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Classification of raw cuts from Iberian and Celta pigs based on lipid analysis and chemometrics

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ABSTRACT

This study explores the fatty acid (FA) profiles of four distinct raw cuts sourced from Iberian and Celta pigs, two prominent and commercially significant autochthonous breeds of the Iberian Peninsula. Significant variations in FA profiles were observed among cuts. Livers exhibited the highest lipid content and concentrations of linoleic and arachidonic acids. In contrast, cheek, presa, and loin were characterised by increased monounsaturated FA, primarily oleic acid. Differences were also found between breeds, albeit less pronounced. Palmitoleic acid was more prevalent in Iberian pigs, with the exception of livers, where no significant differences were found. Conversely, Celta pigs displayed higher levels of linoleic and arachidonic acids, except for livers, where arachidonic acid was higher in Iberian pork. A chemometric study successfully distinguishes between Iberian and Celta pig samples using a PLS-DA model. Variable Importance in Projection (VIP) analysis reveals that linoleic, arachidonic, α-linolenic, eicosenoic and palmitoleic acids, along with some minor FA, are key variables for accurate breed classification in each cut, highlighting the importance of comprehensive FA profile analyses that go beyond major fatty acids or unsaturation levels.

1. Introduction

Pork is the second most widely produced and consumed meat worldwide, trailing only chicken production (FAO, 2023) and represents an important part of the European diet (Verbeke et al., 2011). While intensive breeding systems and more productive pig breeds have dominated the 20th century, there is now a growing resurgence in interest towards recovering autochthonous traditional breeds. These breeds though less productive, offer unique advantages, including adaptation to local conditions, sustainable breeding practices, and superior product quality (Thompson et al., 2023).

The Iberian Peninsula's indigenous pig breeds (*Sus scrofa domestica*) fall into two distinct categories: Mediterranean and Celtic (Gama et al., 2013). These genetic lineages are exemplified by their most prominent representatives, the Iberian and Celta pigs, respectively. The Iberian pig, a highly recognised and consolidated autochthonous breed from the southwest of the Iberian Peninsula, is traditionally reared in the holm oak-dominated *dehesa* agrosystem (Ortiz et al., 2021). During the *montanera* fattening period, acorns fall from the oaks, providing pigs with a natural diet rich in acorns, grass and other foraged components, including fungi, tubers and roots (Martínez-Macipe et al., 2020). This traditional grazing practice contributes significantly to the ecological balance of the *dehesa* ecosystem (García-Gudiño et al., 2020; Plieninger et al., 2021). The Celtic breed group encompasses indigenous pig breeds native to the northern Iberian Peninsula, with the Celta pig (*Porco Celta*) as its most prominent representative (Gama et al., 2013). Characterised by remarkable adaptability to the harsh conditions of the northwest Iberian forests (Temperan et al., 2014), the Celta pig thrives on a traditional diet of woodland pastures, acorns, and chestnuts, complemented with cereals, fruits and vegetables (Domínguez et al., 2015). This unique feeding regime contributes to the succulent quality of Celta pork.

While raised in contrasting ecosystems, Iberian and Celta pigs share a common sustainable, extensive outdoor rearing deeply integrated with the environment. This practice contributes to the superior quality of their highly sought-after products. A common differential trait of these traditional pig breeds is the presence of infiltrated fat in muscle, which contributes to the juiciness, flavour and tenderness of the meat (Scollan

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et al., 2017).

The composition of fatty acids (FA) in meat is not solely determined by genetic factors (Corominas et al., 2013; Valdés-Hernández et al., 2023), but is also significantly influenced by the rearing system and feeding practices (Vehovský et al., 2018; Yi et al., 2023). This FA profile plays a critical role in shaping the overall quality of meat products (Sarmiento-García and Vieira-Aller, 2023; Schumacher et al., 2022; Wood et al., 2008).

Extensive rearing systems have been consistently associated with the production of meats enriched in unsaturated fatty acids (UFA). Iberian pork is renowned for its high content of monounsaturated fatty acids (MUFA), primarily oleic acid. This characteristic is attributed to both the exceptional desaturation capacity of the Iberian breed, which facilitates the conversion of dietary an endogenous saturated fatty acids (SFA) into MUFA (Benitez et al., 2015), and the consumption of acorns, a rich source of oleic acid (González-Domínguez et al., 2020; Pajuelo et al., 2023; Pérez-Palacios et al., 2009). Furthermore, a chestnut-based diet has been demonstrated to produce meats with an elevated concentration of UFA, including oleic acid and various polyunsaturated fatty acids (PUFA), in Celta pigs (Bermudez et al., 2012; Domínguez et al., 2015).

The analysis of FA profiles provides valuable insights into meat's sensory features (Tao and Ngadi, 2018). However, analysing FA content across multiple samples generates a considerable amount of data, which can be challenging to interpret without advanced data analysis tools. Chemometrics emerges as a powerful methodology for regression, discrimination, and classification of chemical systems (Varmuza and Filzmoser, 2009), including food studies (Granato et al., 2018). Chemometric methods have proven valuable for classifying dry-cured pork products and raw meat from Iberian and Celta pigs. Unsupervised exploratory techniques like Principal Component Analysis (PCA) (Bermudez et al., 2012; Lorenzo et al., 2014; Pajuelo et al., 2022; Pérez-Palacios et al., 2010; Pérez-Palacios et al., 2009; Rueda et al., 2020) and supervised methods like Partial Least Squares Discriminant Analysis (PLSDA) and its variants (Arroyo-Manzanares et al., 2018; León et al., 2022; Martín-Gómez et al., 2019; Rodríguez-Hernández et al., 2023) have been successfully employed in previous studies.

Despite their significant standing as representatives of the two distinct genetic groups of autochthonous breeds from the Iberian Peninsula, a comparative study of Iberian and Celta pigs remains remarkably absent. Such an investigation could unveil the distinct characteristics of these highly valued pork varieties, considering their divergent genetic profiles, production and feeding practices, and the contrasting ecosystems they inhabit.

This work aims to provide a comprehensive analysis of the FA profile of four commercially important raw pork cuts (loin -*Longissimus thoracis*-, *presa* -*Serratus ventralis*-, cheek and liver) from Iberian and Celta pigs. By employing GC-FID and chemometric approaches, we aim to identify the key differences in FA profiles between the two breeds and their respective cuts. This study will not only provide valuable information for classifying pork cuts according to breed, but also offer a useful tool for authentication and classification of other pork derivative samples.

2. Materials and methods

2.1. Reagents and standards

All chemicals were of analytical grade and were used as received without any further purification. Chloroform, methanol and sodium sulphate from Scharlau (Barcelona, Spain) were used for the lipid extraction. *n*-Hexane, methanol, sodium methoxide, sulphuric acid from Scharlau (Barcelona, Spain), sodium chloride from Panreac (Barcelona, Spain), Supelco 37-component FAME mix and tridecanoic acid from Sigma (Madrid, Spain) were used in the FA transesterification and analysis.

