Talanta 117 (2013) 463–470

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Short communication

Feasibility of use of fatty acid and triacylglycerol profiles for the authentication of commercial labelling in Iberian dry-cured sausages

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ARTICLE INFO

Article history:
Received 7 June 2013
Received in revised form
6 September 2013
Accepted 17 September 2013
Available online 1 October 2013

Keywords:
Iberian pig
Chorizo
Salchichón
Gas chromatography
Authentication

ABSTRACT

In the present study, fatty acid and triacylglycerol profiles were used to evaluate the possibility of authenticating Iberian dry-cured sausages according to their label specifications. 42 Commercial brand '*chorizo*' and 39 commercial brand '*salchichón*' sausages from Iberian pigs were purchased. 36 Samples were labelled *Bellota* and 45 bore the generic *Ibérico* label. In the market, *Bellota* is considered to be a better class than the generic *Ibérico* since products with the *Bellota* label are manufactured with high quality fat obtained from extensively reared pigs fed on acorns and pasture. Analyses of fatty acids and triacylglycerols were carried out by gas chromatography and a flame ion detector. A CP-SIL 88 column (highly substituted cyanopropyl phase; 50 m × 0.25 mm i.d., 0.2 μm film thickness) (Varian, Palo Alto, USA) was used for fatty acid analysis and a fused silica capillary DB-17HT column (50% phenyl–50% methylpolysiloxane; 30 m × 0.25 mm i.d., 0.15 μm film thickness) was used for triacylglycerols. Twelve fatty acids and 16 triacylglycerols were identified. Various discriminant models (linear quadratic discriminant analyses, logistic regression and support vector machines) were trained to predict the sample class (*Bellota* or *Ibérico*). These models included fatty acids and triacylglycerols separately and combined fatty acid and triacylglycerol profiles. The number of correctly classified samples according to discriminant analyses can be considered low (lower than 65%). The greatest discriminant rate was obtained when triacylglycerol profiles were included in the model, whilst using a combination of fatty acid and triacylglycerol profiles did not improve the rate of correct assignation. The values that represent the reliability of prediction of the samples according to the label specification were higher for the *Ibérico* class than for the *Bellota* class. In fact, quadratic and Support Vector Machine discriminate analyses were not able to assign the *Bellota* class (0%) when combined fatty acids and triacylglycerols were included in the model. The use of fatty acid and triacylglycerol profiles to discriminate Iberian dry-cured sausages in the market according to their labelling information is unclear. In order to ensure the genuineness of Iberian dry-cured sausages in the market, identification of fatty acid and triacylglycerol profiles should be combined with the application of quality standard traceability techniques.

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1. Introduction

Dry-cured sausage production is a major industry in the Spanish economy. The most widely consumed types are '*chorizo*' and '*salchichón*'. Both products are manufactured from a mixture of chopped meat (pork and beef), lard, sugars, authorised additives (nitrate, nitrite, and antioxidants), starter cultures and spices. '*Chorizo*' is characterised by the fact that it includes Spanish paprika and garlic in its formulation, while '*salchichón*' includes black pepper [1,2]. Differences in the formulation mixture, origin of product, process of fermentation and ripening determine the quality of the product. A high-value product is one which includes meat and back fat from Iberian pigs [3].

Iberian dry-cured sausages are meat products manufactured according to traditional methods in the south and west of Spain and regulated by a generic Spanish quality standard for all raw and dry-cured sausage products [1]. Among these products, sausages

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0039-9140/$ - see front matter Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.talanta.2013.09.029

made from the Iberian breed of pig are considered a high-quality food product because of the intrinsic characteristics of the breed and the raw material used in their feeding. Various studies have demonstrated that diet composition during final fattening processes in pigs has an influence on the quality of Iberian pig products [4,5]. According to traditional extensive production systems, Iberian pigs are raised outside in the Mediterranean silvopastoral system known as '*Dehesa*'. This system includes a final fattening phase called '*montanera*', where pigs feed on natural resources, mainly acorns and pasture in extensive conditions [6,7]. However, when acorns are not sufficient, the final fattening diet of pigs is supplemented with commercial feed specifically for swine production. Spain has legislation on the differentiation of ham or subcutaneous fat of Iberian pigs according to their feeding regime [8]. However, there is no specific legislation regarding the differentiation of Iberian dry-cured sausages according to their origin. Hence, dry-cured sausages manufactured with lard obtained from Iberian pigs fattened in '*montanera*' are usually labelled as *Bellota*, whilst those containing fat from animals fed with compound feeds are generically called *Ibérico*. Therefore, a method for authentication of Iberian dry cured products in the marketplace is required.

The sensorial and nutritional attributes of products from animals reared extensively in '*montanera*' mean that they are considered high quality products, while those from animals fed on concentrate are of less quality [9]. Consequently, Iberian pig products labelled as having undergone '*montanera*' fattening have a higher price and this, in turn, increases the possibility of frauds involving other generic Iberian products. In order to discriminate both products, different analytical methods can be found in scientific literature.

Chemical analyses based on gas chromatography to identify the fatty acid profile of the subcutaneous fat of Iberian pigs have been a controversial criterion established for classifying animals according to their feeding system [8–10]. Recently, triacylglycerol composition has been proposed as a method to authenticate Iberian pig fat [11,12] as it has been widely used for oil [13]. In fact, in the study reported by Viera-Alcaide et al. [14] on establishing a method for analysing the triacylglycerol fraction of '*montanera*' and other subcutaneous fat samples by means of gas chromatography and flame ionisation detection, it was concluded that triacylglycerol contents could discriminate between animals from '*montanera*' and concentrate feeding regimes. While determination of fatty acid and triacylglycerol profiles associated with feeding regime has been reported for Iberian subcutaneous fat [15,16] and for Iberian ham [17,18], little information can be found regarding the profiles of Iberian sausages. In fact, generic standards of quality in relation to fatty acid profiles of Iberian ham or subcutaneous fat of Iberian pigs have been regulated [19,8], but there is no generic standard for the classification of dry-cured sausages according to the raw material source.

