Exploratory data analysis for fatty acid composition in pig meat

R. ROS[1], J. REIXACH[2], M. TOR[1] and J. ESTANY[1]
[1]Department of Animal Production - University of Lleida, Spain, rros@prodan.udl.cat
[2]Selección Batallé, Spain

1. Introduction

Fat content and composition are determinant factors affecting pork production and meat quality (Wood et al., 2003). Fat composition is commonly presented as the percentage of each individual fatty acid relative to total fatty acids and then some pork quality traits are described in terms of some fatty acid percentages (see for example the review by Wood et al., 2008). Despite being compositional in nature, to our knowledge there is no reference in the literature where specific compositional data analysis methods had been applied to analyze fatty acid composition. A first objective of this contribution is to analyze fatty acid composition as compositional data.

In meat quality research, it is common to analyze the effect of some influential factors (such as diet, genotype, gender, live weight or age, among others) on fat content and composition, usually the subcutaneous (SF) or the intramuscular (IMF) fats. In these studies, the aim is mostly to estimate and then test the differences among treatments for fatty acid percentages. The pattern of fatty acid deposition may differ among tissue (for instance, SF or IMF) or muscle (Kloareg et al., 2007; Duran-Montgé et al., 2008), and even between localization within a specific tissue. A second objective of this manuscript is to better know the differences between fatty acid deposition pattern between tissues, localization within a tissue, and muscles.

Thus, the purpose of the present study is, first, to describe a data set of fatty acid composition collected specifically for doing research on IMF content and composition and assess the main differences among IMF of three muscles and SF using compositional data methods, and, second, to apply, as a case study, specific compositional data methods to discriminate between samples of IMF and SF by fatty acid composition and compare the results with the obtained when using the traditional approach.

2. Material and methods

2.1 Data

The data set consisted of 943 fatty acid profiles of samples of *gluteus medius* muscle (IMFGM) from Duroc barrows raised in 12 fattening batches. We determined the IMF content (in percentage of dry matter) and fatty acid composition for each sample. Each profile included eleven fatty acids, which were determined in duplicate by quantitative determination by gas chromatography. Then, the saturated (SFA; i.e. C14:0, C16:0, C18:0, and C20:0), monounsaturated (MUFA; i.e. C16:1n-7, C18:1n-9, and C20:1n-9) and polyunsaturated (PUFA; i.e. 18:2n-6, C18:3n-3, C20:2n-6, and C20:4n-6) fatty acid proportions were calculated. Additional samples of the *longissiums dorsi* (IMFLD, n=194) and *semimembranosus* (IMFSM, n=132) muscles, as well as of SF (n=294) were obtained on a subsample of the above barrows and then analyzed for fatty acid composition using the same procedure. Because C20:0 is present at very low levels, it was not detected in some samples and therefore, following Sanford et al. (1993), the corresponding zeros in the raw data were replaced by 0.55 times the lowest measured value.

Egozcue, J.J., Tolosana-Delgado, R. and Ortego, M.I. (eds.)
ISBN: 978-84-87867-76-7

2.2 Exploratory analyses

A descriptive analysis of the fatty acid composition of IMFGM was performed using CoDaPack3D (Thió-Henestrosa and Martín-Fernández, 2005). The descriptive parameters (center, variation array, individual centered log-ratio (clr) variance, and total variance) were calculated. Because we observed a strong effect of batch, data was adjusted for batch (i.e. centered by batch) and the descriptive parameters were recalculated. Then, a biplot for the fatty acid composition in IMFGM was done. The above descriptive parameters were also calculated for IMFLD, IMFSM, and SF.

2.3 Discriminant analyses

A discriminant analysis was performed using data on fatty acid composition of the three muscles (IMFGM, IMFLD, and IMFSM) and SF, either using the raw percentages or their clr-transformed variables (Pawlowsky-Glahn and Egozcue, 2006). For these analyses, we used data from pigs having profiles for SF and, at least, for one muscle (IMFGM, n=290; IMFLD, n=117; IMFSM, n=57). Data in percentages were adjusted for batch using a general linear model. Data as clr-transformed variables were centered by batch before clr-transformation. For both approaches, linear discriminant analyses were performed with JMP® 8 software (SAS Institute Inc., Cary, NC). Either the whole fatty acid profile or only two fatty acids were used as covariates.

3. Results and discussion

3.1 Exploratory analyses

Table 1 shows that the most abundant fatty acids in both tissues were C18:1n-9, C16:0, C18:2n-6, and C18:0. For these fatty acids, the differences among adipose tissues were very low. The main relative differences were found for fatty acids at low concentration, such as C20:4n-6, C18:3n-3, C16:1n-7, and C20:2n-6. The muscle had greater concentrations of SFA and MUFA than SF (except for IMFSM), and lower concentrations of PUFA (except for C20:4n-6). The main PUFA, C18:2n-6 and C18:3n-3, are known to be essential fatty acids, i.e. they have dietary origin, and they are proved to be preferentially deposited in SF (Duran-Montgé et al., 2008). However, the concentration C20:4n-6 was much higher in IMF, showing an opposite trend with respect to other PUFA. This could be explained by the fact that C20:4n-6 can be endogenously synthesized from C18:2n-6 in muscle (Wood et al., 2008). Among muscles, the higher concentration of PUFA and lower of SFA and MUFA in IMFSM could be attributed to the lower fat content in IMFSM (7.6%, SD 3.3 vs. 16.3%, SD 5.2, in IMFGM, and 13.7%, SD 3.9, in IMFLD). It is known that there is a positive relationship between fat and SFA and MUFA contents, but negative between fat and PUFA contents (Cameron and Enser, 1991).

