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# Gas–liquid chromatographic method for analysing complex mixtures of fatty acids including conjugated linoleic acids (*cis*9*trans*11 and *trans*10*cis*12 isomers) and long-chain (n-3 or n-6) polyunsaturated fatty acids Application to the intramuscular fat of beef meat

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**Abstract**

The optimisation and validation of a gas–liquid chromatographic (GLC) method using direct saponification with KOH/methanol followed by a derivatization with (trimethylsilyl)diazomethane was carried out trying to overcome all the difficulties posed by the analysis of complex mixtures of fatty acids (FAs) in animal fat tissues. The presented method allowed sensitive, selective and simultaneous determination of a wide range of different FAs, including short-chain FAs, branched-chain FAs and conjugated linoleic acid isomers in the same GLC run along with other well known saturated, monounsaturated and polyunsaturated FAs. To demonstrate the feasibility of the procedure, the total FA profile of beef meat was characterised.


*Keywords:* GLC; Fatty acid; Beef meat; CLA; n-3; n-6 PUFA

## 1. Introduction

Results published in recent years indicate the importance of food fatty acid (FA) composition in human nutrition and health [1,2]. In general, it is recommended to decrease the intake of saturated and *trans* FAs and to increase the intake of n-3 polyunsaturated FAs (PUFAs). Besides the n-3 PUFA, interest in conjugated linoleic acids (CLAs) has increased considerably over the last years because of their potential beneficial effects on health as they have been linked to a multitude of metabolic effects. However, to date, most of the evidence in relation to the health effects of CLA have been derived from in vitro and animal studies, and the challenge is to define the health effects of CLA in human subjects [3]. CLA refers to a group of positional isomers of octadecadienoic acid with a conjugated double bond system, of which the *cis*9*trans*11 and *trans*10*cis*12 isomers are the most abundant. That is why most of the research studies have focused their studies on these two isomers [4,5]. Both isomers are naturally found in ruminant derived food products, formed as intermediates during the biohydrogenation of linoleic and linolenic acid in the rumen [6,7]. However, endogenous synthesis of *cis*9*trans*11CLA is the predominant production pathway in the mammary gland [8,9], and also occurs in other adipose tissues (i.e. subcutaneous, intramuscular fat) by the action of $\Delta^9$-desaturase enzyme on *trans*-vaccenic acid (C18:1*trans*11), which is another intermediate in ruminal biohydrogenation [10,11]. Evidence suggest that $\Delta^9$-desaturation could also play an important role in providing humans with increased CLA levels derived from dietary *trans*C18:1 precursors by the action of $\Delta^9$-desaturase [12–14]. That is why nutrition has refocused attention on their presence in ruminant tissues. However, the FA composition of ruminant tissues is complex because of the FA synthesis by rumen microorganisms (i.e. branched-chain FAs (BFAs) [15]) as well as by lipolysis followed by hydro-

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genation of dietary PUFAs to more saturated end products [16].

Ruminant fats are comprised of a wide range of lipid classes with complex structures and with most of the FAs bound to chemical structures to form fractions such as triglycerides (TGs) and phospholipids (PLs). Several methodologies are available (i.e. ether or *n*-hexane extraction, chloroform–methanol mixtures) for total lipid extraction from ruminant fats and from foods in general [17–23]. However, using some of these methods, the inclusion of components other than FAs could be found on the lipid fraction. For total FA composition analysis several methods of derivatization are currently employed to esterify free and bonded FAs. Not only it is necessary to optimise this procedure in order to obtain accurate, quantitative and qualitative results, but also to address potential procedural difficulties like incomplete conversion of the FAs to their ester homologues, alterations of the original FA profile during esterification (positional and/or geometrical isomers, i.e. CLA isomers), formation of artefacts and contamination, and subsequent damage of the gas–liquid chromatographic (GLC) column as outlined by Shantha and Napolitano [24] and Aldai et al. [25].