2.2. Experimental design

This study used meat from five Iberian and five Celta pigs (*Sus scrofa domestica*) obtained from two different meat industries. Samples of fresh liver, cheek, *presa* (*Serratus ventralis*) and loin (*Longissimus thoracis*) were collected from each animal. The animals were humanely slaughtered at an industrial abattoir in Guijuelo (Salamanca, Spain) according to approved procedures when they were between 12 and 16 months old and had an approximate weight of 140–160 kg. A piece of 50 g taken from the central part of each pork cut from each animal was minced, vacuum packed and frozen at −80ºC until being analysed. Each of the resulting 40 samples was analysed in triplicate.

2.3. Lipid extraction

The intramuscular fat (IMF) was extracted following the method of Folch et al. (1957) modified by Perez-Palacios et al. (2008). According to this procedure, 5 g of sample was homogenised with 100 mL of chloroform-methanol (2:1 v:v), using an omni mixer homogeniser. The obtained mixture was centrifuged (10 min, 1539 g), and filtered. Distilled water (25 mL) was added to the filtrate, and the resulting mixture was shaken and again centrifuged (10 min, 1539 g). The organic phase was separated and dried by passing it through a small pad of anhydrous sodium sulphate. The solvent was removed in a rotatory evaporator and then under a gentle stream of nitrogen to prevent lipid oxidation. Lipid content was then determined gravimetrically.

Moisture content was determined according to the AOAC method (AOAC Association of Official Analytical Chemists., 2023). Five grams of sample were homogenised with 15 g of sand and a small amount of ethanol in a porcelain capsule and oven dried at 105 °C to constant weight. IMF was quantified relative to the dry sample weight by dividing the weight of fat extracted using the Folch method by the weight of the dry sample and expressing the result as a percentage.

2.4. Determination of fatty acids by gas chromatography

2.4.1. Transesterification

A portion of 10 mg of fat extracted from each sample was transesterified with methanol before being subjected to gas chromatography with flame ionisation detector (GC-FID) analysis. Transesterification of FA was carried out following the method described by Sandler and Karo (1992). Briefly, a 30% solution of sodium methoxide (1 mL) was added to the lipid extract sample (10 mg). The mixture was vortexed briefly and heated in an oven at 80 ºC for 30 min. Then, 5% sulphuric acid in methanol (1 mL) was added, and the mixture was again vortexed and heated at 80 ºC for further 30 min. Then, *n*-hexane (1 mL) and saturated aqueous NaCl (1 mL) were added, and the mixture was shaken and centrifuged at 3464 x g for 2 min. The supernatant phase containing fatty acid methyl esters (FAME) was withdrawn with a Pasteur pipette and placed into a 2 mL GC vial. The solvent was evaporated with a nitrogen flush and the residue was redissolved in *n*-hexane (1 mL).

2.4.2. Gas chromatography analysis and fatty acid quantification

Once FAME were obtained, the gas chromatography analysis was carried out employing a Hewlett–Packard HP-5890A gas chromatograph coupled to flame ionisation detector (GC-FID). Cyanopropyl column (ZEBRON ZB 171 FAME, Phenomenex, California, USA; 20 m × 0.18 mm i.d. × 0.15 µm film thickness) with split injection (100:1) and helium at a constant flow of 2.7 mL/min as the carrier gas were used. Injector and detector temperatures were set at 250 ºC. The temperature profile of the oven started at 150 ºC that increased at 10 ºC/min to 180 ºC; this was held for 1 min and increased again at 7 ºC/min to 205 ºC, which was maintained for 2 min. FAME peaks were analysed and subsequently identified by comparison with suitable standards (Supelco 37-component FAME mix, Merck (Darmstadt, Germany)).

FA quantification was performed employing the external calibration

curve method. A series of five dilutions of the standard mix (Supelco 37-component FAME mix, Merck) was prepared. Calibration curves for each FAME were constructed using the corresponding FAME peak areas versus their respective amounts (Figs. S1-S27). Absolute FAME amounts in milligrams (mg) were converted to relative concentrations by dividing by the total FA content in each sample. Results are expressed as percentages of the total FAME content in the sample.

2.5. Chemometric analysis and statistics

The results from GC-FID quantifications were statistically compared in R v.4.3.1 (R Core Team., 2023) employing the parametric paired Student t-test, in the case of a normal distribution of the data, or the non-parametric U-Mann Whitney test, in the case of non-normally distributed data. A significance value of 95% ($p < 0.05$) was established for all comparisons.

Chemometric analyses were carried out by means of Principal Component Analysis (PCA) (Bro and Smilde, 2014) and Partial Least Squares discriminant analysis (PLS-DA), which were applied to classify pork cuts according to pig breed. The 27 variables per sample corresponding to the concentrations of each FAME derived from GC-FID were subjected to PCA and PLS-DA using the Caret and mixOmics packages in R v.4.3.1 (R Core Team., 2023). Variable Importance in Projection (VIP) in the PLS-DA model was computed using the mixOmics *vip* function in R.

Once models were built, their performance (Cuadros-Rodríguez et al., 2016) was confirmed by four metrics: accuracy, kappa (k), sensitivity (SENS), and specificity (SPEC). These parameters are derived from the confusion matrix built for each model, so they can be calculated as follows:

$$Accuracy = \frac{TP + TN}{TP + TN + FP + FN} \quad (1)$$

$$k = \frac{\Pr_{(a)} + \Pr_{(e)}}{1 - \Pr_{(e)}} \quad (2)$$

$$SENS = \frac{TP}{TP + FN} \quad (3)$$

$$SPEC = \frac{TN}{TN + FP} \quad (4)$$

where, TP and TN are, respectively, the True Positive and True Negative accounting for the samples which have been correctly assigned as belonging (TP) or not belonging (TN) to a specific breed and cut. FP and FN stand for the False Positive and False Negative, respectively, reporting the samples wrongly assigned as belonging (FP) or not belonging (FN) to a specific cut and breed. $\Pr_{(a)}$ is the proportion of agreement between the values obtained by the model and the true values, while $\Pr_{(e)}$ is the proportion of random agreement.

3. Results and discussion

3.1. Fatty acid profile of commercial cuts from Iberian and Celta pigs

The fatty acid (FA) profile of commercial cuts from Iberian and Celta pigs was investigated, with a focus on total fat content and the distribution of polyunsaturated fatty acids (PUFA), monounsaturated fatty acids (MUFA), and saturated fatty acids (SFA). Table 1 summarises the total fat content and the FA profile of liver, cheek, *presa*, and loin from

Table 1
Mean values of the IMF content (expressed as percentage over the dry weight of the sample) and FA profile (expressed as percentages of the total FAME content in the sample) of different cuts from Iberian (n = 5) and Celta pigs (n = 5).

