INCREASE OF GEOMETRICAL AND POSITIONAL FATTY ACID ISOMERS IN DARK MEAT FROM BROILERS FED HEATED OILS

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ABSTRACT. Oxidation of polyunsaturated fatty acids leads to primary and secondary oxidation products. Compounds and amounts of these products vary, depending on the oxidative conditions. As these oxidation products have different absorption and biological effects, we performed two different heating treatments on sunflower oil. The first was heating the oil at 190-195°C for 28 h (i.e. very oxidised oil); and the other, heating at 60°C for 12 days (i.e. peroxidised oil).

In the frame of this study, we compared the fatty acid composition of a refined sunflower oil (fresh oil), the peroxidised oil, the very oxidised oil, and a mixture (1+1) of fresh and very oxidised oil (i.e. oxidised oil). Oil fatty acid compositions were affected by the heating treatments. In addition, different fatty acid isomers were formed during heating at 190-195°C and significant differences were found between their content in the sunflower oils.

We also studied the effect of feeding broilers with these oils and Zn and tocopherol supplements on the fatty acid composition of their raw dark meat. Various *trans* fatty acid isomers increased in dark meat from broilers fed oxidised and very oxidised oils. In addition, discriminant analysis showed that *ditrans*-CLA content was able to distinguish dark chicken meat from chickens fed sunflower oils heated at 190-195°C.

Key words: Heated sunflower oils, fatty acid composition, *trans* isomers, CLA, chicken meat.

INTRODUCTION

Fatty acids lead to different geometrical and positional isomers after thermal treatments (Grandgirard et al., 1984; O'Keefe et al., 1993; Destaillats and Angers, 2002), though only temperatures above 180-190°C yield *trans* fatty acids (TFA) in fats and oils (Martin et al., 1998; Wolff, 1993). During deep-fat frying these temperatures are often reached and sometimes surpassed, which means that TFA, even though they are minor components, increase in frying oils (Gamel et al., 1999; Sébédio et al., 1996a; Romero et al., 2000) and fried products (Sébédio et al., 1996a; Romero et al., 2000).

Conjugated linoleic acids (CLA) are a specific group of different positional and geometrical isomers derived from linoleic acid (LA) that can be also formed by heating, refining or partial hydrogenation of oils (Sébédio et al., 1988; Destaillats and Angers, 2002; Juanéda et al., 2003; Jung et al., 2002; Fritsche et al., 1999; Jung et al., 2001).

Nevertheless, TFA also have a natural origin in the action of bacteria in the rumen of cows and other ruminants and are found in the milk and carcass of these animals being vaccenic acid (*trans*-11-octadecenoic) the main isomer (Craig-Schmidt, 1992) whereas the *cis*-9,*trans*-11-octadecadienoic (9c,11t-CLA) is the main CLA isomer in the milk and carcass of ruminants (Pariza et al., 2001).

Trans and *cis* isomers do not show significant differences in their absorptions (Sébédio and Chardigny, 1998; