50	2.9 ± 0.46	ns	1.6b ± 0.31	1.9a ± 0.38	2.0a ± 0.31	**
Lipid oxidation [b]	0.1b ± 0.04	0.2a ± 0.08	0.2a ± 0.04	***	1.0b ± 0.19	1.3a ± 0.31	1.2a ± 0.36	*
Protein oxidation [c]	166.7a ± 21.98	127.4b ± 14.20	88.1c ± 16.69	***	210.0a ± 16.87	147.8b ± 33.59	138.8b ± 16.18	***
				Fatty acid composition (g/100 g FAMEs)				
C16:0	23.0 ± 1.20	23.6 ± 1.78	23.4 ± 2.32	ns	23.8 ± 2.05	23.5 ± 1.27	23.9 ± 1.90	ns
C16:1	3.8 ± 0.35	3.9 ± 0.43	3.8 ± 0.69	ns	3.9 ± 0.69	3.7 ± 0.38	3.8 ± 0.34	ns
C18:0	11.0 ± 0.81	10.3 ± 1.12	10.8 ± 1.54	ns	11.6 ± 1.78	12.2 ± 0.97	11.2 ± 1.63	ns
C18:1 n-9	52.1 ± 1.39	52.1 ± 0.77	52.3 ± 1.35	ns	51.6 ± 2.83	50.3 ± 1.89	51.0 ± 1.32	ns
C18:2 n-6	6.2a ± 0.31	6.0ab ± 0.54	5.6b ± 0.81	*	5.6ab ± 0.62	5.7a ± 0.43	5.2b ± 0.54	*
C18:3 n-3	0.5a ± 0.01	0.3b ± 0.04	0.4b ± 0.07	***	0.3a ± 0.07	0.2b ± 0.04	0.2b ± 0.04	***
SFA	35.5 ± 1.90	36.4 ± 2.36	36.7 ± 3.72	ns	36.8 ± 3.98	37.9 ± 2.39	38.0 ± 2.59	ns
MUFA	56.9 ± 1.66	56.9 ± 0.93	56.9 ± 1.74	ns	56.2 ± 1.89	56.0 ± 2.01	56.1 ± 1.35	ns
PUFA	7.7a ± 0.43	6.7b ± 0.58	6.4b ± 0.85	***	7.1a ± 0.74	6.1b ± 0.74	5.9b ± 0.54	***
n-6/n-3	11.9c ± 0.77	18.3a ± 2.28	16.4b ± 2.78	***	17.6b ± 2.47	22.5a ± 3.63	21.9a ± 2.40	***

Values were presented as mean values ± standard error. LLU, LLF3 and LLF6 = unfrozen, and frozen during three- or six-months loins (*Longissimus lumborum* muscle), respectively and DCU, DCF3 and DCFF6 = dry-cured loins from *Longissimus thoracis* muscle unfrozen and frozen during three and six months, respectively. [a] expressed as μg of α- or γ-tocopherol/g moisture-free tissue. [b] expressed as mg malondialdehyde/kg DM. [c] expressed as nmol thiol/mg protein. FAMEs, fatty acid methyl esters; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid, C18:3 n-3, linolenic acid, SFA: sum of all saturated fatty acids detected (C14:0, C16:0, C17:0; C18:0, C20:0); MUFA: sum of all monounsaturated fatty acids detected (C16:1, C17:1, C18:1, C20:1); PUFA: sum of all polyunsaturated fatty acids detected (C18:2 n-6, C18:3 n-3; C20:2 n-9; C20:3 n-6; C20:4 n-6; C20:3 n-3); n-6/n-3: PUFA n-6/PUFA n-3 ratio. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to freezing storage time and pre-freezing cure time on fresh and dry-cured loin, respectively, for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

On the contrary, differences in γ-tocopherol content were found among loin sets after curing ($P \leq 0.001$) (Table IV. 2). It can be observed that both α- and γ-tocopherol attained higher values in pre-frozen dry-cured loins (DCF3 and DCF6) with respect to DCU, although only significant differences were found for γ-tocopherol. This finding could be explained by their slightly higher IMF, since all fresh loin sets started the curing process with homogenous values. Our results suggest that a great stability of tocopherols could be predicted not only during curing process, as previous studies have reported (Isabel, López-Bote, Rey, & Sanz Arias, 1999; Rey, López-Bote, Daza, & Lauridsen, 2010), but also after pre-cure freezing.

Lipid oxidation (Table IV. 2) increased ($P \leq 0.001$) after freezing/thawing. This trend is in agreement with Martín Mateos (2013) and Lorido et al. (2016) who found higher oxidation degree after twelve and five months of freezing storage of *Serratus ventralis* and *Longissimus dorsi* respectively, in comparison with unfrozen ones. Likewise, freezing and thawing process, as well as freezing storage time seem to have promoted a higher protein oxidation ($P \leq 0.001$) (Table IV. 2), which is associated with a decrease in thiol groups (Lorido et al., 2016; Utrera et al., 2014). This increase in protein oxidation is consistent with studies carried out by other authors in Iberian meat (Lorido et al., 2016; Martín Mateos, 2013) and could be related to lipid oxidation, since previous studies have reported that products from lipid oxidation may serve as a substrate for protein oxidation (Soyer & Hultin, 2000).

The trend observed for oxidative status remained similar after curing, with pre-frozen dry-cured loin (DCF3 and DCF6) showing the highest oxidation values, probably explained by their higher initial values (Table IV. 2). With respect to lipid oxidation, results obtained in this study are in disagreement with those obtained by Pérez-Palacios et al. (2009) and Lorido et al. (2016) who did not observe any impact of pre-cure freezing on lipid oxidation of Iberian dry-cured hams and loins, respectively. Differences in results among scientific literature could be related to the fatty acid composition, which affects oxidative stability of dry-cured products. Thus, fat from Iberian hams or loins from animals fed with a commercial diet yield high levels of saturated fatty acids (SFA), being a fat with high consistency and therefore being more stable than fat from *Montanera* pigs, in which the high level of polyunsaturated fatty acids (PUFA) makes it more prone to oxidative degradation (Gilles, 2009).

These results would suggest the influence of the productive system on the effect exerted by the pre-cure freezing. Results obtained in this study for protein oxidation are in agreement with Lorido et al. (2016) who reported an increase in protein oxidation of dry-cured loins from frozen/thawed pieces with respect to unfrozen ones.

Regarding fatty acid profile (Table IV. 2), PUFA content was lower in frozen/thawed loins (LLF3 and LLF6) ($P \leq 0.001$), mainly explained by the decrease in C18:2 n-6 and C18:3 n-3 ($P \leq 0.05$), which led to a higher n-6/n-3 relationship ($P \leq 0.001$). It could be hypothesized that the lower content in PUFA in LLF3 and LLF6 after freezing/thawing could be ascribed to its oxidation, which was more intense in these samples (Table IV. 2) ($P \leq 0.001$), and considering that it is generally accepted that oxidation phenomena is initiated by the autooxidation of PUFA (Utrera et al., 2014). Our findings are in agreement with Hernández, Navarro, & Toldrá (1999) and Perez-Palacios et al. (2009) who observed a decrease in PUFA in loin from commercial pig breed and on the polar lipid fraction of Iberian hams after freezing storage.

The differences in the fatty acid profile observed for fresh loin were maintained after curing ($P \leq 0.05$) (Table IV. 2). Our results disagree with those found by Pérez-Palacios et al. (2009) who observed no difference in the fatty acid profile of Iberian dry-cured hams accounting on pre-cure freezing. However, to the best of our knowledge there are no studies in Iberian dry-cured loins to compare our results with.

3.3. Effect of pre-cure freezing time on textural properties

As can be observed in Table IV. 3, freezing affected most of the textural parameters evaluated on fresh loin. More specifically, results from TPA I showed that loins under freezing storage (LLF3 and LLF6) yielded higher hardness, gumminess, and chewiness than unfrozen ones ($P \leq 0.001$). On the contrary, freezing storage time did not exert any effect on these parameters. Contrarily, a previous study by Pérez-Palacios et al. (2011) showed a decrease in hardness and chewiness -in a 50% compression test- in Iberian hams prior to curing after three months of freezing storage. However, loin is a different piece, with a lower IMF and therefore it could be more sensitive to water loss, which is related to tenderness (Traore Aubry, Gatellier, Przybylski, Jaworska, et al., 2012).

Table IV. 3. Effect of freezing on textural properties of fresh loin.

	Fresh loin			Sig.
	LLU	LLF3	LLF6	
Compression test I (TPA-20% compression)				
Hardness (N/cm^2)	1.9b ± 0.50	5.2a ± 1.47	5.5a ± 1.47	***
Springiness (cm)	0.8 ± 0.04	0.8 ± 0.04	0.9 ± 0.04	ns
Cohesiveness	0.7 ± 0.04	0.7 ± 0.04	0.7 ± 0.04	ns
Gumminess (N cm s^2)	1.2b ± 0.19	3.7a ± 1.16	4.1a ± 1.16	***
Chewiness (N cm s^2)	1.0b ± 0.27	3.0a ± 1.08	3.5a ± 1.08	***
Resilience	0.5 ± 0.04	0.5 ± 0.08	0.5 ± 0.04	ns
Compression test II (TPA-80% compression)				
Hardness (N/cm^2)	100.0b ± 10.02	134.3a ± 12.93	132.1a ± 12.03	***
Springiness (cm)	0.5b ± 0.04	0.5a ± 0.04	0.5a ± 0.04	***
Cohesiveness	0.4b ± 0.04	0.5a ± 0.04	0.5a ± 0.04	***
Gumminess (N cm s^2)	41.4b ± 8.05	61.6a ± 11.14	62.8a ± 12.54	***
Chewiness (N cm s^2)	17.8b ± 4.02	32.7a ± 6.85	33.3a ± 7.93	***
Resilience	0.2 ± 0.04	0.3 ± 0.04	0.3 ± 0.04	ns
Warner-Braztler shear force test (WBSF)				
Shear force (N/cm^2)	72.0a ± 13.43	46.2b ± 9.79	49.3b ± 11.22	***

Values were presented as mean values ± standard error. LLU, LLF3 and LLF6 = unfrozen, and frozen during three- or six-months loins (*Longissimus lumborum* muscle), respectively. TPA, textural profile analysis. Sig, significance; a, b: different letters in the same row indicate significant differences due to freezing storage time, for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); *** ($P \leq 0.001$).

Regarding TPA II, LLF3 and LLF6 showed higher values for hardness, springiness, cohesiveness, gumminess and chewiness ($P \leq 0.001$) than unfrozen ones, but no differences were found in resilience ($P > 0.05$) with respect to LLU. As it was observed for TPA I, no differences accounting on freezing storage time were observed. The higher hardness after freezing/thawing in tests with high percentages of deformation could be explained by the greater degree of protein oxidation, specially of the connective tissue (Leygonie, Britz, & Hoffman, 2012). Our results were in agreement with Martín-Mateos (2013) who observed an increase in gumminess, chewiness and hardness in high compression test in *Serratus ventralis* after 365 days frozen.

With respect to WBSF test, LLF3 and LLF6 yielded lower values than those showed by LLU ($P \leq 0.001$). These results could be explained by the rupture of muscle fibers, as well as by the loss of cell membrane resistance after freezing and thawing process. Martín-Mateos (2013) also observed the same trend in shear force, though in *Serratus ventralis,* after one year of freezing storage.

The pre-cure freezing, DCF3 and DCF6, kept maintaining the higher values in hardness, springiness and chewiness ($P \leq 0.05$), when a deformation test (TPA 50%) was applied to them (Table IV. 4), whilst resilience values of DCF3 and DCF6 with respect to DCU decreased ($P \leq 0.001$). No differences were observed for cohesiveness and gumminess in dry-cured loin on account of pre-cure freezing ($P > 0.05$). More specifically, the higher values in hardness for DCF3 and DCF6 could be explained by several factors: (i) by the higher hardness of frozen/thawed raw material ($P \leq 0.001$) (Table IV. 3), (ii) by the increase in protein oxidation, which was more intense in pre-frozen dry-cured loins ($P \leq 0.001$) (Table IV. 2) (Estévez et al., 2011) and (iii) by the higher WL ($P \leq 0.001$) (Table IV. 1) (Ruiz-Ramírez, Arnau, Serra, & Gou, 2005) to which these pieces were subjected during curing. Our results were in agreement with Lorido et al. (2016) who observed higher hardness in Iberian dry-cured loins from frozen/thawed pieces than unfrozen ones, measured by a sensory panel. Regarding WBSF, pre-frozen dry-cured loins (DCF3 and DCF6) yielded lower values than DCU ($P \leq 0.001$) (Table IV. 4), imitating the pattern previously observed in fresh loin ($P \leq 0.001$) (Table IV. 3).

With respect to scientific literature, Pérez-Palacios et al. (2011) reported a lack of differences in instrumental texture accounting on pre-cure freezing on Iberian dry-cured hams, suggesting that the technological process might balance texture differences found in fresh meat. However, no studies dealing with this topic on dry-cured loin were found.

Table IV. 4. Effect of pre-cure freezing on textural properties of dry-cured loins.

	Dry-cured loin			Sig
	DCU	DCF3	DCF6	
Compression test (TPA-50% compression)				
Hardness (N/cm^2)	30.9b ± 2.97	33.2ab ± 1.70	36.0a ± 2.36	*
Springiness (cm)	0.6b ± 0.04	0.7a ± 0.08	0.7a ± 0.04	***
Cohesiveness	0.6 ± 0.04	0.6 ± 0.04	0.6 ± 0.04	ns
Gumminess (N cm s^2)	18.5 ± 4.91	20.1 ± 3.44	21.8 ± 3.64	ns
Chewiness (N cm s^2)	10.7b ± 3.02	13.9a ± 1.97	14.2a ± 2.17	***
Resilience	2.1a ± 0.31	0.2b ± 0.04	0.2b ± 0.04	***
Warner–Braztler shear force test				
Shear force (N/cm^2)	44.1a ± 5.68	33.5b ± 5.46	32.6b ± 3.48	***

Values were presented as mean values ± standard error. DCU, DCF3 and DCFF6 = dry-cured loins from *Longissimus thoracis* muscle unfrozen and frozen during three and six months, respectively. TPA, textural profile analysis. Sig, significance; a, b: different letters in the same row indicate significant differences due to pre-freezing cure time for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); *** ($P \leq 0.001$).

4. Conclusion

Our results suggest that pre-cure freezing (3 and 6 months) had no effect on some quality traits such as lightness, redness, α-tocopherol and MUFA of dry-cured loins from Iberian x Duroc crossed bred pigs reared under *Montanera* system. However, differences were observed in parameters related to PUFA content, oxidative status and textural properties (mainly hardness).

From these results it can be also inferred that quality and technological aptitude of raw material (frozen/thawed vs. unfrozen) had an important effect on weight loss during curing, which could affect the productive and economic performance of the meat industry. Hence, it would be recommendable the pre-cure freezing practice to be considered as a feasible practice by the current Iberian Quality Standard, in order to adjust curing conditions to the type of meat (fresh or frozen/thawed).

The present research was intended as a preliminary study to provide insight into Iberian meat industry. Further researches should be carried out to assess the influence of this practice on shelf life and to explore whether physical-chemical differences are perceived by consumers and therefore the impact in their purchasing decisions.

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Chapter V: Physico-chemical and sensory qualities of Iberian sliced dry-cured loins from various commercial categories and the effects of the type of packaging and refrigeration time

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Physico-chemical and sensory qualities of Iberian sliced dry-cured loins from various commercial categories and the effects of the type of packaging and refrigeration time

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Abstract

The physico-chemical characteristics and sensory attributes of Iberian sliced dry-cured loin from three commercial categories according to the current Spanish Iberian Quality Standard (*Black*, *Red*; purebred Iberian and Iberian x Duroc crossed bred (50%) pigs, respectively, reared outdoor with the final fattening phase based on acorns and grass; *Montanera*, and *White*; Iberian x Duroc crossed bred (50%) pigs reared indoors and fed on commercial fodder) and packaged using different systems (vacuum and modified-atmosphere packaging (MAP)) were evaluated at various stages throughout their long-term storage (12 months). The slices from the *Black* and *Red* categories yielded higher antioxidants, monounsaturated and polyunsaturated fatty acids and higher lipid oxidation at the end of storage time (from T8 to T12). α-tocopherol was better preserved in the case of the slices kept in MAP although, after 8 months of storage, lipid oxidation was seen to increase in the latter ($\Delta_{t12\text{-}t0\ MAP} = 0.4$) compared to the vacuum packaged product. Regarding sensory evaluation, the marbling and texture attributes were affected by commercial category, whereas the influence was insignificant by type of packaging and storage time.

Keywords: modified-atmosphere packaging, vacuum packaging, shelf-life, commercial categories, *Montanera*

1. Introduction

The quality of Iberian dry-cured products is affected by factors such as genetic background (Ramírez & Cava, 2007), the production system and the type of feeding of the animal (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012a). The variety among these production factors leads to various quality standards in the final product, which materialise in different commercial labels according to the current Spanish Iberian Quality Standard (QS) (RD 4/2014): *"Black"* and *"Red"* (100% and at least 50% Iberian breed pigs, respectively, both reared outdoors with the final fattening phase based on acorns and grass; *Montanera*), *"Green"* (at least 50% Iberian breed pigs reared outdoors and fed on commercial fodder and/or acorns and grass) and *"White"* (at least 50% Iberian breed pigs reared indoors and fed on commercial fodder). Likewise, these quality standards are linked to high production costs, especially for dry-cured products with *Black* and *Red* categories because of the long and extensive rearing systems (Mayoral, Dorado, Guillén, Robina, Vivo, et al., 1999) and natural resource-based feeding systems of animals, as well as the long curing times required (RD 4/2014).

On the other hand, sociodemographic changes and recent consumer habits have given rise to new selling formats, such as sliced dry-cured products that are vacuum or modified-atmosphere packaged (MAP) (Parra, Viguera, Sánchez, Peinado, Espárrago, et al., 2012). In the first case, the air surrounding the product is removed, whereas in the case of MAP products, the air is replaced by a gas or a gas mixture without oxygen (Parra et al., 2012) such as CO_2 and N_2.

Previous studies have evaluated the quality traits and how the type of packaging affects dry-cured meat products (Cilla, Martínez, Beltrán, & Roncales, 2006; García-Esteban, Ansorena, & Astiasarán, 2004; Rubio, Martínez, García-Cachán, Rovira, & Jaime, 2008), being those dealing with dry-cured products from Iberian pigs scarcer and only carried out on Iberian ham (Parra, Viguera, Sánchez, Peinado, Espárrago, et al., 2010; 2012). Nevertheless, scientific literature is inexistent when it comes to dry-cured loins.

It is therefore essential to highlight the differences in terms of physicochemical and sensory qualities among commercial categories of Iberian dry-cured loins since the production requirements, and therefore the production costs, for obtaining each of them are different (RD 4/2014). On the other hand, it is necessary to evaluate the influence of these categories as well as new selling formats on shelf life throughout the long-term storage time.

Within this framework, the purpose of this study was to analyse the main quality characteristics of Iberian dry-cured loins according to the commercial categories (*Black, Red* and *White*) compiled by the current QS (RD 4/2014). At the same time, this study is an attempt to evaluate the shelf-life of each commercial category and the influence of two types of packaging (vacuum and MAP) on pre-sliced Iberian dry-cured loins throughout long-term storage (12 months).

2. Materials and methods

2.1. Materials

2.1.1. Iberian dry-cured loins

Iberian *Longissimus thoracis et lumborum* (LTL) muscles from animals of three commercial categories (*Black, Red* and *White*) as defined by the QS (RD 4/2014) were randomly selected and purchased from a pork manufacturing facility. According to these guidelines, the production system conditions required in each commercial category were different and are shown in Figure V. 1.

Figure V. 1. Production system conditions of *Black, Red* and *White* commercial categories according to the current Iberian Quality Standard (RD 4/2014). **Montanera*, typical free-range system from South-West Iberian Peninsula with feeding based in acorn and grass.

The Iberian LTLs were manufactured according to the specifications of QS (RD 4/2014). The LTLs were seasoned in a mixing bowl with a mixture of salt (2.5%), paprika (0.6%) and 0.9% additives (authorised preservatives and stabilisers) specifically prepared for this type of meat products. Subsequently, they were stuffed into 6-7 cm-diameter natural casings, and dry-cured according to industrial manufacturing protocols in a drying chamber. Thus, loins were held for over 30 days at refrigeration temperature (4 ± 2ºC) and high relative humidity (RH) (75 – 80%). Then, loins were ripened at temperatures which ranged from 10 to 16ºC whereas RH decreased from 75 to 65% during the rest of the curing length. The curing time (always over 70 days (RD 4/2014) varied according to the weight and commercial category of the product: 85, 80 and 70 days for *Black*, *Red* and *White* weighting 1.2 ± 0.2; standard error, 1.2 ± 0.3 and 1.4 ± 0.3 kg per piece, respectively.

2.1.2. Experimental design

A total of 72 Iberian dry-cured loins were randomly selected and subsequently distributed into the three categories: *Black* (n = 16 + 8), *Red* (n = 16 + 8) and *White* (n = 16 + 8), for physicochemical and sensory analysis, respectively. The dry-cured loins were sliced into 2 mm-thick slices, homogenously distributed in 100 g-package formats prepared under commercial conditions. A total of 720 packages were obtained, 240 packages of each commercial category (160 + 80 for physicochemical and sensory analysis, respectively) which were distributed homogeneously into the following groups: i) vacuum-packaged (n = 120; 80 + 40 for physicochemical and sensory analysis, respectively), ii) MAP (n = 120; 80 + 40 for physicochemical and sensory analysis, respectively) and stored in a cold chamber (4 ºC) for 12 months. The packages were sampled at: T0 (initial, n = 40 (20 + 20)), T4 (4 months of storage, n = 20 (20 + 0)), T8 (8 months of storage n = 40 (20 + 20)) and T12 (12 months of storage, n = 20 (20 + 0)) for physicochemical and sensory analysis, respectively (Figure V. 2).