	Liver		Cheek		*Presa*		Loin	
Fatty Acid	**Iberian**	**Celta**	**Iberian**	**Celta**	**Iberian**	**Celta**	**Iberian**	**Celta**
IMF	24.37 ± 5.06	25.84 ± 3.67	21.18 ± 2.99	19.23 ± 2.84	23.96 ± 3.81	22.81 ± 4.38	22.49 ± 3.28	23.16 ± 4.41
C10:0	0.98 ± 0.14	0.78 ± 0.23[a]	0.94 ± 0.09	1.08 ± 0.43	0.67 ± 0.16	0.67 ± 0.09	0.73 ± 0.13	0.98 ± 0.10[a]
C11:0	0.05 ± 0.01	0.14 ± 0.17[a]	0.03 ± 0.00	0.07 ± 0.02[a]	0.03 ± 0.01	0.06 ± 0.01[a]	0.03 ± 0.01	0.05 ± 0.01[a]
C12:0	0.93 ± 0.24	0.69 ± 0.22[a]	0.92 ± 0.08	1.06 ± 0.43	0.61 ± 0.12	0.64 ± 0.1	0.66 ± 0.08	0.97 ± 0.11[a]
C14:0	0.58 ± 0.12	0.74 ± 0.3	1.05 ± 0.09	1.05 ± 0.15	1.58 ± 0.15	1.3 ± 0.11[a]	1.56 ± 0.13	1.24 ± 0.05[a]
C14:1	0.78 ± 0.18	0.6 ± 0.27[a]	0.74 ± 0.07	0.88 ± 0.36	0.46 ± 0.11	0.5 ± 0.09	0.53 ± 0.07	0.79 ± 0.09[a]
C15:0	0.11 ± 0.05	0.15 ± 0.07[a]	0.07 ± 0.03	0.08 ± 0.04	0.03 ± 0.03	0.02 ± 0.00	0.02 ± 0.01	0.02 ± 0.01
C15:1	0.07 ± 0.07	0.07 ± 0.09	0.33 ± 0.7	0.25 ± 0.17	0.42 ± 0.18	0.32 ± 0.14	0.38 ± 0.09	0.71 ± 0.16[a]
C16:0	16.16 ± 1.67	18.36 ± 2.23[a]	22.01 ± 0.38	21.61 ± 0.62[a]	25.17 ± 1.15	23.32 ± 0.50[a]	24.42 ± 2.25	22.71 ± 0.34[a]
C16:1	1.11 ± 0.19	1.36 ± 0.59	3.04 ± 0.23	2.48 ± 0.38[a]	3.9 ± 0.32	2.75 ± 0.30[a]	5.29 ± 0.51	3.56 ± 0.29[a]
C17:0	0.7 ± 0.07	0.78 ± 0.14[a]	0.27 ± 0.02	0.27 ± 0.04	0.21 ± 0.04	0.23 ± 0.03	0.17 ± 0.03	0.17 ± 0.02
C17:1	0.24 ± 0.06	0.34 ± 0.17[a]	0.43 ± 0.02	0.49 ± 0.10	0.24 ± 0.04	0.33 ± 0.08[a]	0.26 ± 0.05	0.33 ± 0.04[a]
C18:0	24.57 ± 2.01	22.18 ± 4.96	11.83 ± 0.59	12.93 ± 1.2[a]	12.41 ± 1.22	12.8 ± 1.01	10.61 ± 1.08	11.55 ± 0.55[a]
C18:1n9c	19.51 ± 1.94	21.33 ± 4.21	41.82 + 1.95	39.42 ± 5.59	44.34 ± 2.18	43.32 ± 2.71	47.28 ± 2.44	44.46 ± 1.71[a]
C18:2n6c	12.81 ± 0.98	15.6 ± 1.79[a]	8.99 ± 0.59	11.7 ± 2.75[a]	5.65 ± 1.37	8.78 ± 0.98[a]	4.07 ± 0.4	7.58 ± 0.89[a]
C18:3n6	0.21 ± 0.15	0.47 ± 0.1[a]	0.14 ± 0.14	0.32 ± 0.16[a]	0.02 ± 0.04	0.06 ± 0.11	0.02 ± 0.03	0.1 ± 0.10[a]
C18:3n3	0.35 ± 0.04	0.55 ± 0.2[a]	0.35 ± 0.03	0.44 ± 0.05[a]	0.3 ± 0.05	0.46 ± 0.06[a]	0.23 ± 0.02	0.36 ± 0.05[a]
C20:0	0.04 ± 0.01	0.04 ± 0.01	0.24 ± 0.08	0.36 ± 0.07[a]	0.19 ± 0.1	0.21 ± 0.10	0.26 ± 0.04	0.29 ± 0.02[a]
C20:1	0.75 ± 0.18	0.3 ± 0.21[a]	2.13 ± 0.13	1.02 ± 0.21[a]	1.78 ± 0.16	0.89 ± 0.16[a]	1.82 ± 0.15	0.8 ± 0.08[a]
C20:2	1.54 ± 0.25	0.59 ± 0.16[a]	1.03 ± 0.07	0.54 ± 0.06[a]	0.52 ± 0.21	0.39 ± 0.03[a]	0.44 ± 0.13	0.32 ± 0.03[a]
C20:3n6	0.46 ± 0.1	0.35 ± 0.09[a]	0.3 ± 0.05	0.4 ± 0.15	0.13 ± 0.08	0.2 ± 0.04[a]	0.12 ± 0.06	0.27 ± 0.06[a]
C20:4n6	15.97 ± 1.45	12.83 ± 2.76[a]	2.09 ± 0.19	2.95 ± 1.14[a]	0.85 ± 0.49	1.68 ± 0.46[a]	0.61 ± 0.22	1.95 ± 0.21[a]
C20:3n3	0.06 ± 0.02	0.06 ± 0.02	0.05 ± 0.01	0.05 ± 0.01	0.04 ± 0.01	0.04 ± 0.01	0.03 ± 0.01	0.04 ± 0.01
C20:5n3	0.42 ± 0.05	0.35 ± 0.09[a]	0.16 ± 0.02	0.17 ± 0.05	0.14 ± 0.05	0.16 ± 0.04	0.12 ± 0.03	0.13 ± 0.03
C22:2	0.05 ± 0.11	0.02 ± 0.01	0.01 ± 0	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00
C23:0	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.00	0.01 ± 0.00	0.02 ± 0.00
C24:1	0.94 ± 0.17	0.67 ± 0.14[a]	0.19 ± 0.02	0.21 ± 0.09	0.04 ± 0.04	0.15 ± 0.04[a]	0.03 ± 0.02	0.14 ± 0.04[a]
C22:6	0.44 ± 0.08	0.57 ± 0.35	0.08 ± 0.01	0.1 ± 0.04	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01	0.06 ± 0.01
ω3	1.27 ± 0.12	1.54 ± 0.32[a]	0.64 ± 0.06	0.75 ± 0.11[a]	0.55 ± 0.12	0.73 ± 0.08[a]	0.45 ± 0.07	0.59 ± 0.07[a]
ω6	31.07 ± 1.67	29.88 ± 2.51	12.66 ± 0.63	16.01 ± 4.16	7.32 ± 1.97	11.24 ± 1.36[a]	5.48 ± 0.56	10.36 ± 1.13[a]
PUFA	32.34 ± 1.69	31.42 ± 2.72	13.3 ± 0.66	16.75 ± 4.24[a]	7.87 ± 2.03	11.97 ± 1.39[a]	5.93 ± 0.60	10.95 ± 1.17[a]
MUFA	23.52 ± 2.09	24.69 ± 4.58	48.79 ± 1.83	44.78 ± 5.56[a]	51.21 ± 2.21	48.76 ± 2.47[a]	55.59 ± 2.99	51.06 ± 1.86[a]
SFA	44.15 ± 1.13	43.88 ± 2.78	37.39 ± 0.73	38.48 ± 1.5[a]	40.92 ± 2.23	39.27 ± 1.33[a]	38.48 ± 3.30	37.99 ± 0.86[a]

[a] Significant differences between breeds within each type of cut at a significance level of 95% ($p < 0.05$). IMF: Intramuscular fat. PUFA: Polyunsaturated fatty acids, MUFA: Monounsaturated fatty acids, SFA: Saturated fatty acids.

both breeds. Statistical comparisons were performed to determine whether there were significant differences in total fat and FA content between the two breeds at a significance level of 95% ($p < 0.05$).