Since the determination of fatty acid and triacylglycerol profiles has become a feasible system to identify the origin of Iberian pork products in the case of ham and subcutaneous fat, the aim of this work is to explore the feasibility of using these analytical procedures to authenticate Iberian dry-cured sausages labelled as *Bellota* and generic *Ibérico* in the market. The information obtained could be applied to industrial processing of Iberian dry-cured products in Spain, and contribute to the development of the pig industry as well as to new standards for the control of Iberian dry-cured sausages in the market.

2. Experimental

2.1. Dry-cured sausage samples

A total of 81 Iberian dry-cured sausages (42 '*chorizo*' and 39 '*salchichón*'), belonging to 42 commercial brands were purchased. Dry-cured sausages were acquired from commercial stores located in southern and western Spain (Extremadura, Andalucía and Castilla y León). According to the specifications on the label, 18 samples were labelled as *Bellota chorizo* and 24 as *Ibérico chorizo*. In the case of the '*salchichón*' sausages, 18 were labelled as *Bellota* and 21 as *Ibérico*. Products were manufactured in geographical areas recognised by the Spanish quality standard for the production of *Ibérico* products. The mixes used in the manufacture of these sausages are made up of minced lean pork and beef (50–60%), Iberian pork back fat (20–30%), common salt, Spanish paprika (only in '*chorizo*'), sugar, black pepper (only in '*salchichón*'), and garlic (as well as other authorised additives). Differences between *Bellota* and *Ibérico* labels refer to the origin of the fat used in the sausage manufacturing process. A *Bellota* label should indicate that the fat comes from pigs that have been fed on acorns and pastures during their final fattening period. The *Ibérico* label indicates that the fat included in sausage has been obtained from Iberian pigs which were raised on concentrate feeds.

2.2. Fat extraction procedure

All samples were acquired packed under vacuum and were chopped just before laboratory analysis. According to the method proposed by Folch et al. [20], the extraction of sausage fat was performed as follows: 250–400 g of dry-cured sausages were chopped and minced in a domestic mincer (Illico, Moulinex DJE241, France). After that, 2 g from each sample were accurately weighed into an 80 mL tube to which 30 mL of trichloromethane: methanol (2:1) was added. The mixture was homogenised using a laboratory grinder (Ultraturrax T8 IKA-Werke GMBH & Co.KG, Staufen, Germany) for 1 min at 15,500 rpm. Then, 5 mL of potassium chloride (0.88% in distilled water) was added and the mixture was homogenised again using a laboratory grinder for 1 min at 15,500 rpm. The resulting homogenate was filtered and the liquid phase was collected. After allowing it to settle, the aqueous layer was removed, and 1.5 g of anhydrous sodium sulphate was added. The organic phase was then filtered through a Whatman no. 1 filter paper and the solvent was removed at 35 °C. Two duplicates of each sample were made. Fat samples were stored at −20 °C until analysis.

2.3. Fatty acid analysis

At present there is no Spanish legislation on the fatty acid composition of Iberian dry-cured sausages, in fact, there is no official method for dry-cured sausages fat analysis. Therefore, an adaptation of the chromatographic method used for determining the fatty acid composition of total lipids from subcutaneous adipose tissue has been used. Fatty acid methyl esters (FAMEs) were analysed following the method proposed by Viera-Alcaide et al. [14] and De Pedro et al. [21]. In a 10 mL screw-top test tube, approximately 0.1 g of the extracted fat (melted 1.5 min in a microwave at 360 W) was weighed. 8 mL of hexane was added and the mixture was homogenised by shaking. 0.4 mL of 2 N methanolic potassium hydroxide was added, the cap fitted with a PTFE-joint was put on, and the tube was shaken vigorously for 30 s. After leaving it to stratify until the hexane layer became clear, 1.0 mL of this phase was injected in the gas chromatograph. Separation of FAMEs was carried out using a Varian 3800 (Varian Co, Palo Alto, CA USA) gas chromatograph equipped with a flame ionisation detector (FID) and a CP-SIL 88 (highly substituted cyanopropyl phase) silica capillary column of 50 m × 0.25 mm ID, coated with a 0.2 μm film thickness of stationary phase (Varian, Palo Alto, USA). The oven temperature was kept at 170 °C for 17 min and was then raised to 190 °C at a rate of 5.0 °C min^{-1} and held isothermally for 9 min. The injector and detector temperature was kept at 250 °C. Helium was used as the carrier (1 mL min^{-1}

column constant flow) and make-up (30 mL min^{-1}) gas. Split injection mode was used with a split ratio of 1:80.

Individual FAMEs were identified by comparing their retention times with those of authenticated standards from Sigma (Sigma Chemical Co. Ltd., Poole, UK). In this paper, the list of fatty acids studied was selected according to the Spanish quality standard for fat of Iberian pork that includes 12 molecular species of fatty acids [8]. Quantification of individual fatty acids was carried out by evaluating the corresponding relative percentage according to the area normalisation procedure, assuming an equal response factor for all species.

