Comparison of two gas–liquid chromatograph columns for the analysis of fatty acids in ruminant meat

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1. Introduction

Fatty acid (FA) composition of ruminant meat has been extensively studied due to its importance in human dietary intake and its implications for human health. The highly saturated nature of ruminant edible fat and its relatively high content of trans unsaturated FA might favour the development of cardiovascular diseases. Otherwise, conjugated linoleic acid (CLA) isomers may have beneficial effects on the prevention and treatment of several pathologies [1]. All these features of ruminant fat result from an intense microbrial metabolism of dietary FA occurring in the rumen, where extensive isomerisation and hydrogenation of unsaturated FA take place, as well as de novo synthesis of microbial FA. The result is a very complex FA pattern and consequent particular difficulties concerning its analysis [2,3].

The analysis of FA composition of ruminant fat is commonly performed by gas–liquid chromatography (GLC) of fatty acid methyl ester (FAME) derivatives. Different types of capillary columns with non-polar, polar, and highly polar stationary phases can be used for the separation of FAMES. Flexible fused silica columns coated with highly polar stationary phases of cyanoalkyl polysiloxane have been extensively used for the analysis of samples with complex mixtures of geometric and positional isomers of polyunsaturated fatty acids (PUFA). The CP-Sil 88 (100% cyanopropylsilicone) of 100 m long is the most popular one. The main advantage of these high polar phases compared to non-polar phases is their high-resolution capability of unsaturated FAME, especially for the separation of cis and trans FA isomers [4]. However, the polarity of this type of column shows a stronger dependence on temperature having the disadvantage of less thermal stability compared with other common stationary phases [4,5]. Current stationary phases of intermediate polarity are polyethylene glycol, with DB-Wax, Supelcowax 10 or Carbowax 20M, the most used for the analysis of ruminant fat. More recently, bonded polyethylene glycol phases, like Omegawax 250 and Omegawax 320, were specifically designed for the analysis of omega-3 and omega-6 FAMEs. These columns combine the advantages of a relatively high-resolution capability with those of a relatively high thermal stability [4].

Several valuable research methodologies have been developed over the last decade for FA analysis of oils and fats by GLC. Official methods of analysis supported by collaborative studies have been produced. The American Oil Chemists’ Society (AOCS) released the official methods Ce 1h-05 [6], Ce 1i-07 [7], Ce 1b-89 [8] and Ce 1k-07 [9] and the recommended practices Ce 1j-07 [10] and Ch 2a-94 [11]. The AOCS Ce 1h-05 [6] recommended the CP-Sil 88 of 100 m long or the SP-2560 capillary columns for the determina-
tion of cis, trans, and unsaturated FA in vegetable or non-ruminant animal oils. Moreover, the Supelcowax 10, a capillary column of polyethylene glycol phase, was recommended by the AOCS Ce 11-07 [7] for the determination of FA in marine and other oils containing long-chain PUFA. Additionally, the AOCS Ce 1b-89 [8] suggested the Omegawax for the determination of long-chain omega-3 FAs, as this kind of stationary phase offers a good separation within each even-carbon chain-length and minimal chain-length overlaps [12]. The evaluation and optimization of chromatographic methods developed by official methods have been reported. Ratnayake et al. [13] studied the chromatographic resolutions of FA from margarine samples using the CP-Sil 88 and SP-2560 capillary columns. Furthermore, Kramer and Blackadar [14] compared and characterized the FA separations of milk fat samples using the 100-m CP-Sil 88 and the Supelcowax 10. Despite similarities of the stationary phase of Omegawax 250 and Supelcowax 10, no direct comparisons between Omegawax 250 and CP-Sil 88 have been reported. Additionally, separation and elution order of branched-chain FAs and dimethylacetals on meat fat have not been described in detail in Supelcowax 10 [14].

Determination of FA composition for label purposes usually follows food label regulations that require the use of official methods of analysis. However, for academic research those requirements are not so strict, and chromatographic analysis of complex samples, such as meat fat, may be performed according to the purpose of the study. Current “official methods” (AOCS Ce 1b-89) for the determination of long-chain omega-3 FAs uses carbowax-based liquid phases, as these offers a good separation within each even-carbon chain-length and minimal chain-length overlaps [12]. More recently, bonded polyethylene glycol phases, like Omegawax 250 and Omegawax 320 were designed specifically for the analysis of omega-3 and omega-6 FAMEs. These columns combine the advantages of a relative high-resolution capability with those of a relatively high thermal stability [4].

The objective of this work was to compare the most used GLC capillary column for the analysis of FA composition of ruminant fats, the CP-Sil 88, having a highly polar stationary phase, with the Omegawax 250 capillary column that was specially designed for the analysis of omega-3 and omega-6 FAMEs. These columns combine the advantages of a relative high-resolution capability with those of a relatively high thermal stability [4].

2.2. Standards

**FAME standard 1**: Linolenic acid methyl ester isomer mix (L6031) from Sigma–Aldrich (St. Louis, MO, USA). Ag-ion SPE cartridges (750 mg/6 mL) were purchased from Supelco Inc. (Bellefonte, PA, USA).

2.3. Sample collection

Lamb meat samples used in this study were originated from experiments conducted by Animal Production Unit of Instituto Nacional dos Recursos Biológicos (INRB), Portugal. Ninety-six samples of longissimus dorsi muscle from Merino Branco lambs produced according to Borrego do Alto Alentejo PGI regulation (CE) no. 510/2006 of 20 of March 2006, were used and additionally a few samples of longissimus dorsi of beef cattle originated from a trial where animals were fed with high concentrate diets supplemented with extruded linseed were also used.

2.4. Fatty acid analysis

Lipids from intramuscular fat (<=0.250 g) were extracted using a mixture of dichloromethane and methanol as described in Bessa et al. [18] and FAME were prepared using a sodium methoxide 0.5 M solution in methanol followed by hydrochloric acid in methanol (1:1) [19]. Nonadecanoic acid methyl ester was used as internal standard at concentration of 1 mg per sample. Separation of cis and trans 18:1 isomers by Ag-ion SPE was conducted as described in Alves et al. [20].

2.5. Gas–liquid chromatography

2.5.1. System A

A gas chromatograph HP6890A (Hewlett-Packard, Avondale, PA, USA), equipped with a flame-ionization detector (GC–FID) and a CP-Sil 88 capillary column (100 m; 0.25 mm I.D.; 0.20 μm film thickness; Chrompack, Varian Inc., Walnut Creek, CA, USA) was used. The column temperature of 100°C was held for 15 min, increased to 150°C at a rate of 10°C/min and held for 5 min, then increased to 158°C at 1°C/min and held for 30 min, and finally increased to 200°C at a rate of 1°C/min and maintained for 65 min. Helium was used as carrier gas at a flow rate of 1 mL/min, the split ratio was 1:50 and 1 μL of sample was injected. The injector and detector temperatures were 250 and 280°C, respectively.

2.5.2. System B

A gas chromatograph Varian 3800GC equipped with a flame-ionization detector and an Omegawax250 capillary column (30 m; 0.25 mm I.D.; 0.25 μm film thickness Supelco, Bellefonte, CA) was used. The column temperature of 150°C was held for 11 min, then increased to 210°C at a rate of 3°C/min and maintained for 30 min. Helium was used as carrier gas at a flow rate of 1.3 mL/min, the split ratio was 1:50 and 1 μL of sample was injected. The injector and detector temperatures were 250 and 220°C, respectively.

2. Materials and methods

2.1. Chemicals

All reagents and solvents were analytical and chromatographic grade, and were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ag-ion SPE cartridges (750 mg/6 mL) were purchased from Supelco Inc. (Bellefonte, PA, USA).