3.1.1. Intramuscular fat (IMF)

Unsurprisingly, liver presented the highest fat content, followed by *presa*, loin and cheek. However, the variation across cuts is lower than 25%. Intramuscular fat (IMF) is a characteristic trait of native pig breeds and is one of the most important factors defining the quality and distinct organoleptic traits of the meat (Mayoral et al., 1999; Pugliese and Sirtori, 2012; Schumacher et al., 2022). IMF content was similar for the same cut across breeds and no statistical differences were found.

3.1.2. Overall FA profile

Previous research has shown substantial variations in fatty acid (FA) profiles of pork derived from native and commercial pig hybrids (Kasprzyk et al., 2015; Li et al., 2021; Nevrkla et al., 2017; Nevrkla et al., 2023; Seo et al., 2023; Touma et al., 2017). However, direct comparisons of FA profiles between different autochthonous breeds (Debrecéni et al., 2018; Fortina et al., 2005; Pugliese and Sirtori, 2012) or strains (Estévez et al., 2003; Garrido et al., 2023) have been less common, yielding less pronounced differences. Notably, the FA profiles of Iberian and Celta pork remain unexplored in direct comparison. Our comparative analysis of Iberian and Celta pork FA profiles revealed significant differences between the two breeds, particularly in the distribution of polyunsaturated fatty acids (PUFA) and monounsaturated fatty acids (MUFA). However, these breed-specific differences are less marked than the variations observed across different pork cuts. Statistically significant differences ($p < 0.05$) between the two breeds were observed in all FA categories (PUFA, MUFA, and SFA) across all cuts except liver.

In general, Iberian pork exhibits a higher MUFA content, with oleic acid as the predominant FA in both breeds. This is attributed to the diet of Iberian pigs, which typically includes acorns and other high-fat plant-based sources. Conversely, Celta pork displays a higher PUFA content, with linoleic acid (C18:2) as the major PUFA.

3.1.3. Liver

The FA profiles of different pork cuts vary significantly, with distinct patterns observed across breeds. The FA profiles of livers from Iberian and Celta pigs differ significantly from those of muscle tissues. Liver is the cut that shows the higher content of saturated fatty acids (SFA), where they are the main lipid components, accounting for approximately 44% of the total FA content. Stearic acid (C18:0) constitutes 22–25% and palmitic acid (C16:0) content stands at approximately 16–18% (Table 1). Palmitic acid is significantly higher in Celta livers. Remarkably, livers from both Iberian and Celta pigs also exhibit a relatively high proportion of polyunsaturated fatty acids (PUFA), with linoleic (C18:2n6c) and arachidonic acids (C20:4n-6) as the primary contributors. PUFA play a crucial role in liver structure and function, contributing to both metabolic processes and cell membrane integrity. Among PUFA, arachidonic acid is the most prevalent in Iberian livers, constituting approximately 16% of the FA content, followed by linoleic acid at around 13%. Conversely linoleic acid prevails over arachidonic acid in Celta livers, accounting for approximately 16% and 13% of FA content, respectively. The substantial presence of PUFA in pork liver elevates its health-promoting potential, as these PUFA have been linked to various health benefits, including the mitigation of cardiovascular diseases. Notably, linoleic acid plays a crucial role in regulating low-density lipoprotein (LDL) metabolism (Froyen and Burns-Whitmore, 2020), while arachidonic acid participates in diverse structural and metabolic processes, particularly as a key second messenger molecule (Tallima and El Ridi, 2018). Monounsaturated fatty acids (MUFA) are present in a lower proportion, with oleic acid (C18:1n9c) contributing 20–21% and palmitoleic acid (C16:1) contributing just 1%. No statistical differences were displayed in stearic, oleic, and palmitoleic acids between Iberian and Celta livers. Previous studies have observed consistent proportions of SFA, MUFA and PUFA, along with characteristic FA, such as linoleic, arachidonic, oleic, palmitoleic, stearic and palmitic acids in Iberian (Estévez et al., 2004) and Celta (Domínguez et al., 2015) pig livers. Conversely, crossbred commercial pigs usually show lower MUFA and higher SFA contents (Babicz et al., 2018). Therefore, autochthonous pig breeds, such as Celta and Iberian, emerge as a healthier alternative for human consumption. This is corroborated by their liver PUFA/SFA ratios of 0.7, well above the recommended minimum of 0.4 for optimal human health (Wood et al., 2008). Nevertheless, the ω6/ω3 ratios in both breeds surpass the recommended guidelines, suggesting a potential intake excess of omega-6 fatty acids (Hammad et al., 2016; Russo, 2009).

3.1.4. Cheek

In stark contrast to the liver, the remaining tissues exhibit a distinct fatty acid pattern, characterised by a prominence of monounsaturated fatty acids (MUFA), particularly oleic acid. In the cheeks MUFA account for nearly half of the total fatty acids, with oleic acid contributing around 39–42% and palmitoleic acid contributing around 3%. Intriguingly, no statistically significative differences between breeds were found for oleic acid; however, palmitoleic acid predominates in Iberian cheeks. Linoleic acid emerges as the predominant PUFA, constituting 9–12% of the total FA content, while arachidonic acid is present in a relatively minor proportion, ranging up to 3%. Celta cheeks display significantly higher contents of these two PUFA compared to Iberian cheeks. The major SFA in pork cheeks are palmitic acid, which constitutes 22%, being more abundant in Iberian pigs, and stearic acid, which accounts for 12–13% and is more abundant in Celta pigs (Table 1). Although pork cheek has gained renewed culinary popularity (Sanchez del Pulgar et al., 2012), to our knowledge, no previous studies have investigated its FA profile. Notably, pork cheek harbours a PUFA content exceeding that of other cuts, excluding liver. Additionally, its total UFA surpasses that of liver and the PUFA/SFA ratio for Iberian and Celta cheeks is 0.36 and 0.44, respectively, approaching the recommended dietary value.

