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RESEARCH ARTICLE

Lipidomic analysis and classification of Iberian dry-cured hams with low field NMR

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Abstract

Iberian ham is a high-quality food that is traditionally produced from black pigs that are native to the Iberian Peninsula. The taste and texture of Iberian ham are largely determined by its lipid content and composition. In this work, low field ^{1}H-NMR spectroscopy was used for the first time to analyze the lipid profile of Iberian hams as a determinant of quality. The quantification of fatty acids (FAs) is readily obtained from the integration of the spectral peaks. The FA profile is highly influenced by the diet of the pigs, with monounsaturated FAs being more prominent in hams from acorn-fed pigs. The NMR data was analyzed using a sparse partial least squares multivariate data analysis multivariate model, which allowed for the categorization of Iberian hams according to the pig's feeding regime. The main variables on which this classification is based are FA unsaturation and triacyl glyceride hydrolysis. On the other hand, there are no significant differences in the phospholipid content of the different categories of Iberian hams as determined by colorimetric and inductively coupled plasma optical emission spectroscopy methods.

Benchtop ^{1}H-NMR spectroscopy has thus revealed as an operationally simple and powerful tool for the lipid analysis and classification of Iberian hams according to the diet of pigs.

KEYWORDS
benchtop NMR, chemometrics, cured meat, fatty acids, lipidomics, nuclear magnetic resonance spectroscopy, phospholipids

1 | INTRODUCTION

Iberian ham is a traditional and highly appreciated food produced from black pigs native to the Iberian Peninsula. The quality of Iberian ham is determined by crossbreed and rearing system of the Iberian pig and the processing conditions. The Spanish government strictly controls the quality of Iberian ham establishing four categories, labeled with different colors: "Black tag" is for 100% Iberian pigs exclusively fed on acorns and grass in an extensive system (*montanera*); "Red tag" is also an acorn ham, at least 50% Iberian breed; "Green tag" corresponds to at least 50% Iberian breed pigs, reared in extensive system and feed with concentrate feeds; and "White tag" is used for pigs that are at least 50%

Iberian breed reared in an intensive system (Boletín Oficial del Estado [BOE], 2014).

Feeding is crucial to define the lipid content and fatty acid (FA) profile of pig tissues (Pérez-Palacios et al., 2009; Tejeda et al., 2002), which give Iberian hams their characteristic texture, aroma and flavor (Carrapiso et al., 2003; Cava et al., 2000). Pigs fed on acorns and grass in extensive regime (black and red labels) produce hams with superior sensory traits that reach the highest prices in the market.

Most of the methods for the quality control and classification of Iberian hams rely on the analysis of their lipid profile, the most used being gas chromatography with flame ionization detector (GC-FID) of the lipidic fraction after transmethylation in acid medium (Petron et al., 2004).

Nuclear magnetic resonance (NMR) spectroscopy has become a popular tool in food analysis in recent years. This is mainly because it is a fast, reproducible, and nondestructive technique that provides detailed information on the chemical composition with little or no treatment required in food samples (Cao et al., 2021). Proton nuclear magnetic resonance (^{1}H-NMR) spectroscopy has been widely used for lipid analysis of different types of food (Alexandri et al., 2017; Lankhorst & Chang, 2017; Li et al., 2017), and we have recently developed a method for the analysis of the lipid profile of Iberian dry-cured hams using high field ^{1}H-NMR spectroscopy (Pajuelo et al., 2022). Although high field NMR has proven to be a very useful and powerful technique, it suffers from serious drawbacks, such as the need for expensive cryogenic spectrometers and highly specialized personnel.

Interestingly, benchtop NMR spectrometers present a viable alternative to high field equipment. Although they work at lower fields, benchtop spectrometers are characterized by excellent permanent magnet homogeneity, low maintenance cost, robustness, and operational simplicity (Yu et al., 2021). When combined with chemometric techniques, low field NMR spectroscopy provides useful information on the composition of different foods and a tool for their classification. For instance, this method has been utilized to analyze wine and other fermented beverages (Matviychuk et al., 2021), to determine oil authenticity and adulteration (Gunning et al., 2020; McDowell et al., 2019), to asses fruit acceptability (Migues et al., 2022), and to identify the species of origin of meat products (Jakes et al., 2015). However, this methodology has never been used to analyze the lipid profile of dry-cured pork products.