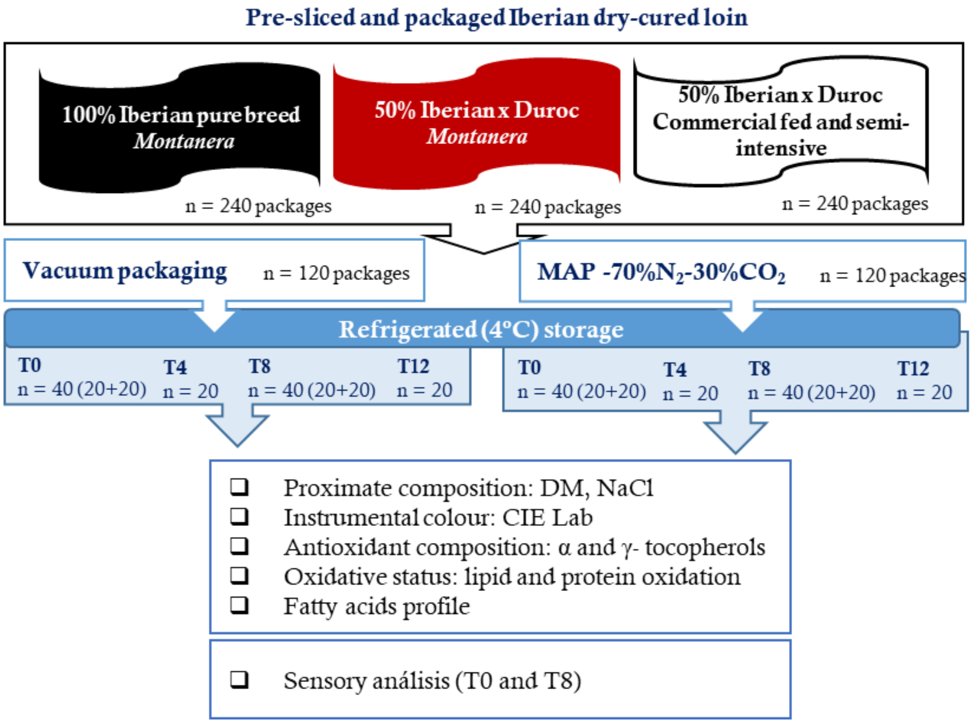

Figure V. 2. Experimental design. MAP, modified atmosphere packaging; T0, T4, T8, T12, initial, 4, 8 and 12 months of cold storage, respectively; n, number of samples; DM, dry matter; NaCl, salt content.

2.1.3. Packaging and storage

Some slices of dry-cured loin were vacuum-packaged using laminated film (O_2 permeability, 9.3 cm^3 $O_2/m^2/24$ h at 4 °C) with an EGARVAC® packaging machine. Some slices of dry-cured loin were packaged under MAP with a mixture of gases used commercially in the general manufacturing industry (70% N_2 - 30% CO_2) using an Ulma® SMART 300 packaging machine and placed in polystyrene trays (150 mm thick) with oxygen permeability of 3.2 $cm^3/m^2/24$ h/atm at 4°C and sealed with 70 mm-thick polyethylene film (VIDUCA, Alicante, Spain) with oxygen permeability of 1 $cm^3/m^2/24$ h (4°C; 50% RH), 5.5 $cm^3/m^2/24$ h (4°C; 50% RH) to CO_2 and 2.2 $g/m^2/24$ h (4°C; 90% RH) to H_2O. All packages were stored in dark cold chambers (4°C), except during the last 72 h before their analysis, at which time, they were placed under fluorescent lights (616 LUX) to simulate commercial conditions. For the purposes of their physicochemical analysis, the packages were opened after their storage time and the whole content of each tray was homogenised (IKA ULTRA-TURRAX Homogenizer T-25), except for the instrumental colour (which was measured on unopened packagings) for subsequent analysis. For the purposes of the sensory analysis, the intact slices of dry-cured loins from the trays designed for this purpose were used.

2.2. Methods

2.2.1. Proximate composition

Intramuscular fat (IMF) was carried out according to Folch, Lees, & Sloane-Stanley (1957). Dry matter (DM) was measured following the AOAC method (AOAC, 2003) and the Chloride content (NaCl) was measured using the Volhard method (AOAC, 2000). The results were expressed as g/100 g sample, as mean values.

2.2.2. Instrumental colour measurement

The following colour coordinates were determined: lightness (L*), redness (a*, red ± green) and yellowness (b*, yellow ± blue) according to the recommendations for colour determination of the American Meat Science Association (AMSA, 2011) using a Minolta CR-400 colorimeter (Minolta Camera, Osaka, Japan) with D65 diffuse illuminant/0º viewing (specular component included) and a 2.5 cm port/viewing area. The colorimeter was standardised before use with a white tile. Before reading the colour coordinates, the samples were exposed to room temperature during 30 min.

Additionally, the saturation index or chroma (C*), defined as ($C = (a^2 + b^2)^{0.5}$) and hue angle (Hº) -which measures the divergence from the true red axis of the colour space (Brewer, Novakofski, & Freise, 2006)- as arctangent (b*/a*) was determined (Wyszcecki & Stiles, 1982). The measurements were repeated at five randomly selected sites on each sample and subsequently averaged.

2.2.3. Antioxidant composition

The tocopherols (α and γ) were extracted and quantified following the method proposed by Liu, Scheller, & Schaeffer (1996). Tocopherol determination was performed on an Agilent Technologies HPLC Series 1100 instrument (Agilent Technologies, Santa Clara, CA, USA), equipped with a Kromasil Silica column (5 μm particle size, 150 x 4.6 cm) (Symta, Madrid, Spain) and a Kromasil Silica Guard Column (10 μm) (Symta, Madrid, Spain). The mobile phase was hexane:isopropanol:etanol (98.5:1:0.5 v/v), at a flow rate of 1 mL/min and the fluorescence detector (Agilent Technologies Series 1200) was fixed at λ-excitation: 295 nm and λ-emission: 330 nm. Identification and quantification of the peaks were performed by comparison with α-tocopherol and γ-tocopherol standards (0.2-14 μg/mL). The results were expressed as μg of α- or γ-tocopherol/g sample, as mean values.

2.2.4. Lipid oxidation

Lipid oxidation was measured using 2-thiobarbituric acid (TBA) according to Salih, Smith, Price, & Dawson (1987). TBA-RS values were calculated from the standard (1,1,1,3- tetraethoxypropane, TEP) curve and expressed as μg malondialdehyde (MDA)/g of sample, as mean values.

2.2.5. Protein oxidation

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

2.2.6. Fatty acids profile

The fatty acid profile was determined from the intramuscular fat extracted according to Folch et al., (1957) which was dissolved in 2 ml of hexane. Out of this, 210 μl were taken and mixed with 4 ml of hexane and 200 μl of KOH (85% in MetOH). It was vortexed and after centrifugation (10 minutes at 3000 rpm) the organic phase was collected in vials. One microlitre was injected into a gas chromatograph (model 4890 Series II; Hewlett-Packard, Palo Alto, CA, USA) equipped with a split/split-less injector and a flame ionisation detector. Fatty acid methyl esters (FAMEs) were separated on a Carbowax™ fused silica capillary column (30 m × 0.25 mm id; 0.25

μm film thickness (Ohio Valley, Marietta, OH, USA)). The oven temperature was held at 200°C. The injector and detector were set at 250°C. The carrier gas was nitrogen at 1.8 mL min^{-1}. The identification of individual FAME was based on a standard mixture of 37 Component FAME Mix (Sigma–Aldrich, Supelco 37 Component FAME Mix- CRM47885, St. Louis, MO, USA).

The amount of each fatty acid and the various fatty acid groups was calculated on the total of fatty acids detected, and the results were expressed as percentages of FAMEs, as mean values.

2.2.7. Sensory analysis

A sensory analysis was performed at the beginning (T0) and after 8 months in cold storage (T8), after ensuring microbial safety of the slices through microbiological analysis. For ensuring microbial safety, total mesophiles, coliforms, *E. coli*, *Clostridium perfringens* and *Staphylococcus aureus* counting, as well as presence/absence of *Listeria monocytogenes* and *Salmonella sp* were carried out on 5 random packages before sensorial analysis. Microbiological counts determined that the samples at the time of sensory analysis were suitable for consumption according to Commission Regulation (EC) Nº 1441/2007 and the technical regulation of the customs union 021/2011. Dry-cured loins were assessed by a trained panel of 10 members, using a quantitative-descriptive analysis method. Two of the most representative slices of dry-cured loin were selected from each sample package and presented to each panellist. Subsequently, "visual appearance" was assessed against two parameters: *redness* (intensity of red colour in the lean meat) and *marbling* (level of visible intramuscular fat). The "aroma/odour" was evaluated through parameter *aroma intensity*. The "texture" perceived while chewing (texture in the mouth) was assessed looking at *hardness* (perception of firmness during chewing), *juiciness* (impression of lubricated food during chewing) and *fibrosity* (extent to which fibre strands are noticed when chewing). "Intensity of tastes" such as *salty*, *sweet* and *sour* were also evaluated. Other parameters evaluated were the *persistence of the typical flavour* of dry-cured loin (length of time you are perceiving the flavour), *rancidity* (intensity of the rancid flavour) and *unpleasant or strange tastes*. All sessions were conducted at room temperature in a sensory room equipped with white fluorescent lighting. Water (100 mL) at room temperature was provided to the panellists between samples.

2.2.8. Statistical analysis

The statistical analysis of the data consisted of a multivariate analysis of variance (two-way ANOVA) using software package SPSS.PC+ v. 20.0, to analyse the influence of the commercial category (*Black, Red* and *White*), the type of packaging (vacuum vs. MAP) and the interaction of the two on the proximate composition, instrumental colour, antioxidant and fatty acid profile, oxidative status and sensory analysis. Thus, the model used was as follows:

$$Yijk = \mu + CCi + TPj + CCi * TPj + eijk,$$

where Yijk is the variable considered; μ is the mean value; CCi is the fixed effect of commercial category (i = 1: *Black*, i = 2: *Red,* i = 3: *White*); TPj is the fixed effect of type of packaging (j = 1: MAP and j = 2: vacuum); CCi * TPj is the interaction between the commercial category and type of packaging and eijk is the random residual.

Additionally, a one-way ANOVA test was used to study the effect of cold storage time (T0, T4, T8 and T12 months) in each commercial category and packaging individually. Also, for the sensory analysis, one-way ANOVA was used to study the effects of cold storage time (T0 and T8), according to the following model:

$$Yij = \mu + STi + ei(j),$$

where Yijk is the variable considered; μ is the mean value; STi is the effect of storage time (i =1: T0, i = 2, T4, i = 3, T8 and i = 4: T12) and ei(j) is the residual error.

Mean and standard error of mean (SEM) were reported. Tukey's HSD test was applied to compare the mean values of each group. Statistical significance was set at $P \leq 0.05$.

3. Results

With regard to proximate composition, differences of IMF between the samples from the *Black*, *Red* and *White* commercial categories (12.7, 13.5 and 21.3 ± 0.596; (standard error of the mean; SEM) g/100 g, respectively) were identified ($P \leq 0.05$), whilst no differences were identified due to packaging (15.78, 16.11 ± 0.596, for vacuum and MAP, respectively) nor cold storage (T0, T4, T8 and T12) (data not shown) ($P > 0.05$).

The initial DM and salt content (Table V. 1) showed differences deriving from commercial category ($P \leq 0.05$). Thus, DM was higher in loin slices from the *White* category ($P \leq 0.05$) compared to the *Red* and *Black* ones. These differences were maintained throughout the full storage period ($P \leq 0.05$). In general, the type of packaging did not affect DM with the exception of T8, in which slices in vacuum-packaging showed higher dry matter content than slices in MAP ($P \leq 0.01$). Also, there was an interaction effect between both factors; commercial category and packaging, at the middle and at the end of storage (T8 and T12). In refer to salt content, slices from the *Black* category yielded the highest value ($P \leq 0.001$) along the full storage period. In contrast, no differences were identified for NaCl due to the type of packaging.

As far as instrumental colour is concerned (Table V. 1), in general, there were differences amongst the various commercial categories ($P \leq 0.05$) in L*, b* and Hue, while the type of packaging affected all the coordinates ($P \leq 0.05$). The most remarkable difference throughout the storage was the decrease in a* observed in all categories as well as MAP samples ($P \leq 0.05$). Lightness, was significantly dependent on both commercial category and type of packaging. Thus, the slices from the *White* category yielded higher values than those from the *Black* category, while the *Red* category showed intermediate values ($P \leq 0.001$). This trend was maintained until the end of storage (T12), when they decreased in the slices from the *Black* and *White* ($P \leq 0.05$) categories. On the other hand, slices in the MAP showed higher lightness than those from vacuum packaging (from T0 to T12). The decrease of this parameter during storage was no significant ($P > 0.05$) for any packages. No differences were found in redness ($P > 0.05$) accounting on commercial category. In contrast, the packaging exerted a significant effect, yielding slices from MAP higher values than those in vacuum packaging ($P \leq 0.01$). Redness decreased throughout the storage time ($P \leq 0.05$) regardless commercial category and package ($P \leq 0.01$), but this was not significant for slices from vacuum packaging. With regards to yellowness, differences were identified by commercial category and packaging, with the slices from the *White* category and slices packed in MAP yielding higher values in comparison with the rest of categories and vacuum packaging, respectively, throughout the full storage time ($P \leq 0.05$). Chroma and Hue changes can be attributed to the modifications of the previous parameters. Specifically, Chroma was higher in MAP slices than in vacuum-packaged products for the full storage time. Hue yielded higher values in slices from the *White* category than those from the *Black* category, whereas the *Red* category showed intermediate values from T0 to T8. With regard to commercial category x packaging interaction, its effect on instrumental colour was slight with the exception of b* coordinate, where it was significant for almost the whole storage period ($P \leq 0.05$).

Table V. 1. Effects of commercial category and package type during the cold storage time on proximate composition and instrumental colour of sliced Iberian dry-cured loin.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
		Proximate composition (g/100 g)								
DM	T0	60.9b	60.6b	63.3a	61.7	61.5	0.200	***	ns	ns
	T4	61.3b	60.7b	62.7a	61.4	61.7	0.188	***	ns	ns
	T8	60.9b	61.0b	63.1a	61.2	62.1	0.206	***	**	**
	T12	59.9b	61.7a	62.4a	61.1	61.6	0.390	*	ns	*
	SEM	0.143	0.18	0.174	0.171	0.138				
	Sig.	0.107	0.491	0.357	0.645	0.348				
NaCl	T0	3.7a	3.4b	3.3b	3.4	3.5	0.034	***	ns	ns
	T4	3.7a	3.4b	3.4b	3.5	3.5	0.032	***	ns	ns
	T8	3.9a	3.5b	3.4b	3.6	3.5	0.037	***	ns	ns
	T12	3.7a	3.3b	3.2b	3.4	3.4	0.061	***	ns	ns
	SEM	0.027	0.028	0.031	0.023	0.030				
	Sig.	0.051	0.323	0.226	0.155	0.428				
		Instrumental colour coordinates								
L*	T0	34.8cA	37.3b	40.3aA	38.9	36.1	0.366	***	***	ns
	T4	34.9bA	36.1b	39.0aAB	37.6	35.7	0.320	***	***	ns
	T8	34.9cA	36.8b	39.5aAB	38.2	35.9	0.315	***	***	ns
	T12	34.3bB	37.1a	38.6aB	37.4	35.9	0.615	**	*	*
	SEM	0.194	0.284	0.300	0.270	0.221				
	Sig.	0.049	0.351	0.042	0.269	0.932				
a*	T0	20.5A	19.6A	19.7A	21.1A	18.8	0.242	ns	***	ns
	T4	20.0AB	19.6A	19.7A	20.9A	18	0.229	ns	***	***
	T8	19.7AB	19.2AB	18.5B	19.9AB	18.4	0.228	ns	***	ns
	T12	18.5B	18.6B	18.6B	19.5B	17.6	0.35	ns	**	*
	SEM	0.217	0.254	0.18	0.155	0.166				
	Sig	0.047	0.043	0.021	0.002	0.175				
b*	T0	12.3b	13.6ab	14.1a	14.1A	12.5	0.232	**	***	*
	T4	12.0b	12.2b	14.0a	13.3AB	12.1	0.21	***	***	***
	T8	12.1b	12.6b	13.7a	13.7AB	11.8	0.211	***	***	ns
	T12	12.1b	12.5b	13.3a	12.8B	12.4	0.355	*	ns	**
	SEM	0.180	0.238	0.158	0.141	0.173				
	Sig.	0.876	0.133	0.464	0.046	0.470				
C*	T0	24	23.9	24	25.3A	22.6	0.279	ns	***	ns
	T4	23.3	23	24.2	24.6AB	21.8	0.266	ns	***	***
	T8	23.1	23.3	23.1	24.5AB	21.9	0.303	ns	***	*
	T12	22.2	22.5	22.8	23.4B	21.6	0.39	ns	*	*
	SEM	0.229	0.344	0.202	0.175	0.209				
	Sig.	0.172	0.189	0.058	0.021	0.311				
H°	T0	30.8c	34.4b	36.0a	34.1	33.4	0.423	***	ns	**
	T4	30.7c	33.3b	35.4a	32.6	33.7	0.361	***	ns	**
	T8	31.5c	32.9b	36.5a	34.6	32.7	0.37	***	**	ns
	T12	33.1	33.9	35.5	33.3	35.1	0.782	ns	ns	**
	SEM	0.392	0.300	0.276	0.303	0.304				
	Sig.	0.392	0.252	0.514	0.054	0.22				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; SEM, standard error of the mean; DM, dry matter; NaCl, salt content; L*, lightness; a*, redness; b*, yellowness; C*, Chrome; H°, hue angle (dimensionless instrumental colour coordinates measured in CIELab space); T0, T4, T8, T12, initial, 4, 8 and 12 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category; A, B: different letters in the same column indicate significant differences due to cold storage time, both for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Results for the antioxidant composition showed a marked influence of commercial category (Table V. 2). Thus, α- and γ-tocopherol yielded the highest values in slices from the *Black* category ($P \leq 0.001$) for the full storage time (T0-T12). In general, no differences were observed due to packaging. However, during the cold storage time, both α and γ- tocopherols decreased ($P \leq 0.001$), regardless of the commercial category and type of packaging. Significant interactions between both commercial category and packaging were observed for α- and γ-tocopherol, in the former for the full storage time ($P \leq 0.05$).

Table V. 2. Effects of commercial category and package type during the cold storage time on antioxidant composition and oxidative status of sliced Iberian dry-cured loin.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
Antioxidant composition (μg g-1)										
α-Tocopherol	T0	11.8aA	7.9bA	5.9cA	8.3A	8.8A	0.295	***	ns	***
	T4	9.6aB	6.1bB	4.8cB	7.3AB	6.4B	0.248	***	**	***
	T8	9.3aB	6.3bB	4.4cB	6.8B	6.5B	0.242	***	ns	*
	T12	10.5aB	6.7bB	4.0cB	6.8B	6.3B	0.476	***	ns	*
	SEM	0.194	0.159	0.146	0.192	0.232				
	Sig.	0.000	0.000	0.000	0.013	0.000				
ɣ-Tocopherol	T0	1.5aA	1.0abA	0.7bA	1.0A	1.0A	0.050	***	ns	***
	T4	1.0aBC	0.9abB	0.7bA	0.9A	0.8A	0.034	***	*	***
	T8	1.2aB	0.8abC	0.5bB	0.7AB	0.7AB	0.040	***	ns	ns
	T12	0.9aC	0.8abC	0.5bB	0.5B	0.6B	0.054	***	ns	ns
	SEM	0.036	0.012	0.019	0.030	0.035				
	Sig.	0.000	0.000	0.000	0.007	0.030				
Oxidative status										
Lipid oxidation [a]	T0	0.9C	0.9C	0.9B	0.9C	1	0.012	ns	***	ns
	T4	1.0BC	0.9C	1.0AB	0.9C	1	0.018	ns	***	***
	T8	1.1aB	1.1aB	1.0bAB	1.1B	1	0.016	**	***	***
	T12	1.2aA	1.2aA	1.1bA	1.3A	0.9	0.056	*	***	ns
	SEM	0.021	0.018	0.013	0.019	0.010				
	Sig.	0.000	0.000	0.008	0.000	0.062				
Protein oxidation [b]	T0	3.1cB	3.3bB	3.4aB	3.3B	3.3B	0.039	***	ns	***
	T4	3.8aA	3.5bAB	3.8aA	3.7A	3.7A	0.037	***	ns	*
	T8	3.7aA	3.4bAB	3.8aA	3.6A	3.7A	0.043	***	ns	**
	T12	3.6A	3.6A	3.7A	3.6A	3.7A	0.070	ns	ns	ns
	SEM	0.047	0.034	0.038	0.035	0.034				
	Sig.	0.000	0.002	0.000	0.000	0.000				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; SEM, standard error of the mean. [a] expressed as μg malondialdehyde/g dry-cured sample. [b] expressed as nmol carbonyls/mg protein. T0, T4, T8, T12, initial, 4, 8 and 12 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category; A, B, C: different letters in the same column indicate significant differences due to cold storage time, both for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

As far as oxidative status is concerned, results show that the rate of lipid oxidation in dry-cured loin slices at T0 and T4 was similar in all commercial categories, however, for longer storage times the commercial category exerted a significant effect, with it being higher in the *Black* and *Red* categories than in the *White* one ($P \leq 0.05$) (Table V. 2). The type of packaging also had an impact on TBARS values ($P \leq 0.001$) and in this case at all storage times (T0-T12). At the earlier stages (T0 and T4), the TBARS values were noticed to be higher in vacuum than in MAP packaging. But, at later stages (T8-T12), lipid oxidation was higher in slices from MAP than vacuum packaging ($P \leq 0.001$).

With regard to protein oxidation (Table V. 2), the highest level was obtained in the slices from the *White* category, whereas those from the *Black* category yielded the lowest values, and the slices from the *Red* category revealed an intermediate pattern at T0 ($P \leq 0.001$). For the rest of times, no differences were observed between the slices from *White* and *Black* categories, being the oxidation values in these higher than those from *Red* category ($P \leq 0.001$). In contrast, the type of packaging did not affect protein oxidation ($P > 0.05$). Storage time increased the amounts of carbonyls in dry-cured loin slices ($P \leq 0.01$) regardless of commercial category and packaging. A marked effect of commercial category x packaging interaction was observed for both lipid ns protein oxidation ($P \leq 0.05$).