3.1.5. Presa

Presa and loin show a similar FA profile. Oleic acid is again the major FA in presa, representing more than 43% of the total FA content, although no significative differences in this FA were found between breeds. Together with palmitoleic acid (3%-4%), they make up the main part of MUFA, which represent nearly 50% of the total FA content. *Presa* samples from both breeds showcase lower proportions of palmitoleic acid and higher proportions of palmitic acid (23–25%) compared to loin samples. The proportion of these two FA is significantly higher in Iberian pigs. Stearic acid represents approximately 12% of the FA content. The contribution of linoleic acid is around 6–9%, whereas arachidonic acid only reaches less than 2%, and in both cases is higher in Celta pigs (Table 1). Notably, *presa* samples from both breeds exhibit statistical differences in almost all major FA, with the exceptions of oleic acid and stearic acid. *Presa* is a traditional pork cut very appreciated in native breeds, especially Iberian pig. Our results are in line with previously reported FA profiles of Iberian pig *presa* (Tejerina et al., 2012a; Tejerina et al., 2012b).

3.1.6. Loin

Loin samples exhibited statistical differences in all major FA constituents. Loin samples from both Iberian and Celta pigs demonstrate a higher proportion of MUFA, with oleic acid as the primary FA contributor, as it was in cheek and *presa*. However, Iberian loin exhibits a significantly higher content of oleic acid (approximately 47%) compared to Celta loin (approximately 45%). Other major FA are palmitic acid and stearic acid, which account for approximately 23–24% and 11%, respectively. Linoleic acid values reach up to 8%, while arachidonic acid is present in less than 2% (Table 1). Compared to loins from commercial breeds reared in intensive system, our results show

higher MUFA and lower PUFA contents (Bragagnolo and Rodriguez-Amaya, 2002; Estévez et al., 2003). Intriguingly, supplementation of the animal's diet with natural or fermented herbs has been shown to enhance the MUFA content, particularly oleic acid, and reduce PUFA levels in loin meat, approaching those observed in Iberian and Celta pigs. This highlights the influence of feeding practices on the lipid profile of pork (Ahmed et al., 2016). Moreover, our results corroborate previous reports on the fatty acid composition of loin meat from Iberian (Estévez et al., 2003; Rey et al., 2006) and Celta (Domínguez et al., 2015) pigs.

A comparison of the different pork cuts reveals distinct FA profiles, reflecting the varying biological roles of these tissues (Corominas et al., 2013). The liver, a major FA metabolism hub, regulates the balance between fat storage and utilisation, whereas FA in skeletal muscles play a multifaceted role function, contributing to different functions, such as energy production, membrane structure and signalling. In terms of FA composition, liver exhibits a higher content of PUFA, while cheek, *presa* and loin contain a higher proportion of MUFA. PUFA content diminishes from cheeks to presa and loin, while MUFA content increases correspondingly. Omega-3 FA constitute approximately 1.5% of the total FA content in liver and less than 1% in other cuts. Omega-6 FA, on the other hand, are the predominant PUFA in all cuts and account for the observed differences in PUFA distribution. Although SFA levels remain almost constant across cuts, small differences between cuts are statistically significant in most cases, except between cheeks and loin in Iberian pigs and cheeks and presa and cheeks and loin in Celta pigs ($p > 0.05$). Remarkably, no significant differences were observed in ω3 FA content between *presa* and cheek and in ω6 FA content between presa and loin in Celta pigs ($p > 0.05$). All other comparisons revealed statistically significant differences in PUFA, ω3, ω6, MUFA and SFA levels between cuts ($p < 0.05$). In general, the FA profile for loins, cheeks and liver reported in this study aligns well with findings from previous research (Daza et al., 2007; Estévez et al., 2003; Rey et al., 2006).

In summary, the FA profiles of Iberian and Celta pork demonstrate distinct breed-specific characteristics, with Iberian pork characterised by higher MUFA and lower PUFA content compared to Celta pork. However, these breed-specific differences are less pronounced than the variations observed across different pork cuts. In general, linoleic acid and arachidonic acid contents are greater for Celta samples. MUFA dominates in Iberian samples, with oleic acid being the main contributor in both breeds, while palmitoleic acid is statistically different between breeds for each cut except in liver. Palmitic acid is the major SFA in both breeds (except in liver), with its values being higher for Iberian pig. On the other hand, stearic acid content is either predominant in Celta pig or shows no significant differences between breeds, depending on the particular cut. Loin, presa, and cheek samples from both breeds exhibit distinct FA profiles, reflecting their unique metabolic and functional roles. Liver samples from both breeds harbour unique FA profiles, influenced by their metabolic functions and nutrient uptake.

3.2. Chemometric results of fatty acid composition

The FAME percentages obtained from the GC-FID analysis of all samples were subjected to principal component analysis (PCA) as an initial data exploration (Fig. 1). The PCA model was constructed using 27 principal components. The first two and three components account for 65.04% and 74.00% of the total variance respectively (50.71% for PC1, 14.34% for PC2 and 8.95% for PC3), which is over the recommended minimum 65% (Bro and Smilde, 2014). Each of the remaining 24 components contribute less than 5% to the total variance.

The bidimensional scores plot that represents the two first principal components (Fig. 1) already shows some sample aggregation tendencies. Not surprisingly, liver samples cluster in a group that is clearly differentiated from the rest of the cuts, with positive values for PC1 and positive or near-zero values for PC2. However, this model does not allow us to distinguish Iberian from Celta pig liver samples. Cheeks are represented in the lower part of the graph, with mostly negative values for PC2. Celta cheeks show considerably higher dispersion, while Iberian cheeks cluster in a quite compact group with slightly negative values for PC1 and PC2. The rest of the cuts lay in the middle and the left of the scores plot. Loins samples from Celta and Iberian pigs cluster in completely differentiated groups, while *presa* samples from both breeds tend to form different groups that however show some overlapping. Iberian *presa* samples present higher dispersion, partially overlapping Celta *presa* samples. Notably, Iberian loin samples cluster together with Iberian presa samples, suggesting that both cuts have similar FA profiles.

Fig. 1B shows direction vectors representing the FA loadings associated with the two first components of the PCA model. Liver samples are characterised by high loadings of PUFA such as linoleic, arachidonic, EPA and DHA, among others. Conversely, *presa* and loin are associated with high loadings of MUFA such as oleic and palmitoleic acids. Cheek has also important contributions from minor FAs, such as C10:0, C12:0,