The aim of the present study was to evaluate the feasibility of low field NMR spectroscopy to analyze the composition and FA profile of intramuscular lipids from different categories of Iberian hams. In addition, multivariate discriminant analysis applied on low field ^{1}H-NMR data was done.

2 | MATERIALS AND METHODS

2.1 | Reagents and standards

Chloroform, methanol, and sodium sulfate from Scharlau were used for the lipid extraction. Deuterochloroform ($CDCl_3$) containing 1% TMS from Sigma was used in the NMR analysis.

2.2 | Experimental design and sample preparation

The samples used in this study were a selection of Iberian ham lipid extracts obtained during the development of the original method at 500 MHz (Pajuelo et al., 2022). Samples of Iberian ham (sliced in 90 g packages) of different categories (black, red, green, and white) and commercial brands were purchased from an Iberian ham industry. In this study, two commercial brands from each category were used, making a total of eight samples. The samples were minced and homogenized, and three portions of 5 g were taken from each one, resulting in 24 samples, which were vacuum packed and frozen until analysis.

Intramuscular fat was extracted following the method of Folch (Folch et al., 1957) modified by Pérez-Palacios (Pérez-Palacios et al., 2008). According to this procedure, each sample (5 g) was homogenized with 100 mL of chloroform–methanol (2:1, v:v), using an omni mixer homogenizer. 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 sulfate. The solvent was removed in a rotary evaporator and then under a gentle stream of nitrogen to prevent lipid oxidation.

2.3 | Low field ^{1}H-NMR

Samples (25 mg of the extracted lipid fractions) were dissolved in 500 μL of $CDCl_3$ containing 1% TMS as internal reference and placed in standard 5 mm NMR tubes. ^{1}H-NMR spectra were recorded in an 80 MHz Magritek Spinsolve Ultra 80 spectrometer at 298 K, using a 90° pulse and a spectral width of 5000 Hz. A total of 65,536 data points were collected after 256 scans, with a repetition time of 7 s and acquisition time of 6.4 s.

Spectra were processed using the MestReNova software (Mestrelab Research, 2019) applying an exponential apodization of 0.3 MHz and conserving the phase and baseline corrections determined by the spectrometer. The spectra were referenced to the TMS signal (0 ppm), cut between 6.5 and 0.5 ppm, and normalized to the total area. Integration of the different signals was carried out using MestReNova algorithm. The signals corresponding to the different FAs were normalized to the peak of the methylene protons α to the carboxyl group (peak E, Figure 1). The relative values of the integrals were then used to determine the relative proportions of the different lipid constituents.

^{1}H-NMR signals were assigned according to the assignations previously made on the high field spectra and to previous studies (Tsiafoulis et al., 2019; Nieva-Echevarría et al., 2015). The low field spectra allowed the quantification of polyunsaturated (PUFA), monounsaturated (MUFA), and saturated FAs (SFA), according to the equations described in Section 3.

2.4 | Phospholipid quantification

Samples of extracted lipids (20–30 mg) were exactly weighed and placed in PTFE tubes. HNO_3 (4 mL) and distilled water (4 mL) were

added, and the tubes were placed in a microwave digestor, heated up to 200°C for 10 min and then maintained at 200°C for 15 min. Phospholipids (PLs) were quantified in the digested samples using two different methods:

Colorimetric determination of PLs was carried out by a modified ascorbic acid/molybdenum blue method (American Oil Chemists' Society [AOCS], 2009; Watanabe & Olsen, 1965). Briefly, Reagent A (0.1 M ascorbic acid and 0.5 M trichloroacetic acid; 10 mL) was placed in a 25-mL volumetric flask and an aliquot of the digested lipid sample was transferred to the flask. Reagent B (0.01 M ammonium molybdate; 2 mL) and Reagent C (0.1 M sodium citrate, 0.2 M sodium arsenite and 5% acetic acid; 5 mL) were immediately added, and the contents of the flask were mixed and diluted to volume with distilled water. After 10 min, the absorbance at 700 nm was measured and compared with calibration curves constructed from different dilutions of KH_2PO_4 to quantify phosphorous.