The most common fatty acids found were oleic acid (C18:1 n-9) and palmitic acid (C16:0) (Table V. 3). More specifically, the slices from the *White* category revealed the highest values of C16:0, stearic acid (C18:0) and the saturated fatty acid group (SFA) (Table V.3 bis), as well as palmitoleic acid (C16:1) ($P \leq 0.01$). Whilst the slices from the *Black* and *Red* categories yielded higher values for C18:1 n-9 oleic acid, linoleic acid (C18:2 n-6) and linolenic acid (C18:3 n-3) (Table V. 3), as well as the monounsaturated (MUFA) and (PUFA) fatty acid groups (Table V. 3 bis) ($P \leq 0.001$). In terms of the influence of the type of packaging, the vacuum package yielded higher SFA and lower MUFA and PUFA than the MAP, after T4 (Table V. 3 bis) ($P \leq 0.05$). Similarly, throughout storage time, the most remarkable effect was related to unsaturation. Thus, an increase in SFA, together with a decrease in the percentages of MUFA and PUFA can be seen regardless to commercial category and type of packaging (Table V. 3 bis) ($P \leq 0.05$). As far as commercial category x packaging interaction is concerned, its effect on the saturated fatty acids was slight, but it was more marked on the poly unsaturated fatty acids (C18:2 n-6 and C18:3 n-3) and therefore in the group that comprise them ($P \leq 0.05$).

Table V. 3. Effects of commercial category and package type during the cold storage time on fatty acids profile of sliced Iberian dry-cured loin.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
		Fatty acid composition (g/100g FAMEs)								
C16:0	T0	24.2cC	25.8bB	27.5aC	25.9B	25.8C	0.166	***	ns	ns
	T4	24.9cB	25.9bB	27.6aC	26.4B	25.9C	0.147	***	*	ns
	T8	25.2cB	26.4bB	28.4aB	26.3B	27.0B	0.154	***	***	ns
	T12	26.0cA	28.4bA	29.7aA	27.5A	28.5A	0.338	***	*	ns
	SEM	0.094	0.129	0.103	0.129	0.135				
	Sig.	0.000	0.000	0.000	0.004	0.000				
C16:1	T0	3.8bA	4.3aA	4.3aA	4.1A	4.1A	0.035	***	ns	***
	T4	3.4bC	3.9aB	3.9aB	3.7B	3.8B	0.035	***	ns	***
	T8	3.6bBC	4.0aB	4.0aB	3.9B	3.8B	0.033	***	ns	ns
	T12	3.7bAB	4.2aAB	4.0aB	3.6B	3.8B	0.062	**	*	ns
	SEM	0.024	0.033	0.029	0.027	0.031				
	Sig.	0.000	0.000	0.000	0.000	0.000				
C18:0	T0	10.8cB	11.4bB	12.7aB	11.6B	11.6B	0.113	***	ns	ns
	T4	11.6cA	12.3bA	13.4aA	12.4A	12.4A	0.111	***	ns	***
	T8	11.3cA	12.0bAB	13.2aA	12.0AB	12.3A	0.094	***	**	*
	T12	11.1cAB	11.9bAB	13.3aA	11.7B	12.6A	0.200	***	***	ns
	SEM	0.065	0.092	0.074	0.083	0.089				
	Sig.	0.000	0.002	0.001	0.001	0.001				
C18:1 n-9	T0	52.7aA	51.3bA	49.5cA	50.9	51.4A	0.190	***	ns	ns
	T4	51.7aB	50.1bAB	48.8cA	50.1	50.3AB	0.179	***	ns	***
	T8	52.1aAB	50.7bAB	48.9cA	51	50.2B	0.173	***	***	ns
	T12	51.8aB	49.3bB	47.8cB	50.3	48.9C	0.405	***	*	ns
	SEM	0.113	0.162	0.130	0.149	0.147				
	Sig.	0.006	0.002	0.004	0.051	0.000				
C18:2 n-6	T0	4.9aA	3.6bB	2.6cB	3.8	3.6AB	0.103	***	ns	ns
	T4	4.9aA	4.1bA	2.9cA	4	4.0A	0.095	***	ns	***
	T8	4.6aB	3.7bAB	2.5cBC	3.7	3.5AB	0.096	***	*	***
	T12	4.4aB	3.1bC	2.3cC	3.4	3.1B	0.170	***	**	*
	SEM	0.043	0.065	0.035	0.080	0.075				
	Sig.	0.000	0.000	0.000	0.219	0.006				
C18:3 n-3	T0	0.4aA	0.2bA	0.2cA	0.3A	0.3A	0.013	***	ns	***
	T4	0.4aB	0.3aA	0.2bAB	0.3A	0.3AB	0.007	***	ns	***
	T8	0.4aB	0.2bA	0.2cAB	0.3A	0.3AB	0.009	***	**	*
	T12	0.4aB	0.2bB	0.1bB	0.2B	0.2B	0.017	***	*	ns
	SEM	0.008	0.006	0.005	0.007	0.008				
	Sig.	0.000	0.000	0.000	0.038	0.040				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; SEM, standard error of the mean. FAMEs, fatty acid methyl esters; C16:0, palmitic acid; C16:1, palmitoleic acid; C18:0, stearic acid; C18:1 n-9, oleic acid; C18:2 n-6, linoleic acid, C18:3 n-3, linolenic acid. T0, T4, T8, T12, initial, 4, 8 and 12 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category; A, B, C: different letters in the same column indicate significant differences due to cold storage time, both for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Table V. 3 (bis). Effects of commercial category and package type during the cold storage time on fatty acids profile of sliced Iberian dry-cured loin.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
		Fatty acid composition (g/100g FAMEs)								
SFA	T0	36.9cB	39.4bB	42.3aC	39.7B	39.4C	0.278	***	ns	ns
	T4	39.0cA	40.9bA	43.7aB	41.4A	41.0B	0.242	***	ns	ns
	T8	39.0cA	40.9bA	44.1aAB	40.8AB	41.9AB	0.247	***	***	ns
	T12	38.8cA	42.4bA	44.9aA	41.1AB	43.0A	0.545	***	**	ns
	SEM	0.154	0.202	0.156	0.210	0.215				
	Sig.	0.000	0.000	0.000	0.012	0.000				
MUFA	T0	57.8aA	56.8bA	54.9cA	56.3A	56.7A	0.197	***	ns	ns
	T4	55.8aB	54.7bB	53.2cB	54.4B	54.8B	0.178	***	ns	***
	T8	56.1aB	55.1bB	53.3cB	55.3AB	54.4B	0.171	***	***	ns
	T12	56.4aB	54.4bB	52.6cB	55.3AB	53.7B	0.401	***	**	ns
	SEM	0.136	0.194	0.143	0.152	0.163				
	Sig.	0.000	0.000	0.000	0.000	0.000				
PUFA	T0	5.3aA	3.8bB	2.8cA	4	3.9AB	0.115	***	ns	ns
	T4	5.3aA	4.4bA	2.7cAB	4.2	4.2A	0.100	***	ns	***
	T8	4.9aB	3.9bAB	2.7cAB	3.9	3.7AB	0.104	***	*	***
	T12	4.8aB	3.2bC	2.5cB	3.7	3.3B	0.186	***	**	*
	SEM	0.047	0.071	0.039	0.086	0.082				
	Sig.	0.001	0.000	0.000	0.238	0.011				
n-6/n-3	T0	11.6cB	16.5a	14.8bC	14.6	14.0B	0.275	***	ns	***
	T4	12.7bA	16.4a	16.0aB	15.2	16.6A	0.343	***	*	**
	T8	12.5bA	15.9a	15.3aB	14.4	14.7B	0.177	***	ns	*
	T12	13.0bA	17.9a	17.3aA	15.5	16.7A	0.507	***	ns	*
	SEM	0.302	0.223	0.224	0.183	0.250				
	Sig.	0.000	0.104	0.000	0.171	0.000				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; SEM, standard error of the mean. FAMEs, fatty acid methyl esters; SFA, sum of all saturated fatty acids detected (C12:0 + C14:0 + C16:0 + C17:0 + C18:0 + C20:0); MUFA, sum of all monounsaturated fatty acids detected (C16:1 + C17:1 + C18:1 n-9 + C20:1); PUFA, sum of all polyunsaturated fatty acids detected (C18:2 n-6 + C18:3 n-3); n-6/n-3, ratio PUFA n-6/PUFA n-3. T0, T4, T8, T12, initial, 4, 8 and 12 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category; A, B, C: different letters in the same column indicate significant differences due to cold storage time, both for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Concerning to sensory analysis (Table V. 4 and V. 4 bis), redness only showed differences due to the type of packaging at T0, with the values being higher in MAP slices ($P \leq 0.01$). In contrast, marbling only varied by commercial category, with the slices from the *White* category yielding the highest value ($P \leq 0.001$). Neither the commercial category nor the type of packaging influenced the aroma intensity ($P > 0.05$).

In terms of texture traits, the slices from the *White* category showed less hardness and fibrosity (T4) and more juiciness (T0) ($P \leq 0.05$) than the *Red* and *Black* categories. Hardness and juiciness were also affected by the type of packaging (T0), with the MAP product yielding higher values for hardness and lower for juiciness ($P \leq 0.05$). The taste and flavour descriptors were not affected by commercial category nor packaging ($P > 0.05$). On the other hand, in general, juiciness decreased, and marbling, sweetness, acidity, and rancidness, increased during storage in all categories and both vacuum and MAP ($P \leq 0.05$). No significant effect was observed for sensory attributes with regard to the commercial category x type of packaging interaction, except for hardness (T8) ($P \leq 0.05$).

Table V. 4. Effects of commercial category and package type during the cold storage time on sensory analysis of Iberian dry-cured loin slices.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
				Visual appearance						
Redness	T0	7.2	7.1	7.0	7.2	6.9	0.061	ns	*	ns
	T8	7.4	7.1	7.4	7.4	7.2	0.095	ns	ns	ns
	SEM	0.104	0.098	0.087	0.080	0.074				
	Sig.	0.696	0.792	0.051	0.369	0.074				
Marbling	T0	4.2c	5.3b	6.1a	5.0	5.4	0.147	***	ns	ns
	T8	5.4 b	6.1b	7.6a	6.2	6.6	0.167	***	ns	ns
	SEM	0.181	0.190	0.177	0.156	0.168				
	Sig.	0.000	0.019	0.000	0.000	0.000				
				Odour/Aroma						
Intensity	T0	6.7	6.7	6.8	6.8	6.7	0.080	ns	ns	ns
	T8	6.8	6.8	6.8	6.7	6.9	0.104	ns	ns	ns
	SEM	0.112	0.112	0.109	0.091	0.090				
	Sig.	0.849	0.664	0.933	0.960	0.688				
				Texture						
Hardness	T0	6.2a	5.9a	5.2b	6.0	5.5	0.101	***	**	ns
	T8	5.7a	5.5ab	5.1b	5.5	5.4	0.110	*	ns	*
	SEM	0.119	0.123	0.134	0.110	0.100				
	Sig.	0.063	0.083	0.694	0.013	0.600				
Juiciness	T0	5.7b	5.9b	6.5a	5.8	6.2	0.098	***	*	ns
	T8	5.3	5.1	5.8	5.2	5.2	0.137	ns	ns	ns
	SEM	0.140	0.148	0.139	0.124	0.112				
	Sig.	0.155	0.007	0.013	0.022	0.002				
Fibrosity	T0	5.4 a	5.4 a	4.8 b	5.3	5.1	0.097	*	ns	ns
	T8	5.0	5.1	4.7	5.0	4.8	0.143	ns	ns	ns
	SEM	0.143	0.143	0.147	0.121	0.117				
	Sig.	0.115	0.205	0.589	0.137	0.218				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; T0, T8, initial and 8 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

Table V. 4 (bis). Effects of commercial category and package type during the cold storage time on sensory analysis of Iberian dry-cured loin slices.

	Cold storage time	Commercial category (1)			Package (2)		SEM	Sig.		
		Black	*Red*	*White*	MAP	Vacuum		1	2	1x2
				Taste						
Sweetness	T0	2.7	2.6	2.7	2.6	2.7	0.087	ns	ns	ns
	T8	3.2	3.1	3.3	3.1	3.2	0.152	ns	ns	ns
	SEM	0.149	0.140	0.152	0.115	0.125				
	Sig.	0.074	0.086	0.057	0.024	0.029				
Salty	T0	5.4	5.2	5.0	5.2	5.1	0.079	ns	ns	ns
	T8	5.5	5.4	5.1	5.5	5.3	0.116	ns	ns	ns
	SEM	0.125	0.113	0.112	0.096	0.096				
	Sig.	0.521	0.319	0.581	0.239	0.553				
Sour	T0	2.4	2.3	2.2	2.3	2.3	0.060	ns	ns	ns
	T8	3.4	3.6	3.1	3.2	3.5	0.173	ns	ns	ns
	SEM	0.158	0.164	0.145	0.121	0.133				
	Sig.	0.001	0.000	0.002	0.000	0.000				
				Flavour						
Rancidity	T0	1.1	1.1	1.0	1.1	1.0	0.024	ns	ns	ns
	T8	1.6	1.7	1.5	1.6	1.7	0.101	ns	ns	ns
	SEM	0.096	0.079	0.085	0.072	0.069				
	Sig	0.004	0.000	0.001	0.002	0.000				
Flavour persistence	T0	6.3	6.0	6.1	6.1	6.1	0.108	ns	ns	ns
	T8	6.2	6.1	6.3	6.2	6.2	0.154	ns	ns	ns
	SEM	0.160	0.153	0.162	0.126	0.133				
	Sig.	0.776	0.606	0.545	0.881	0.603				
Strange tastes	T0	1.4	1.8	1.4	1.6	1.4	0.072	ns	ns	ns
	T8	1.6	2.0	1.5	1.7	1.7	0.092	ns	ns	ns
	SEM	0.091	0.114	0.089	0.083	0.080				
	Sig.	0.259	0.347	0.632	0.448	0.173				

Values were presented as mean values. *Black* (100% Iberian pig and reared outdoors with acorns and pasture); *Red* (50% Iberian pig and reared outdoors with acorns and pasture); *White* (50% Iberian pig and reared indoors and fed on commercial fodder); MAP, modified atmosphere packaging; T0, T8, initial and 8 months of cold storage, respectively. Sig, significance; a, b, c: different letters in the same row indicate significant differences due to commercial category for $P = 0.05$ according to Tukey´s HSD test. ns ($P > 0.05$); * ($P \leq 0.05$); ** ($P \leq 0.01$); *** ($P \leq 0.001$).

4. Discussion

The results obtained for IMF in the current research disagree with previous studies on both fresh (Tejerina et al., 2012a) and dry-cured loins (Ventanas, Ventanas & Ruiz, 2007), in which meat and meat products from Iberian purebred pigs reared in the *Montanera* system (corresponding to *Black* category in the current study) yielded higher IMF than those from Iberian x Duroc crossed pigs reared in *Intensive* systems and fed on commercial fodder (*White* category). Discrepancies observed among studies may derive from the variability and origin of the samples in each commercial category.

Regarding the highest DM content observed in slices from the *White* category, it could be associated to its higher IMF content (Noidad, Limsupavanich, Suwonsichon, & Chaosap 2019). In turn, the lower IMF content of slices from the *Black* category together to the lower weight of the pieces before technological process of curing may have led to the higher values of salt content observed on these, since the seasoning was similar among all the commercial categories (Ventanas et al., 2007).

The differences in the IMF content could have also promoted the lightness differences among commercial categories. Thus, the higher IMF values yielded by slices form *White* category could be responsible of the higher lightness of these. Additionally, not only the amount of IMF should be taken into account, but also the fatty acid profile of that. Thus, the higher content of saturated fatty acids in the IMF of slices with the *White* category could have also led to their higher lightness values (Carrapiso & García, 2005). With respect to the higher lightness observed in slices from MAP in comparison with those from vacuum packaging during the full storage period (T0-T12), it could be due to a whitening layer identified in the former, although as far as we know, there is no scientific literature to support this. In contrast, previous studies carried out on Iberian dry-cured ham did not report variation in lightness associated to packaging (García-Esteban et al., 2004; Parra et al., 2012).

In refer to redness as affected by type of packaging, the higher permeability of oxygen of the vacuum package could be an explanation for the lower redness values observed in vacuum packaging samples with respect to MAP ones, which was maintained during the full storage period. On the other hand, the redness decrease throughout the storage period in samples from MAP may be explained by the presence of residual oxygen in the headspace of the latter (García-Esteban et al., 2004), since efficiency in the meat industry's packaging techniques has its limits, and residual oxygen levels achieved values from 2% to 5%. The decrease in a* observed in all categories was no unexpected. Colour fading of dry-cured meat products during storage is normally attributed to the oxidation of red pigments such as nitrosyl myoglobin, which is highly unstable in the presence of oxygen, resulting in the formation of metmyoglobin, which is primarily responsible for meat browning.

With regard to yellowness, the higher values found in slices with the *White* category may be attributed to the higher IMF content of these (Olivares, Navarro, Salvador, & Flores, 2010). The lack of differences in yellowness during storage (T0-T12 months), was unexpected, since previous studies have related lipid oxidation with the decrease of b* (Carrapiso & García, 2005), which increased with storage time. Maybe the differences in the lipid oxidation values identified in this study along the storage period were not enough as to allow to establish differences in this colour coordinate.

In refer to differences in tocopherol content among the commercial categories, these were not unexpected, since there is scientific consensus about the effect of the systems in which the animals are reared and more specifically of the feed provided on antioxidant content (Tejerina et al., 2012a). Thus, the higher content of α and γ-tocopherol in dry-cured loin slices from animals reared in *Montanera (Black* and *Red* categories) could be attributed to their intake of grass and acorns, respectively (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2011). On the other hand, differences in the tocopherol content between the *Black* and *Red* categories could be due to factors relating to the feeding provided during the *Montanera* period, such as the variety of the natural resources (acorn and grass) or the quality or length of feeding (Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012b), since crossbreeding did not prove to be of great influence on antioxidant content (Ventanas, Estevez, Tejeda, & Ruiz, 2006).

In the same way, the feeding provided would explain differences observed in the fatty acid profile accounting on commercial category. Thus, the higher MUFA -and specially C18:1 n-9- and PUFA as well as fatty acids which comprise this group; C18.2 n-6 and C18:3 n-3- of slices with *Black* and *Red* categories with respect to slices with *White* one would be associated to the acorns and grass intake, respectively, of animals during the finishing period in *Montanera* (Tejerina et al., 2012a, 2012b). The differences found between the *Black* and *Red* categories for these fatty acids' groups and the individual fatty acids that comprise them could be attributed to both the variability of the *Montanera*´s sources (Tejerina et al., 2012b) and the racial percentage. In this sense, Fuentes, Ventanas, Ventanas and Estévez, (2014) reported a lower MUFA content in m. *biceps femoris* in dry-cured ham from Iberian x Duroc crossed bred pigs in comparison with Iberian purebred ones, but in animals reared indoors and fed on commercial fodder. The decrease in the unsaturation within the storage time with regard to commercial category and packaging could be attributed to lipolysis or oxidation processes, usually affecting PUFA (Ventanas et al., 2006). This decreased affected in a higher extent to the vacuum-packed slices due to the higher permeability of oxygen, despite the fact that lipid oxidation was higher in slices packed in MAP ($P \leq 0.05$). In a similar way, the higher decrease of PUFA and C18:2 n-6 within storage time in the slices from the *Black* and *Red* category with respect to those from *White* one, may be explained by several factors, such as lipolytic activity, as a consequence of genetic factors (Muriel, Andrés, Petrón, Antequera, & Ruiz, 2007) and the differences in lipid oxidation processes due to both fatty acid and antioxidant profile (Daza, Rey, Ruiz, & López-Bote, 2005). Indeed, higher lipid oxidation were observed in these categories, and also previous studies have reported that lipolysis and oxidation reactions affect PUFA preferentially (Gandemer, 2002).

With respect to the lipid oxidation according to the type of packaging, the higher values of slices vacuum packaged at the earlier stages (T0 and T4) may be due to the higher permeability of oxygen in this type of packaging (Berruga, Vergara, & Gallego, 2005). Nevertheless, at the middle and at the end of storage (T8 and T12), the greater conservation of lipid oxidative stability was observed in vacuum packaging, which is consistent with the findings in previous studies on sliced dry-cured ham (Cilla et al., 2006) and Iberian dry-cured ham (Parra et al., 2010; 2012), although the latter considered lower storage times than the present study.

The lipid and protein oxidation followed the same trend along storage period, which would indicate a likely relationship between them, since both are affected by unsaturated fatty acids, pigments, transition metals and other compounds (Estévez, Ventanas, Heinonen, & Puolanne, 2011). Since lipid oxidation did not increased until after eight months of storage, our results suggest that protein oxidation could have begun earlier than lipid oxidation reactions, as has been previously reported in muscle by Davies & Goldberg (1987), since proteins are present at the aqueous phase in which many radicals are formed (Soyer & Hultin, 2000).

In refer to sensory attributes, the visual appearance was partially affected by the commercial category, being the higher marbling reported for slices with *White* category in line with the results observed for IMF.

The lack of differences on redness on account on commercial category is in agreement with the findings of Ventanas et al. (2007), in which the redness of Iberian dry-cured loins was not influenced by either crossbreeding or rearing system. Meanwhile, the lack of differences in aroma intensity may be partly explained by the use of paprika and garlic during the seasoning of loins, which might have masked the possible differences in aroma due to the commercial categories and packaging given by the differences in the fatty acid profile (Muriel, Ruiz, Martin, Petrón, & Antequera, 2004; Ruiz, Cava, Antequera, Martín, Ventanas, et al., 1998).

The less hardness and fibrosity and more juiciness observed in the slices from the *White* category could be explained by the higher values of IMF (Ruiz, Ventanas, Cava, Andrés, & García, 2000) and marbling of these. On the other hand, the general increase of rancidness along storage period with regard to commercial category and packaging could be linked to the increase in TBARS.

With regard to the commercial category x type of packaging interaction, its effect on the proximate composition and instrumental colour was slight, but it was marked on the antioxidant and fatty acid profile as well as oxidative status. Henceforth, significant interaction would indicate that depending on the commercial category of pre-sliced packaged dry-cured loin considered -which include genetic background and production system- one of the packaging could be more appropriate than another, especially to retard the lipolysis and oxidative phenomena. More specifically, slices from *Black* and *Red* categories may be more suitable to be vacuum packaged because of its greater conservation of lipid oxidative stability in comparison with MAP ($\Delta_{T12\text{-}T0}$ Vacuum packaging = 0.1, $\Delta_{T12\text{-}T0}$ MAP = 0.4), given the higher susceptibility to lipid oxidation of these two categories with respect to those from the *White* one ($\Delta_{T12\text{-}T0}$ *Black* = 0.3; *Red* = 0.3; *White* = 0.2).