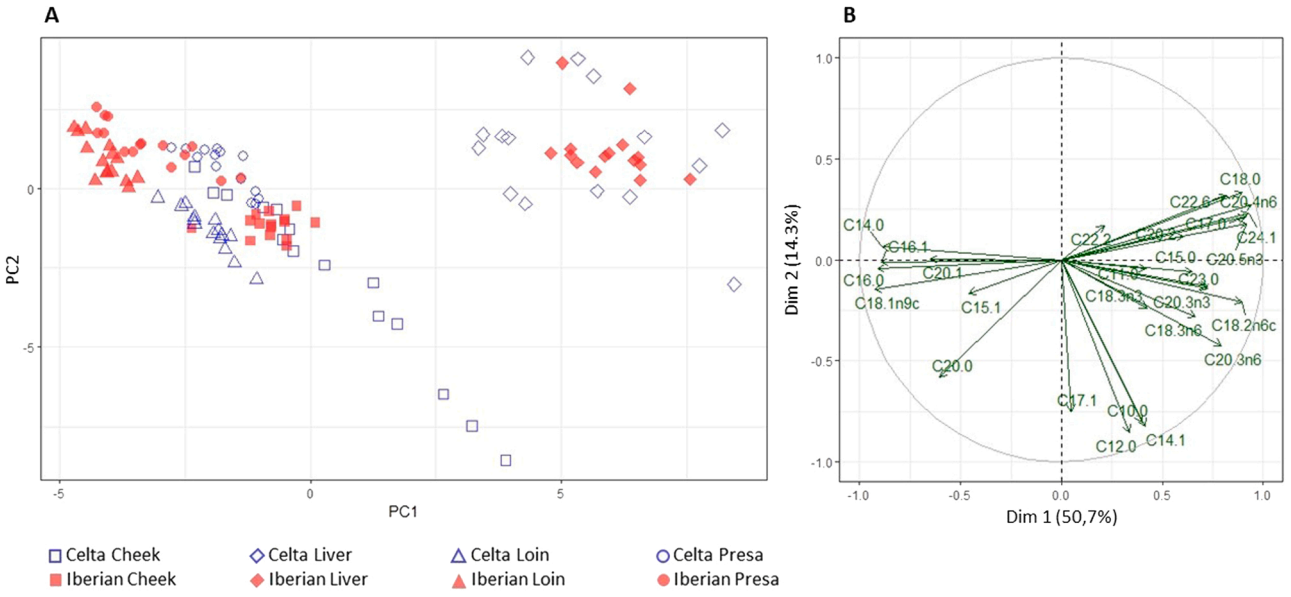

Fig. 1. A. PCA scores plot of Iberian and Celta pig samples from the FA proportions in the FA profile. B. Loadings plot representing the correlation between the FA proportions and the principal components.

C14:1 and C17:1, particularly in Celta samples. Notably, stearic acid seems to be relevant in defining liver FA profile, while palmitic acid is mainly represented in *presa* and loin.

Remarkably, the eight sample groups corresponding to the four cuts of the two pig breeds already exhibit clear clustering tendencies with the first two components of PCA. This suggests that it is possible to classify pork cut samples based on their breed and location using a chemometric approach that relies on their FA profiles. Thus, a supervised Partial Least Square Discriminant Analysis (PLS-DA) was performed with the aim of achieving accurate discrimination and prediction ability. The optimal PLS-DA model was selected using resampling with a 10-fold cross-validation and achieved the highest accuracy (92%), Kappa (0.91), sensitivity (0.93), and specificity (0.98) for 10 components.

The PLS-DA model's classification performance was evaluated by the confusion matrix comparing the predicted and actual classes (Fig. 2). Correctly classified samples are represented on the diagonal of the graphic, while misclassified samples are located outside it. The number and percentage of classified samples with respect to the total are displayed in the centre of each square in the matrix. At the bottom of each tile is represented the column percentage and, at the right side of each tile, the row percentage. The row percentage of the tiles on the diagonal of the graphic represents the classification accuracy for each class. It is the percentage of correct predictions over the total predictions of the corresponding class. For example, the accuracy for Iberian presa is 93.3%, because, out of the total predictions made for Iberian presa, 14 samples were correctly classified while 1 was erroneously classified as Iberian loin.

Most of the samples were correctly classified. Misclassifications occur just for one sample of Iberian *presa* that was categorised as Iberian loin, and two samples of Celta cheek that were predicted as Celta *presa*. Overall, despite the heterogeneity of the data and the relatively high number of classes, 97.5% samples were correctly discerned for a specific tissue and breed.

Furthermore, separate PLS-DA models were constructed for each cut, also performing a 10-fold cross-validation procedure to build each model and prevent overfitting issues. Practically all metrics (accuracy, kappa, sensitivity, and specificity) (Cuadros-Rodríguez et al., 2016) were above 0.90 in all pieces (Table 2).

The accuracy for all the classification in cross-validation and prediction was over 0.95, while the Kappa statistic, which accounts for the agreement between predicted and true values, is above 0.90 for the different models. This is considered very good agreement between real and predicted values (McHugh, 2012).

Sensitivity and specificity are important metrics used to characterise the performance of the models. Sensitivity measures the ability of the models to correctly identify a sample to correspond to a particular cut (true positive rate), while specificity is the ability to correctly discern a sample as not belonging to a particular class (true negative rate). The cross-validation metrics of PLS-DA models have sensitivities and specificities higher than 0.95, which are considered very good (Cuadros-Rodríguez et al., 2016). Prediction metrics of PLS-DA models for cuts are all 1.00.

The classification of samples generated by these models is represented in Fig. 3, which shows the two-dimensional scores plots for the

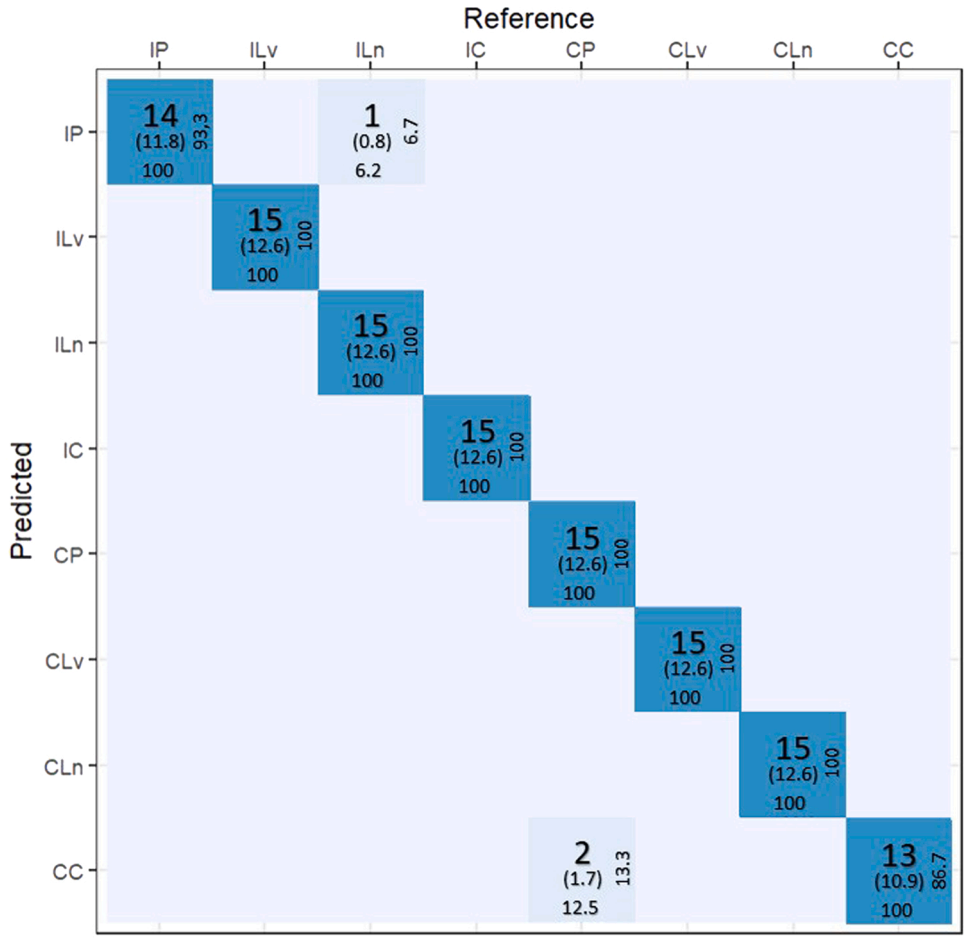

Fig. 2. Confusion matrix for the PLS-DA model. IP: Iberian Presa, ILv. Iberian Liver, ILn: Iberian Loin, IC: Iberian Cheek, CP: Celta Presa, CLv: Celta Liver, CLn, Celta Loin, CC: Celta Cheek. In each tile, the number and (percentage) of classified samples with respect to the total are displayed in the centre, the column percentage is represented at the bottom and the row percentage at the right side.