2.4. Analysis of triacylglycerols

Identification of triacylglycerol species was carried out using a Varian 3800 gas chromatograph equipped with a flame ionisation detector, a fused silica capillary column of 30 m × 0.25 mm I. D. coated with a 0.15 μm film of DB-17HT stationary phase (50% phenyl–50% methylpolysiloxane). A Varian 8400 automatic injector was used. The oven temperature was kept at 320 °C and was then raised to 350 °C at a rate of 2.0 °C min^{-1} and held isothermally for 10 min. The injector temperature was kept at 360 °C, while the detector temperature was 370 °C. Hydrogen was used as the carrier gas (2.1 mL min^{-1} column constant flow), while the make-up gas was nitrogen at 30 mL min^{-1}. Split injection mode was used in a ratio of 1:40. The extracted fat was dissolved in hexane 3.0% (m/v). Aliquots of 2 μL of this sample solution were injected into the gas chromatograph and the peak area of the analyte was used as an analytical signal. The quantification of individual triacylglycerols was carried out by evaluating the corresponding relative percentage according to the area normalisation procedure assuming an equal response factor for all species. Sixteen triacylglycerol species were identified. The assignment of the chromatographic peaks was performed by means of standards of trilinolein (LLL), triolein (OOO), tripalmitin (PPP) and tristearin (SSS), which allowed the carbon number of the components associated with each peak group to be deduced, as well as the differences between triglyceride retention times. Palmitindioleine was used as a reference to calculate the relative retention times.

2.5. Data analysis

According to the standard method of analysis for determining the fatty acid composition of subcutaneous adipose tissue from Iberian pigs, 12 fatty acids [8] and 16 triacylglycerols have been analysed in dry-cured sausages. The statistical analysis was performed using the statistical R package version 2.15-1 [22]. Both groups of variables (fatty acids and triacylglycerols) were log-transformed to reduce skewness. The chisquare plot function in the R mvtoulier library was used to identify multivariate outliers [23]. This function plots the ordered robust Mahalanobis distances of the data against the quantiles of the chi-squared distribution [24]. By user interaction this plotting was iterated, each time leaving out the observation with the greatest distance. In this way six samples were identified as outliers and were removed from the data set prior to the subsequent statistical analysis, which meant 75 samples remained in the data set. Firstly, a one-way analysis of variance was performed to compare the effect of label class (*Bellota* or *Ibérico*) for total fatty acids and triacylglycerols. Secondly, a Hotelling two sample *T*2-test comparing the population mean vectors for *Bellota* and *Ibérico* was carried out with the ICNSP library [25]. Given that multivariate normality and homoscedasticity assumptions may be questionable, a non-parametric test was also performed with the Cramer test function in the Cramer library [26]. A Principal Component Analysis (PCA) was applied to the data matrix with the *princomp* function. This model allows the reduction of the multidimensional data set to fewer dimensions for further analysis or to reveal its internal structure. PCA provides the user with a lower dimensional picture, in which the scores of samples can be computed. Several classification rules were also trained to predict the class of a sample. The accuracy of each rule was assessed with the percentage of correct predictions for each of the two classes and for both categories using leave-one-out-validation (jackknife test).

3. Results and discussion

3.1. Fatty acid profile of Iberian dry-cured sausages

Given that the mixture composition and manufacturing of '*chorizo*' and '*salchichón*' is similar, both products have been grouped to discuss the results. In fact, no significant differences between the fatty acid profiles of '*chorizo*' and '*salchichón*' have been observed. Twelve fatty acid molecular species were identified according to their relative retention times (Table 1). The fatty acid profile of samples from Iberian dry-cured sausages is in line with other dry-cured sausages produced in Spain [27,28]. Fig. 1 shows the individual chromatograms corresponding to *Bellota* and *Ibérico* dry-cured sausages, where 12 peaks were identified. In this figure, the peaks of fatty acids eluted according to the atom carbon number and unsaturation number (Table 1), except C18:3 fatty acid, which eluted after C20:0 fatty acid. In fact, the C18:3 and C20:1 fatty acids co-eluted at the end of the chromatogram between 36.2 and 36.9 min. C18:1 was the most abundant fatty acid in both products (around 48%) because the formulation included an Iberian pork fat mixture [4]. Other saturated fatty

Table 1
Means and standard deviation values of the fatty acid composition (expressed as a percentage of the total fatty acids detected) of Iberian dry-cured sausages.

Fatty acids	Retention time	*Bellota*				*Ibérico*				*p*-Value
		Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	
C12:0	5.4	0.08	0.01	0.06	0.09	0.08	0.01	0.06	0.09	0.969
C14:0	6.7	1.45	0.10	1.25	1.62	1.45	0.12	1.25	1.78	0.937
C16:0	9.10	25.46	1.52	23.10	28.00	25.50	1.41	22.83	27.77	0.899
C16:1	10.4	2.98	0.36	2.29	3.63	2.90	0.33	2.31	3.71	0.309
C17:0	10.9	0.22	0.04	0.14	0.29	0.21	0.04	0.13	0.33	0.626
C17:1	12.4	0.24	0.04	0.17	0.31	0.24	0.04	0.15	0.33	0.530
C18:0	13.3	12.62	1.24	10.47	14.68	12.73	1.06	10.80	14.83	0.677
C18:1	15.3	48.36	2.38	42.97	52.59	48.02	2.24	44.07	52.07	0.529
C18:2	18.4	6.88	1.36	4.54	9.84	7.17	1.16	4.89	10.34	0.320
C20:0	19.7	0.21	0.02	0.18	0.27	0.21	0.02	0.16	0.28	0.544
C18:3	21.3	0.75	0.25	0.38	1.05	0.73	0.23	0.38	1.14	0.612
C20:1	21.4	0.74	0.18	0.41	1.08	0.76	0.16	0.45	1.09	0.612