2.2. Standards

**FAME standard 1**: Linolenic acid methyl ester isomer mix (L6031) from Sigma–Aldrich.

**FAME standard 2**: Commercial standard FAME mix C4:0 to C24:0 from Supelco Inc. (Bellefonte, PA, USA).

**FAME standard 3**: Synthesized by partial hydrogenation of linseed oil as described by Christie [15] and adapted by Alves and Bessa [3]. Briefly, 0.5 g of linseed oil was stirred with 100 mL of 10% hydrazine hydrate solution in methanol for 2.5 h at 45°C. After that time, the solution was diluted with 100 mL H2O and acidified with 40 mL 6N HCl. Lipids were extracted 3× with a mixture of 50 mL ether:petroleum ether (1:1), dried with Na2SO4 and concentrated under N2. Finally, methyl esters were prepared with 1% sulphuric acid in methanol [16].

**CLA standard 1**: Prepared by sigmatropic rearrangement of CLA cis-9,trans-11 as described by Destaillats and Angers [17]. Briefly, 500 μL of CLA cis-9,trans-11 was mixed with 1.5 mL of hexadecane and heated under N2 in a sealed glass vial at 190°C during overnight, in a CombiPal incubator heater (Varian Inc., Walnut Creek, CA, USA). After cooling to room temperature, 1 mL of hexane was added prior to GLC analysis.

**CLA standard 2**: Prepared by catalysis with selenium of CLA cis-9,trans-11 standard [17].

**CLA standard 3**: Prepared by sigmatropic rearrangement and catalysis with selenium of CLA trans-10,cis-12 standard [17].

A gas chromatograph Varian 3800GC equipped with a flame-ionization detector and an Omegawax 250 capillary column (30 m; 0.25 mm I.D.; 0.25 μm film thickness Supelco, Bellefonte, CA) was used. The column temperature of 150°C was held for 11 min, then increased to 210°C at a rate of 3°C/min and maintained for 30 min. Helium was used as carrier gas at a flow rate of 1.3 mL/min, the split ratio was 1:50 and 1 μL of sample was injected. The injector and detector temperatures were 250 and 220°C, respectively.
2.6. Quantification of fatty acid methyl esters

Quantification of total FAME was done using nonadecanoic acid methyl ester as internal standard. Results for each FA were expressed as a percentage of the sum of total FA.

2.7. Statistical analysis

The data obtained from running 96 samples of lamb meat with both chromatographic systems were analysed by a general linear model considering the chromatographic system as the only factor using the GLM procedure of SAS (SAS Inst. Inc. 2002, Cary, NC, USA) and by regression analysis using the REG procedure of SAS, for testing if $a$ parameter (y-intercept) was significantly different from 0 and if $b$ parameter (slope) was significantly different from 1.

A subset of eight samples with FA concentration higher than 100 mg/g muscle dry matter were used in a crossover design with a $2 \times 2$ factorial arrangement to test the effects of the type of equipment, column and equipment $\times$ column interaction. The samples were processed analytically using both columns mounted in both gas chromatographic equipments. Data were analysed using the MIXED procedure of SAS, considering the equipment, column and its interaction as fixed effects and the sample as random effect.

3. Results and discussion

Considerable differences in resolution and elution order were observed between the columns which are attributed to different stationary phases and polarities, as well as temperature program.

The analysis of FAs using the 30-m Omegawax column required 60 min, while the CP-Sil 88 of 100 m long required about 160 min for elute all FAs (Fig. 1). Programmed temperature columns were chosen in order to obtain the best resolution, in one run, of the majority of FAMES, with the emphasis on the octadecadecenoate cis and trans isomers and non-conjugated 18:2 and 18:3 isomers. Resolution of these isomers is particularly important in research concerning ruminant lipid metabolism. Kramer [21] described a combination of GLC programmed and isothermal temperature programs, using hydrogen as carrier gas to resolve complex mixtures of geometric and positional isomers of monounsaturated and polyunsaturated FAMES in total milk and beef fats in about 100 min each. The capillary column length and diameter are the major determinants of the time of analysis, although other operating parameters such as carrier gas type and velocity can also influence it [22]. It is known that in order to reduce time of analysis hydrogen should be used as carrier gas instead of helium [23,24], although it requires safety precautions in the event of a leak and during discharge from the split vent in the split-injection mode [25].