Despite all different methods actually employed for total FA analyses in animal adipose tissues, there are still contradictory opinions about the most suitable and satisfactory methods for overcoming all the difficulties posed by the analysis of complex mixtures including CLA isomers. Accordingly, trying to approach previous considerations, the objective of this study was the optimisation and validation of a GLC method using direct saponification with KOH in methanol followed by a derivatization with (trimethylsilyl)diazomethane (TMS–DM), which is a convenient alternative source of diazomethane, safer to handle, more stable with minimal side reactions, and which allows to analyse and quantify in a single chromatogram a complex mixture of FAs. And then to apply that method in analysing the total FA composition of beef meat. The FA composition of meat (intramuscular fat) exerts a strong influence on meat quality because of the implications of these components on human health [1,26,27] and on organoleptic properties through the oxidative breakdown during ageing [28] and the formation of volatiles during cooking [29,30].

## 2. Experimental

### 2.1. Animals

Intensively fed yearling bulls of Asturiana de los Valles beef breed were employed. The diet of the animals was composed by concentrate meal (84% barley meal, 10% soya meal, 3% fat, minerals, vitamins and oligoelements) and barley straw, both ad libitum.

Animals were slaughtered on reaching an average live weight of 550 kg in a commercial abattoir according to standard procedures.

### 2.2. Sample preparation

A steak from the *Longissimus thoracis* (LT) muscle, previously vacuum packed and frozen at −80 °C when 24 h postmortem, was thawed overnight at 4 °C. The lean muscle was chopped up after eliminating subcutaneous and intermuscular adipose tissue and visible collagen, minced with an electrical grinder, and manually homogenised.

### 2.3. Chemicals and gases

All reagents and solvents were of analytical grade (Merck, Panreac, Sigma–Aldrich, Spain) except for *n*-hexane, that was of higher purity grade, special for chromatography (Suprasolv, Merck, Spain). Free FA standards (see Table 3) were of 99% purity (Sigma–Aldrich, Spain), except for individual *cis*9*trans*11 and *trans*10*cis*12 isomers of octadecadienoic acid (98+% purity) that were purchased from Matreya (USA). The ultra pure water was obtained from a Milli-Q system from Millipore (Mildford, USA). All gases employed ($N_2$, He, $H_2$, Air) were of 99.999% purity (Alphagaz, Air Liquide, Spain).

### 2.4. Total FA determination

Total FAs were saponified using KOH in methanol and derivatized using TMS–DM based on a modification of the method by Elmore et al. [30] as outlined in Aldai et al. [25].

#### 2.4.1. Extraction procedure

Duplicate 1 g quantity from each sample was accurately weighed out into 50 mL conical centrifuge tubes and 1 mg of the internal standard (I.S.) (free heneicosanoic acid, 100 μL of 10 mg/mL C21:0 in methanol:toluene (1:1, v/v)) was added before saponification. Then, 6 mL of saponification solution (5 M KOH in methanol:water (50:50, v/v)) was added. Tubes were flushed with $N_2$, sealed and shaken for 10 min prior to placing in a 60 °C waterbath during 60 min for direct saponification. After that, the reaction mixture was diluted with 12 mL 0.5% NaCl and 5 mL of a non-polar solvent (i.e. petroleum spirit). Samples were vortexed for 5 min, few drops of absolute ethanol added and centrifuged at 800 × *g* for 5 min at 20 °C for layer separation. The top layer (containing the non-saponifiable extract, i.e. cholesterol, *N*-acyl lipids) was removed and discarded. Then, 3 mL of glacial acetic acid was added to neutralise the KOH fraction. And then, 5 mL of a non-polar solvent (petroleum spirit) was added and the tubes vortexed for 10 min. This step also resulted in protonation of the FA carboxyl groups, hence they would partition more rapidly into non-polar solvents such as petroleum spirit. Samples were centrifuged again (800 × *g* for 5 min at 20 °C) and the top layer was kept and transferred to clean screw-cap glass tubes. Once again 5 mL of a non-polar solvent was added for second clearance. Centrifugation and layer transference steps were repeated again. Then 100 μL of 2,2-dimethoxypropane, which acts as water scavenger reacting with water to produce acetone, were added to each tube and vortexed for 2 min.

#### 2.4.2. Derivatization procedure

For methylation of free FAs, first of all, samples were reduced to dryness under $N_2$ at 40 °C and then redissolved in 1 mL of methanol: toluene (2:1 vol.) and vortexed for 5 min. Methanol is a catalyst for the TMS–DM reaction and drives the reaction

in favour of methyl ester formation. At this stage, methylation reagent was added in molar excess of 2 M TMS–DM in *n*-hexane (120 μL) and the reaction proceeded at 40 °C for 10 min without capping the tubes. The samples were dried down under gentle stream of $N_2$ at 40 °C for approximately 20 min. Finally, each sample was reconstituted in 2 mL of *n*-hexane (with 50 ppm of BHT), centrifuged at 20,000 × *g* for 5 min at 7 °C and transferred into vials for storage at −20 °C. Before GLC injection, samples were diluted in *n*-hexane and 1 μL was injected onto GLC column and run under an optimised temperature program with optimised gas flow rate.

### 2.5. GLC equipment

A Varian Star CX3400 GLC (Varian, Spain) equipped with a FID detector, an automatic sample injector (SPI) in on-column mode, and a Chrompak CP-SIL 88 for FA methyl esters (FAMEs) (WCOT FUSED SILICA 100 m × 0.25 mm i.d., 0.2 μm film thickness) with a retention gap (FUSED SILICA TUBING 4 m × 0.25 mm i.d., methyl deactivated) was used. He was used as the carrier gas with a column head pressure of 355 kPa (and a flow rate of approximately 2 mL/min measured at 100 °C). The GLC conditions were as follows: temperature programmable (100 °C, at 2 °C/min to 170 °C, hold for 15 min, at 0.5 °C/min to 180 °C, at 10 °C/min to 200 °C, hold for 10 min, at 2 °C/min to 230 °C, hold for 10 min); injector temperature, 250 °C; detector temperature, 300 °C. Results were evaluated with a conventional integrator program (Saturn GC Workstation 5.51 Software).

### 2.6. Quantification of individual FAMEs

The individual FAMEs were identified according to similar peak retention times using standards, and quantified using the chromatographic peak area according to the I.S. method. The response factors (rf) of the individual FAs were calculated. For these calculations, solutions of 0.05 mg/mL in *n*-hexane of pure FA standards (Table 3) were used. Each one was methylated with the previously described method, and a 1 μL volume was analysed seven times in the GLC.

### 2.7. Validation procedure

Method validation included establishment of FID detector response linearity, detection and quantification limits, recovery and precision of the analytical procedure. For response linearity, detection and quantification limits, and recovery criteria, five FAs were selected in relation to different chain length and constitution (branched or straight), and double bond positions: *iso*C15:0, C18:0, C18:3n-6, *cis*9*trans*11CLA and C20:5n-3. In all cases, free FAs but methylated with the aforementioned derivatization method were used.

The linearity of the response was determined from the analysis in triplicate of aforementioned pure FA solutions in *n*-hexane, where injected quantities were from 0.004 to 15 μg (5 points). Linear regression analysis of absolute areas versus injected quantities of the FAs were used.

The detection and quantification limits of the analytical method were calculated from the calibration curves of the selected pure FAs (*iso*C15:0, C18:0, C18:3n-6, *cis*9*trans*11-CLA, C20:5n-3) and the mean noise value of eight repetitions of the blank (*n*-hexane) analysis.

The recovery percentage of the method was established from the complete analysis in triplicate of beef meat samples (1 g) fortified with the selected pure FAs (*iso*C15:0, C18:0, C18:3n-6, *cis*9*trans*11CLA, C20:5n-3) in different quantities ranging from 0.04 to 1.80 mg.

The precision of the method was established from five complete analyses of the same beef steak sample.