Adipose tissue	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:4
IMFGM	1.68	23.04	3.77	11.09	45.03	11.92	0.73	0.14	0.82	0.59	1.19
IMFLD	1.57	24.26	4.10	11.95	45.78	9.22	0.45	0.15	0.84	0.42	1.27
IMFSM	1.61	21.55	3.01	10.38	43.98	14.05	0.61	0.08	0.77	0.58	3.38
SF	1.48	21.37	2.10	10.89	44.46	16.05	1.23	0.14	1.12	0.89	0.26

Table 1. Centers of the fatty acid composition (in percentage of total fatty acid content) of intramuscular fat of *gluteus medius* (IMFGM), *longissimus dorsi* (IMFLD) and *semimembranosus* (IMFSM), and subcutaneous fat (SF).

The total variance of the IMFGM composition was 0.54 while the individual clr-variances ranged from 0.03, for C18:1n-9, to 0.12, for C20:4n-6. After adjusting for batch, the total variance was reduced to 0.30 and the individual clr-variances to 0.02, for C18:1n-9, and to 0.06, for C20:4n-6 (Table 2). Clr-variances in IMFLD were similar to those in IMFGM, whereas IMFSM had the highest and SF the lowest ones. Fatty acid composition as a whole resulted to show low variation. The high clr-variances of C20:0 were due to its low content but probably also to the presence of adjusted zeros. In general, C20:4n-6 showed the highest relative variability in all cases, except for IMFSM.

Adipose tissue	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:0	C20:1	C20:2	C20:4
IMFGM	0.026	0.017	0.026	0.020	0.017	0.022	0.023	0.052	0.021	0.020	0.057
IMFLD	0.028	0.019	0.024	0.023	0.018	0.025	0.024	0.049	0.021	0.024	0.060
IMFSM	0.053	0.050	0.162	0.054	0.051	0.064	0.055	0.178	0.057	0.051	0.136
SF	0.015	0.015	0.018	0.017	0.013	0.015	0.020	0.035	0.016	0.015	0.023

Table 2. Individual centered log-ratio variances, after centering by batch, of the fatty acid composition of intramuscular fat of *gluteus medius* (IMFGM), *longissimus dorsi* (IMFLD) and *semimembranosus* (IMFSM), and subcutaneous fat (SF).

The biplot of the two first components of IMFGM (Figure 1) explained 68% of total variance. The analysis of the biplot showed that the angles between links corresponding to the individual SFA_i/SFA_j, $MUFA_i/MUFA_j$ and $SFA_i/MUFA_j$ component log-ratios (where i and j are two any SFA, MUFA or PUFA) were close to zero, especially for the main fatty acids (C16:0, C16:1n-7, C18:0, C18:1n-9), and with the only exception of C14:0. The angles between links corresponding to $PUFA_i/PUFA_j$ log-ratios were also close to zero, except for C20:2n-6. However, the links of these two groups of log-ratios were almost perpendicular when compared among them. This revealed high correlations among individual SFA_i/SFA_j, $MUFA_i/MUFA_j$ and $SFA_i/MUFA_j$ components log-ratios, as well as among the log-ratios of individual $PUFA_i/PUFA_j$ components, but generally low correlations among both groups of log-ratios. This correlation structure (similar to the results in Cameron and Enser, 1991) indicates that SFA and MUFA behave similarly to each other but differently from PUFA, a result which is consistent with their different deposition patterns, being PUFA mostly dietary while SFA and MUFA mostly derived from endogenous synthesis (Duran-Montgé et al., 2008; Wood et al., 2008).