![Fig. 1. Total GLC chromatogram of a lamb meat fat sample, using a 30-m Omegawax 250 and a 100-m CP-Sil 88 capillary column.](image-url)
Chromatographic FA evaluation using the CP-Sil 88 and the Supelcowax 10 column coated with a stationary phase similar to the Omegawax 250, was reported by Kramer and Blackadar on milk fat [14]. Despite similarities between Supelcowax 10 and Omegawax 250, the elution order of branched-chain and dimethylacetals, as far as we know, has not been reported on meat fat.

Analysis of FA using the 30 m Omegawax capillary column requires smaller analysis time compared with the 100 m CP-Sil 88 capillary column (Fig. 1). Due to different stationary phases and polarities of each capillary column the order of elution of FAs may be quite distinctive. The column temperature program may also affect retention time and some times order of elution and resolution of FAMEs.

3.1. Qualitative chromatographic resolution

3.1.1. Branched-chain fatty acids and dimethylacetals

Fig. 2 shows a partial chromatogram of the branched-chain and dimethylacetals (DMA) region using the Omegawax and the CP-Sil 88 capillary column. Branched-chain FAs are mainly derived from de novo microbial synthesis in the rumen [26]. DMA are formed from cleavage of alkenyl chains linked to ether bonds in plasmalogenic lipids when acid conditions are used to prepare FAME derivatives. The order of elution and resolution of branched-chain FA with odd-chain (iso- and anteiso-15:0 and 17:0) and even-chain-lengths (iso-16:0 and iso-18:0) can be distinctive depending on the column used. However, iso and anteiso branched-chain configurations usually elute prior to the corresponding saturated FAME in both columns. Iso-15:0 and anteiso-15:0 have small retention times than the saturated 15:0, as well iso-16:0, iso-17:0 and iso-18:0 elute earlier than the saturated 16:0, 17:0 and 18:0, respectively. Co-elutions of both branched 17:0 with 16:1 isomers may be a problem when the CP-Sil 88 capillary column is used. The resolution of both iso-17:0 and anteiso-17:0 from the 16:1 isomers can be improved by using Ag-ion TLC or SPE fractionation previous to GC analysis, as described by Destaillats et al. [27] and Kramer et al. [14,21].

Omegawax capillary column is a good alternative for the analysis of branched-chain FAs and also for 16:1 isomers, showing a clear resolution of 16:1 geometric (trans-9 and cis-9) and positional isomers (cis-9 and cis-7).

DMA elute in both columns earlier to the FAME with the same number of carbon atoms. DMA-16:0 elutes at 17.8 min while 16:0 FAME elutes at 19.0 min, in the Omegawax column and using our chromatographic conditions. On the CP-Sil 88 capillary column the DMA-16:0 and the 16:0 FAME elute at 51.2 and 57.6 min, respectively. The DMA-18:0 elutes near 18:0 FAME in the Omegawax column while in CP-Sil 88 capillary column it elutes 7.7 min before 18:0 and even earlier than iso-18:0 and 17:1 isomers. The monounsaturated DMA-18:1 can also be unequivocally quantified in both columns, eluting just before 18:0 FAME, using our chromatographic conditions.