## 3. Results and discussion

In Fig. 1, a chromatogram of total FA analysis on beef meat (LT muscle) is represented. For this analysis the saponification/derivatization procedure, outlined before, was used. The complete analysis took 97 min and 47 peaks were detected ranging from C10:0 until C22:6n-3. From these 47 peaks, the one eluting at retention time (rt) 31 min corresponded to the antioxidant (BHT) used in the analysis, and the one eluting at rt 42 min corresponded to the sum of C16:1*cis*9 and *anteiso*C17:0. These aforementioned FAs (C16:1*cis*9, *anteiso*C17:0) were easily separated (see rf, Table 3) when similar pure FA quantities were injected, but in real samples, we could not separate them probably because of the higher proportion of C16:1*cis*9 in comparison to *anteiso*C17:0 in beef meat. Another two peaks eluting at rt 51.5 and 53.5 min, were respectively named as C18:1*trans*10 and C18:1*cis*12 but their identification was just corroborated with bibliographical data [31] and not with pure standards. The I.S. (C21:0) added to the sample eluted at rt 70 min. As a result, 43 peaks were identified as detectable FAs in beef meat samples, 13 saturated FAs (SFAs) (6 were odd-chain FAs (OFAs)), 5 BFAs, 11 monounsaturated FAs (MUFAs), 12 PUFAs and the 2 major CLA isomers (*cis*9*trans*11 and *trans*10*cis*12). From the PUFA group, 7 FAs were n-6 type and 5 were n-3 type. Moreover, several isomers of the C18:1 as *trans*9, *trans*11, *cis*9, *cis*11, and *cis*13 could be separated. Most of the actual studies based on beef fat depots' FA composition carry out the quantification of the two aforementioned CLA isomers along with major FAs. Some other works focus their studies just on the separation of several CLA isomers [32–34]. And few works included short-chain or BFAs in their beef fat depots' characterisation studies [35–38]. In this sense, there are scarce works on the meat field [39], which carried out the separation of all the aforementioned type of FAs together in the same GLC run and without using any other auxiliary separation. Even some research studies did achieve the determination of a wide range of FAs on samples like milk and rumen fluid [40,41]. In general, the presented method of analysis allows sensitive, selective and simultaneous determination of a wide range of different FAs, including short-chain FAs, BFAs and CLA isomers in the same GLC run along with some other chiefly known FAs as SFAs, MUFAs and PUFAs, and without previous auxiliary lipid fractionation.

For the validation of the quantitative method, response linearity of the pure FAs, detection and quantification limits, recov-