In contrast, regarding commercial category x type of packaging interaction on parameters results from the sensory analysis, only hardness (T8) was affected, so interaction would not have a marked practical repercussion on consumer evaluation and purchasing.

Recently, Carrapiso, Tejeda, Noguera, Ibáñez-Escriche, & González, (2020) reported an effect on fatty acid profile from subcutaneous back fat and mainly on sensory characteristics on Iberian dry-cured shoulder due to interactions derived from a mixed model, but considering the Iberian genetic line and diet composition of pigs reared indoors. But, to our knowledge, there is no information about the effect of interaction between commercial categories compiled by the current QS or even genetic background and production system to which animals are subjected with type of packaging used in the derived meat nor dry-cured products to compare our results.

5. Conclusion

The results obtained in the current research indicate differences in pre-sliced packaged Iberian dry-cured loin quality traits amongst commercial categories, especially in terms of physicochemical qualities, and for which the trend along the long-term storage was similar. Differences in antioxidant and fatty acid profile among commercial categories would support the stronger influence exerted by the production system -*Montanera*- compared to the racial factor.
Also, our results suggest a better preservation of the oxidative status of vacuum with respect to MAP.

In a similar way, the commercial category x type of packaging interaction should be considered when selecting the more appropriate packaging to package a particular commercial category because of its repercussion on the oxidative status.

Further studies should be conducted in the improvement of these packaging types (i.e., active packaging; packages permeability to oxygen) and how could increase the shelf-life of sliced Iberian dry-cured loin.

Credit author statement

R. Contador: Methodology, Formal analysis, Writing - original Draft. A. Ortiz: Writing - original Draft. M. R. Ramírez: Review & Editing. S. García-Torres; Review

& Editing. M. M. López-Parra: Review & Editing. D. Tejerina: Conceptualization, Funding acquisition, Investigation, Review & Editing.

Declaration of competing interest

All authors state no actual or potential conflict of interest including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work.

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Chapter VI: Near Infrared Spectroscopy (NIRS) as tool for classification into official commercial categories and shelf-life storage times of pre-sliced modified atmosphere packaged Iberian dry-cured loin

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Near Infrared Spectroscopy (NIRS) as tool for classification into official commercial categories and shelf-life storage times of pre-sliced modified atmosphere packaged Iberian dry-cured loin

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Abstract

The Iberian sector needs to assure the quality standards and to protect the authenticity of its products. The potential of Near Infrared Spectroscopy (NIRS) combining different spectral treatments and classification models was assessed for the classification of individual pre-sliced Iberian dry-cured loin packaged under modified atmosphere packaging (MAP) into three official commercial categories (which involve the breed purity and production system) according to the current Iberian Quality Standard (*Black, Red* and *White*) and for the assignment to the shelf-life time (0, 4, 8 and 12 months). External validation results provided acceptable results, with up to 100% of samples correctly assigned to *Black* and *White* official commercial category and into all storage times. Actually the 100% assignments were obtained using more than one approach (SIMCA and LDA for commercial categories and PLS-DA, SIMCA and LDA for storage times). These results might contribute to support the on-line control of authenticity of official commercial category and to facilitate the monitoring of the quality of pre-package dry-cured products throughout the shelf-life prediction.

Keywords: PLS-DA, LDA, SIMCA, authentication, food control, breed purity, feeding regime.

1. Introduction

Iberian dry-cured products, are widely appreciated and very valuable in the European market (Pugliese & Sirtori, 2012) due to their sensory attributes. These coming from *Montanera* system (outdoor rearing with the finishing phase based on natural resources, mainly acorns and grass) attain the highest quality (Díaz-Caro, García-Torres, Elghannam, Tejerina, Mesías, et al., 2019). Nevertheless, given to the geographical limitations and seasonality to which *Montanera* production system is subjected, the use of crossbreeding with Duroc breed (Ramírez & Cava, 2007) and intensive system (indoor rearing and fed based on commercial fodder) have been included in the Iberian production schemes to fulfil the growing demand of Iberian products (Pugliese & Sirtori, 2012). The variety in these production factors leads to various quality standards degrees in the derived products, which are compiled by the current Spanish Iberian Quality Standard (QS) (RD 4/2014). Thus, four official quality categories are contemplated, which commercially are labelled with different colours; "*Black*" (100% Iberian pigs finished in *Montanera*), "*Red*" (at least 50% Iberian pigs finished in *Montanera*), "*Green*" (at least 50% Iberian pigs reared in outdoor system and fed with commercial fodder and/or acorns and grass) and "*White*" (at least 50% Iberian pigs reared indoor and fed based on commercial fodder) (RD 4/2014).

The control authentication of these official commercial categories begins to be guaranteed at the farm level by farm inspections, resulting in high time-consuming practices and in the impossibility to provide individual animal traceability. Recently, the feasibility of the near infrared spectroscopy technology (NIRS) for the commercial category discrimination of individual Iberian pig carcasses according to the previous RD 1469/2007 (Zamora-Rojas, Pérez-Marín, De Pedro-Sanz, Guerrero-Ginel, & Garrido-Varo, 2012) and the current QS (RD 4/2014) (Horcada, Valera, Juárez, & Fernández-Cabanás, 2020; Pérez-Marín, Fearn, Riccioli, De Pedro, & Garrido, 2021) have consolidated NIRS technology as a tool to support the official control of the Iberian pig carcasses, and also Horcada et al., (2020) obtaining good classification results measuring in live animals.

On the other hand, new selling formats for Iberian dry-cured products such as vacuum and modified atmosphere packaging (MAP), have gained importance due to the convenience in the purchasing and use up, at the expense of the traditional commercialization as a whole piece. In this regard, studies carried out on Iberian dry-cured ham highlighted the relevance of MAP format over the vacuum-packaging because the product presentation is more similar to the original hand-sliced and solves the problems of slice adherence of the latter (García-Esteban, Ansorena, & Astiasarán, 2004; Parra, Viguera, Sánchez, Peinado, Espárrago, et al., 2010 et al., 2010, Parra, Viguera, Sánchez, Peinado, Espárrago, et al., 2012). So, rigorous control procedure is required to ensure the official commercial category of these pre-sliced packaged products, which might be susceptible to fraudulent practices because of its spread with regards to the original whole piece, and where to the best of our knowledge, the scientific literature is inexistent.

Additionally, guarantying the quality of pre-sliced packaged Iberian dry-cured products evaluating its shelf-life at different refrigerated storage times is essential, especially at industrial scale. In fact, the latter might be reduced mainly due to the increase of oxidative processes and alterations of fatty acid profile, colour and other nutrients after slicing, especially in MAP, as concluded Parra et al., (2010) for pre-sliced packaged Iberian dry-cured ham. In consequence, a more frequent and suitable quality control is required in these new selling formats, and, given the high value added, the sector requires that these are by faster and non-destructive methods. Thus, it would be also interesting that NIRS technology could be used as an alternative tool to conventional analysis, to discriminate between products from different refrigerated stored time obtaining a feasible method for quality control in production line.

However, although NIRS has been widely applied to predict several quality parameters such as acid profile in live Iberian pigs (Pérez-Marín, De Pedro-Sanz, Guerrero-Ginel, & Garrido-Varo, 2009), carcasses, subcutaneous back fat (Gonzalez-Martín, Gonzaíez-Pérez, Hernández-Ménde, & Alvarez-García, 2003; Pérez-Marín et al., 2009), fresh meat (Cáceres-Nevado, Garrido-Varo, De Pedro-Sanz, & Pérez-Marín, 2019; González-Martín et al., 2003) and dry-cured products (Fernández-Cabanás, Polvillo, Rodríguez-Acuña, Botella, & Horcada, 2011; Tejerina, García-Torres, Cabeza de Vaca, Ortiz, & Romero-Fernández, 2018) from

Iberian pigs, there is not scientific literature dealing with NIRS feasibility to monitories the shelf-life of pre-sliced packaged Iberian dry-cured products without opening the packaging as non-destructive form.

In this framework, the purpose of this piece of research is to evaluate the feasibility use of NIRS technology to classify into various official commercial category (*Black, Red* and *White*) defined by the current QS (RD 4/2014) pre-sliced MAP Iberian dry-cured loin, and to identify several refrigerated storage times (T0, T4, T8 and T12) by various classification approaches on unopened packages, combining different spectral pre-treatments and classification models.

2. Material and methods

2.1. Exterimental design

A total of 24 Iberian dry-cured loins belonging to three commercial categories defined by the QS (RD 4/2014); *Black, Red* and *White* (n = 8 per category) were randomly selected and purchased from a pork manufacture industry. The fourth category defined in the QS (*Green* category) was not included in the study because of its great variability, since compile animals from production systems with intermediate characteristics between intensive and extensive farming and breed purity from purebred to Duroc-crossed. The production system conditions of animals from which Iberian dry-cured loins derived were those compiled in the QS (RD 4/2014) guidelines. Thus, animals from *Black* and *Red* categories were reared extensively: under free-range system in *dehesa* ecosystem (rangelands with evergreen oaks and pastures that are found in the Southwest of the Iberian Peninsula), for at least 60 days, with a minimum weight increase in this finishing phase of 46 kg from the *ad libitum* acorns and pasture consumption. In both, the minimum age at slaughter was 14 months, being the Iberian breed purity of 100% Iberian and 50-75% Iberian x Duroc for animals with *Black* and *Red* category, respectively. The minimum carcass weight were 108 kg and 115 kg, for animals with *Black* and *Red* category, respectively. Animals from *White* category were reared under a minimum area of 2 m^2/animal (semi-intensive conditions) for at least 60 days previous to slaughtering, with the *ad libitum* intake of commercial fodder, and slaughtered with a minimum age of 10 months. The minimum carcass weight was 115 kg.

Iberian dry-cured loins were manufactured according to the specifications of the QS (RD 4/2014). Raw material (muscle *Longissimus thoracis et lumborum*) was seasoned with a mixture of salt (2.5%), paprika (0.6%) and 0.9% of authorised preservers and stabilisers specially prepared for this type of meat products (E-250, E-252, E-331). Afterwards, loins were kept at a temperature of 4ºC for 48 h -in darkness- to allow the seasoning mixture to distribute into the meat. Subsequently, they were stuffed into in 6-7 cm diameter natural casing, and dry-curing according to industrial manufacturing process in a drying chamber. The curing length (always over 70 days (RD 4/2014)) varied according to the weight and commercial category; 85, 80 and 70 days for *Black*, *Red* and *White*, weighting an average of 1.2 ± 0.2; standard error (SE), 1.2 ± 0.3 and 1.4 ± 0.3 kg per piece, respectively. The technological process of curing started holding loins for over 30 days at 4 ± 2ºC and a relative humidity between 75 and 80%. Then, loins were ripened at temperatures which ranged from 10 to 16ºC whereas relative humidity decreased from 75 to 65% during the rest of the curing length.

Subsequently, dry-cured loins were sliced in 2 mm thick slices and homogeneously distributed in 100 g modified atmosphere packaging (MAP) (70% N_2-30% CO_2) in an Ulma® SMART 300 packaging machine, using polystyrene trays (150 mm thick) with an oxygen permeability of 3.2 $cm^3/m^2/24$ h/atm at 4ºC and sealed with 70 mm thick polyethylene film (VIDUCA, Alicante, Spain) with an oxygen permeability of 1 $cm^3/m^2/24$ h (4ºC; 50% RH), 5.5 $cm^3/m^2/24$ h (4ºC; 50% RH) to CO_2 and 2.2 $g/m^2/24$ h (4ºC; 90% RH) to H_2O. A total of 168 packages were used for the present research (n = 55, 56 and 57 for *Black, Red* and *White* category, respectively). All packages were stored in refrigeration chambers (4ºC) in darkness and sampled at: T0 (initial, n = 51), T4 (4 months of storage, n = 51), T8 (8 months of storage n = 50) and T12 (12 months of storage, n =16). T12 included lower samples due to the collapse of the packaging of some of them, which did not allow for an adequate NIR measurement. Thus, only those that showed an intact packaging were used.

2.2. NIRS spectra collection and spectral pre-treatment

NIRS reflectance spectra were collected using a LabSpec 2500 (ASD Inc., USA) NIRS spectrometer fitted with an ASD fibre-optic Contact Probe® (21-mm window diameter), previously calibrated using a spectralon tile as the white reference. Since the aim of this study was to obtain predictive models without opening the packages, the calibration was carried out with the spectralon tile covered with the same material with which the product was packaged (polystyrene; 150 mm thick). One spectrum (which was an average of 40 scans over the range of 1000 to 2500 nm) per sample was collected with ASD contact probe® by direct contact sensor-sample (package) and making a zigzag scanning throughout the surface to increase the sampling area and reducing the sampling error. The outer temperature was kept at 24 ± 1°C in the laboratory to avoid interferences in sensitiveness of NIR spectra acquisitions. Instrument control and initial spectral manipulation were performed with Indico TM Pro software package (Analytical Spectral Device-ASD Inc., Boulder, CO). Subsequently, data were imported into Unscrambler X vs 10.5 (CAMO® Trondheim, Norway) for successive chemometric analysis.

Outlier were identified during model development by principal component analysis (PCA) and removed. The criteria for deleting outliers in this study were 1) samples with residuals higher than 2; 2) samples with large leverage (H): samples with leverage higher than 3 times the average leverage, where the average leverage was calculated as (Faber, 1999):

$$H = \frac{1}{\mathrm{n} + \frac{number\ of\ principal\ components}{n}},$$

being "n" the number of samples.

The remaining spectres (164) and the distribution according to the calibration and validation sets for both commercial category and storage time factors was as follow: *Black*: n = 52 (37 + 15), *Red*: n = 56 (41 + 15), *White*: n = 56 (40 + 16), T0: n = 50 (36 + 14), T4: n = 50 (38 +12), T8: n = 48 (36 +12), T12: n = 16 (8 + 8), for calibration and validation sample sets, respectively. The data split between calibration and validation subsets (70% and 30%, approximately of total samples) was carried out by manual and random selection, ensuring the representation in each subset of samples from all classes (*Black*, *Red, White* and T0, T4, T8, T12) from both commercial category and refrigeration storage time factors, respectively, in order to maximize variability in both calibration and validation sample tests.

The cleaned data set was subjected to several mathematical pre-treatments in order to optimize the accuracy of calibration models, including SNV (Standard Normal Variate), detrend correction (DE) (Barnes, Dhanoa, & Lister, 1989) in order to reduce scattering effect of light, and first or second order Savitzky-Golay derivatives (Savitzky & Golay, 1964). Specifically, the first order derivative with: 4 smoothing left and right-side points (symmetric Kernel), and first polynomial order (1,4,4,1) and the second order derivative: with 5 smoothing left and right-side points, and second polynomial order (2,5,5,2) were performed. Furthermore, combinations of these abovementioned pre-treatments (SNV-DE; SG 1,4,4,1-SNV; SG 1,4,4,1-SNV-DE; SG 2,5,5,2 -SNV and SG 2,5,5,2- SNV-DE) were also tested within the two spectral range; 1000 to 2300 nm and 1000 to1800 nm.

2.3. NIRS classification

Various classification models were evaluated; Partial least squares-discriminant analysis (PLS-DA), soft independent modelling of class analogies (SIMCA) and linear discriminant analyse (LDA) using Unscrambler X vs 10.5 software (CAMO® Trondheim, Norway) in order to classify pre-sliced Iberian dry-cured loin packaged under MAP according to its official commercial category (*Black, Red* and *White*) and refrigerated storage time (T0, T4, T8 and T12).

2.3.1. PLS-DA

PLS-DA algorithm correlates spectral variations and category classes, maximizing the covariance between them. The independent variables (X) were the NIRS spectrum of each sample. The dependent variables (Y) were categorical or "dummy" variables (commercial categories or storage times) defined by assigning different values to the different classes (*Black, Red* and *White* or T0, T4, T8 and T12, respectively). Thus, a value of 1 is assigned when the sample belongs to the class and 0 when not (Naes, Isaksson, Fearn, & Davies, 2002). Under these assumptions, it is possible to use traditional regression methods to operate classification, computing a calibration model relating the matrix of predictors and this dummy matrix of responses. The core of the PLS-DA approach is the use of Partial Least Squares regression (Wold, Martens, & Wold, 1983), which operates a bilinear decomposition of both the X- and Y-spaces, under the assumption that a relationship between the two internal spaces exists, to compute the model parameters. Cross-validation with the leave-one-out method was performed to determine the number of factors or latent variables (LVs) in the models, avoiding the overfitting. The predictive ability of the model was analysed based on the highest value of the determination coefficient (1-VR) and the lowest root mean square error of cross validation (RMSECV) and number of LVs.

2.3.2. SIMCA

In contrast with PLS-DA, in problems like those concerning the fast-classification of quality meat on-line, for real-time decision making, we consider interesting to assess whether the unknown sample is compatible with the model of a specific category, which is exactly what SIMCA does.

Thus, SIMCA, is a class-modelling method in which each class (*Black, Red* and *White* and T0, T4, T8 and T12, for commercial category and storage time, respectively) is independently modelled throughout a PCA model, determining the centroid and dispersion of the samples for each class. The dimension of the individual model is given by the number of principal components (PCs) that have been considered. Each sample may be classified into one, several or any of the classes, depending on the distance of the samples to the centre of the model (leverage), reporting on the placement of the sample projected on the PCs and by the distance of the sample from the model defined by the PCs (S distances). The evaluation of the models was based in both the percentage of samples in a class that the model correctly recognized or *sensitivity* (SE) and the percentage of samples which did not belong to a class and were correctly rejected or *specificity* (SP) in the calibration sample set (Oliveri, Malegori, & Casale, 2019). If a sample belonged to two or more groups, it was classified in the one with the lowest values of leverage and S distances.

2.3.3. LDA

LDA is used to find the linear combination of features which best separates the different classes. For LDA, a previous reduction of variables using PCA was applied, since data set contained more variables (spectra) than samples. Thus, differences between classes (*Black, Red* and *White* and T0, T4, T8 and T12, for commercial category and storage time, respectively) were maximized, whilst variance within-class is minimized by running a PCA-LDA, guaranteeing maximal separability (Chen, Zhao, & Cai, 2008; Liu, Cozzolino, Cynkar, Gishen, & Colby, 2006). Mahalanobis method was used in the present study as a measure of class distance (Naes et al., 2002) from the optimal number of factors in the performance of the PCA-LDA discrimination model. The evaluation of the models was based in both SE and SP in the calibration sample set (Oliveri et al., 2019).

3. Results and discussion

3.1. NIR spectral features

The Figures VI. 1S, and VI. 2S (supplementary material) shows the raw NIR data set spectra from both calibration and validation sets, respectively, collected from unopened packages of sliced Iberian dry-cured loin and grouped according to the commercial category (*Black*, *Red* and *White*) (Figures VI. 1S A and VI. 2S A) and storage time (T0, T4, T8 and T12) (Figures VI. 1S B and VI. 2S B).

Obtaining spectra with high-quality is essential for the construction of reliable discriminant models and consequently to discriminate amongst commercial categories and storage time, respectively.

In general terms, spectra data adhered to a similar shape regardless of commercial categories or storage time, given by the coincidence of optical features along the whole spectra range (1000-2300 nm), even though there were differences in the intensity absorbance. More specifically, absorption bands frequencies around 1050 and 1260 nm showed the two main absorption dominated bands. Also, a slightly absorption band was identified at 1700 nm.

On the other hand, it can be observed (Figure VI. 3S A) that in general, spectra data from *Black* and *Red* category overlapped throughout the whole wavelength range (1000-2300 nm), showing lower absorbance intensity than spectra with *White* category.

Furthermore, spectra from the different storage times considered displayed a gradual absorbance intensity from T0 (lowest value) to T12 (highest value) over the whole range (1000-2300 nm), mainly in the main absorption band frequencies; 1050 and 1260 nm. This pattern was more visible observing mean spectra from calibration set (Figure VI. 3S B).

Spectra also displayed areas with low signal/noise ratio, specifically from 1800 to 2300 nm.

3.2. NIRS qualitative predictive models of commercial categories

PLS-DA, SIMCA and LDA results within the official commercial categories (*Black, Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model are shown in Tables VI. 1, VI. 2 and VI. 3, respectively.

3.2.1. PLS-DA of commercial categories

The pre-processing transformations considered were evaluated empirically. Thus, results within the official commercial categories of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model developed by PLS-DA are summarized in Table VI. 1, whereas the Table VI. 1S of the supplementary material shows the PLS-DA full results for all math pre-treatments and spectra range.

Table VI. 1. PLS-DA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

				Cross-validation			External validation		
Commercial category	pre-treatment	Range	LVs	n	1-VR	RMSECV	n	SE	SP
Black	SG 1,4,4,1 SNV-DE	1000-1800	7	37	0.729	0.244	15	60.00	74.19
Red	SNV-DE	1000-2300	12	41	0.676	0.274	15	46.67	54.84
White	SNV-DE	1000-1800	11	40	0.634	0.289	16	43.75	73.33

SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; SNV, standard normal variate; DE, de-trending; *Black, Red* and *White,* official commercial categories according to the current Iberian Quality Standard (RD 4/2014); LVs, latent variables or number of partial least square terms; n, number of samples used for cross-calibration and external validation, respectively; 1-VR, coefficient of determination in cross-validation; RMSECV, root mean square error of cross validation; SE, sensitivity; SP, specificity.

The best fitting prediction model for *Black* category was obtained with the first order Savitzky-Golay derivative pre-treatment (SG 1,4,4,1) in combination with the SNV-DE, and using the spectra range from 1000 to 1800 nm, being the 1-VR and the RMSECV 0.729 and 0.244, respectively (Table VI. 1). For *Red* and *White* categories, the best models were obtained after SNV-DE, but using the full range (1000-2300 nm) and the bounded one (1000-1800 nm), respectively (Table VI. 1). The cross-validation statistics for the latter two categories were both similar and slightly lower than those obtained for the *Black* one. The PCA plots after these abovementioned pre-treatments and ranges are represented in Figure VI. 4S (supplementary material), which graphically present the sample grouping according to official commercial categories. The prevalence of the bounded range for obtaining the best models could be explained by the fact that most useful data was available between the 1000 and 1800 nm of the spectral range, given that this area displayed high signal/noise ratio. Above this wavelength, low signal/noise areas with limited useful spectral information were observed (Figure VI. 1S).

As far as external validation results are concerned, the best SE and SP were obtained for *Black* category with a 60% and 74.2%, respectively (Table VI. 1). Regarding to external validation results for *Red* and *White* categories, the SE decreased to 46.7% and 43.8%. The SP obtained from the model for *White* category maintained similar SP to that observed for *Black*, whilst it was lower for the *Red* one.