Fig. 2. Partial GLC chromatogram of the branched-chain and dimethylacetal (DMA) region of a lamb meat fat sample, using a 30-m Omegawax 250 and a 100-m CP-Sil 88 capillary column.
3.1.2. Octadecadecenoate cis and trans isomers

Complex 18:1 isomeric pattern are very characteristic of ruminant fats and results from the hydrogenation pathways of C18 PUFA and from cis/trans isomerisation and double bond migration through the acyl chain catalysed by microbial population in the rumen [28,29].

Fig. 3 shows chromatograms of the 18:1 isomers region from a total sample and the cis and trans fractions after Ag-ion SPE, using the Omegawax and the CP-Sil 88 capillary columns. The Omegawax column with its medium polarity stationary phase did not accurately separate geometric isomers (cis and trans) of 18:1, on the contrary the CP-Sil 88 capillary column showed a much better resolution between trans and cis 18:1 isomers. In both capillary columns, FAME retention times increase with increasing the double bond position counted from the carbonyl group.

Chromatograms from the Omegawax column show that even after Ag-ion fractionation the trans-9 to trans-12 18:1 isomers can not be completely resolved, although these trans octadecadecenoic FAME can be resolved on the CP-Sil 88 column. Both trans-13 and 

trans-14 co-elute together in the two columns, however, several authors had described that partial resolution of these isomers could be achieved using an isothermical temperature of 120 °C [2,21,30], using the CP-Sil 88 column. Trans isomers had lower retention times than cis isomers on the CP-Sil 88 capillary column. These cis octadecadecenoic acids can be fully resolved after Ag-ion fractionation on both capillary columns. The omegawax only requires 27 min to elute all cis 18:1 isomers, usually present in ruminant fat, while the CP-Sil 88 requires about 85.2 min, using our chromatographic conditions.

If nonadecanoic acid methyl ester (19:0) is used as internal standard, it elutes close to the 18:1 cis-15 isomer on the CP-Sil 88 capillary column. Moreover, the linoleic acid elutes between cis-15 and trans-16 18:1 on the Omegawax capillary column.

3.1.3. Non-conjugated 18:2 and 18:3 isomers

Most of these FAs result from microbial biohydrogenation pathways in the rumen, although the two FAs quantitatively more representative are the linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) both from exclusive dietary origin [18].

Figs. 4 and 5 show partial GLC chromatograms of the non-conjugated 18:2 and 18:3 isomers region by using the Omegawax and the CP-Sil 88 capillary column, respectively. Both figures present a sample of a beef meat fat from animals fed with concentrate diets supplemented with extruded linseed.

**Non-conjugated 18:2 and 18:3 region**

Fig. 4. Partial GLC chromatograms of non-conjugated 18:2 and 18:3 isomers region analysed using a 30-m Omegawax 250 capillary column, showing from top to bottom: FAME standard 1; FAME standard 2; FAME standard 3; and beef meat fat sample from a trial where animals were fed with concentrate diets supplemented with extruded linseed.

Fig. 5. Partial GLC chromatograms of non-conjugated 18:2 and 18:3 isomers region analysed using a 100-m CP-Sil 88 capillary column, showing from top to bottom: FAME standard 1; FAME standard 2; FAME standard 3; and beef meat fat sample from a trial where animals were fed with concentrate diets supplemented with extruded linseed.

Fig. 3. Partial GLC chromatograms of 18:1 isomers region and their trans and cis Ag-ion SPE fractions analysed using a 30-m Omegawax 250 and a 100-m CP-Sil 88 capillary column of a beef meat fat sample from a trial where animals were fed with concentrate diets supplemented with extruded linseed.
Fig. 5. Partial GLC chromatograms of non-conjugated 18:2 and 18:3 isomers region analysed using a 100-m CP-Sil 88 capillary column, showing from top to bottom: FAME standard 1; FAME standard 2; FAME standard 3; and beef meat fat sample from a trial where animals were fed with concentrate diets supplemented with extruded linseed.

son we show in Fig. 5, chromatograms obtained using the CP-Sil 88 of the same samples presented in Fig. 4.