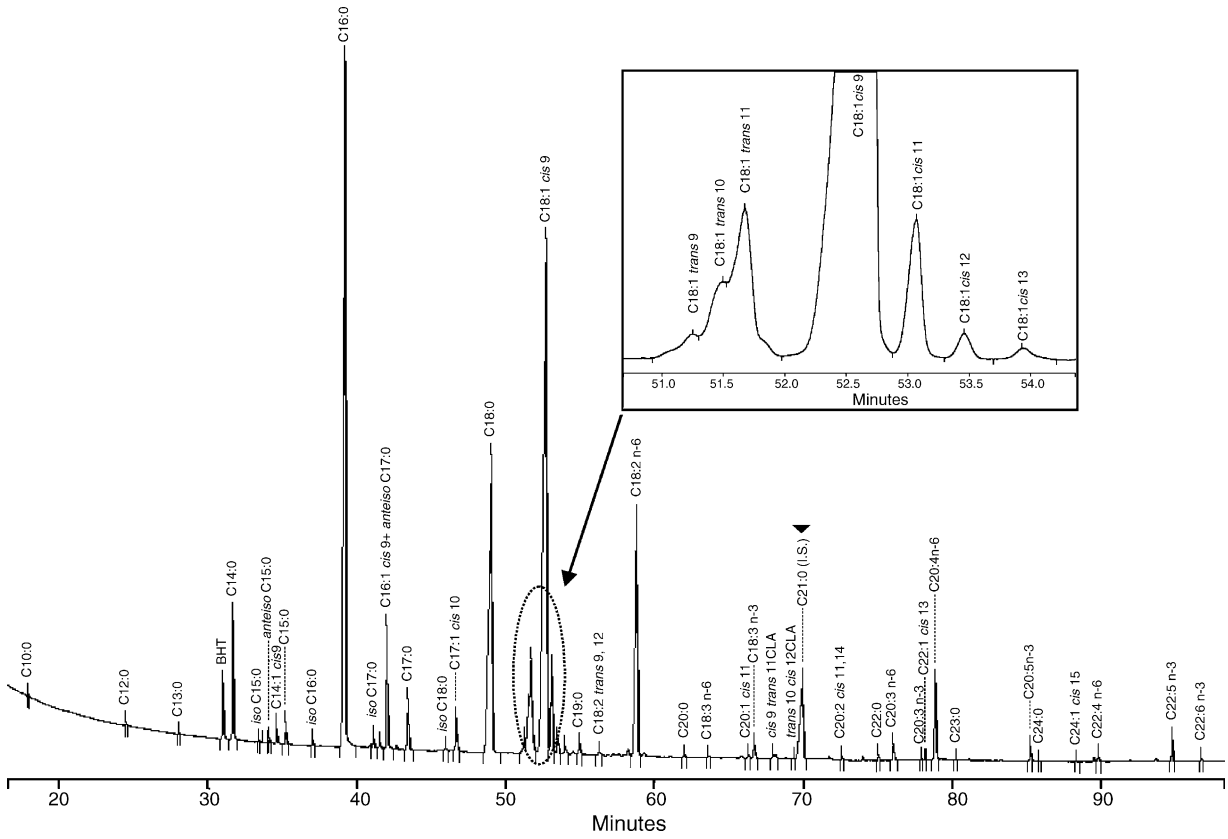


Fig. 1. Gas–liquid chromatogram for beef intramuscular total FAs saponified with KOH in methanol and derivatized with TMS–DM employing the following conditions of analysis: CP Sil-88 fused silica capillary column (100 m × 0.25 mm i.d.) connected in series packed with a retention gap (4 m × 0.25 mm i.d.); temperature program (100 °C, at 2 °C/min to 170 °C, hold for 15 min, at 0.5 °C/min to 180 °C, at 10 °C/min to 200 °C, hold for 10 min, at 2 °C/min to 230 °C, hold for 10 min); injector temperature, 250 °C; detector temperature, 300 °C; He as the carrier gas with a column head pressure of 355 kPa and a flow rate of 2 mL/min. I.S.: internal standard.

Table 1
Estimated regression parameters for the selected standard FAs in the linearity study of the FID detector

| Fatty acid | Equation | $R^2$ |
|---|---|---|
| *iso*C15:0 | $A = 1.43E+06c + 1.80E+03$ | 0.999 |
| C18:0 | $A = 1.20E+06c + 4.20E+04$ | 0.999 |
| C18:3n-6 | $A = 1.30E+05c + 4.35E+01$ | 0.999 |
| *cis*9*trans*11CLA | $A = 1.41E+06c - 3.56E+01$ | 0.999 |
| C20:5n-3 | $A = 1.37E+06c + 5.03E+03$ | 0.998 |

$R^2$, coefficient of correlation; $A$, area; $c$, quantity in μg.

ery, and precision of the analytical procedure were calculated. Table 1 presents the calculations of the response linearity for the different selected pure FA standards used. Linear functions of chromatographic peak area ($A$) and the quantity of the FA injected ($c$) were used to calculate the linear response of the FAs in the FID detector. The slopes of the regression lines for the FAs were similar for all FAs (mean value of 1.35E + 06 ± 104682.06, $n = 4$) except for C18:3n-6 which showed a 10 times lower value (1.30E + 05), indicative of differing detector calibration sensitivity for the last FA. An adequate linearity was obtained for all analytes, with $R^2$-values higher than 0.99.

Table 2 presents the results of the calculations of detection and quantification limits for the different pure FAs considered. Detectable peaks were defined as peaks with an area at least three times the mean noise (this was 35.89 mV × min), and quantifiable peaks were defined as peaks with an area at least ten times the mean noise (this was 119.64 mV × min). In this sense, in the analysed beef meat samples, 38 FAs were quantifiable (Table 5).

Because of the complexity of the beef intramuscular total FA composition and in order to achieve an accurate quantification of the different types of FAs, the quantification method was signifi-

Table 2
Mean ($\bar{x}$) and standard deviation (SD) values for the detection and quantification limits for the analysis in the chromatograms, estimated for selected pure FAs

| Noise[a] (mV × min) | $\bar{x}$ ± SD (11.964 ± 0.982) | | | | |
|---|---|---|---|---|---|
| | *iso*C15:0 | C18:0 | C18:3n-6 | *cis*9*trans*11CLA | C20:5n-3 |
| Detection limit (mg) | 0.015 | 0.015 | 0.115 | 0.015 | 0.015 |
| Quantification limit (mg) | 0.022 | 0.022 | 0.167 | 0.022 | 0.022 |

[a] Noise was estimated as the mean area of the signal obtained on analysing eight blanks.