To our knowledge, the literature concerning the application of qualitative models obtained by NIRS to the official commercial categories compiled by the current QS is very scarce and inexistent on dry-cured products. The external validation results obtained in the current research were lower than those obtained by Horcada et al., (2020) in a similar study but in fresh meat (*psoas major* muscle), except for the SE of the *Red* commercial category. In contrast, the trend observed in the current research was consistent with these latter authors, in which better SE was obtained for fresh meat with the *Black* category in comparison with that with the *Red* and *White* ones. The possible lower degree of variability within pre-sliced packaged Iberian dry-cured samples with *Black* category (since the only genetic background for animals reared under *Black* category was 100% Iberian) with respect to those with *Red* and *White* categories (in which percentage of Iberian breed can range from 50 to 75%) might be responsible for the better discriminant ability for the former (Horcada et al., 2020; McDevitt, Gavin, Andres, & Murray, 2005).

The ability to discriminate among commercial categories could be attributed to spectra differences. Thus, the spectra from the *Black* and *Red* categories samples practically overlapped over the entire range, while the spectra from the *White* category attained higher absorbance intensity (Figure VI. 1S A). These minimal differences may be sufficient to predict the commercial category of pre-sliced Iberian dry-cured loin packaged under MAP. More specifically, absorbance differences observed at around 1270 nm (Figure VI. 1S), which would correspond to the second overtone of the C-H bonds (Murray & Williams, 1987), could reflect differences in the lipids, specifically to fatty acid profile accounting on the commercial category (Alamprese, Casale, Sinelli, Lanteri, & Casiraghi, 2013; Dumalisile, Manley, Hoffman, & Williams, 2020; Silva, Folli, Santos, Barros, Oliveira, et al., 2020). These results are also consistent with previous studies dealing with the influence of animal feeding regime on fatty acid profile of the meat and meat products derived (Garrido & De Pedro, 2007; Pérez-Marín, De Pedro-Sanz, Guerrero-Ginel, & Garrido-Varo, 2009; Tejerina, García-Torres, Cabeza de Vaca, Vázquez, & Cava, 2012), and would confirm the higher influence of production system (*Black* and *Red* vs. *White*) compared to the breed purity (*Black* vs. *Red*) on the compositional differences and final quality of meat and meat products (Horcada et al., 2020; Zamora-Rojas et al., 2012).

Several studies have previously evaluated the potential use of NIRS technology in combination with PLS-DA as classification model to authentication-classification of individual Iberian pig carcasses into different official commercial categories compiled by the previous (RD 1469/2007) (Zamora-Rojas et al., 2012) and the current QS (RD 4/2014) (Horcada et al., 2020) concluding a high predictive ability. Also, PLS-DA models have recently proven a successful tool to discriminate between different muscle types within species of game animals and to classify species regardless of the muscle (Dumalisile et al., 2020) or to classify merino lamb perirenal fat according their feeding during fattening phase (Agudo, Delgado, López and Rodríguez, 2020). Results obtained in the current research could provide the basis for the authentication-classification into official commercial categories of pre-sliced Iberian dry-cured packaged products. It should be also highlighted the benefit of the higher sensitivity for the pre-sliced Iberian dry-cured loin packaged under MAP with the *Black* category, but even more the ability to discriminate the samples that do not belong to this category (SP) from the market point of view. Products belonging to *Black* category are the most appreciated by consumers (Diaz-Caro et al., 2019) and attain the highest prices in the market. In consequence, products with the *Black* category may be the most exposed to fraudulent practices. Therefore, efforts should be aimed at ensuring that this category is not supplanted by any of the other categories. According to the results obtained in the present study, this could be possible with the PLS-DA model obtained with SG 1,4,4,1 SNV-DE pre-treatment in the bounded range (1000 -1800 nm).

3.2.2. SIMCA of commercial categories

The results related to the best fitting models obtained by SIMCA for commercial categories are shown in Table VI. 2. Additionally, results obtained according to various spectral math pre-treatments and spectra range are shown in Table VI. 2S of the supplementary material. The best model for pre-sliced Iberian dry-cured loin packaged under MAP with *Black* category was obtained with raw spectra (Log (1/R)) using the bounded range (1000-1800 nm). The best fitting models for *Red* and *White* were obtained after SNV-DE math pre-treatment (1000-1800 nm). SE results were around 100% for all categories, whereas results for SP were quite lower; ranged between 24.4% for *White* and 44.2% for *Red,* both results referred to calibration (Table VI. 2). Figures VI. 5S, VI. 6S and VI. 7S of the supplementary material represent graphically the sample-to-model distance (Si) and the sample leverage (Hi), including the class membership limits for projection of samples to *Black* PCA Log (1/R), *Red* PCA SNV-DE (Log (1/R)) 1000-1800 1000-1800 and *White* PCA SNV-DE (Log (1/R)) 1000-1800, respectively, in calibration and validation sample sets. Predictive models developed for all categories resulted in similar values of both SE and SP in the external validation to those obtained in the calibration sample test, with the exception of SE and especially SP of *Red* category which decreased from 100% to 86.67% and 44.2% to 22.6%, respectively (Table VI. 2). In any case, the SP values of the external validation results decreased strongly with the models developed by SIMCA compared to the models obtained by PLS-DA.

Table VI. 2. SIMCA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

Commercial category	pre-treatment	Range	PCs	Calibration			External validation		
				n	SE	SP	n	SE	SP
Black	Absorbance	1000-1800	1	37	97.30	32.10	15	100.00	32.26
Red	SNV-DE	1000-1800	6	41	100.00	44.16	15	86.67	22.58
White	SNV-DE	1000-1800	7	40	97.50	24.36	16	100.00	23.33

SNV, standard normal variate; DE, de-trending; *Black, Red* and *White,* official commercial categories according to the current Iberian Quality Standard (RD 4/2014); PCs, number of principal components that have been considered for each model; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

Pieszczek, Czarnik-Matusewicz, & Daszykowski (2018) obtained classification models for different types of fresh meat from SIMCA with similar SE values. On the opposite, these latter authors observed quite higher SP values than those obtained in the present study. In our case, the low SP could be explained by the similarity between products, especially between those with *Black* and *Red* categories, since the only difference is found in the breed purity of the animals to which they proceed (100% for *Black* and 50-75% for *Red*). Therefore, this makes it difficult for the model to correctly reject samples which not belong to a determined class. In any case, higher values of SP would be desirable, since in the purpose of this research, SP is of capital importance. A higher SP would support the monitoring and control of the samples with respect to the authentication of the official commercial category to which they correspond, avoiding fraudulent practices.

3.2.3. LDA of commercial categories

Regarding to LDA results, the best fitting prediction models for all categories were obtained with the bounded range (1000-1800 nm), after the SG 1,4,4,1 in combination with SNV-DE for *Black* and after SG 1,4,4,1 for *Red* and *White* categories (Table VI. 3). In general, good calibration results were obtained, with values of SE and SP higher than 75%. More specifically, the highest SE was obtained for *White* category with a 95%, whereas *Black* and *Red* categories models attained SP higher than 97% (Table VI. 3). SP values did not suffer significant variations when the external validation was applied, but SE showed a decrease for *Black* and *Red* categories, especially for the latter, dropping from 75.61% to 66.67%. LDA results within the official commercial categories for the rest of pre-treatments and spectra range are shown in Table VI. 3S of the supplementary material.

The results of our study regarding the best fitting models obtained through LDA are consistent with Agudo et al., (2020), who obtained good results in the lamb perirenal fat classification according to their feeding during fattening by LDA-Mahalanobis.

Table VI. 3. LDA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

Commercial category	pre-treatment	Range	Calibration			External validation		
			n	SE	SP	n	SE	SP
Black	SG 1,4,4,1 SNV-DE	1000-1800	37	91.89	97.53	15	86.67	93.55
Red	SG 1,4,4,1	1000-1800	41	75.61	97.40	15	66.67	100.00
White	SG 1,4,4,1	1000-1800	40	95.00	82.05	16	100.00	83.33

SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; SNV, standard normal variate; DE, de-trending; *Black, Red* and *White,* official commercial categories according to the current Iberian Quality Standard (RD 4/2014); n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

3.3. NIRS qualitative predictive models of storage times

PLS-DA, SIMCA and LDA results for the several refrigeration storages times (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model are shown in Tables VI. 4, VI. 5 and VI. 6, respectively.

3.3.1. PLS-DA of storage times

The best model to predict storage time of pre-sliced Iberian dry-cured loin packaged under MAP by PLS-DA was observed for T0, in which 0.924 and 0.128 values for 1-VR and RMSECV, respectively were obtained. Additionally, 100% of samples from validation set were correctly classified as T0 (SE) as well as the total of samples that did not belong to this time were correctly rejected (SP). These results were obtained after applying the second order of Savitzky-Golay derivative pre-treatment (SG 2,5,5,2) in combination with SNV-DE in the full range (1000-2300 nm) (Table VI. 4). At middle storage times (T4 and T8), the best fitting models were attained in the raw spectra (Log (1/R)) and after applying SG 1,4,4,1 pre-treatment, respectively. However, cross-validation statistics decreased, as did both SE and SP in the external validation with respect to T0 (Table VI. 4). In these both cases, the full range was used. The external validation results for T12 were not satisfactory, since the best model obtained after SG 1,4,4,1 pre-treatment in the bounded range (1000-1800 nm), was not able to identify any of the samples with 12 months of storage. However, 100% SP was obtained. The PCA plots with sampling grouping according to refrigeration storage times considering the pre-treatment and range from which the best model fitting is obtained in each time are represented in Figure VI. 8S (supplementary material). Additionally, PLS-DA results within the refrigeration storage times according to the rest of spectral pre-treatments and ranges are shown in Table VI. 4S of the supplementary material.

Table VI. 4. PLS-DA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

				Cross-validation			External validation		
Refrigeration storage time	pre-treatment	Range	LVs	n	1-VR	RMSECV	n	SE	SP
T0	SG 2,5,5,2 SNV-DE	1000-2300	4	36	0.924	0.128	14	100.00	100.00
T4	Absorbance	1000-2300	12	38	0.679	0.267	12	58.33	76.47
T8	SG 1,4,4,1	1000-2300	8	36	0.617	0.288	12	41.67	55.88
T12	SG 1,4,4,1	1000-1800	9	8	0.294	0.213	8	0.00	100.00

SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; SNV, standard normal variate; DE, de-trending; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage, respectively; LVs, latent variables or number of partial least square terms; n, number of samples used for cross-calibration and external validation, respectively; 1-VR, coefficient of determination in cross-validation; RMSECV, root mean square error of cross validation; SE, sensitivity; SP, specificity.

During the refrigeration storage the oxidative status is altered, as well as antioxidants content and percentages of fatty acids are modified, the latter especially due to the oxidation of the polyunsaturated fatty acids (PUFA) (Domínguez, Pateiro, Gagaouad, Barba, Zhang, et al., 2019). Furthermore, the oxidative phenomena take on special relevance in MAP with respect to vacuum-packed products as concluded Parra et al., (2010) and (2012). Thus, the variability of these oxidative reactions throughout the storage period and the consequent compositional differences might have been reflected in absorbance differences, allowing the discrimination among the shelf life of pre-sliced MAP Iberian dry-cured loin. Indeed, absorbance intensity discrepancies can be observed (Figure VI. 1S B, VI. 2S B and VI. 3S B; supplementary material) among times spectres at around 1260 nm, which is a region corresponding to the second overtone of the C-H bonds (Murray & Williams, 1987). More specifically could attend to CH_3 groups, which is part of the composition of alpha and gamma tocopherols, presents in Iberian dry-cured products (Ortiz, García-Torres, González, De-Pedro Sanz, Gaspar, et al., 2020), as well as lipids (Alamprese et al., 2013; Dumalisile et al., 2020). Thus, the lowest absorbance intensity at 1260 nm was attained by T0, whilst the highest was observed for T8, showing T4 and T12 an intermediate behaviour (Figure VI. 3S B).

Regarding to scientific literature dealing with such topic, only Tejerina et al., (2018) evaluated NIRS technology for quality control in Iberian dry-cured ham vacuum-packed by setting predictive models (by PLS-DA) to quantify some parameters such as antioxidants content, fatty acid profile and lipid oxidation as quality-determining markers. Nevertheless, to our knowledge, the possibility of establishing qualitative models to predict the storage time of the product has not been explored yet.

3.3.2. SIMCA of storage times

The best fitting SIMCA model for T0 was obtained after SG 2,5,5,2 and with the range between 1000 and 1800 nm, in which 100% of SE and SP for calibration and validation sample sets were obtained (Table VI. 5). For T4 and T8, the best models were also obtained with the bounded range; for the former after SG 2,5,5,2 SNV-DE pre-treatment and the latest with the SG 1,4,4,1 one. For T12, the best fitting model was obtained using the raw spectra in the whole range (1000-2300 nm). SE and SP results observed in calibration for these times were in general maintained after external validation. Figures from VI. 9S to VI. 12S of the supplementary material represent graphically the sample-to-model distance (Si) and the sample leverage (Hi), including the class membership limits for projection of samples T0 PCA SG 2,5,5,2 (Log (1/R)) 1000-1800, T4 PCA SG 2,5,5,2 SNV-DE (Log (1/R)) 1000-1800 nm, T8 PCA SG 1,4,4,1 (Log (1/R)) 1000-1800 and T12 PCA Log (1/R) 1000-2300 nm, respectively, in calibration and validation sample sets.

Table VI. 5. SIMCA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

Refrigeration storage time	pre-treatment	Range	PCs	Calibration			External validation		
				n	SE	SP	n	SE	SP
T0	SG 2,5,5,2	1000-1800	4	36	100.00	100.00	14	100.00	100.00
T4	SG 2,5,5,2 SNV-DE	1000-1800	3	38	94.74	45.00	12	100.00	41.18
T8	SG 1,4,4,1	1000-1800	3	36	100.00	54.88	12	100.00	41.18
T12	Absorbance	1000-2300	2	8	100.00	58.18	8	100.00	50.00

SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; SNV, standard normal variate; DE, de-trending; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage, respectively; LVs, latent variables or number of partial least square terms; PCs, number of principal components that have been considered for each model; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

In general, external validation results obtained by SIMCA showed higher SE (100%) and lower SP for all storage times considered than those obtained by PLS-DA (Table VI. 5). It should be also highlighted the sharp increase in SE obtained in the models developed by SIMCA with respect to those by PLS-DA for samples belonging to T12. SIMCA results within the refrigeration storage times according to the rest of spectral pre-treatments and ranges are shown in Table VI. 5S of the supplementary material.

3.3.3. LDA of storage times

LDA results within the refrigeration storage time of pre-sliced Iberian dry-cured loin packaged under MAP according to the various spectral pre-treatments and ranges are presented in Table VI. 6S of the supplementary material, whereas those from the best fitting prediction model are summarized in Table VI. 6. When the ability of discriminant models to classify samples according to storage times was explored with LDA, the results showed a fairly balanced pattern between both parameters; SE and SP, as well as throughout the storage period with respect to models obtained by PLS-DA and SIMCA in both, calibration and external validation (Table VI. 6).

Table VI. 6. LDA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to the best fitting prediction model.

Refrigeration storage time	Pre-treatment	Range	Calibration			External validation		
			n	SE	SP	n	SE	SP
T0	SG 2,5,5,2 SNV-DE	1000-1800	36	100.00	100.00	14	100.00	100.00
T4	SG 1,4,4,1 SNV-DE	1000-1800	38	100.00	88.75	12	66.67	88.24
T8	SG 1,4,4,1 SNV-DE	1000-1800	36	83.33	95.12	12	66.67	82.35
T12	SG 2,5,5,2 SNV-DE	1000-1800	8	100.00	95.45	8	37.50	94.74

SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; SNV, standard normal variate; DE, de-trending; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage, respectively; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

In a first stage, it can be observed again, the great ability of the model to identified and discriminate among samples belonging to the beginning of storage (T0). In this case, the best fitting model was obtained after SG 2,5,5,2 SNV-DE pre-treatment and with the spectra range comprised between 1000 and 1800 nm. In both calibration and external validation, the SE and SP results were 100% (Table VI. 6). For the subsequent times (T4 and T8) the best predictive models were obtained after SG 1,4,4,1 SNV-DE (1000-1800 nm), which yielded SE over 80% and SP higher than 88% in calibration. Regarding external validation, a slightly decrease in both SE and SP values were observed. The best accurate model for the prediction of T12 was achieved after SG 2,5,5,2 in combination with SNV-DE in the 1000-1800 nm range, in which despite the excellent results observed for calibration, a sharp decline was subsequently obtained for external validation in SE, whilst SP kept a similar value (Table VI. 6). The low value of SE after validation for T12 was also observed in the model obtained by PLS-DA, in fact for the latter it was 0, and could be associated to the reduced number of samples from T12 used.

Thus, classificatory capacity of the models constructed by LDA would appear more satisfactory than those obtained by PLS-DA and SIMCA in general terms. Henceforth, LDA would offer clear practical advantages for the routinely quality control at industrial and retail level, either to dispose products that are at the end of their shelf life or to prevent the presence of products that have already exceeded their shelf life.

To the best of our knowledge, the application of LDA classification algorithms in storage times has been only conducted by Chen, Cai, Wan, & Jiewen Zhao, (2011), who evaluated its potential ability to discriminate among various pork storage times associated with its freshness (from 1 to 6 days), obtaining good results.

4. Conclusion

The present research covers the recent applications of NIRS in pre-sliced MAP Iberian dry-cured products to identified commercial categories and storage times. Our results suggest that NIRS technology in combination with spectral pre-processing and classification models could help to support the control authenticity of the individual packages samples within the official commercial categories compiled by the current Spanish Iberian Quality Standard (*Black*, *Red* and *White*). Furthermore, good accuracy was obtained for the assignments into storage times throughout long-term storage (0, 4, 8 and 12 months).

As limitations that might have an influence on the obtained results of the current research are the composition of the atmosphere and the surrounding plastic material, as well as the limited number of samples from T12 sampling time used.

Future work should be carried out on other packaging types such as vacuum and active packaging, as well as in other Iberian dry-cured products (Iberian dry-cured ham, sausages), to explore the classifying ability of NIRS on these, allowing the development of robust NIRS models that can be implemented as a routine control in the Iberian sector.

Credit authorship contribution statement

David Tejerina: Conceptualization, Funding acquisition, Investigation. **Rebeca Contador**: Methodology, Formal analysis, Writing - original draft. **Alberto Ortiz:** Conceptualization, Methodology, Formal analysis, Writing - original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Table VI. 1S. PLS-DA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	LVs	Cross-validation							External validation						
			n	*Black*		*Red*		*White*		n	*Black*		*Red*		*White*	
				1-VR	RMSECV	1-VR	RMSECV	1-VR	RMSECV		SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	12	118	0.665	0.270	0.599	0.304	0.548	0.321	46	46.67	83.87	40.00	54.84	25.00	63.33
	1000-1800	12	118	0.643	0.279	0.579	0.312	0.631	0.290	46	40.00	61.29	26.67	48.39	37.50	66.67
SNV-DE	1000-2300	12	118	0.702	0.256	**0.676**	**0.274**	0.637	0.287	46	60.00	70.97	46.67	54.84	43.75	70.00
	1000-1800	11	118	0.727	0.244	0.662	0.279	**0.634**	**0.289**	46	60.00	70.97	26.67	51.61	43.75	73.33
SG 1,4,4,1	1000-2300	7	118	0.554	0.312	0.492	0.342	0.590	0.306	46	40.00	61.29	26.67	41.94	31.25	70.00
	1000-1800	7	118	0.632	0.284	0.579	0.311	0.649	0.283	46	46.67	77.42	40.00	45.16	31.25	70.00
SG 1,4,4,1 SNV-DE	1000-2300	9	118	0.689	0.261	0.592	0.307	0.567	0.313	46	60.00	67.74	26.67	45.16	37.50	66.67
	1000-1800	7	118	**0.729**	**0.244**	0.621	0.296	0.635	0.288	46	60.00	74.19	33.33	41.94	43.75	63.33
SG 2,5,5,2	1000-2300	1	118	0.110	0.441	0.041	0.470	0.322	0.393	46	0.00	16.13	0.00	6.45	0.00	43.33
	1000-1800	6	118	0.322	0.385	0.088	0.459	0.520	0.331	46	6.67	38.71	26.67	38.71	12.50	60.00
SG 2,5,5,2 SNV-DE	1000-2300	4	118	0.116	0.440	0.090	0.458	0.264	0.410	46	13.33	29.03	6.67	25.81	18.75	26.67
	1000-1800	4	118	0.416	0.358	0.203	0.429	0.468	0.348	46	0.00	19.35	26.67	16.13	12.50	43.33

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; *Black*, *Red* and *White*, official commercial categories according to the current Iberian Quality Standard (RD 4/2014); LVs, latent variables or number of partial least square terms; n, number of samples used for cross-validation and external validation, respectively; 1-VR, coefficient of determination in cross-validation; RMSECV, root mean square error of cross validation; SE, sensitivity; SP, specificity.