Additionally, the digested samples were quantified by inductively coupled plasma optical emission spectroscopy (ICP-OES) in an Agilent 5900 spectrometer.

To transform the resulting phosphorus concentrations into approximate PL concentrations, we considered an average molecular weight of approximately 800 for PLs. Therefore, phosphorus concentrations were multiplied by 25 (Carelli et al., 2002).

2.5 | Statistical analysis and chemometrics

Statistics were carried out using the R software (R Core Team, 2021). PL measures obtained by the colorimetric and ICP-OES methods were subjected to Shapiro–Wilk normality test and compared by the Student *t*-test when the distribution is normal or the test *U* of Mann–Whitney when it is not. The PL values of all samples were also compared by the Kruskal–Wallis test. It is accepted that there are no significative differences between data when the *p*-value >.05.

Principal component analysis (PCA), partial least square discriminant analysis (PLS-DA) and sparse partial least squares multivariate data analysis (sPLS-DA) were used to assess the ability of benchtop NMR to classify the samples into different categories. The normalized spectra were binned into 0.04 ppm segments, and the values of the integrals corresponding to each bin were collected for data analysis. The resulting 150 variables per sample were Pareto scaled and subjected to PCA and sPLS-DA [3] using the mixOmics package (Rohart et al., 2017) in R v.4.2.3 (R Core Team, 2021).

3 | RESULTS AND DISCUSSION

3.1 | NMR peak assignment and quantification

All the samples were analyzed by low field ^{1}H-NMR spectroscopy in an 80 MHz spectrometer. Some high field ^{1}H-NMR spectra were also recorded in a 500 MHz spectrometer as a means of comparison.

Figure 1 shows overimposed high field and low field ^{1}H-NMR spectra of the organic extract of an Iberian ham sample, where the principal bands were labeled with letters from A to K. Identification of the different types of protons from FAs and glyceride species is easily achieved in both spectra; however, signal overlapping in the 80 MHz spectrum makes the quantification of some species more difficult. The signals on the low field spectra were assigned according to the chemical shifts determined on the high field spectra (Pajuelo et al., 2022) and to previous reports on the ^{1}H-NMR analysis of fats (Nieva-Echevarría et al., 2015; Tsiafoulis et al., 2019) and are summarized in Table 1.

Peaks A–G correspond to CH, CH_2, and CH_3 protons of FAs, both from glycerides and free FAs (FFA) and can be integrated on the low field spectra to calculate the proportion of SFA, MUFA, and PUFA. Peak E, due to methylene protons α to the carboxyl group, is common to all SFA and unsaturated FA and thus was used as a reference to normalize the rest of the peaks in the spectrum. As peak E corresponds to two protons, the normalization factor equals the integral of E divided by 2, and therefore, to obtain the ratios of the different lipids, their corresponding peak integrals were divided by E/2. Peak F corresponds to bis-allylic protons of PUFA. As the vast majority of PUFAs in ham have only two C–C double bonds separated by a single methylene group, it is a reasonable approximation to assume that they all have two bis-allylic protons, which allows the quantification of these lipids, according to Equation (1). Peak D corresponds to the allylic protons of both PUFA and MUFA, and its value always corresponds to 4 protons. Thus, the percentage of MUFA can be calculated, dividing the value of the integral D by 4 and subtracting the value obtained for the PUFA (Equation 2). Finally, as the values are normalized to 100, the amount of SFA is calculated by subtracting the value of PUFA and MUFA from 100 (Equation 3). It is worth noting that the terminal methyl group of omega-3 FAs can be identified by a minor triplet at 0.98 ppm (A') in the high-field spectra. However, this signal is not visible in the low-field spectra because it is completely overlapped with peak A, which corresponds to the terminal methyl group of all FAs:

$$\text{PUFA} = 100 \times (F/E) \quad (1)$$

$$\text{MUFA} = 100 \times (D - 2 \times F) / (2 \times E) \quad (2)$$

$$\text{SFA} = 100 - \text{PUFA} - \text{MUFA} \quad (3)$$

The presence of other minor signals on the low field spectrum evidences the occurrence of different products of the hydrolysis of triglycerides. It is well known that the concentration of DAG, MAG, and FFA increases during the curing process (Narváez-Rivas et al., 2007), and this can be determined from the integrals of the corresponding signals on the high field ^{1}H-NMR spectra (Pajuelo et al., 2022). Unfortunately, only peak H, corresponding to the glycerol methylene group attached to a free OH in 1,2-DAG, is well resolved on the 80 MHz ^{1}H-NMR spectra. Other signals corresponding to the glycerol framework of 1,2-DAG and 1,3-DAG overlap with the TAG signals in the region of