Table 3
Mean response factor (rf) values relative to the value of the internal standard (C21:0, heneicosanoic acid) for pure FAs

| Fatty acid | | rf | CV (%) |
|---|---|---|---|
| C10:0 | Capric acid | 1.64 | 1.78 |
| C11:0 | Hendecanoic acid | 1.47 | 1.93 |
| C12:0 | Lauric acid | 1.59 | 2.34 |
| C13:0 | Tridecanoic acid | 1.42 | 2.39 |
| C14:0 | Myristic acid | 1.22 | 2.86 |
| C14:1*cis*9 | Myristoleic acid | 1.19 | 2.74 |
| C15:0 | Pentadecanoic acid | 1.08 | 2.73 |
| *iso*C15:0 | 13-Methyltetradecanoic acid | 1.16 | 2.66 |
| *anteiso*C15:0 | 12-Methyltetradecanoic acid | 1.12 | 2.85 |
| C16:0 | Palmitic acid | 1.13 | 2.70 |
| *iso*C16:0 | 14-Methylpentadecanoic acid | 1.04 | 2.63 |
| C16:1*c*9 | Palmitoleic acid | 0.88 | 3.61 |
| C17:0 | Margaric acid | 0.82 | 3.29 |
| *iso*C17:0 | 15-Methylhexadecanoic acid | 0.75 | 2.22 |
| *anteiso*C17:0 | 14-Methylhexadecanoic acid | 0.73 | 4.29 |
| C17:1*cis*10 | *cis*-10-Heptadecenoic acid | 0.80 | 3.33 |
| C18:0 | Stearic acid | 0.74 | 1.64 |
| *iso*C18:0 | 16-Methyheptadecanoic acid | 0.73 | 2.58 |
| C18:1n-9 | Oleic acid | 0.74 | 1.64 |
| C18:1*cis*11 | *cis*-Vaccenic acid | 0.72 | 2.54 |
| C18:1*cis*13 | *cis*-13-Octadecenoic acid | 0.79 | 7.54 |
| C18:1*trans*9 | Elaidic acid | 0.82 | 2.43 |
| C18:1*trans*11 | *trans*-Vaccenic acid (TVA) | 0.73 | 2.80 |
| C18:2n-6 | Linoleic acid | 0.94 | 3.59 |
| C18:2*trans*9,12 | Linolelaidic acid | 0.78 | 3.93 |
| C18:3n-3 | α-Linolenic acid | 0.96 | 5.97 |
| C18:3n-6 | γ-Linolenic acid | 5.37 | 4.35 |
| C19:0 | Nonadecanoic acid | 0.77 | 1.81 |
| C20:0 | Arachidic acid | 0.79 | 1.54 |
| C20:1*cis*11 | Gondoic acid | 0.79 | 1.55 |
| *cis*9*trans*11CLA | *cis*-9, *trans*-11-Octadecadienoic acid | 0.79 | 3.31 |
| *trans*10*cis*12CLA | *trans*-10, *cis*-12-Octadecadienoic acid | 0.93 | 3.13 |
| C20:2n-6 | *cis*-11,14-Eicosadienoic acid | 1.05 | 3.51 |
| C20:3n-3 | *cis*-11,14,17-Eicosatrienoic acid | 1.11 | 3.79 |
| C20:3n-6 | Dihomo-γ-linolenic acid | 1.07 | 2.25 |
| C20:4n-6 | Arachidonic acid | 0.71 | 1.43 |
| C20:5n-3 | *cis*-5,8,11,14,17-Eicosapentaenoic acid (EPA) | 0.70 | 5.77 |
| C22:0 | Behenic acid | 1.05 | 1.73 |
| C22:1*cis*13 | Erucic acid | 0.88 | 2.69 |
| C22:4n-6 | *cis*-7,10,13,16-Docosatetraenoic acid | 1.33 | 0.27 |
| C22:5n-3 | *cis*-7,10,13,16,19-Docosapentaenoic acid (DPA) | 0.89 | 0.23 |
| C22:6n-3 | *cis*-4,7,10,13,16,19-Docosahexaenoic acid (DHA) | 0.71 | 0.45 |
| C23:0 | Tricosanoic acid | 1.10 | 1.13 |
| C24:0 | Lignoceric acid | 1.58 | 2.25 |
| C24:1*cis*15 | Nervoic acid | 1.34 | 2.49 |

CV: variation coefficient for seven replicate analysis.