Table VI. 2S. SIMCA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	Calibration										External Validation						
		n	*Black*			*Red*			*White*			n	*Black*		*Red*		*White*	
			PCs	SE	SP	PCs	SE	SP	PCs	SE	SP		SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	118	2	97.3	35.80	2	95.12	35.06	2	97.50	16.67	46	93.33	19.35	100.00	6.45	100.00	3.33
	1000-1800	118	**1**	**97.3**	**32.10**	2	90.24	35.06	2	97.50	17.95	46	100.00	32.26	100.00	12.90	100.00	13.33
SNV-DE	1000-2300	118	9	100.00	28.39	7	100.00	28.57	9	97.50	21.79	46	80.00	22.58	93.33	12.90	100.00	23.33
	1000-1800	118	7	100.00	29.63	**6**	**100.00**	**44.16**	**7**	**97.50**	**24.36**	46	86.67	16.13	86.67	22.58	100.00	23.33
SG 1,4,4,1	1000-2300	118	2	97.30	19.75	4	100.00	18.18	4	100.00	0.00	46	100.00	12.90	100.00	3.23	100.00	0.00
	1000-1800	118	2	97.30	30.86	4	100.00	19.48	2	100.00	0.00	46	100.00	25.81	100.00	3.23	100.00	13.33
SG 1,4,4,1 SNV-DE	1000-2300	118	6	97.30	1.23	5	100.00	0.00	5	97.50	10.26	46	100.00	0.00	100.00	0.00	100.00	13.33
	1000-1800	118	4	97.30	17.28	4	100.00	2.60	6	100.00	24.36	46	100.00	16.13	93.33	3.23	100.00	26.67
SG 2,5,5,2	1000-2300	118	4	91.89	1.23	5	97.56	1.30	5	97.50	0.00	46	100.00	0.00	100.00	0.00	100.00	33.33
	1000-1800	118	8	94.59	0.00	7	97.56	5.19	6	100.00	0.00	46	100.00	0.00	100.00	0.00	100.00	33.33
SG 2,5,5,2 SNV-DE	1000-2300	118	2	97.30	1.23	4	97.56	0.00	2	97.50	0.00	46	100.00	0.00	100.00	0.00	100.00	33.33
	1000-1800	118	2	97.30	14.81	2	92.68	0.00	2	100.00	5.13	46	93.33	12.90	100.00	0.00	100.00	6.67

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; *Black*, *Red* and *White*, official commercial categories according to the current Iberian Quality Standard (RD 4/2014); PCs, number of principal components that have been considered for each model; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

Table VI. 3S. LDA results within the official commercial categories (*Black*, *Red* and *White*) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	Calibration							External Validation						
		n	*Black*		*Red*		*White*		n	*Black*		*Red*		*White*	
			SE	SP	SE	SP	SE	SP		SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	118	54.05	91.36	75.61	88.31	90.00	80.77	46	20.00	93.55	86.67	67.74	75.00	30.00
	1000-1800	118	54.05	93.83	73.17	89.61	95.00	78.21	46	33.33	93.55	73.33	70.97	81.25	80.00
SNV-DE	1000-2300	118	56.76	86.42	68.29	79.22	75.61	87.18	46	33.33	87.10	66.67	64.52	75.00	86.67
	1000-1800	118	83.78	85.16	63.41	94.81	82.50	85.9	46	73.33	83.87	46.67	77.42	75.00	86.67
SG 1,4,4,1	1000-2300	118	59.46	93.83	24.39	58.44	20.00	53.85	46	20.00	90.32	73.33	61.29	87.50	90.00
	1000-1800	118	83.78	98.77	**75.61**	**97.4**	**95.00**	**82.05**	46	86.67	93.55	66.67	100.00	100.00	83.33
SG 1,4,4,1 SNV-DE	1000-2300	118	51.35	80.91	75.61	72.73	85.00	92.31	46	40.00	93.55	86.67	61.29	75.00	96.67
	1000-1800	118	**91.89**	**97.53**	87.8	93.51	87.50	92.31	46	86.67	93.55	86.67	83.87	81.25	100.00
SG 2,5,5,2	1000-2300	118	40.54	87.65	41.46	76.79	75.00	61.54	46	26.67	90.32	33.33	67.74	81.25	63.33
	1000-1800	118	54.05	80.91	65.85	85.71	90.00	78.21	46	33.33	90.32	60.00	77.42	87.50	73.33
SG 2,5,5,2 SNV-DE	1000-2300	118	32.43	88.89	73.17	58.44	55.00	83.33	46	13.33	87.10	60.00	41.94	56.25	86.67
	1000-1800	118	64.86	86.42	82.93	71.43	60.00	96.15	46	26.67	90.32	86.67	45.16	43.75	93.33

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; *Black*, *Red* and *White*, official commercial categories according to the current Iberian Quality Standard (RD 4/2014); n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

Table VI. 4S. PLS-DA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	LVs	Cross-validation									External validation								
			n	T0		T4		T8		T12		n	T0		T4		T8		T12	
				1-VR	RMSECV	1-VR	RMSECV	1-VR	RMSECV	1-VR	RMSECV		SE	SP	SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	12	118	0.625	0.284	**0.679**	**0.267**	0.652	0.274	NA	0.257	46	57.14	71.88	58.33	76.47	33.33	64.71	0.00	89.47
	1000-1800	11	118	0.507	0.326	0.543	0.319	0.548	0.312	0.133	0.236	46	21.43	62.50	33.33	61.76	25.00	58.82	0.00	84.21
SNV-DE	1000-2300	12	118	0.525	0.320	0.687	0.264	0.506	0.326	0.127	0.237	46	50.00	43.75	41.67	82.35	25.00	58.82	0.00	97.37
	1000-1800	11	118	0.409	0.357	0.615	0.292	0.539	0.315	NA	0.255	46	35.71	25.00	41.67	61.76	16.67	64.71	0.00	92.11
SG 1,4,4,1	1000-2300	8	118	0.534	0.317	0.582	0.305	**0.617**	**0.288**	0.274	0.216	46	71.43	29.17	33.33	79.41	41.67	55.88	0.00	100.00
	1000-1800	9	118	0.554	0.310	0.623	0.289	0.619	0.287	**0.294**	**0.213**	46	57.14	46.88	33.33	73.53	41.67	64.71	0.00	100.00
SG 1,4,4,1 SNV-DE	1000-2300	6	118	0.553	0.311	0.637	0.284	0.266	0.398	0.293	0.213	46	92.86	53.13	25.00	73.53	8.33	47.06	0.00	94.74
	1000-1800	8	118	0.643	0.278	0.676	0.268	0.432	0.350	0.298	0.212	46	85.71	50.00	33.33	79.41	25.00	52.94	0.00	100.00
SG 2,5,5,2	1000-2300	4	118	0.252	0.402	0.189	0.424	0.060	0.450	NA	0.263	46	50.00	18.75	0.00	50.00	0.00	47.06	0.00	100.00
	1000-1800	5	118	0.362	0.371	0.468	0.344	0.252	0.402	0.168	0.231	46	64.29	31.25	8.33	70.59	0.00	41.18	0.00	97.37
SG 2,5,5,2 SNV-DE	1000-2300	4	118	**0.924**	**0.128**	0.316	0.390	0.118	0.436	0.054	0.247	46	100.00	100.00	8.33	50.00	0.00	38.24	0.00	100.00
	1000-1800	5	118	0.919	0.132	0.571	0.309	0.325	0.382	0.111	0.239	46	100.00	24.24	16.67	67.65	0.00	29.41	0.00	100.00

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage, respectively; LVs, latent variables or number of partial least square terms; n, number of samples used for cross-validation and external validation, respectively; 1-VR, coefficient of determination in cross-validation; RMSECV, root mean square error of cross validation; SE, sensitivity; SP, specificity.

Table VI. 5S. SIMCA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	Calibration													External validation								
		n	T0			T4			T8			T12			n	T0		T4		T8		T12	
			PCs	SE	SP	PCs	SE	SP	PCs	SE	SP	PCs	SE	SP		SE	SP	SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	118	2	94.44	41.25	2	100.00	6.25	2	97.22	65.00	4	**100.00**	**58.18**	46	92.86	31.25	100.00	2.94	100.00	38.24	100.00	50.00
	1000-1800	118	2	97.22	43.90	2	92.11	1.25	2	97.22	67.91	1	100.00	53.64	46	92.86	31.25	100.00	11.76	100.00	47.06	100.00	31.58
SNV-DE	1000-2300	118	7	100.00	79.27	9	97.37	10.00	8	100.00	34.15	6	100.00	20.00	46	85.71	53.13	83.33	8.82	100.00	17.65	75.00	15.79
	1000-1800	118	5	100.00	46.34	6	97.37	6.25	8	100.00	23.17	6	100.00	26.36	46	85.71	40.63	83.33	5.88	100.00	14.71	75.00	23.68
SG 1,4,4,1	1000-2300	118	3	100.00	98.78	2	97.37	3.75	4	100.00	39.02	2	100.00	18.18	46	100.00	100.00	100.00	0.00	100.00	14.71	100.00	0.00
	1000-1800	118	3	100.00	91.25	2	97.37	2.50	3	**100.00**	**54.88**	2	100.00	20.00	46	100.00	93.75	100.00	2.94	100.00	41.18	100.00	5.26
SG 1,4,4,1 SNV-DE	1000-2300	118	6	100.00	100.00	5	100.00	0.00	5	100.00	13.41	5	100.00	0.00	46	85.71	100.00	100.00	0.00	100.00	5.88	100.00	0.00
	1000-1800	118	5	**100.00**	**100.00**	4	100.00	1.25	5	97.22	19.51	6	100.00	0.00	46	92.86	100.00	100.00	8.82	100.00	8.82	100.00	0.00
SG 2,5,5,2	1000-2300	118	4	100.00	100.00	5	100.00	0.00	4	100.00	0.00	5	100.00	0.00	46	100.00	100.00	100.00	0.00	100.00	0.00	100.00	0.00
	1000-1800	118	4	100.00	100.00	3	94.74	0.00	6	100.00	0.00	5	100.00	0.00	46	100.00	100.00	100.00	0.00	100.00	0.00	100.00	0.00
SG 2,5,5,2 SNV-DE	1000-2300	118	4	100.00	100.00	6	97.37	21.25	9	100.00	20.73	4	100.00	20.91	46	100.00	100.00	100.00	23.53	100.00	20.59	100.00	26.32
	1000-1800	118	5	100.00	100.00	3	**94.74**	**45.00**	7	100.00	43.90	4	100.00	34.55	46	100.00	100.00	100.00	41.18	100.00	41.18	100.00	36.84

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage; PCs, number of principal components that have been considered for each model; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

Table VI. 6S. LDA results within the refrigeration storage time (T0, T4, T8 and T12) of pre-sliced Iberian dry-cured loin packaged under MAP according to various spectral math treatments and spectra ranges.

Pre-treatment	Range	n	Calibration								n	External validation							
			T0		T4		T8		T12			T0		T4		T8		T12	
			SE	SP	SE	SP	SE	SP	SE	SP		SE	SP	SE	SP	SE	SP	SE	SP
Absorbance	1000-2300	118	66.67	96.34	94.74	77.50	61.11	92.68	50.00	95.45	46	71.43	96.88	91.67	76.47	75.00	85.29	25.00	94.74
	1000-1800	118	63.89	96.34	94.74	75.00	61.11	92.68	50.00	96.36	46	71.43	96.88	91.67	73.53	75.00	85.29	50.00	97.37
SNV-DE	1000-2300	118	41.67	95.12	94.74	83.75	61.11	86.59	37.50	87.27	46	28.57	90.63	66.67	76.47	75.00	61.76	12.50	89.47
	1000-1800	118	30.56	90.24	89.47	81.25	58.97	81.71	37.50	92.73	46	14.29	96.88	75.00	58.82	50.00	73.53	25.00	92.11
SG 1,4,4,1	1000-2300	118	50.00	96.34	84.21	80.00	63.89	85.37	62.50	94.55	46	71.43	96.88	83.33	88.24	91.67	100.00	100.00	94.74
	1000-1800	118	75.00	97.56	86.84	90.00	80.56	87.80	75.00	97.27	46	64.29	93.75	75.00	79.41	75.00	79.41	25.00	97.37
SG 1,4,4,1 SNV-DE	1000-2300	118	66.67	100.00	97.37	85.00	69.44	82.93	50.00	98.18	46	64.29	100.00	66.67	82.35	50.00	58.82	12.50	94.74
	1000-1800	118	94.44	100.00	**100.00**	**88.75**	**83.33**	**95.12**	37.50	98.18	46	92.86	100.00	66.67	88.24	66.67	82.35	50.00	92.11
SG 2,5,5,2	1000-2300	118	0.00	100.00	92.11	38.75	22.22	90.24	75.00	89.09	46	7.14	100.00	83.33	35.29	33.33	82.35	12.50	94.74
	1000-1800	118	19.44	100.00	97.37	55.00	44.44	95.12	75.00	90.00	46	14.29	100.00	83.33	41.18	33.33	97.06	62.50	89.47
SG 2,5,5,2 SNV-DE	1000-2300	118	100.00	100.00	86.84	72.50	44.44	92.68	62.50	99.09	46	100.00	100.00	75.00	64.71	58.33	94.12	0.00	100.00
	1000-1800	118	**100.00**	**100.00**	83.33	83.75	55.55	93.90	**100.00**	**95.45**	46	100.00	100.00	41.67	73.53	58.33	82.35	37.50	94.74

SNV, standard normal variate; DE, de-trending; SG, Savitzky-Golay derivatives, with the first number corresponding to order derivative, second and third one indicating the smoothing pints on the left and right sides and the last number corresponding to the polynomial order; T0, T4, T8, T12, initial, 4, 8 and 12 months of refrigeration storage; n, number of samples used for calibration and external validation, respectively; SE, sensitivity; SP, specificity.

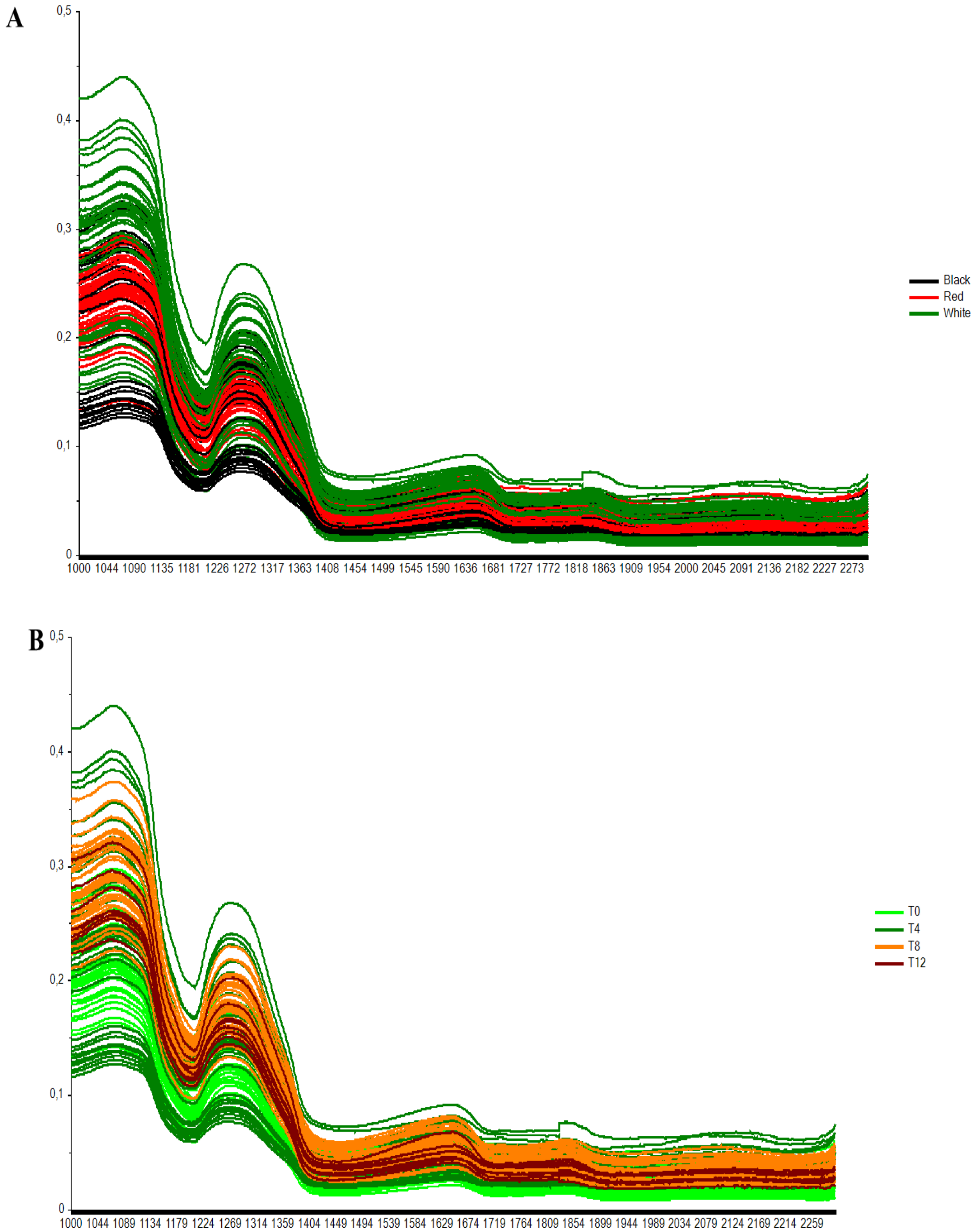


Figure VI. 1S. Raw spectra (reflectance) from calibration sample set of pre-sliced Iberian dry-cured loin packaged under MAP (1000-2500 nm) grouped according to official commercial categories (*Black, Red* and *White*) (A) and refrigeration storage time (T0, T4, T8 and T12) (**B**).

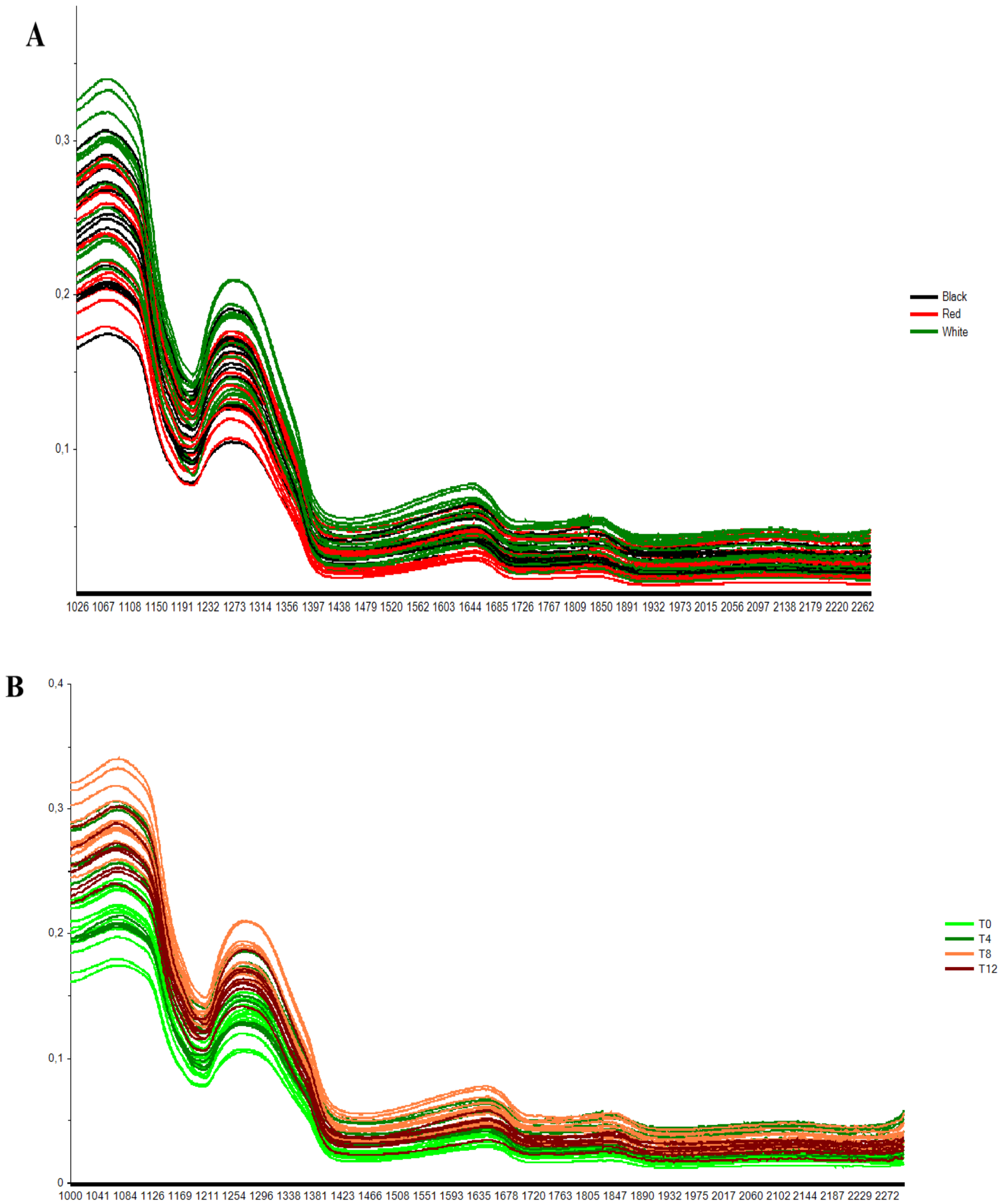


Figure VI. 2S. Raw spectra (reflectance) from validation sample set of pre-sliced Iberian dry-cured loin packaged under MAP (1000-2500 nm) grouped according to official commercial categories (*Black, Red* and *White*) (A) and refrigeration storage time (T0, T4, T8 and T12) (**B**).