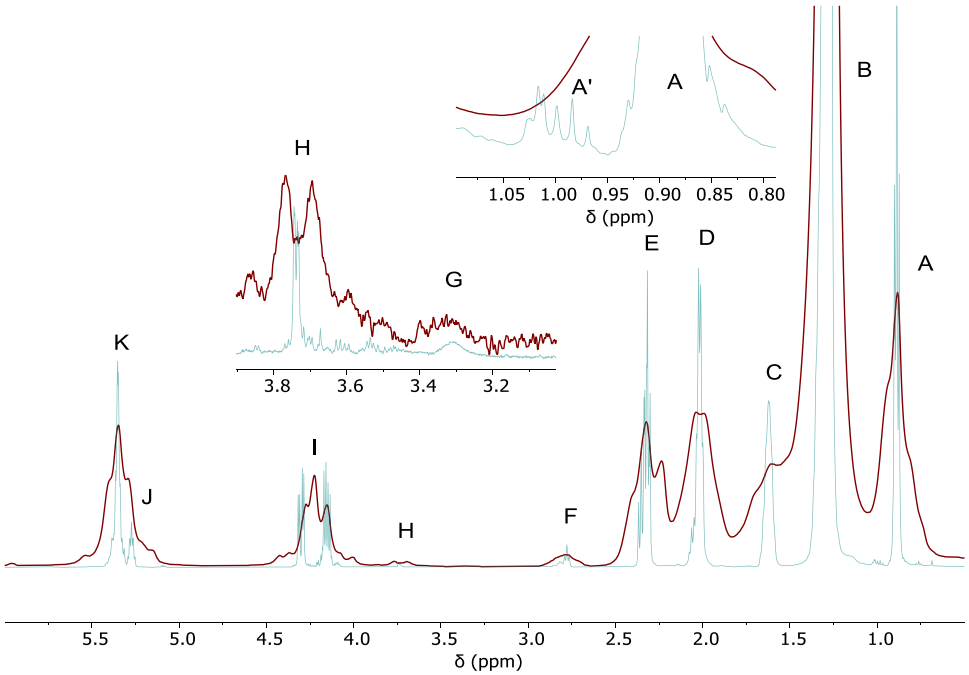

FIGURE 1 Comparison of the high field (500 MHz, green line) and low field (80 MHz, red line) proton nuclear magnetic resonance (^{1}H-NMR) spectra of a typical organic extract of Iberian ham. (A) Fatty acid (FA) terminal CH_3, (B) FA $(CH_2)_n$, (C) β-CH_2, (D) monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) allyl CH_2, (E) FA α-CH_2, (F) PUFA bis-allyl CH_2, (G) phosphatidylcholine $N(CH_3)_3$, (H) 1,2-DAG glyceryl CH_2OH, (I) TAG glycerol CH_2, (J) 1,2-DAG glyceryl CHOR, (K) MUFA and PUFA vinyl CH.

TABLE 1 Low-field proton nuclear magnetic resonance (^{1}H-NMR) peak assignment of a typical lipid extract from Iberian ham.

Peak	δ (ppm)	Multiplicity, J (Hz)	Integration interval	Assignment
A	0.88	t, $J = 5.0$ Hz		FA terminal CH_3
B	1.26	m		FA $(CH_2)_n$
C	1.61	m (overlapped)		FA β-CH_2
D	2.01	m	1.82–2.16	=CH–CH_2 (MUFA and PUFA)
E	2.32	m	2.17–2.60	FA α-CH_2
F	2.77	m	2.69–2.92	=CH–CH_2–CH=(PUFA)
G	3.33	s		Phosphatidylcholine $N(CH_3)_3$
H	3.73	d, $J = 5.7$ Hz		1,2-DAG glyceryl CH_2OH
I	4.22	m		TAG glyceryl CH_2
J	5.21	m (overlapped)		1,2-DAG glyceryl CHOR
K	5.35	m	5.23–5.50	CH=CH (MUFA and PUFA)

Note: Peak labels (A–K) agree with those given in Figure 1.
Abbreviations: FA, fatty acid; MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid.