On the Omegawax column, the region between linoleic acid and the internal standard 19:0 comprises most of the non-conjugated octadecadienoic isomers, as well as the trienoic 18:3n−6. FAME standard 2 from Fig. 4 shows that 18:2 isomers with cis configuration elute first than total trans isomers, additionally the n−6 series elute before the n−3 series, as established by the retention time of 18:3n−6 and 18:3n−3. On the contrary, in the CP-Sil 88 column (Fig. 5, FAME standard 2) non-conjugated 18:2 isomers with cis,cis configuration elute after trans,trans isomers. As example, the retention times of 18:2 trans-9,trans-12 and 18:2n−6 isomers were, 87.2 and 90.9 min when the CP-Sil 88 column was used, whereas when the Omegawax column was used their retention times were, 26.9 and 26.6 min, respectively.

FAME standard 3 shows the retention times of 18:2 cis-9,cis-15 and 18:2 cis-12,cis-15 isomers. On the CP-Sil 88 capillary column (Fig. 5) these two isomers elute on a clear region, otherwise on the Omegawax column (Fig. 4) they elute on a complex region, comprising most of the non-conjugated 18:2 isomers, as demonstrated by the partial chromatogram of the beef fat sample. So, care should be taken with samples having high levels of 18:2 biohydrogenation intermediates, because they may co-elute with each other and with the internal standard 19:0 when the Omegawax column is used.

The CP-Sil 88 capillary column shows a partial resolution of the non-conjugated cis/trans 18:2 isomers commonly present in ruminant fat samples (Fig. 5, sample), although these isomers may also co-elute with the monounsaturated 18:1 cis-16 and the 17-cyclo (methyl 11-cyclohexylundecanoate).

FAME standard 1 (Figs. 4 and 5) shows a mixture of eight geometric isomers of 18:3n−3, varying between all cis and trans configurations. The order of elution of these geometric isomers (Fig. 4) is cct < ccc < ttt < ttc < tct < ctc < tcc < ctt on the Omegawax column (c meaning cis and t meaning trans), whereas on the CP-Sil 88 (Fig. 5), trans isomers elute first than the c,c,c-18:3 isomer (18:3n−3), being the elution order ttt < tct + ctt < ttc + ctc < ccc < ctt. Three 9, 12, 15−18:3 isomers can be fully resolved when the Omegawax column is used, although two cluster containing the ttt + ctt and ttc + ctc + tcc cannot be resolved using our chromatographic conditions. However, on the CP-Sil 88 capillary column 4 geometric isomers of 18:3n−3 can be fully resolved, being those the ttt, ctc, tcc and ccc. Some authors [13,33] have reported that resolution between 18:3n−3 and 20:1 cis-11 may be a problem when the CP-Sil 88 capillary column is used, however in our chromatographic conditions these two FAs can be baseline resolved.

3.2. CLA isomers

Several CLA isomers have been reported as occurring in ruminant fat. The major CLA isomer, rumenic acid (18:2 cis-9,trans-11), is produced in the rumen during the microbial biohydrogenation of dietary 18:2n−6 and in the tissues through delta-9 desaturation of 18:1 trans-11 [34]. Fig. 6 shows the order of elution of the
common CLA isomers present in beef meat fat, together with three synthesized standards, using the Omegawax column. The pattern of CLA isomers on the CP-Sil 88 capillary column is well known [35–41], however, as far as we know, there is no reports showing CLA isomers distribution on the Omegawax capillary column. On this column, CLA isomers with cis,trans configuration elute first, followed by trans,cis and cis,cis isomers. Their retention times increase with increasing the double bond position counted from the carboxyl group. The trans-8, cis-10 isomer co-elutes with cis-9, trans-11, although trans-9, cis-11 can be resolved from cis-9, trans-11. Moreover, the cis-10, trans-12 elutes between both 9,11 CLA isomers. Furthermore, the cis-11, trans-13 co-elutes between the 10,12 isomers, so in samples rich in trans-10, cis-12, its quantification may be overestimated due to inappropriate resolution between these isomers. Predominant cis,cis CLA isomers in ruminant fat can be fully resolved on the Omegawax column, with the cis-9, cis-11 eluting before cis-10, cis-12, followed by cis-11, cis-13 CLA isomer.