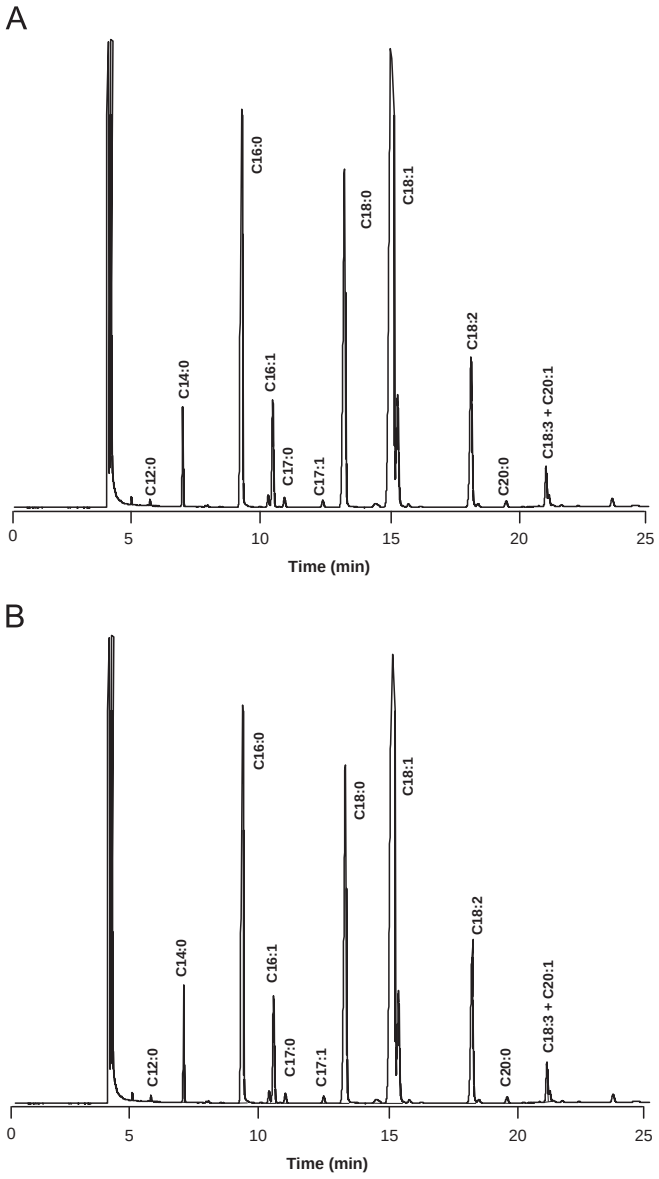

Fig. 1. Gas-chromatogram of the fatty acid profile of Iberian dry-cured sausage samples labelled as '*Bellota*' (A) and '*Iberico*' (B).

acids such as C16:0 and C18:0 were also the most abundant in the composition of the sausages (around 25.5% and 12.5%, respectively). No significant effect of sample origin has been observed for all of the fatty acids detected (Table 1). Furthermore, the multivariate population mean vectors for *Bellota* and *Ibérico* including fatty acids variables were compared by means of Hotelling's *T*2 and Cramer's tests, revealing a non-significant effect of the samples' origin (*p*-values obtained were $p=0.821$ and $p=0.675$, respectively).

3.2. Triacylglycerol profile of Iberian dry-cured sausages

Sixteen triacylglycerol molecular species were identified according to their relative retention times to palmitindioleine (Table 2). The individual chromatograms corresponding to *Bellota* and *Ibérico* dry-cured sausages are shown in Fig. 2.

The peaks of the triacylglycerols identified can be observed grouped by their atom carbon number (CN) and unsaturation number. The first group of peaks included PPP and MOP (CN=48) which elute at a retention time of between 5.0 and 6.0 min. The peaks with CN=50 (PPS, POP, POPo, PLP, PLPo and MLO) elute between 6.9 and 8.0 min. POPo and PLP (with two unsaturations) and PLPo and MLO (with three unsaturations) co-eluted together around 7.4 and 7.7 min, respectively. The triacylglycerol group with 52 carbons (PSS, PSO, POO, PLO, PLL and PoLO) elutes between 9.0 and 10.5 min. In this group, the PLL and PoLO (with four unsaturations) co-eluted together at 10.5 min. Finally, the peaks corresponding to SOS, SOO, OOO, SOL and OOL (CN=54) elute between 11.0 and 13.0 min. Four triacylglycerols accounted for at least 75% of the total (POP, PSO, POO and PLO). POO was the most abundant triacylglycerol (around 38% of total triacylglycerols), followed by PSO (around 21%) and POP (around 10%). The high proportion of these triacylglycerols observed in dry-cured sausage has also been reported by Viera-Alcaide et al. [14] in subcutaneous fat of Iberian pigs and by Petron et al. [15] in dry-cured hams.

For the *Bellota* and *Ibérico* classes no significant differences between mean triacylglycerols were observed (Table 2). However, triacylglycerol variables provided a significant *p*-value for Hotelling's *T*2-test ($p=0.037$), but this was accompanied by a non-significant *p*-value for Cramer's test ($p=0.523$). Therefore, the possibility of using only triacylglycerols to discriminate *Bellota* and *Ibérico* dry-cured sausage samples could be considered. On the other hand, in the Hotelling's *T*2 and Cramer's tests applied to the

Table 2
Means and standard deviation values of triacylglycerols (expressed as percentage of the total triacylglycerols detected) of Iberian dry cured sausages.

Triacylglycerols	Retention time	*Bellota*				*Ibérico*				*p*-Value
		Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.	
PPP	5.2	0.61	0.13	0.38	0.83	0.59	0.12	0.32	0.86	0.531
MOP	5.4	1.40	0.22	1.01	1.82	1.38	0.19	0.85	1.74	0.704
PPS	6.9	1.82	0.38	1.07	2.60	1.78	0.33	0.97	2.44	0.609
POP	7.1	10.02	1.26	7.47	12.03	9.92	1.23	6.91	12.30	0.721
POPo+PLP	7.4	6.20	0.44	5.05	7.02	6.08	0.40	5.22	6.95	0.26
PLPo+MLO	7.7	0.75	0.13	0.51	1.15	0.73	0.11	0.55	1.04	0.342
PSS	9.0	1.73	0.38	1.06	2.62	1.69	0.32	0.95	2.41	0.642
PSO	9.3	20.82	2.13	15.84	23.93	20.54	2.12	15.38	24.19	0.581
POO	9.6	37.74	1.86	34.96	41.56	38.09	1.72	34.43	41.81	0.391
PLO	10.0	7.58	1.01	5.56	9.59	7.63	0.99	5.88	10.19	0.826
PLL+PoLO	10.5	0.77	0.28	0.29	1.70	0.68	0.24	0.24	1.62	0.159
SOS	11.8	0.79	0.13	0.50	1.15	0.83	0.14	0.65	1.49	0.272
SOO	12.1	3.08	0.39	2.16	3.91	3.22	0.41	2.44	4.05	0.140
OOO	12.4	4.62	1.33	3.01	8.25	4.69	1.34	2.79	8.86	0.814
SOL	12.6	1.08	0.24	0.62	1.53	1.13	0.22	0.68	1.55	0.353
OOL	13.0	1.00	0.48	0.24	2.25	1.01	0.43	0.47	2.29	0.892