3.9–4.6 ppm, making it difficult to quantify lipolysis products, especially FFA.

The means of the results of each label of dry-cured Iberian hams, obtained by 80 MHz ^{1}H-NMR and their respective deviations, are summarized in Table 2.

The results indicate that MUFAs are major lipidic components of Iberian ham. The proportion of MUFA is significantly higher in the hams obtained from pigs that have been fed with acorns and grass (black and red labels), being around 55%. In contrast, green and white labeled hams have a lower content of MUFA, roughly 51%. Conversely, the

TABLE 2 Mean values of the molar percentages of fatty acid (FA) in dry-cured Iberian ham samples from different labels obtained by 80 MHz nuclear magnetic resonance (NMR) and the *p*-values derived from statistical comparison between labels.

Label[a]	Black	Red	Green	White	*p*-Value
PUFA	7.42 ± 1.43	8.39 ± 1.03	7.87 ± 1.22	7.08 ± 1.56	.396
MUFA	55.1 ± 1.93	55.20 ± 2.37	50.9 ± 2.87	51.90 ± 3.35	.022[b]
SFA	37.5 ± 0.73	36.40 ± 1.79	41.20 ± 2.35	41.00 ± 2.63	<.001[c]

Abbreviations: MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; SFA, saturated fatty acid.
[a]$n = 6$ samples per label.
[b]Different at a significance level of 95%.
[c]Different at a significance level of 99.9%.

TABLE 3 Results of the Tukey test to compare polyunsaturated fatty acid (PUFA), monounsaturated fatty acid (MUFA), and saturated fatty acid (SFA) proportions between each pair of ham labels.

Categories[a]	PUFA (*p-value*)	MUFA (*p-value*)	SFA (*p-value*)
Green–black	.930	.054	.018[b]
Red–black	.585	1.000	.807
White–black	.974	.230	.039[b]
Red–green	.904	.051	.002[c]
White–green	.754	.910	.996
White–red	.380	.220	.006[c]

[a]$n = 6$ samples per label.
[b]Different at a significance level of 95%.
[c]Different at a significance level of 99%.

proportion of SFA is significantly lower in black and red labeled hams, ranging from 36% to 38%, contrasting with the near to 41% of green and white labeled hams. The contents of PUFA show no significant differences between labels. A post hoc analysis (Table 3) also shows that the differences in MUFA and SFA between black and red labels and between green and white labels are not significant, which strongly suggest that the FA profiles of Iberian hams are mainly dependent on the diet.

Previous studies have already emphasized the importance of feeding to determine the FA profile of Iberian hams. The higher content of unsaturated FAs (principally oleic acid) in acorn is the main dietary contribution of pigs in *montanera*, which explains why the meat of pigs fed on acorns has consistently higher contents of MUFA (Pérez-Palacios et al., 2009; Tejeda et al., 2002).

Our results are consistent with those reported for the analysis by GC-FID and high field NMR of Iberian hams of different labels (Pajuelo et al., 2022). Furthermore, there are no significant differences in the results obtained by the three methods for a significance level of 95% (*p*-value >.05), which suggests that benchtop NMR spectroscopy is a reliable method for the quantification of MUFA, PUFA, and SFA in the lipidic fraction of Iberian hams. However, high field NMR spectroscopy has shown to be superior as it allowed to calculate the proportion of $\omega - 3$ and $\omega - 6$ PUFA, which was not possible from the low field spectra.

The degree of hydrolysis of TAG in Iberian hams can also be determined using high field ^{1}H-NMR spectra, as reported by Pajuelo et al. (2022). However, low field NMR spectroscopy has lower resolution and cannot accurately quantify the ratio of TAG, 1,2-DAG, 1,3-DAG, and FFA. Although most signals corresponding to hydrolyzed glycerides cannot be discerned, they still contribute to the final shape of the spectra and chemometric techniques can be used to extract this information and classify the samples.