CLA isomers with configuration trans, trans have longer retention times than both cis,cis and cis,trans isomers. These t,t CLA isomers elute on the Omegawax column between the saturated 20:0 and the unsaturated 20:1, as shown by the meat fat sample in Fig. 6.

### 3.3. Long-chain polyunsaturated fatty acids

Fig. 7 shows the PUFA region of a ruminant meat fat sample, using the Omegawax or the CP-Sil 88 capillary column. The order of elution of PUFAs in both capillary columns is quite similar, increasing the retention time, primarily by the increase of carbon chain-length and secondarily by level of unsaturation. The n−9 series have retention times lower than n−6 series, and the n−6 series have retention times lower than n−3 series. As example, the retention times of the 20:3 n−9, 20:3 n−6 and 20:3 n−3 are 33.6, 34.4 and 35.8 min in the Omegawax column, and 108.8, 111.9 and 114.8 min in the CP-Sil 88 column, respectively.

Omegawax column can be used up to 280 °C, providing an excellent and highly reproducible resolution of FAMEs. However, we used a temperature column of 210 °C for eluting all long-chain PUFAs with satisfactory resolution, in about 60 min. The CP-Sil 88 required about 160 min for eluting all FAMEs, using a temperature column of 200 °C, showing analogous resolution to the obtained with the Omegawax column. The 21:0 and 19:0 are the internal standards more frequently used for quantification of FAs in ruminant fats. As pointed out by Cruz-Hernandez et al. [2], both FAs raise problems and for most of the individual FAs did not differ between chromatographic system, although some minor FAs, branched (iso-14:0; iso-15:0; iso-16:0; iso-17:0; iso-18:0) and unsaturated (16:1 cis-7; 16:1 cis-9; 16:1 trans-9; 17:1 cis-9, 20:1 n−9; 20:2 n−6; 20:3 n−9) did present some small, but significant differences. However, for the 21:0 and 22:0 the concentrations observed with the CP-Sil 88 were 4 and 3.5 higher than the concentrations observed with the Omegawax, which suggests that the 21:0 and 22:0 might co-elute with other minor compounds.

### 3.4. Quantitative comparisons

The effect of both chromatographic system used on the quantification of FAMEs was studied using the database resulting from running 96 lamb meat samples in both chromatographic systems. Table 1 presents the average values obtained with each chromatographic system. The averages obtained for the total FA content and for most of the individual FAs did not differ between chromatographic system, although some minor FAs, branched (iso-14:0; iso-15:0; iso-16:0; iso-17:0; iso-18:0) and unsaturated (16:1 cis-7; 16:1 cis-9; 16:1 trans-9; 17:1 cis-9, 20:1 n−9; 20:2 n−6; 20:3 n−9) did present some small, but significant differences. However, for the 21:0 and 22:0 the concentrations observed with the CP-Sil 88 were 4 and 3.5 higher than the concentrations observed with the Omegawax, which suggests that the 21:0 and 22:0 might co-elute with other minor compounds.
Table 1
Total fatty acids (mg/g muscle dry matter) and individual fatty acids (mg/g total fatty acids) of 96 samples of lamb meat quantified using the CP-Sil 88 column (system A) or the Omegawax column (system B).