Table 4
Mean recovery percentages ($\bar{x}$) and coefficient of variation (CV) estimated for selected pure FAs

| Fatty acid | Recovery (%) | CV (%) |
|---|---|---|
| *iso*C15:0 | 143.70 | 23.97 |
| C18:0 | 112.31 | 8.57 |
| C18:3n-6 | 80.78 | 7.68 |
| *cis*9*trans*11CLA | 93.67 | 5.81 |
| C20:5n-3 | 102.85 | 9.66 |

cantly improved by means of an I.S. Mean response factors were employed using heneicosanoic acid (C21:0) as the I.S. This FA was selected as I.S. because it was not detected in the fat fraction of beef meat. In Table 3 mean values of the relative rf of each individual FA analysed are shown. As it can be observed, the relative rf were in general proximate to 1.0, except for C18:3n-6 (5.4). The coefficients of variation obtained were in general lower than 5%, which confirmed the response linearity of the FAs at the concentration range used.

The recovery of the method was determined according to the recovery percentages of selected pure FAs quantities added to the meat sample. In the Table 4 mean values of recovery percentages and coefficients of variation of pure FAs employed are shown. In general, the recovery values were next to 100% except for *iso*C15:0 (143.70%). This high recovery of *iso*C15:0 could be probably resulting from the low concentration of this FA in meat samples.

Replications on a real sample were used to measure the precision of the quantitative method. The reproducibility of the quantities obtained (mg of each FA/100 g of raw beef meat) were calculated by five analysis (five subsamples) carried out in the same beef steak and on day (Table 5). Apart from some exceptions, the value of the coefficient of variation (CV) were lower than 20%, which is a slightly high value that we regarded as acceptable bearing in mind the nature of the fat extraction process and the complexity of chromatographic analysis of beef meat FA profile. Moreover, the highest values of CV were obtained for the FAs at lowest concentration as found by Carrapiso et al. [42] when employing some other extraction/derivatization methods.

Major FAs were represented by C16:0 (30%), C18:1*cis*9 (26.5%), C18:0 (13.8%) and C18:2n-6 (9.5%), which accounted an overall percentage of 80%. Individually, C14:0, C16:1*cis*9, C17:0, C18:1*trans*11, C18:1*cis*11 and C20:4n-6 accounted between 1 and 3.5%, while the rest of the FAs with individual contributions not exceeding 1%. Referring to CLA isomers, *cis*9*trans*11 represented a 6.5 times higher quantity than *trans*10*cis*12 isomer, but the overall CLA content was of 0.24%. Looking at the FA groups, SFA represented the major percentage (49.2%) and BFA accounted the minor proportion (0.6%) while MUFA and PUFA represented 36.7 and 13.5%, respectively. Furthermore, the sum of unsaturated FAs (MUFA + PUFA) (50.2%) accounted a slightly higher proportion than SFA.

In this study, a P/S ratio of 0.21 and a n-6/n-3 ratio of 10.34 were obtained. According to nutritional guidelines, individuals should maintain the P/S ratio at about 0.45 and the n-6/n-3 ratio

Table 5
Mean total FA content (mg/100 g of raw meat) of beef meat obtained from five replicate analyses