3.2 | Quantification of phospholipids in Iberian hams

PLs play an essential role in the development of oxidation reactions and the characteristic flavor of cured meat products. During the curing process of Iberian ham, PLs undergo hydrolysis and oxidation, resulting in the production of volatile aromatic compounds that contribute to its unique flavor (Toldrá, 1998). Studies estimate that 66%–90% of the PLs present in fresh pork are hydrolyzed at the end of the dry-curing process (Larrea et al., 2007; Martıń et al., 1999). However, only a few works report the PL content of Iberian dry-cured hams, where it was determined by chromatographic fractionation of the lipid fraction (Martıń et al., 1999) or HPLC analysis (Pérez-Palacios et al., 2010).

Conventional lipid transesterification followed by GC-FID analysis does not provide information about the PL fraction. High field ^{1}H-NMR spectroscopy can determine the proportion of FA in free form or being part of different glycerides in Iberian hams, but not PLs (Pajuelo et al., 2022). Although a peak at 3.33 ppm corresponding to

phosphatidylcholine, the major PL in pork meat, is sometimes evident in the high field ^{1}H-NMR spectra, the low amount of PLs remaining in cured ham makes it difficult to quantify them accurately. Some of the low field ^{1}H-NMR spectra measured show this phosphatidylcholine peak, but it is not useful for quantification. As a result, it was not possible to quantify PLs using low-field NMR in this study.

Here, the PL content of Iberian hams from different labels was determined to provide a complete profile of their lipid composition. To quantify PLs, both a molybdenum-based spectrophotometric method (AOCS, 2009) and ICP-OES were used as alternatives. The mean percentages of PL in the lipid fraction of the different categories of Iberian hams obtained by the two methods are summarized in Table 4. The statistical comparison shows that there are no significant differences in the values obtained by both methods. The percentage of PL in the lipid fraction of all samples is less than 1%. As PLs represent 8%–9% of the lipids in fresh pork *Biceps femoris* (Tejeda et al., 2002), this indicates that there has been extensive lipolysis of PLs during the maturation process, which is consistent with previous research (Martıń et al., 1999; Pérez-Palacios et al., 2010).

On the other hand, a Kruskal–Wallis test on the whole set of data yields a *p* value of .258 for colorimetric measures and .246 for ICP-OES measures, indicating that there are no statistically significant differences for the different categories of Iberian ham in terms of PLs.

From this, data we can conclude that PLs are minor components of the lipid fraction of Iberian hams and do not allow for the discrimination of different classes of hams. Accordingly, the lipid profile obtained from the low field ^{1}H-NMR spectra provides a complete picture of the acyl glycerides composition of Iberian hams and can be used on its own as a mean of classification.

3.3 | Chemometric approach for classification and authentication of Iberian hams

A preliminary exploration of the low field NMR data was carried out using PCA. The scores plot of the three first components (Figure 2) does not show a clear separation of samples from different labels. However, some grouping of the samples according to diet is evident. Ham samples from acorn-fed animals (black and red labels) cluster on the right side of the plot, whereas samples from concentrate-fed pigs (green and white labels) tend to cluster on the left side.

Then, PLS-DA was carried out on the data obtained from binning of the ^{1}H-NMR spectra. Resampling using a 10-fold cross-validation was used to select the optimal model. The highest accuracy (100%) was obtained with a number of components equal to 10, with sensitivity and specificity values of 100%. This model was able to correctly classify all the samples, as shown in Figure 3.

Then, the sPLS-DA (Lê Cao et al., 2011) was performed to enable the selection of the most discriminative features in the NMR data to classify the samples. Using sPLS-DA, two components allow the graphical discrimination of hams according to the pigs' feeding (Figure 4, 5).

Variables contributing to components 1 and 2 of the sPLS-DA model (Figure 5)are, in order of importance, spectral bins among 1.83–

TABLE 4 Mean values of the percentages of phospholipid (PL) in the lipid fraction of dry-cured Iberian ham samples from different labels, obtained by Mo colorimetric method and inductively coupled plasma optical emission spectroscopy (ICP-OES, and the *p*-values resulting from the statistical treatment comparing both methods.