Table 2
Performance metrics of the PLS-DA models constructed for each pork cut.

	Cross-Validation				Prediction			
Cut	**Accuracy**	**Kappa**	**Sensitivity**	**Specificity**	**Accuracy**	**Kappa**	**Sensitivity**	**Specificity**
Liver	0.98	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Cheek	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Presa	0.95	0.90	0.95	1.00	1.00	1.00	1.00	1.00
Loin	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

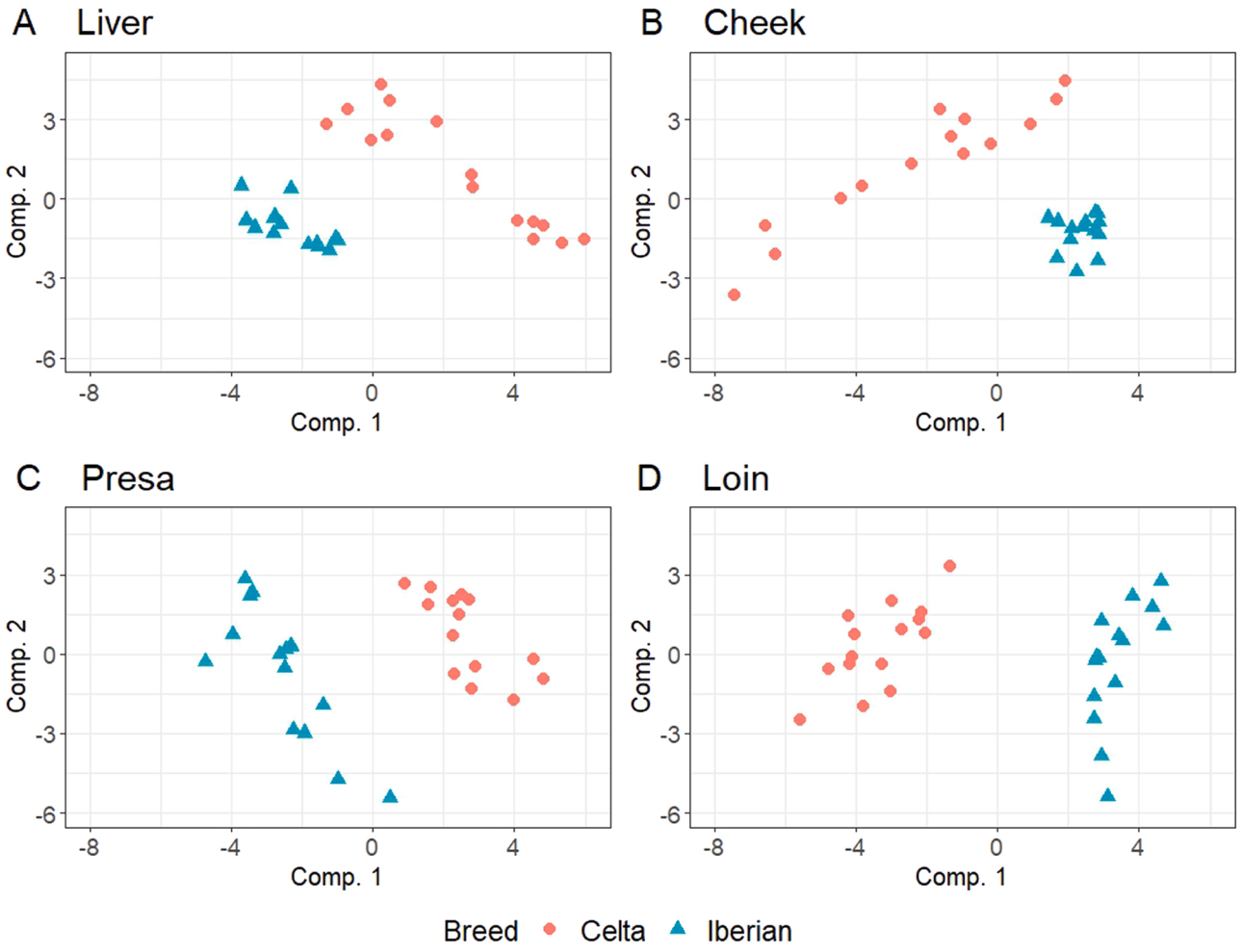

Fig. 3. PLS-DA two-dimensional scores plots for each cut. Samples cluster according to breed.

two first components of each cut. Celta and Iberian samples of each cut are correctly discerned. Liver samples (Fig. 3A) show higher dispersion in the Celta samples, while Iberian liver samples form a compact group at negative component values. A similar picture is shown for cheeks (Fig. 3B). *Presa* and loin samples (Fig. 3C-D) also show a clear separation, with the Iberian samples clustered on one side of the graph and Celta samples on the other.

To identify the FA that most effectively differentiate between Celta and Iberian pork cuts, we employed a Variable Importance in Projection (VIP) analysis. Fig. 4 summarises the ten principal FAs that contribute to the segregation of the samples in each cut, showing that all have VIP values over one and p-values < 0.05 (Table 1).

The VIP analysis revealed that unsaturated fatty acids (UFAs) play a pivotal role in distinguishing the two breeds. Linoleic (C18:2n6c), arachidonic (C20:4n6), α-linolenic (C18:3n3) and eicosenoic (C20:1) acids emerged as crucial variables in the classification of all the cuts. This finding aligns with previous studies demonstrating the influence of the diet and rearing system on pork PUFA content (Gómez et al., 2017; Pérez-Palacios et al., 2009; Wood et al., 2008). The discriminatory capability of minor α-linolenic and arachidonic acids was reported by Pérez-Palacios et al. (2009) to differentiate Iberian pigs fattened outdoors on acorns and grass from those fed compound feeds. Palmitoleic acid (C16:1) also emerged as a significant classifying factor in the three muscular cuts (Fig. 4B-D), but not in liver (Fig. 4A). This observation is consistent with previous studies highlighting the importance of palmitoleic acid to distinguish between native Chinese black pigs from hybrid breeds (Li et al., 2021) and as a differentiating factor between Iberian lines (Caballero et al., 2018).