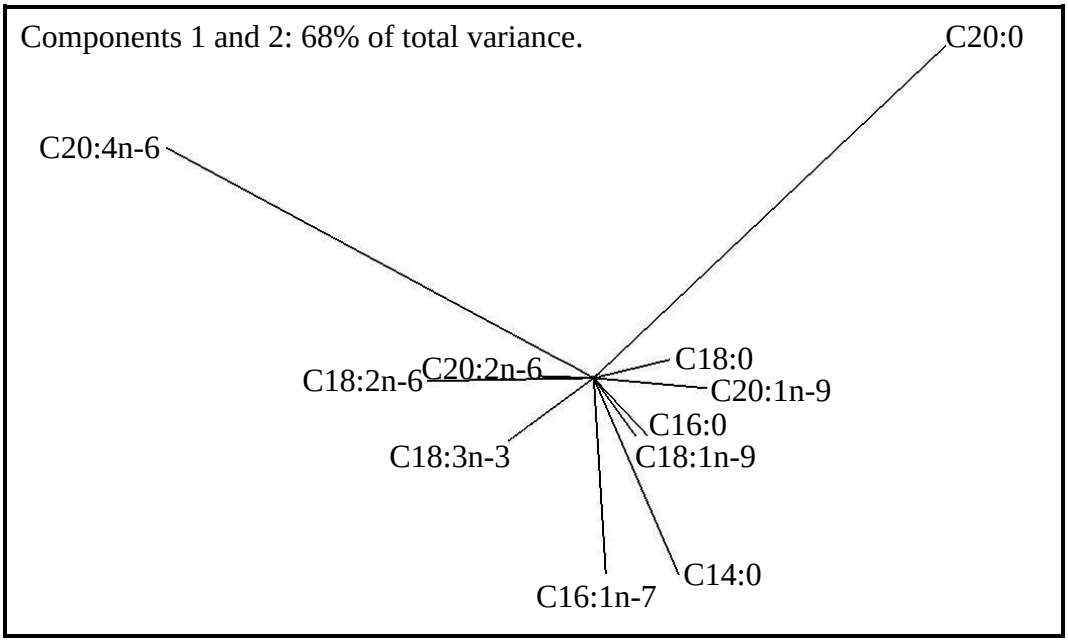

Figure 1. Biplot of the components 1 and 2 for the fatty acid composition of the intramuscular fat of *gluteus medius*.

3.2 Discriminant analyses

Samples were discriminated by fatty acid composition using either the whole fatty acid profile or only the two most influential fatty acids (C18:3n-3 and C20:4n-6) as covariates. When using all the fatty acids as covariates, discrimination among tissues (SF vs. muscle) was almost complete (Table 3). Only two samples were misclassified when using percentages of fatty acids (Table 3a). Overall, the percentage of misclassification was 19.5% and 16.9% for percentages and clr-transformed variables. This result proved that IMF and SF can be totally discriminated by their fatty acid profile.

		a) Predicted tissue using percentages of total fatty acids				b) Predicted tissue using clr-transformed variables			
		IMFGM	IMFLD	IMFSM	SF	IMFGM	IMFLD	IMFSM	SF
Actual tissue	IMFGM	182	86	21	1	206	55	29	0
	IMFLD	16	97	4	0	18	90	9	0
	IMFSM	11	8	38	0	15	2	40	0
	SF	0	1	0	293	0	0	0	294

Table 3. Classification of intramuscular fat in *gluteus medius* (IMFGM), *longissiums dorsi* (IMFLD) and *semimembranosus* (IMFSM), and subcutaneous fat (SF) samples using the whole fatty acid profile as covariates, expressed either as (a) fatty acid percentages or (b) clr-transformed data. Count of actual samples (rows) by predicted tissue (columns), with the correctly classified samples on the diagonal.

When using only the information of the two most influential fatty acids, i.e. C18:3n-3 and C20:4n-6, misclassification of samples according to tissue (SF vs. muscle) was still low (Table 4). It ranged from 5%, if fatty acids were expressed as raw percentages of total fatty acids (Table 4a), to 0.7%, if the data were clr-transformed (Table 4b). This result proved that IMF and SF can be very well discriminated by their fatty acid profile, especially by the PUFA concentrations. In particular, C18:3n-3 and C20:4n-6 resulted to be informative enough to discriminate among adipose tissues. In all cases, discrimination among muscles was less effective, but it was better when using clr-transformed variables instead of the raw percentages. Globally, the total percentage of misclassification also decreased with clr-transformed variables (23.2%) in relation to raw percentages (28.2%). The use of clr-transformed variables improved the discrimination with respect to the percentages.

		a) Predicted tissue using percentages of total fatty acids				b) Predicted tissue using clr-transformed variables			
		IMFGM	IMFLD	IMFSM	SF	IMFGM	IMFLD	IMFSM	SF
Actual tissue	IMFGM	151	103	24	12	158	60	67	5
	IMFLD	23	89	5	0	14	90	13	0
	IMFSM	10	11	36	0	11	6	40	0
	SF	24	2	0	268	0	0	0	294

Table 4. Classification of intramuscular fat in *gluteus medius* (IMFGM), *longissiums dorsi* (IMFLD) and *semimembranosus* (IMFSM), and subcutaneous fat (SF) samples using C18:3n-3 and C20:4n-6 as covariates, expressed either as (a) fatty acid percentages or (b) clr-transformed data. Count of actual samples (rows) by predicted tissue (columns), with the correctly classified samples on the diagonal.

4. Implications

Fatty acid composition in pig fat shows low variation, although large enough to discriminate between adipose tissues. Compositional data analysis resulted to improve the discrimination between tissues and muscles by fatty acid composition and therefore it may lead towards a better understanding of the differential development adipose tissues.

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Acknowledgements

Work funded by CICYT, Spain (grant AGL2009-09779). R. Ros acknowledges a FPI grant from MICINN (BES-2010-034607).