Label	N	Mo	ICP	*p*-Value
Black	9	0.45 ± 0.31	0.49 ± 0.21	.767
Red	8	0.30 ± 0.09	0.43 ± 0.18	.161[a]
Green	9	0.43 ± 0.18	0.67 ± 0.39	.190[a]
White	10	0.43 ± 0.15	0.57 ± 0.15	.054

[a]Non-normal distribution.

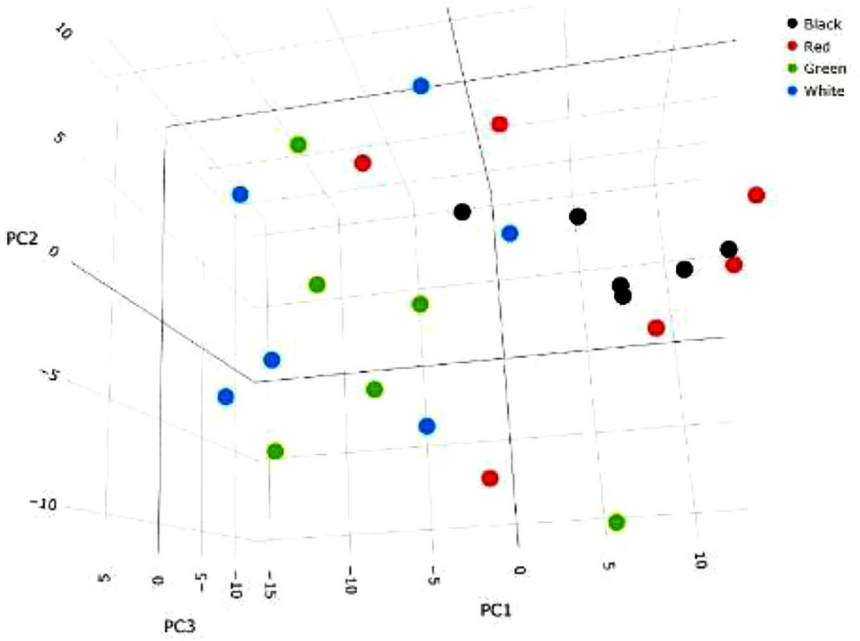

FIGURE 2 Principal component analysis (PCA) 3D scores plot of the proton nuclear magnetic resonance (^{1}H-NMR) data obtained from the spectral bins.

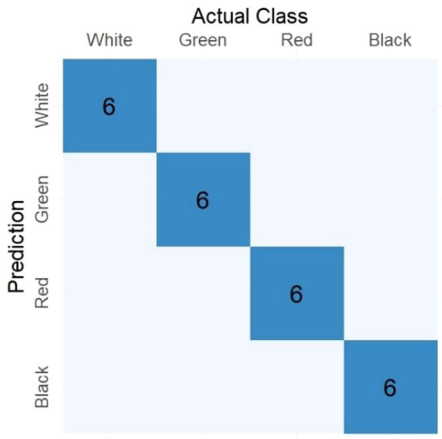

FIGURE 3 Confusion matrix showing the actual sample classes and the sample classes predicted by the partial least square discriminant analysis (PLS-DA) model with 10 components.

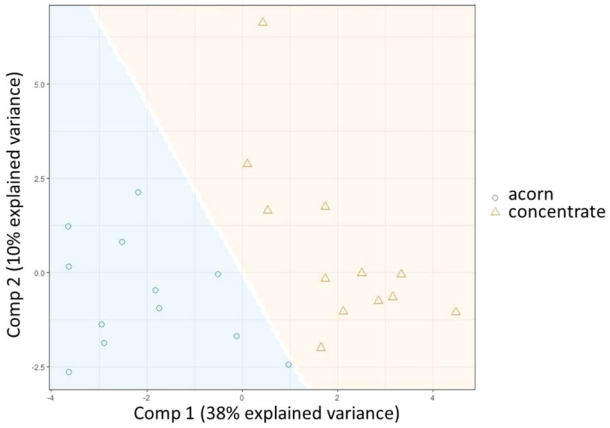

FIGURE 4 Sparse partial least squares multivariate data analysis (sPLS-DA) classification of Iberian hams according to feeding.