Other minor FA also played crucial roles in differentiating the breeds across various cuts. Among PUFA, γ-linolenic (C18:3n6) and eicosadienoic (C20:2) acids were found to be significant in liver (Fig. 4A) and cheek (Fig. 4B), while dihomo-γ-linolenic (C20:3n6) acid was crucial in liver (Fig. 4A) and loin (Fig. 4D). Minor MUFA, such as nervonic (C24:1), heptadecenoic (C17:1), and myristoleic (C14:1), and SFA, such as arachidic (C20:0), myristic (C14:0), lauric (C12:0), undecanoic (C11:0) and capric (C10:0), also contributed to the discrimination

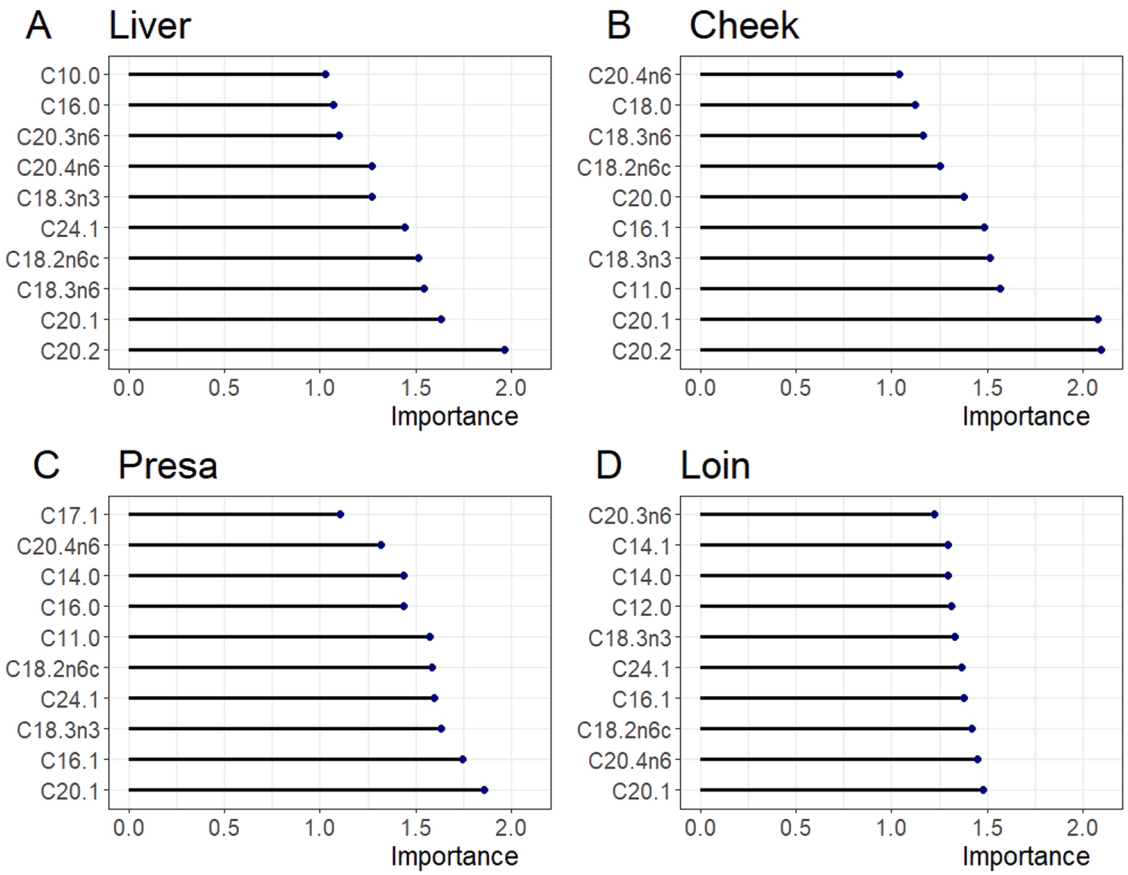

Fig. 4. Top ten FA as important variables for discrimination between traditional breeds in PLS-DA model for different pig tissues, according to VIP scores.

between cuts.

Eicosadienoic acid exhibited the highest VIP scores in liver and cheek, with significantly higher concentrations in Iberian samples. Eicosenoic was the second most important in these cuts and the most important in presa and loin, with its concentration in Iberian samples more than doubling that of Celta samples (Table 1). These two FA were identified as key differentiating factors in PLS-DA models built to distinguish between native Chinese black pigs from hybrid breeds (Li et al., 2021), and to classify Bamaxiang, Erhualian and Laiwu Chinese indigenous pig breeds (Huang et al., 2020).

Interestingly, major MUFA and SFA identified as key predictors of differentiation between native and hybrid pigs (Estévez et al., 2003; Kasprzyk et al., 2015; Nevrkla et al., 2017; Nevrkla et al., 2023; Seo et al., 2023), were not found to be significant in differentiating Iberian and Celta pork. Consequently, oleic acid (C18:1n9c) did not rank among the top ten most influential variables in any cut. Stearic (C18:0) acid seemed to be relevant solely in differentiating Iberian and Celta cheek cuts, while palmitic acid (C16:0) proved important in *presa* and liver. These findings indicate that Iberian and Celta pork share relatively similar FA profiles, which contrast sharply with those of commercial hybrid pigs. Nevertheless, the subtle differences between these two native Iberian breeds are substantial enough to make fatty acid analysis a valuable tool for distinguishing between Celta and Iberian pork cuts. Specifically, VIP analysis revealed that UFAs, particularly linoleic, arachidonic, α-linolenic, and eicosenoic acids, play a crucial role in differentiating the two breeds.

4. Conclusion

This study comparatively analysed the FA profile of four valuable commercial cuts (liver, cheek, *presa*, and loin) from two autochthonous Spanish pig breeds. Differences in the FA profiles between the different cuts are evident. Notably, liver has a higher PUFA content, whereas cheek, *presa* and loin have a higher MUFA content. Differences between the two breeds were also found, although not as noticeable, specifically related to the level of FA unsaturation. PUFAs were more prevalent in Celta breed, whereas MUFAs were higher in Iberian pigs.

A chemometric study of the FA profile was able of correctly discriminating between pieces from both pigs using a PLS-DA model. Distinct fatty acids contribute to the segregation in the different pieces. Linoleic, arachidonic, α-linolenic, eicosenoic and palmitoleic acids were involved as discriminatory variables in most cases. Importantly, the determination of the VIP of the PLS-DA models reveals the significant influence of some minor fatty acids in classification. This suggests the importance of performing comprehensive fatty acid profile analyses and not just focusing on major fatty acids or the sum of fatty acid concentrations.

CRediT authorship contribution statement

Teresa Antequera: Writing – review & editing, Validation, Resources, Funding acquisition, Data curation, Conceptualization. **Abraham Pajuelo:** Methodology, Investigation, Data curation. **Ana G. Neo:** Writing – review & editing, Supervision, Methodology, Data curation, Conceptualization. **Trinidad Pérez-Palacios:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **José Luis Ramiro:** Writing – original draft, Software, Methodology, Investigation, Formal analysis, Data curation. **Carlos F. Marcos:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jfca.2024.106173.

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