M: miristic; P: palmitic; Po: palmitoleic; S: stearic; O: oleic; and L: linoleic.

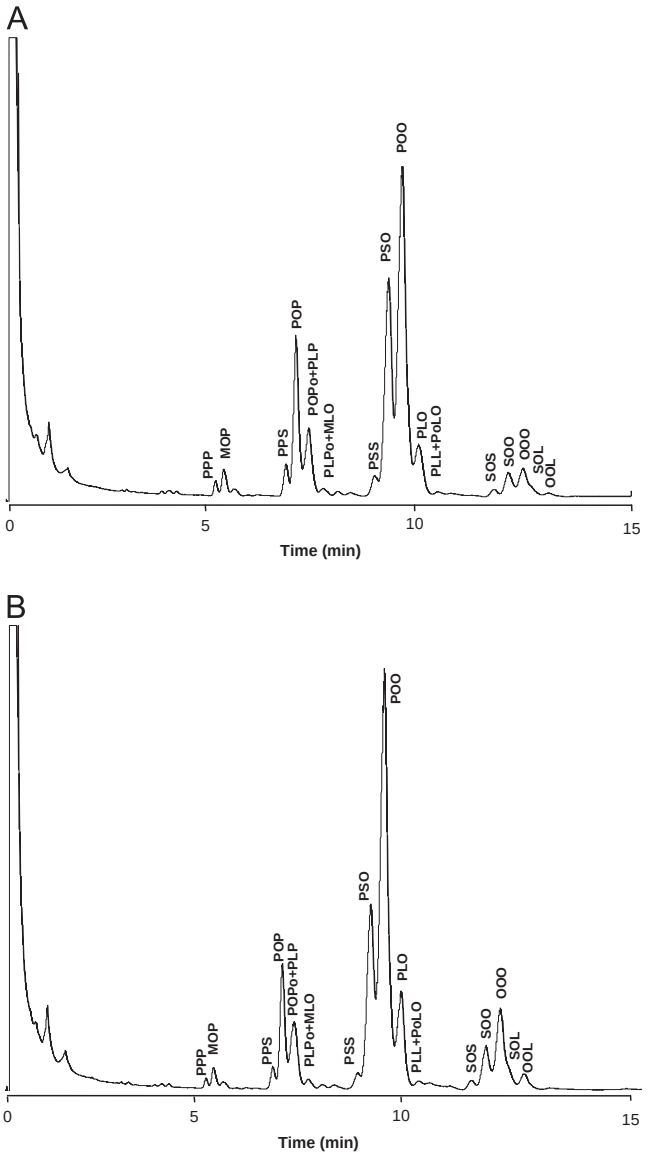

Fig. 2. Chromatograms of the triacylglycerol profile of Iberian dry-cured sausage samples labelled as '*Bellota*' (A) and '*Iberico*' (B).

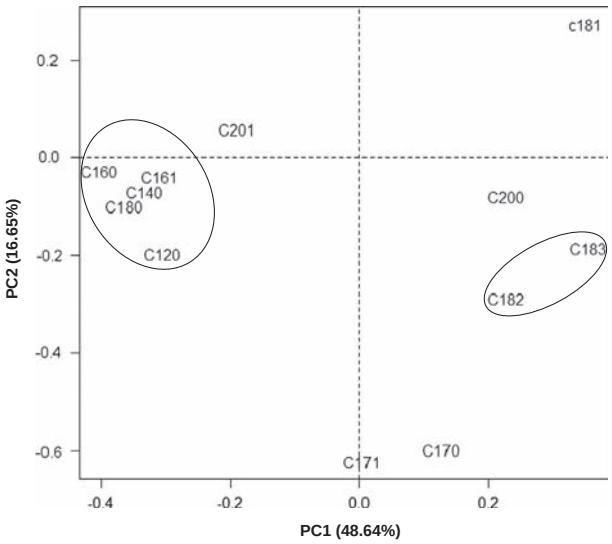

Fig. 3. Loadings plot for the first two principal components of fatty acids.

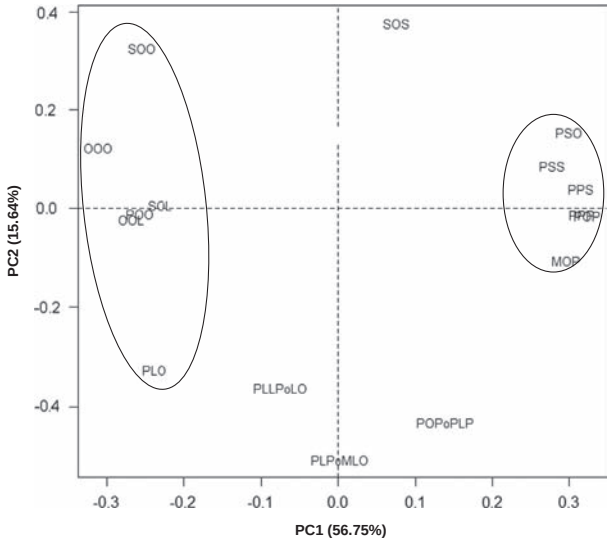

Fig. 4. Loadings plot of the first two principal components of triacylglycerols.

model including fatty acids and triacylglycerols and to the whole set of variables, non-significant differences between *Bellota* and *Ibérico* samples were observed ($p=0.359$ and $p=0.545$ respectively).