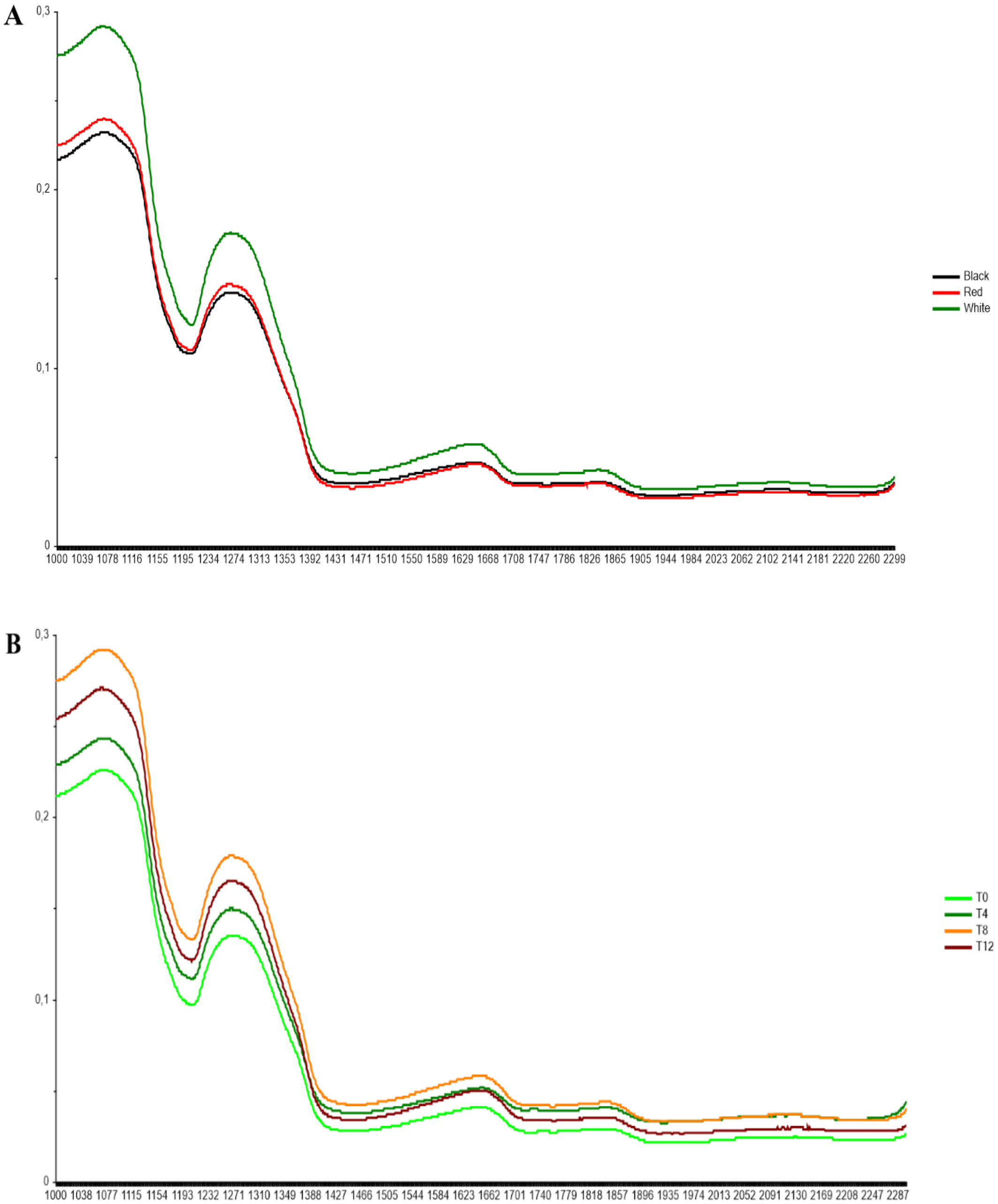


Figure VI. 3S. Raw (reflectance) mean spectra from calibration sample set of pre-sliced Iberian dry-cured loin packaged under MAP (1000-2300 nm) grouped according to official commercial categories (*Black, Red* and *White*) (A) and refrigeration storage time (T0, T4, T8 and T12) (**B**).

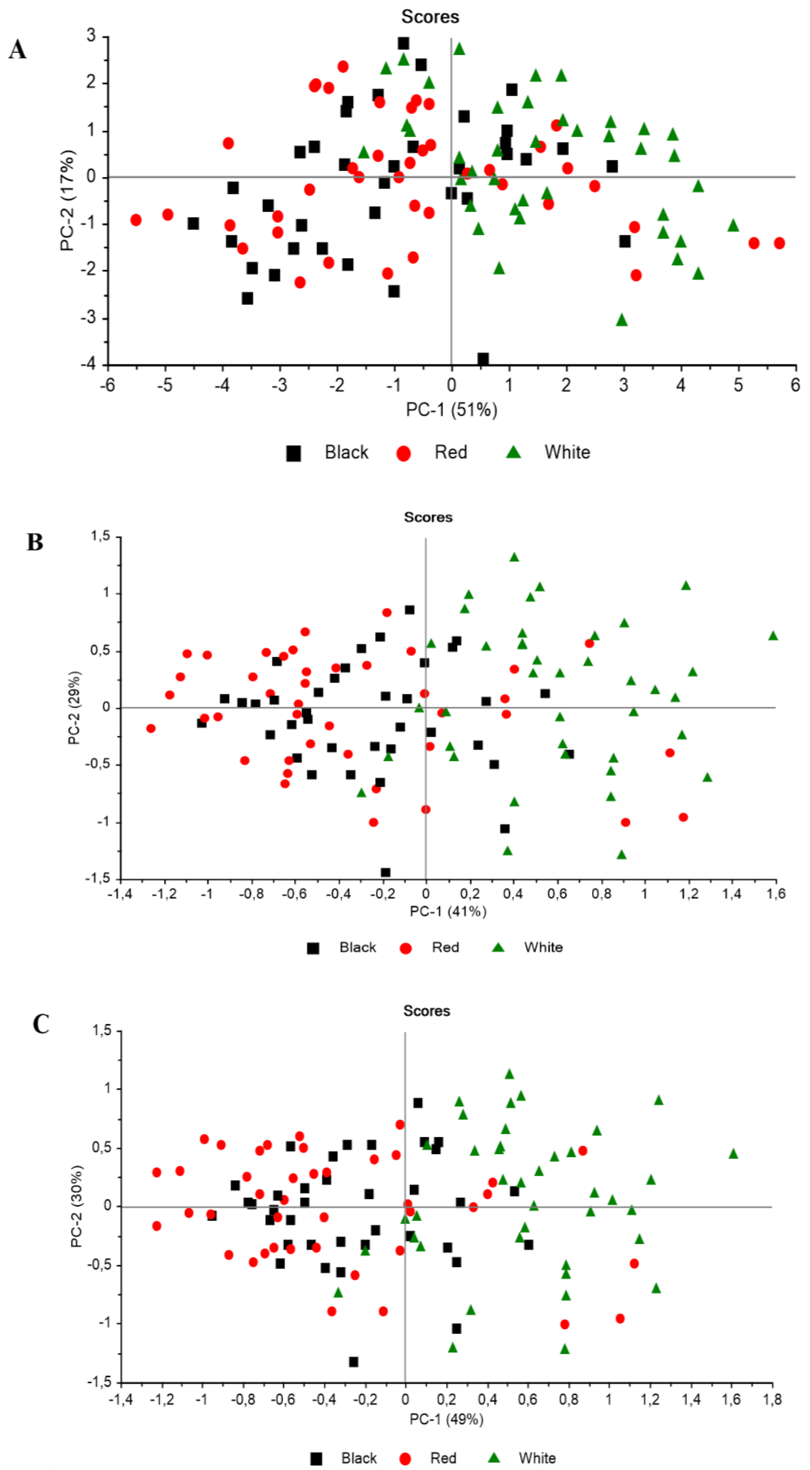


Figure VI. 4S. PCA analysis of calibration sample set after SG 1,4,4,1 (Log (1/R)) at 1000-1800 nm (A), SNV-DE (Log (1/R)) at 1000-2300 nm (B) and SG 1,4,4,1 SNV-DE (Log (1/R)) at 1000-1800 nm (C). Sampling grouping according to official commercial categories (*Black, Red* and *White*). Graphical representation of PC1 (51%, 41%, 49%, respectively) vs. PC2 (17%, 29%, 30%, respectively).

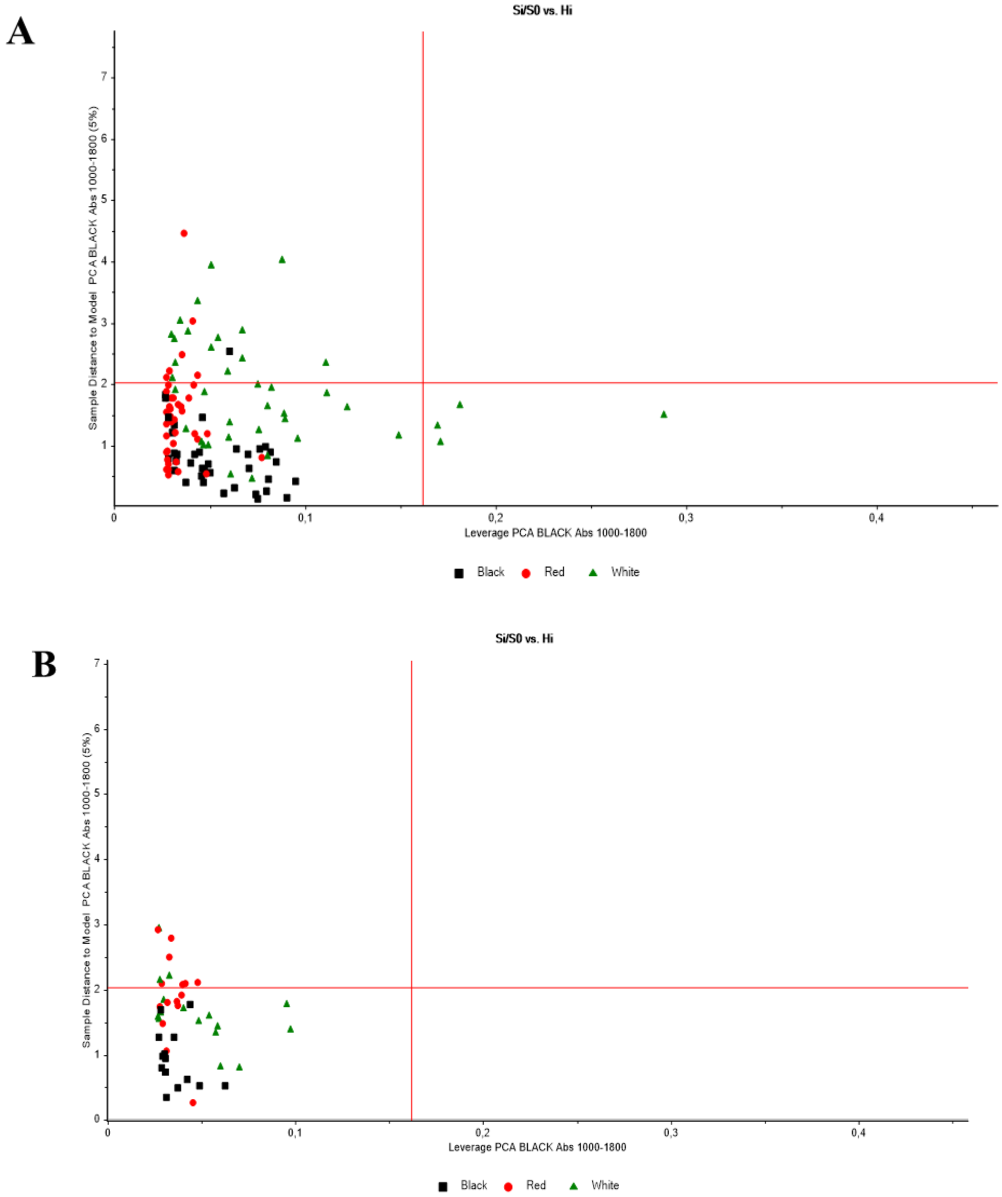
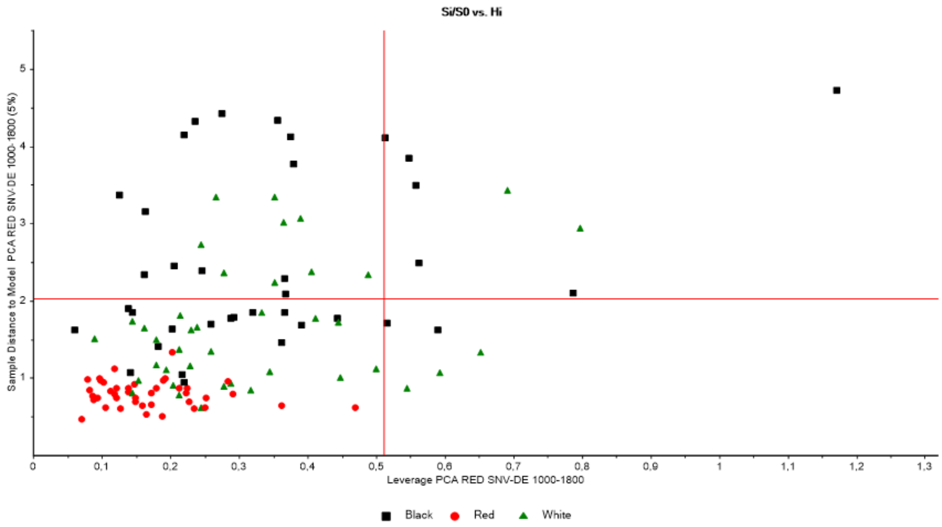


Figure VI. 5S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to *Black* PCA Log (1/R) 1000-1800 nm model in calibration (**A**) and external validation (**B**) sample sets.

A



B

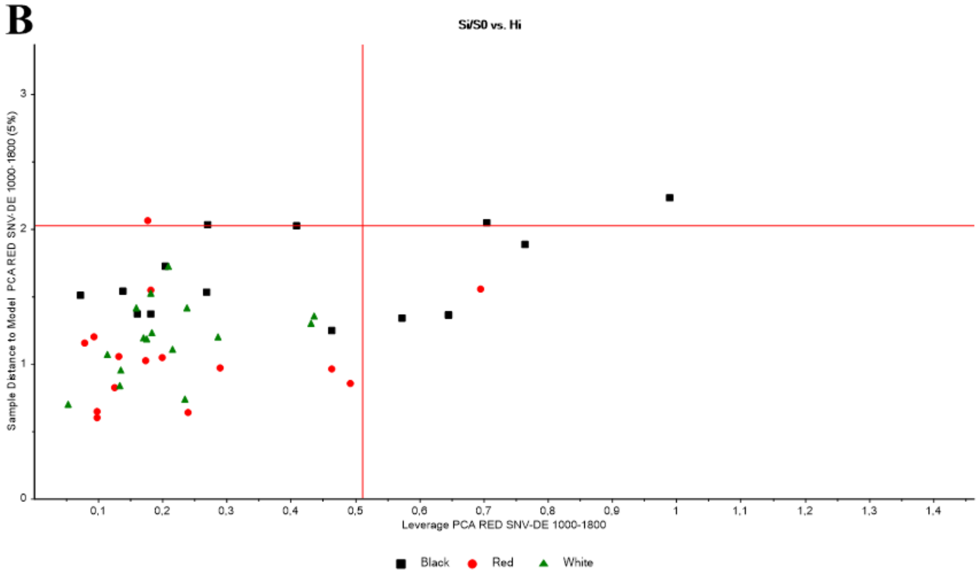


Figure VI. 6S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to *Red* PCA SNV-DE (Log (1/R)) 1000-1800 nm model in calibration (**A**) and external validation (**B**) sample sets.

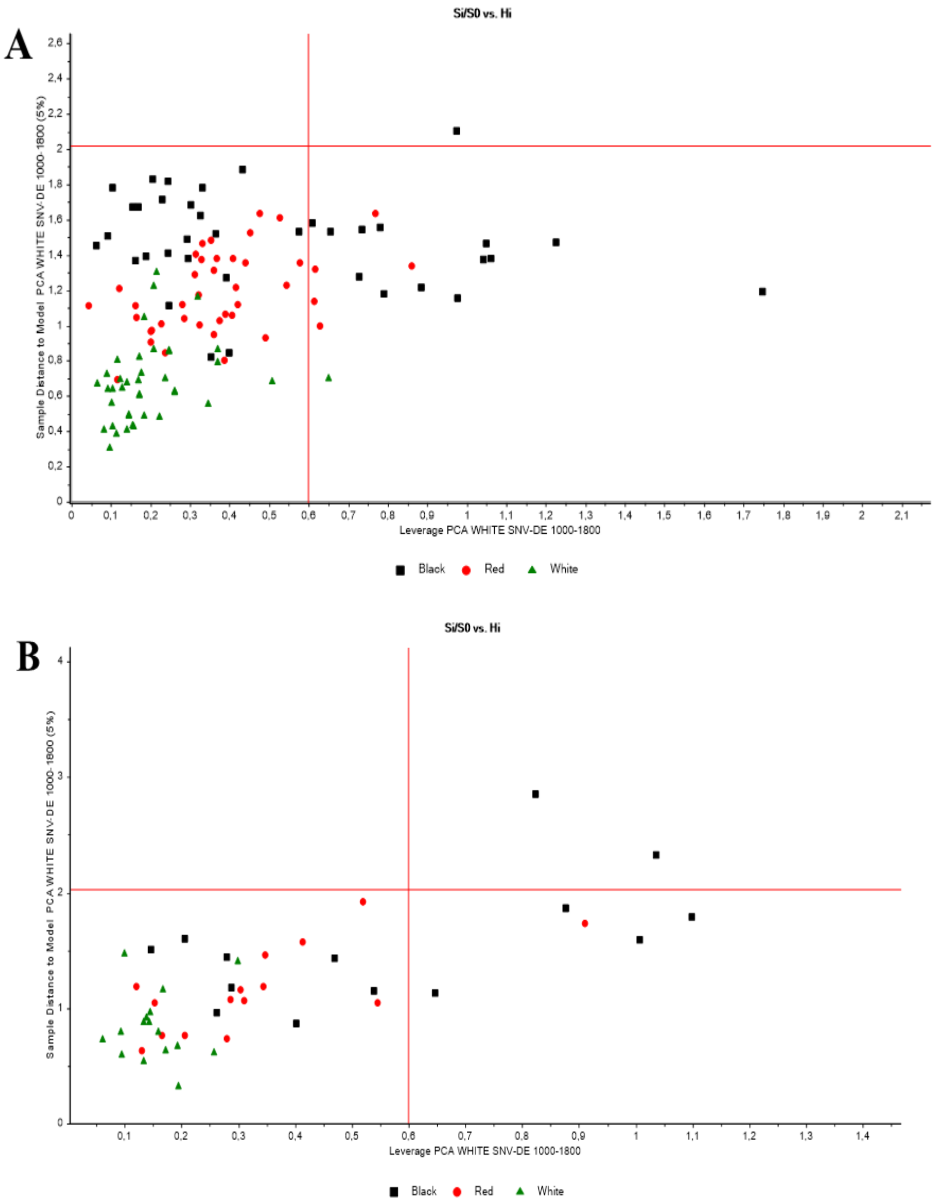


Figure VI. 7S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to *White* PCA SNV-DE (Log (1/R)) 1000-1800 nm model in calibration (**A**) and external validation sample (**B**) sets.

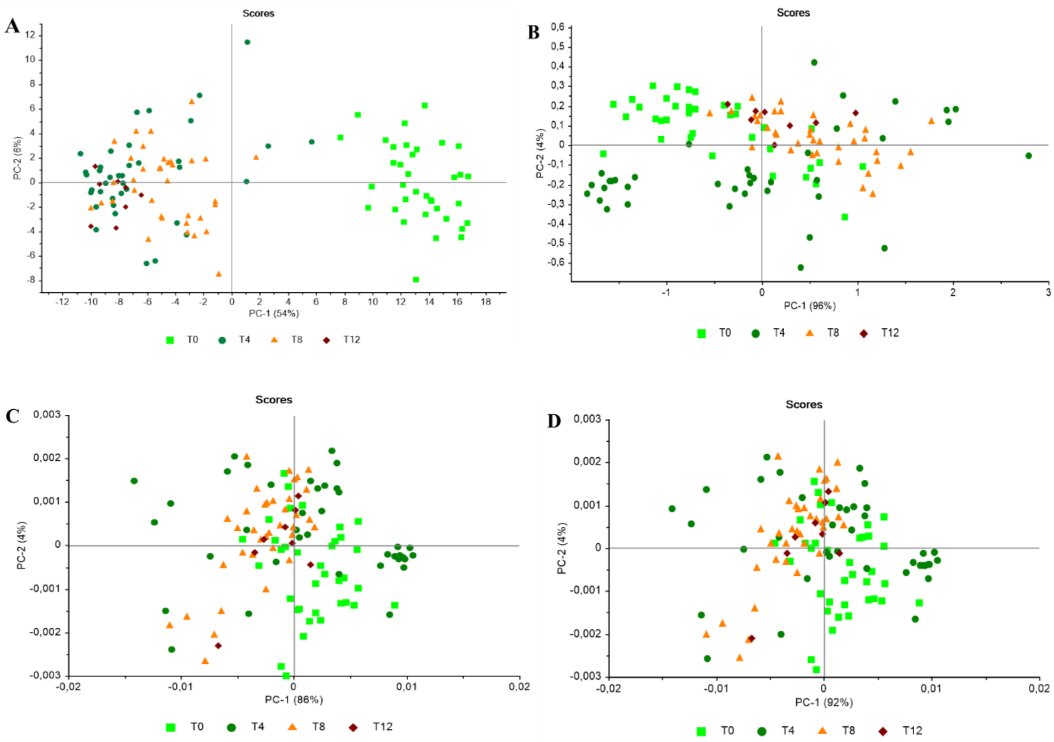


Figure VI. 8S. PCA analysis of calibration sample set after SG 2,5,5,2 SNV-DE (Log (1/R)) at 1000-2300 nm (A), Log (1/R) at 1000-2300 nm (B), SG 1,4,4,1 (Log (1/R)) at 1000-2300 nm (C) and SG 1,4,4,1 (Log (1/R)) at 1000-1800 nm (D). Sampling grouping according to refrigeration storage time (T0, T4, T8 and T12). Graphical representation of PC1 (54%, 96%, 86%, 92%, respectively) vs. PC2 (6%, 4%, 4%, 4%, respectively).