| Fatty acid | mg/100 g | CV (%) |
|---|---|---|
| C10:0 | 0.85 | 18.77 |
| C11:0 | BD | |
| C12:0 | 1.54 | 24.40 |
| C13:0 | 0.26 | 25.36 |
| C14:0 | 41.82 | 13.75 |
| *iso*C15:0 | 0.67 | 10.84 |
| *anteiso*C15:0 | 1.57 | 17.51 |
| C14:1*cis*9 | 6.38 | 15.45 |
| C15:0 | 6.36 | 14.23 |
| *iso*C16:0 | 1.68 | 17.80 |
| C16:0 | 405.69 | 12.95 |
| *iso*C17:0 | 2.75 | 11.24 |
| C16:1*cis*9 + *anteiso*C17:0 | 36.88 | 13.08 |
| C17:0 | 16.70 | 14.70 |
| *iso*C18:0 | 1.06 | 22.76 |
| C17:1*cis*10 | 10.74 | 13.07 |
| C18:0 | 186.15 | 14.13 |
| C18:1*trans*9 | 8.23 | 16.36 |
| C18:1*trans*11 TVA | 45.33 | 18.37 |
| C18:1*cis*9 | 358.58 | 14.43 |
| C18:1*cis*11 | 25.92 | 12.40 |
| C18:1*cis*13 | 3.28 | 14.46 |
| C19:0 | 3.69 | 20.75 |
| C18:1*trans*9,12 | BQ | |
| C18:2n-6 | 128.96 | 14.67 |
| C20:0 | 0.88 | 20.99 |
| C18:3n-6 | 3.17 | 31.36 |
| C20:1*cis*11 | 1.69 | 17.79 |
| C18:3n-3 | 5.78 | 12.66 |
| *cis*9*trans*11 CLA | 2.83 | 18.76 |
| *trans*10*cis*12 CLA | 0.43 | 21.53 |
| C20:2n-6 | 1.12 | 12.09 |
| C22:0 | 1.78 | 17.23 |
| C20:3n-6 | 6.58 | 15.33 |
| C20:3n-3 | 0.32 | 33.48 |
| C22:1*cis*13 | BQ | |
| C20:4n-6 | 20.86 | 14.67 |
| C23:0 | BQ | |
| C20:5n-3 EPA | 3.10 | 15.37 |
| C24:0 | BQ | |
| C24:1*cis*15 | BQ | |
| C22:4n-6 | 3.05 | 13.06 |
| C22:5n-3 DPA | 6.34 | 16.19 |
| C22:6n-3 DHA | 0.61 | 17.24 |
| Σ SFA | 665.72 | 13.25 |
| Σ BFA | 7.73 | 14.83 |
| Σ MUFA | 497.04 | 14.25 |
| Σ PUFA | 183.16 | 14.05 |
| P/S | 0.21 | 14.26 |
| n-6/n-3 | 10.34 | 8.79 |

BD: below detection limit; BQ: below quantification limit; Σ SFA: sum of all saturated FAs; Σ BFA: sum of all branched FAs; Σ MUFA: sum of all monounsaturated FAs; Σ PUFA: sum of all polyunsaturated FAs; n-6:sum of C18:2, C18:3, C20:2, C20:3, C20:4, C22:4; n-3: sum of C18:3, C20:5, C22:5, C22:6; P/S = (C18:2n-6 + C18:3n-3)/(C14:0 + C16:0 + C18:0).

close to 4.0 [43,44]. The low P/S ratio was probably due to the content of SFA and MUFA that increases faster with increasing animal fatness than does the content of PUFA, leading to a decrease in the relative proportion of PUFA and consequently in the P/S ratio [45–47]. Regarding n-6/n-3 ratio of the total lipid fraction, this may vary depending on the n-6/n-3 ratio of the PL and TG fractions though the n-6/n-3 ratios are much more affected by feeding regimes than by genetics [48]. In this sense, this meat showed high C18:2n-6 content, what could be due to the high linoleic proportion on the concentrate feed given to the animals (C16:0, C18:1*cis*9 and C18:2n-6 represented 90% of the total FAs in the concentrate meal), and which could be directly influencing the n-6/n-3 ratio. In general, these results were similar to the findings reported for beef intramuscular fat of intensively fed animals by other researchers [49,50].

## 4. Conclusion

In our experience, this analytical method involves a step forward in this field. It allows the determination of free and esterified FAs, including two CLA isomers, with a high degree of accuracy and reproducibility. It yields satisfactory results on analysing a complex mixture of FAs spanning a broad range of molecular types as found in beef meat (major and minor FAs), in a single GLC run and without the need for auxiliary separations. And what it is more, this derivatization method did not change the original isomer distribution nor alter the geometric configuration of conjugated double bonds, and we did not find evidence of artefacts interfering on FA chromatographic peaks. Therefore, the application of this method to the analysis of meat samples could be an effective tool for the study of different management factors (i.e. breed, gender and feeding) that can influence the FA profile of beef meat.

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