3.3. PCA-based display method

A PCA was applied to the data matrix according to fatty acids, triacylglycerols and to the whole set. In the PCA performed using fatty acids (Fig. 3), PC1 and PC2 explained up to 65.29% of total variance, 48.64% being explained by PC1 and 16.65% by PC2. The variables corresponding to short and medium chain saturated fatty acids C12:0, C14:0, C16:0 and C18:0 appear in the left side of PC1, while the polyunsaturated fatty acids (C18:3 and C18:2) are located on the opposite side. Accordingly, the score plot obtained by selecting the variables that most contribute to PC1 is that of saturated fatty acids and polyunsaturated fatty acids. Regarding score plot PC2, fatty acid C18:1 is located on the positive side, unlike all the other fatty acids. According to this plot C18:1 fatty acid could differentiate between the *Bellota* and *Ibérico* classes, although the variability explained by PC2 could be considered to be low. These results may be due to the fact that the difference between pigs raised on acorns (*Bellota*) and the *Ibérico* samples is that the former include more C18:1 in their fat composition than the latter [4].

The loadings for variables of triacylglycerols were represented in the space of the two principal components obtained from the PCA (Fig. 4). Total variability explained using triacylglycerols in the matrix was 72.39%. PC1 accounted for 56.75% of total variability and was mainly determined by triacylglycerols that include palmitic fatty acids (PSO, PSS, PPS, PPP, POP and MOP) in their molecule, on the right side, whilst triacylglycerols that contain oleic fatty acid (SOO, OOO, SOL, POO, OOL and PLO) are located on the opposite side. PC2 accounted for 15.64% of total variability and was mainly determined by SOS on the top side and variables that included palmitoleic fatty acid (PLL+PoLO, POPo+PLP and PLPo+MLO) on the bottom side. Among the most promising features, the triacylglycerols that include oleic fatty acid were the most characteristic ones. In fact, SOS, SOO and PLO are clearly depicted separately from other triglycerides. These results are in agreement with Viera-Alcaide et al. [14] who reported a clear separation of samples of subcutaneous fat from *montanera* pigs and *concentrate* pigs using OOO and PSO triacylglycerols.

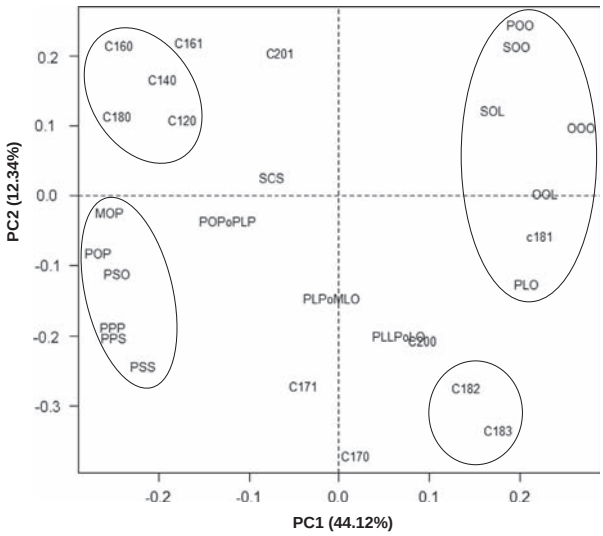

Fig. 5. Loadings plot for the first two principal components of fatty acids and triacylglycerols.

To achieve a better separation of the groups according to product labelling, a PCA including both fatty acids and triacylglycerols was carried out. Fig. 5 shows two principal components extracted from the PCA of fatty acids and triacylglycerols together. PC1 and PC2 explain up to 56.46% of total variance, 44.12% being explained by function 1 and 12.34% by function 2. In PC1 the most contributing variables are on the right side, the triacylglycerols including C18:1 fatty acid (POO, SOO, SOL, OOO, OOL and PLO). These triacylglycerols appear correlated with C18:1 on the right side. Furthermore, on the left side triacylglycerols including C16:0 fatty acid are located (MOP, POP, PSO, PPP, PPS and PSS). Most of the saturated fatty acids appear on the left side, near to the triacylglycerols, which include C16:0, while on the opposite side (right side) polyunsaturated fatty acids are isolated. PC2 was determined mainly by monounsaturated fatty acids (C16:1 and C20:1) located at the top, unlike the polyunsaturated fatty acids (C18:2 and C18:3), which were located at the bottom. Moreover, triacylglycerols that include C16:1 fatty acid (POPo+PLP, PLP+MLO and PLL+PoLO) are seen to have a low contribution to variability. As can be observed, SOS appears isolated from the rest of triacylglycerols. It thus follows that an acceptable separation of the product classes could be achieved from the PCA. Among the promising features for the discrimination of product classes, the most interesting parameters would seem to be, on the one hand, triacylglycerols POO, SOO, SOL, OOO and OOL, as reported by Viera-Alcaide et al. [14] in subcutaneous adipose tissue of Iberian pig and, on the other, C18:1 fatty acid [9].

To display data trends the scores for the samples were depicted in the space of two principal components selected from each PCA. Fig. 6 graphically displays the scores for the samples, identified by labels 'B' (*Bellota*) and 'I' (*Ibérico*) according to the variable selection: fatty acids (A), triacylglycerols (B) and the whole set (C). As can be observed, distribution of samples portrayed in the space of the first two principal components using fatty acid variables, triacylglycerols and the whole set did not show a clear separation between *Bellota* and *Ibérico* samples. The negative results obtained for differentiation of samples based on their fatty acid content are probably due to the use of concentrates with the same fatty acid contents present in acorns (mainly C18:1). Furthermore, the similarity in the processes of maturation of fermented sausages and the inclusion of fat from other animal species (e.g. beef) could also reduce the differences between the two classes studied.