<table>
<thead>
<tr>
<th>FAME</th>
<th>Column</th>
<th>SEM</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP-Sil 88</td>
<td>Omegawax</td>
<td></td>
</tr>
<tr>
<td>Total FA</td>
<td>65.4</td>
<td>67.2</td>
<td>2.14</td>
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<tr>
<td>Branched-chain</td>
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<td></td>
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<tr>
<td>i-14:0</td>
<td>0.5</td>
<td>0.4</td>
<td>0.02</td>
</tr>
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<td>i-15:0</td>
<td>1.2</td>
<td>1.0</td>
<td>0.03</td>
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<td>a-15:0</td>
<td>2.0</td>
<td>2.1</td>
<td>0.06</td>
</tr>
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<td>i-16:0</td>
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<td>1.4</td>
<td>0.03</td>
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<td>4.1</td>
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<td>1.1</td>
<td>0.02</td>
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<td>Saturated-chain</td>
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<td>12:0</td>
<td>4.0</td>
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<td>1.73</td>
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<td>20:0</td>
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<tr>
<td>21:0</td>
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<tr>
<td>22:0</td>
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Significance: ns, P>0.05; SEM, standard error of mean.
** P<0.01
*** P<0.001.

The results of the regression analysis of HP/CP-Sil 88 (system A) and Varian/Omegawax (system B) data are presented in Table 2. Values obtained with both chromatographic systems are highly correlated and the R² of regression equations for total FA and 14 of the 39 individual FAs quantitated, including all major FAs, are higher than 0.99. Nevertheless only 7 of those 14 FAs have slopes ("b") not significantly different than 1. Also for the total FA the slope is different (P<0.001) than 1, although very close to 1. The implication of this small but significant deviation of slope from 1 is that values obtained with each column will be equal in an intermediate concentration range and starts to deviate in samples with high and low FA concentrations (Fig. 8). Computing the confidence intervals for the estimates (for confidence level of 99%), we observed that total FA concentration quantified with HP/CP-Sil 88 starts to be significantly lower than total FA concentration quantified with Varian/Omegawax when the FA concentration is higher than ~66 mg/g muscle dry matter (i.e. ratios mg FA/ mg PI above 16:1). In these data base 43% of the samples had total FA concentration higher than 66 mg/g muscle dry matter. In the lower limit, the values obtained with both columns start to be significantly different from each other when the concentration of FA is bellow ~4 mg/g muscle dry matter, that is well bellow than what is commonly observed in ruminant meat samples.

For some FAs both slope and R² of the regression equations are quite low. In this case we have, iso-14:0, iso-17:0, 21:0, 16:1 cis-7, 18:3n-6 – 6, 16:1 trans-9. This is probably the case of 18:3n-6 – 6, 20:3n-6 – 6, and 20:3n-9. The lower slopes suggest that there are co-elutions with other compounds on CP-Sil 88. This is probably the case of 18:3n-6 – 6, 20:3n-6 – 6, and 20:3n-9. The implica-
differences observed were due to the column or to the equipment, we analysed eight samples (with more than 100 mg FA/g muscle dry matter) in both columns and equipments. The results are presented in Fig. 9. The interaction between equipment and column was highly significant as the quantifications obtained with both columns were not different when the HP6890 was used and differs significantly when the Varian 3800 was used. Comparisons of CP-Sil 88 mounted in the HP6890 and the Omegawax 250 mounted in the Varian 3800 were fully consistent with those obtained in the large database (96 samples).

4. Conclusions

The Omegawax column of 30 m long with an intermediate polarity stationary phase of polyethylene glycol is a good alternative to the most frequently used, the CP-Sil 88 capillary column of 100 m long, for the analysis of FAME in ruminant meat fat. Excepting for cis and trans 18:1 isomers, the Omegawax column showed good resolution of FAME. Both chromatographic systems showed that averages obtained for total FA content and for most of the individual FA did not differ between columns. Furthermore, regression analysis of Omegawax and CP-Sil 88 data is highly correlated. However, some quantitative differences, associated with the chromatographic system, could be detected in samples where FA concentration is higher than 66 mg/g muscle dry matter.

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References