2.07 ppm (bins 112–117, allylic methylene groups in MUFA and PUFA), 4.23–4.51 ppm (bins 51–57, glycerol CH_2 from TGA), 0.71–0.91 ppm (bins 141–145, coincident with the signals of cholesterol and terminal methyl groups of non $\omega-3$ FA), 0.95–1.07 ppm (bins 137–139, terminal methyl groups of non $\omega-3$ FA), 1.27–1.39 ppm (bins 129–131, $(CH_2)_n$), 4.03–4.11 ppm (bins 61–62, 1,3-DAG), and 2.35–2.51 ppm (bins 101–104, α CH_2 of FFA and DAG). Therefore, the discrimination of hams according to the diet of the pigs seems to be mainly due to the degree of unsaturation of the FAs, i.e. the amount of PUFA and MUFA, as well as the abundance of TAG hydrolysis products.

This is consistent with a higher ratio of MUFA (mainly oleic acid) in hams from acorn-fed animals, as also reported in previous studies (Pérez-Palacios et al., 2009). On the other hand, although the degree of lipolysis is related to the duration and conditions of the curing period (Narváez-Rivas et al., 2007), it also seems to be highly influenced by the diet of the pigs (Pajuelo et al., 2022).

4 | CONCLUSION

Benchtop ^{1}H-NMR spectroscopy has proven to be a valuable and operationally simple analytical method to determine the FA profile of Iberian hams. The quantitative information obtained is comparable to that provided by high field NMR, as the amounts of PUFA, MUFA, and SFA can be directly obtained from the integration of the corresponding spectral peaks. However, the products of lipolysis cannot be quantified due to peak overlapping in the 80 MHz spectra.

The low PL contents in cured Iberian hams are possibly due to extensive PL hydrolysis during the curing process. This makes its

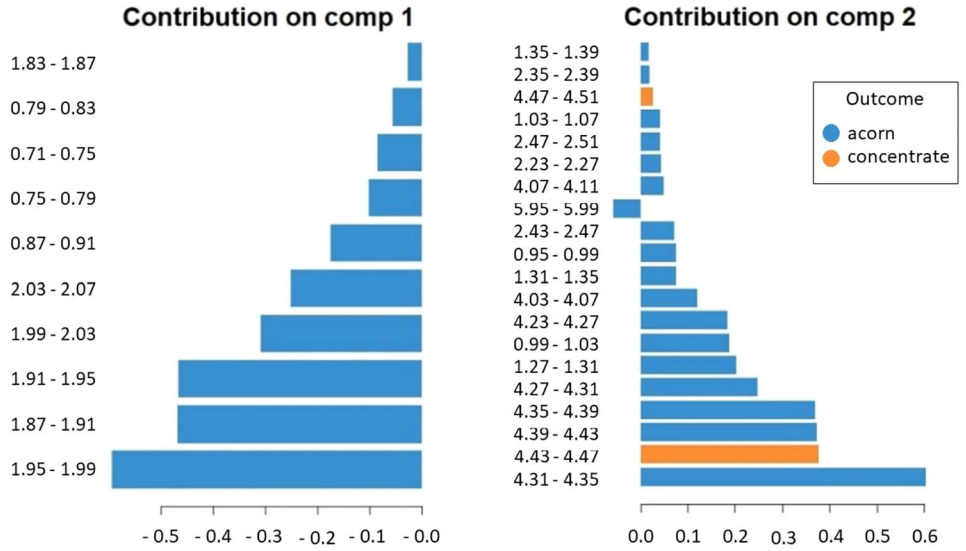

FIGURE 5 Variables contributing to components 1 and 2 of the sparse partial least squares multivariate data analysis (sPLS-DA) model.

quantification by ^{1}H-NMR difficult. The alternative colorimetric and ICP-OES quantification of PL showed that there are no statistically significant differences in the PL contents for the different categories of Iberian ham.

The multivariate chemometric approaches on the low field spectral data allow hams to be neatly classified according to the diet of the pigs. This is mainly based on the higher content of unsaturated FAs (presumably mainly oleic acid) in hams obtained from pigs fed on acorns and other natural sources.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no known conflicts of interest or personal relationships that could have appeared to influence the work reported in this paper.

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