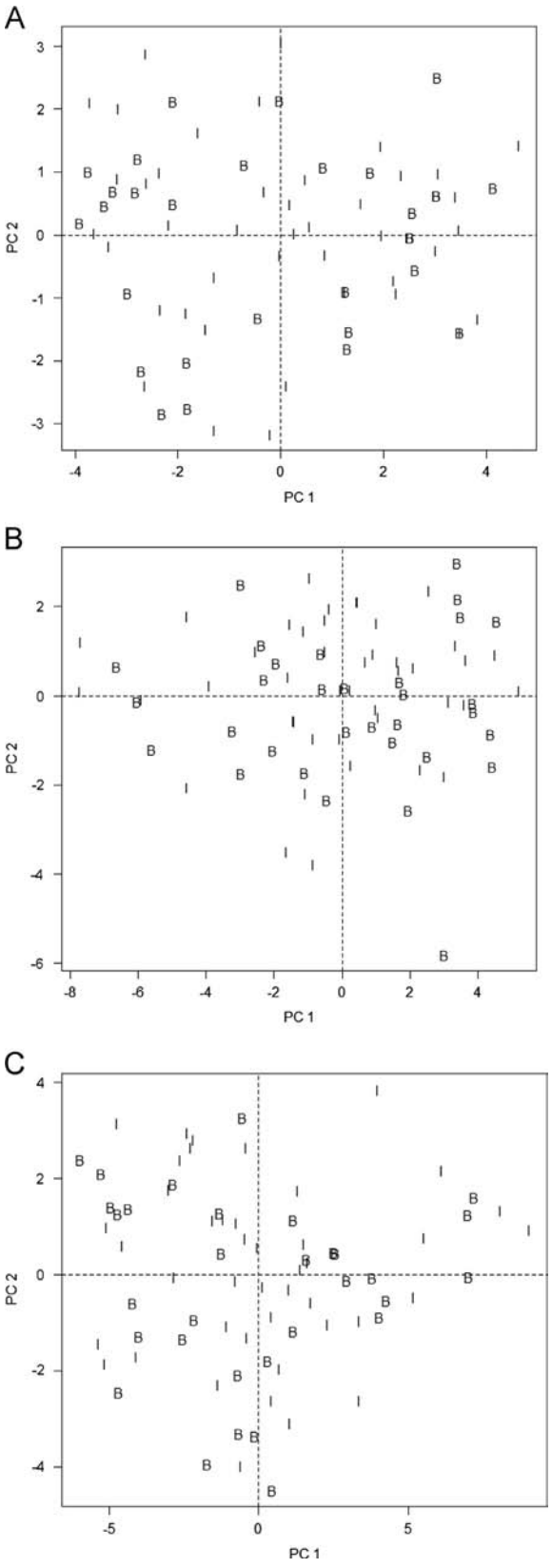

Fig. 6. Scores plot of the first two first principal components for fatty acids (A), triacylglycerols (B) and fatty acids and triacylglycerols together (C). B='*Bellota*' and I='*Ibérico*'.

3.4. Discriminant analysis

Finally, classification rules including fatty acids, triacylglycerols and combined fatty acid and triacylglycerol profiles were trained

Table 3
Jackknife measures of accuracy for Linear and Quadratic Discriminant analysis (% reliability of predicting).

Constituents	Linear discriminant			Quadratic discriminant		
	Total	*Bellota*	*Ibérico*	Total	*Bellota*	*Ibérico*
Fatty acids	41.34	15.62	60.47	49.33	34.38	60.46
Triacylglycerols	61.33	56.25	65.12	50.67	34.38	62.80
All the variables	53.33	37.50	65.12	45.33	0.00	79.07

Table 4
Jackknife measures of accuracy for Logistic Regression and Support Vector Machine analysis (% reliability of predicting).

Constituents	Logistic Regression			Support Vector Machine		
	Total	*Bellota*	*Ibérico*	Total	*Bellota*	*Ibérico*
Fatty acids	40.00	15.62	58.14	54.67	18.75	81.39
Triacylglycerols	64.00	59.37	67.44	44.00	0.00	76.74
All the variables	52.00	40.63	60.47	56.00	0.00	97.67

to predict the class (*Bellota* or *Ibérico*) of a sample. The accuracy of each rule was assessed with the percentage of correct prediction for each of the classes. The three groups of variables were computed by the leave-one-out validation (jackknife procedure). As a first classification model, linear discriminant analysis (Table 3) shows that triacylglycerol variables alone have the greatest discrimination ability, although the estimated measures do not exceed 62% accuracy. While the use of fatty acids in the model achieved the lowest discrimination ability (lower than 42%), in the linear discriminant model the values that represent the reliability of predicting samples according to their origin were higher in the *Ibérico* than in the *Bellota* class. It is well known that linear discriminant analysis performance is optimum in homoscedastic Gaussian populations, a questionable assumption in our data matrix. When the covariance matrices are not assumed to be equal, quadratic discrimination functions are computed. Therefore, quadratic discrimination classification [29] was also computed and discrimination results are shown in Table 3. The number of correctly classified samples according to a quadratic discriminant analysis can be considered to be low (below 51%). The greatest discriminant rates were obtained when triacylglycerols (61.33%) were used and the lowest when all of the variables together (45.33%) were used. As occurred in the linear discriminant model, in the quadratic model prediction reliability was higher for the *Ibérico* class than for the *Bellota* class, in fact, in the quadratic model *Bellota* class assignment ability using all of the variables was null (0%).