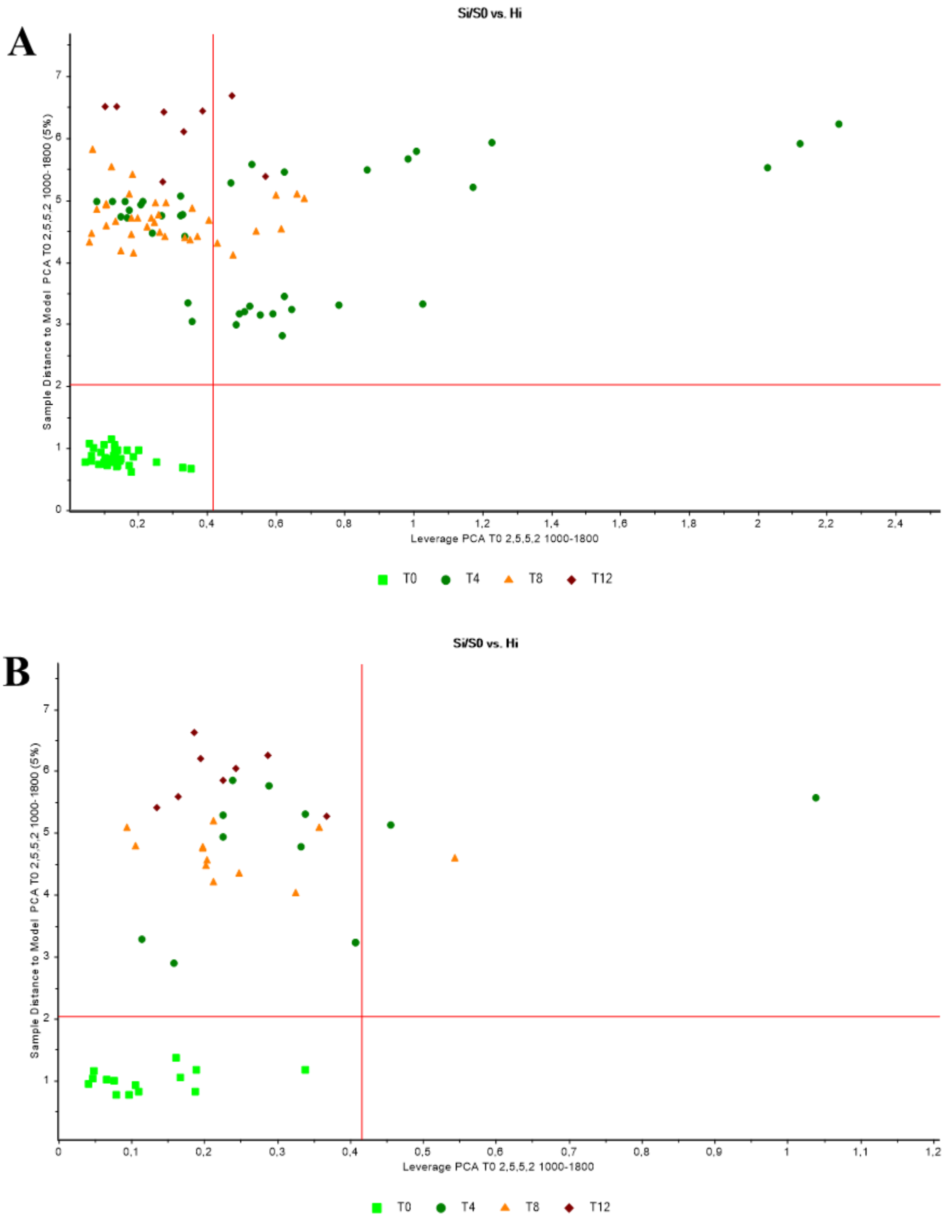


Figure VI. 9S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to T0 PCA SG 2,5,5,2 (Log (1/R)) 1000-1800 nm model in calibration (**A**) and external validation (**B**) sample sets.

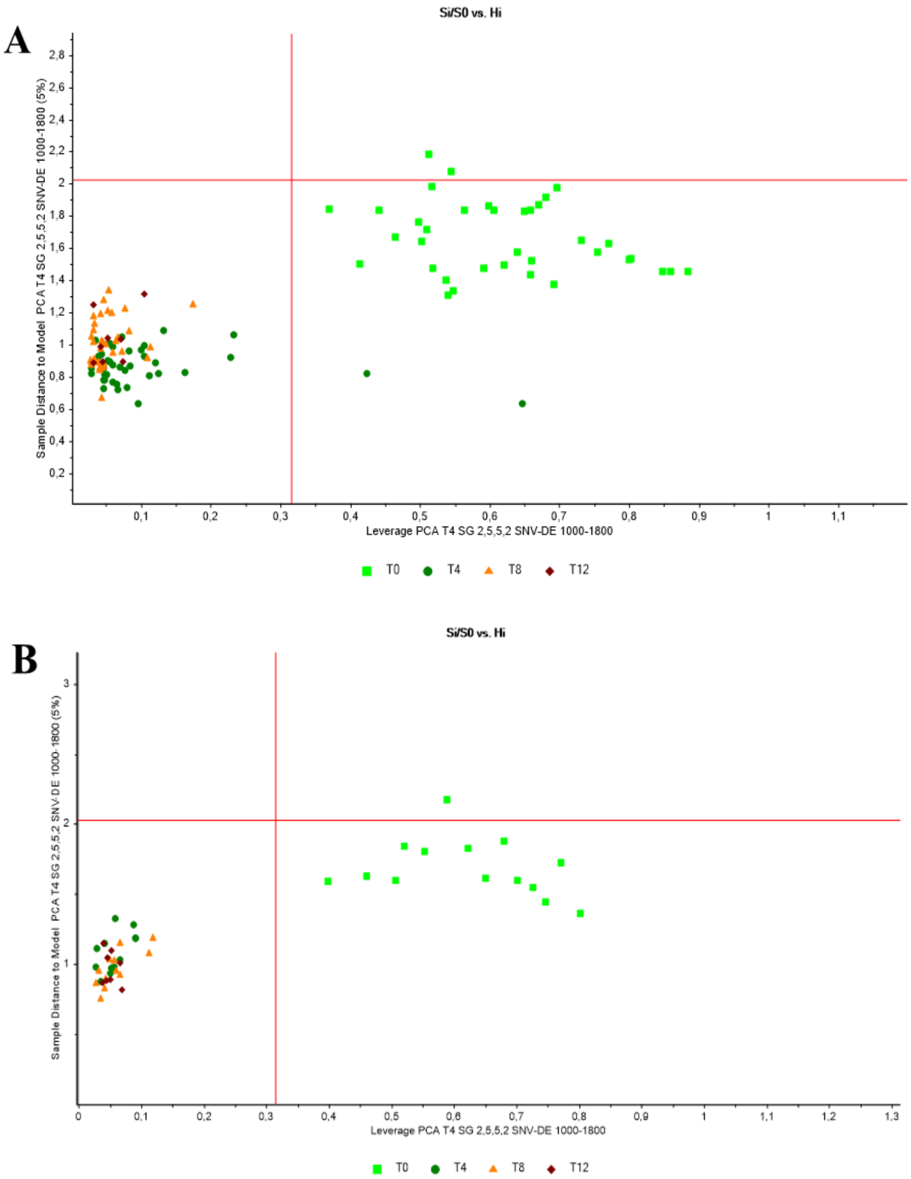


Figure VI. 10S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to T4 PCA SG 2,5,5,2 SNV-DE (Log (1/R)) 1000-1800 nm model in calibration (**A**) and external validation (**B**) sample sets.

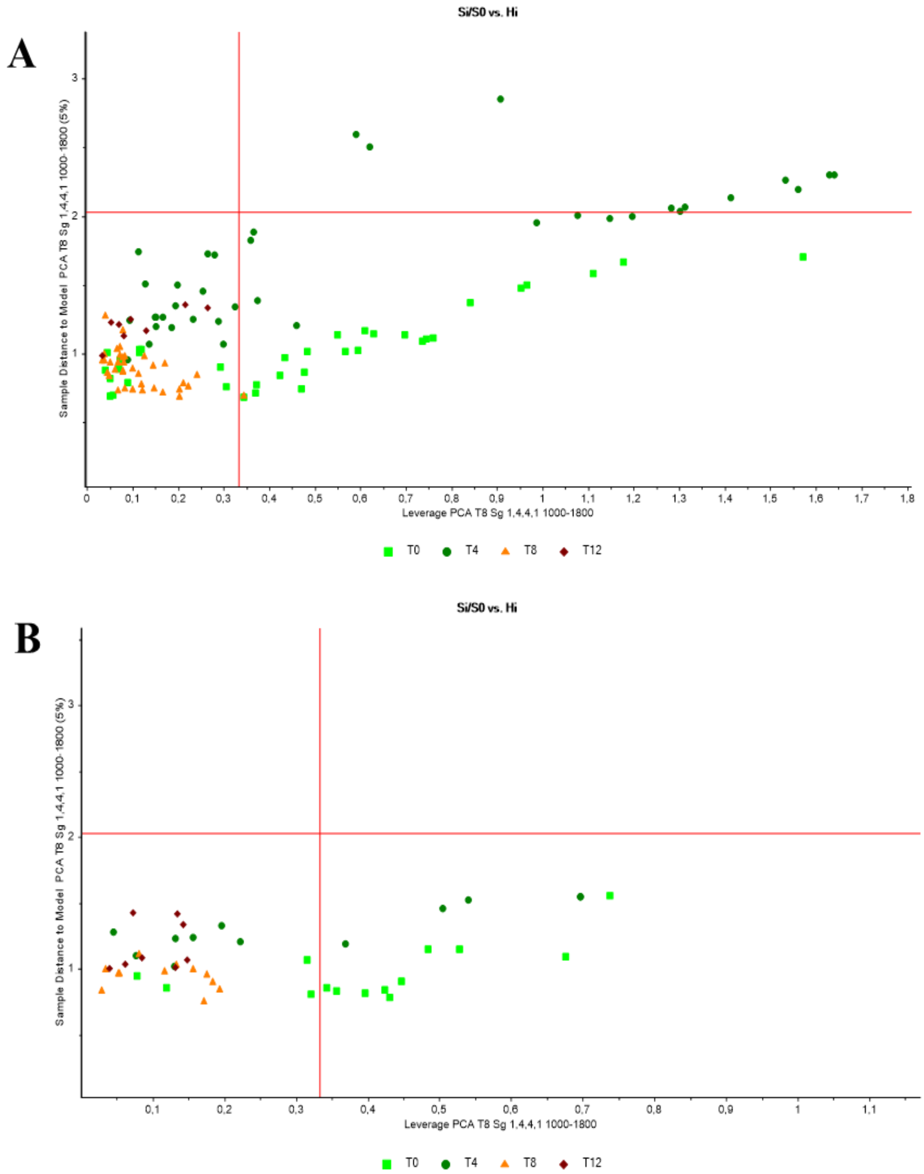


Figure VI. 11S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to T8 PCA SG 1,4,4,1 (Log (1/R)) 1000-1800 nm model in calibration (**A**) and external validation (**B**) sample sets.

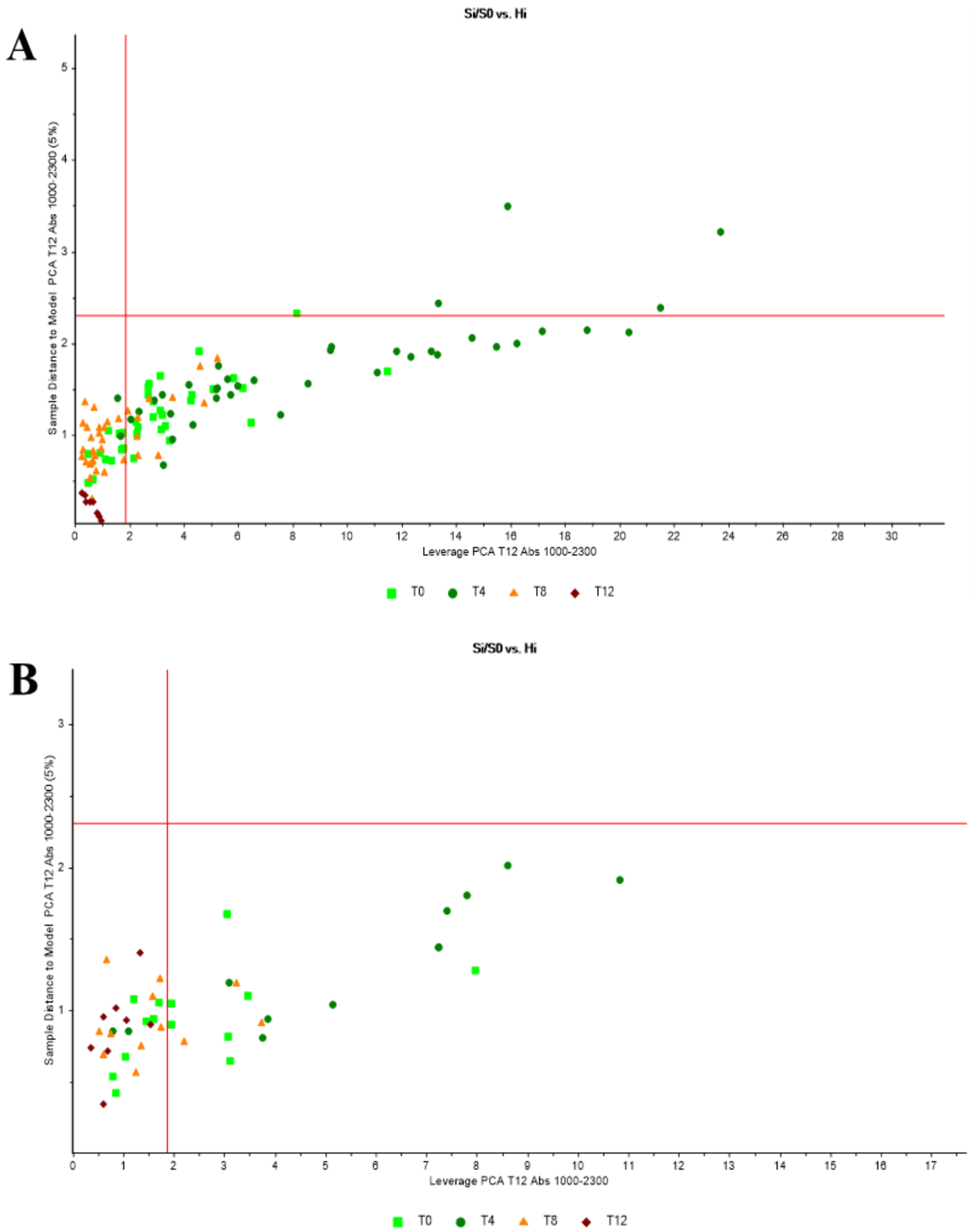


Figure VI. 12S. SIMCA results for spectra data. This plot gives a view of both the sample-to-model distance (Si) and the sample leverage (Hi) for a given model. It includes the class membership limits for projection of samples to T12 PCA Log (1/R) 1000-2300 nm model in calibration (**A**) and external validation (**B**) sample sets.

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CONCLUSIONS

1. The in-depth interviews with the main Iberian pig industry stakeholders, i.e. farmers and industrial entrepreneurs, and their analysis, allowed us to identify the main opportunities and limitations in the production and commercialization of Iberian products under the legal framework of Royal Decree 4/2014 of 10th of January, which approved the quality standard for Iberian pork meat, ham leg, ham shoulder and loin.

They stated their dissatisfaction with the variability provided in the Quality Standard for the production factors of animals under the *Green* label commercial category. Additionally, the age at slaughter for the *White* label animals was thought to be too high to meet the interests of farmers and industrial entrepreneurs. On the other hand, industrial entrepreneurs stated that freezing the commercial cuts before they are cured could be an effective measure in balancing production and demand, either applicable to cuts produced throughout the year or those considered as seasonal such as is the case of the *Montanera* products.

2. The age of Iberian x Duroc crossed bred pigs at the beginning of the finishing phase in the *Montanera* system had an impact on some production parameters, with younger animals having lower ADGs, and therefore yielding lower weight increases at the end of this phase. On the other hand, these animals proved to have better carcass yields with lower fat thickness values and smaller ham legs. This might be an interesting factor for the industry to take into consideration, since there is a market niche for smaller hams, which are hard to obtain through the *Montanera* traditional production system.

Gender (surgically-castrated males and immunologically-castrated females) had no significant impact on the production parameters.

3. The age at slaughter of Iberian x Duroc crossed bred pigs finished in the *Montanera* system had an impact on some quality parameters, both in fresh (m. *Longissimus thoracis et lumborum*) and dry-cured loin. Specifically, fresh loins from older animals showed more redness and more hardness as well as higher natural antioxidant and unsaturated fatty acid contents. However, in dry-cured loins there were no differences in colour or in texture parameters, whereas the antioxidant content and the fatty acid profile followed a similar behaviour to fresh loins. This fact suggests that the older the animals are, the higher the nutritional quality of the product.

4. Freezing the meat prior to the technological curing process had a significant effect on some *Montanera* Iberian dry-cured loins' quality parameters. Specifically, the loins that were previously frozen experienced greater weight loss during curing, which were also greater as the freezing storage time was longer (6 against 3 months), a fact that caused more hardness. Additionally, an increase in lipid and protein oxidation was identified, although with values below the limits detectable by consumers.

Considering the pre-cure freezing practice as a tool to overcome production seasonality in dry-cured products as Iberian dry-cured loin of animals reared in the *Montanera* system could be an interesting procedure for the industry. But it would be convenient to adapt the conditions of the curing process to the characteristics of the initial raw material (fresh or frozen/thawed).

5. The commercial categories compiled by the current Iberian Quality Standard *Black, Red and White* suggest differences in the quality of the products. Thus, for sliced packaged dry-cured loin, the differences were mainly found in the antioxidant content and the fatty acid profile, with the categories deriving from the *Montanera* system (*Black* and *Red*) showing higher alpha and gamma tocopherol and unsaturated fatty acid values.

The quality of the dry-cured loins of the commercial categories under study (*Black, Red* and *White*) experienced a similar behaviour during storage throughout the 12 months in refrigeration conditions, showing a decrease in lightness, redness, antioxidant content, unsaturated fatty acids and an increase in lipid and protein oxidation, which caused an increase in rancidness after 8 months evaluated by a trained panel.

The type of packaging (vacuum versus modified-atmosphere packaging) had an impact on some of the parameters such as colour, with the modified-atmosphere packed slices showing more lightness and redness, although they also revealed higher lipid oxidation from 8 months of storage onwards. Despite this, the trained panel did not identify such differences.

6. The use of NIRS technology proved to be a reliable tool to discriminate and authenticate amongst the *Black, Red* and *White* commercial categories compiled by the current Iberian Quality Standard in modified-atmosphere packed slices of Iberian dry-cured loin samples, without the need to open the packaging. This means a technological challenge for the industry in its continuous search of fast non-destructive solutions for the control, traceability and homogeneity of products.

During refrigeration storage, the discriminating models obtained could be considered as reliable for the classification of the samples by storage times: 0, 4, 8 and 12 months, which is interesting at the industrial level for the logistic control of its stocks.

CONCLUSIONES

1. Las entrevistas en profundidad a los principales actores del sector ibérico; ganaderos e industriales, y su análisis cualitativo, permitieron conocer las oportunidades y principales limitaciones a las que están sujetas tanto la producción como la comercialización de productos ibéricos bajo el marco legislativo del Real Decreto 4/2014, del 10 de enero, por el que se aprobó la norma de calidad para la carne, el jamón, la paleta y la caña de lomo.

Éstos señalaron su insatisfacción con la variabilidad recogida por la Norma en los factores de producción de los animales bajo la categoría comercial asociada con la etiqueta *Verde*. Además, la edad de sacrificio de los animales asociados a la etiqueta *Blanca* se consideró elevada para los intereses de ganaderos. Por otra parte, los industriales pusieron de manifiesto que la congelación previa a la curación de las piezas comerciales puede ser una medida para equilibrar producción y demanda, ya sea en las producciones que se generan durante todo el año, como aquellas ligadas a una estacionalidad como es el caso de la *Montanera.*

2. La edad de los cerdos Ibéricos cruzados con Duroc al comienzo de la fase de acabado en *Montanera* tuvo influencia en algunos parámetros productivos, siendo la GMD menor en los animales de menor edad, dando lugar a menores incrementos de peso al final de la misma. Por el contrario, estos animales tuvieron mayor rendimiento en canal con menores valores de espesor de grasa y jamones de menor tamaño. Esto podría ser de interés para el sector, ya que existe un nicho de mercado que demanda jamones más pequeños y, generalmente, esto es difícil de conseguir con el sistema de producción tradicional de *Montanera*.
El factor sexo (los machos castrados quirúrgicamente y las hembras castradas inmunológicamente) no tuvo influencia en los parámetros productivos de forma mayoritaria.

3. La edad al sacrificio de los cerdos Ibéricos cruzados con Duroc acabados en *Montanera* influyó sobre algunos parámetros de calidad, tanto en el lomo fresco (m. *Longissimus thoracis et lumborum*) como curado. Concretamente, los lomos frescos de animales de mayor edad mostraron coloraciones más rojas y mayor dureza, así como mayor contenido en antioxidantes naturales y ácidos grasos insaturados. Sin embargo, en lomos curados no se observaron diferencias ni en el color ni en los parámetros de textura, mientras que el contenido en antioxidantes y el perfil de ácidos grasos siguió un comportamiento similar al del lomo fresco. Esto sugiere que llevar los animales a mayor edad está ligado a productos con mayor calidad nutricional.

4. La congelación previa a la curación tuvo un efecto significativo sobre algunos parámetros de calidad del lomo Ibérico de *Montanera*. En concreto, los lomos previamente congelados sufrieron mayores mermas durante la curación, que además fueron mayores con el tiempo de almacenamiento en congelación (6 frente a 3 meses), lo que provocó mayor dureza. También se observó un incremento de la oxidación lipídica y proteica, aunque con valores inferiores a los límites detectables por el consumidor.

Por tanto, considerar la práctica de congelación previa a la curación, como una herramienta para solventar la estacionalidad de las producciones en los productos curados como el lomo ibérico de *Montanera*, podría ser de interés a nivel industrial. Pero sería conveniente adaptar las condiciones del proceso de curación a las características de la materia prima de partida (fresca o congelada/descongelada).

5. Las categorías comerciales *Negra*, *Roja* y *Blanca* de la Norma sugieren diferencias en la calidad final de los productos. Así, en el caso del lomo curado loncheado y envasado, éstas fueron observadas principalmente en el contenido en antioxidantes y perfil de ácidos grasos, siendo las categorías procedentes de *Montanera* (*Negra* y *Roja*) las que mostraron mayores valores de alfa y gamma tocoferol y ácidos insaturados.

La calidad de los lomos de las tres categorías comerciales estudiadas (*Negra*, *Roja* y *Blanca*) experimentó un comportamiento similar durante la conservación a lo largo de 12 meses en refrigeración; mostrando un descenso en la luminosidad, color rojo, contenido en antioxidantes, ácidos grasos insaturados y un aumento de las oxidaciones lipídica y proteica, lo que provocó un aumento de la rancidez a los 8 meses, evaluado por un panel de catadores.

El tipo de envasado (vacío vs. atmósfera modificada) tuvo influencia en algunos parámetros de color, siendo las lonchas envasadas en atmósfera modificada las que mostraron mayor luminosidad y color rojo, aunque también mayor oxidación lipídica, a partir de los 8 meses de almacenamiento, aunque el panel de jueces entrenados no observó estas diferencias.

6. El uso de la tecnología NIRS demostró ser una herramienta viable para la discriminación y autentificación de las categorías comerciales *Negra*, *Roja* y *Blanca* definidas por la Norma en muestras de lomo Ibérico loncheado y envasado en atmósfera modificada, sin necesidad de abrir el envase. Esto supone un reto tecnológico a nivel industrial en su constante búsqueda de soluciones rápidas y no destructivas para control, trazabilidad y homogeneidad de sus productos.

Durante la conservación en refrigeración, los modelos discriminantes obtenidos podrían ser fiables para clasificar las muestras por sus distintos tiempos de almacenamiento; 0, 4, 8 y 12 meses, lo cual es interesante a nivel industrial para el control logístico de sus stocks.

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