In order to determine if other classification models could provide better performance than linear and quadratic discriminant analyses, other statistical classification models were tested. Taking into account that only 75 samples were available in our data set, two models that could be trained in this limited scenario were tested: Logistic Regression [29] and Support Vector Machines [30]. The number of correctly classified samples using Logistic Regression and Support Vector Machines is shown in Table 4. As observed in other statistical models, in the Logistic Regression model the use of triacylglycerols has the greatest ability to discriminate between samples (64.0%) whilst the worst discrimination ability was observed when the model contained fatty acids only (40%), although this model showed the greatest discrimination ability in the case of samples included in the *Ibérico* class. The Support Vector Machine model shows greater discrimination ability when all variables (fatty acids and triacylglycerols) are included in the model (56.0%). It is evident that there is a misclassification in the assignment of category in the case of *Bellota*, in fact 0% correct assignment of *Bellota* class occurred when triacylglycerols were included in the model. These analyses can produce false positive results, which means that there are samples which obtain the high quality denomination (*Bellota*), although they really do not posses it, due to the fact that *Ibérico* samples have shown the same fatty acid and triacylglycerol profiles as *Bellota* samples.

4. Conclusions

The effectiveness of using fatty acid and triacylglycerol profiles to discriminate between Iberian dry-cured sausages according to their labelling information is unclear. The set of variables that includes triacylglycerol performs the best, particularly in the linear discriminant analysis model. However, its estimated accuracy to discriminate between *Bellota* and *Ibérico* dry-cured sausages on the market is far from being practical due to the very high error rate. In order to guarantee the genuineness of the Iberian dry-cured sausages in the market, the identification of fatty acid and triacylglycerol profiles should be combined with other quality control techniques. Other methods of certification, according to feeding and genetic origin of pigs, should be provided in quality policies to regulate the market of Iberian dry-cured sausages. Specific rules regarding labelling of Iberian dry-cured sausages should include references to product traceability.

Acknowledgements

This research was partially funded by the Junta de Andalucía (Consejería de Innovación, Ciencia y Empresa), the European Commission (European Regional Development Fund), and Ministerio de Educación y Ciencia of Spain (PTQ04-3-0716).

References

[1] Boletín Oficial del Estado 70 (1980) 6280.
[2] J. Chasco, M.J. Beriain, J. Bello, Meat Sci. 34 (1993) 191.
[3] V. Ortiz-Somilla, F. España-España, A.J. Gaitán-Jurado, J. Pérez-Aparicio, Food Chem. 101 (2007) 1031.
[4] R. Cava, J. Ruiz, C. López-Bote, L. Martín, C. García, J. Ventanas, T. Antequera, Meat Sci. 45 (1997) 263.
[5] M. Narváez, M. León-Camacho, I.M. Vicario, Grasasy Aceites 60 (2009) 238.
[6] E. Cantos, J.C. Espin, C. Lopez-Bote, L. de la Hoz, J.A. Ordoñez, F.A. Tomas, J. Agric. Food Chem. 51 (2003) 6248.
[7] C.J. Lopez Bote, Meat Sci. 49 (Suppl) (1998) 17.
[8] Boletín Oficial del Estado 300 (2007) 51655.
[9] I. Franco, S. Iglesias, B. Prieteo, J. Carballo, Grasas y Aceites 55 (2007) 273.
[10] E. Gallardo, M. Narváez-Rivas, F. Pablos, J.M. Jurado, M. León-Camacho, J. Agric. Food. Chem. 60 (2012) 1645.
[11] I. Viera-Alcaide, I.M. Vicario, M.L. Escudero-Gilete, E. Graciano-Constante, M. León-Camacho, Grasas y Aceites 59 (2008) 327.
[12] J.F. Tejeda, G. Gandemer, T. Antequera, M. Viau, C. García, Meat Sci. 60 (2002) 357.
[13] D.L. García, M. Viera, N. Tena, R. Aparicio, Grasas y Aceites 58 (2007) 344.
[14] I. Viera-Alcaide, I.M. Vicario, E. Graciani, M. León-Camacho, Anal. Chim. Acta 596 (2007) 319.
[15] M.J. Petrón, E. Muriel, M.L. Timón, L. Martín, T. Antequera, Meat Sci. 68 (2004) 71.
[16] L. Arcea, A. Domínguez, V. Rodríguez, S. López, M.J. Ayora, M. Valcárcel, Anal. Chim. Acta 636 (2009) 183.
[17] S. Ventanas, J. Ventanas, J. Tovar, C. García, M. Estévez, Meat Sci. 77 (2007) 246.
[18] M. Narváez-Rivas, I.M. Vicario, M.J. Alcalde, M. León-Camacho, Talanta 81 (2010) 1224.
[19] Boletín Oficial del Estado 264 (2007) 45087.
[20] J. Folch, M. Lees, G.H. Sloane-Stanley, J. Biol. Chem. 226 (1957) 497.
[21] E. De Pedro, M. Casillas, C.M. Miranda, Meat Sci. 45 (1997) 45.
[22] R. Core, ⟨http://www.R-project.org/⟩, 2012.
[23] P. Filzmoser, M. Gschwandtner, ⟨http://CRAN.R-project.org/package=mvoutlier⟩, 2013.

[24] R.G. Garrett, J. Geochem. Explor. 32 (1989) 319.
[25] K. Nordhausen, S. Sirkia, H. Oja, D.E. Tyler ⟨http://CRAN.R-project.org/package=ICSNP⟩, 2012.
[26] C. Franz, R package version 0.8-1, 2006.
[27] M.J. Beriain, M.P. Pefia, J. Bello, Food Chem. 48 (1993) 31.
[28] S. Bañón, M. Bedia, E. Almela, P.J. Martínez, Agric. Food Sci. 19 (2010) 240.
[29] W.N. Venables, B.D. Ripley, Modern Applied Statistics with S-PLUS, Springer, New York, 2002.
[30] R. Pino, M.D. Cubiles, M.A. Romero, A.P. Acosta, A.J. López, N. Bellinfantei, Environ. Modelling Software 25 (2010) 826.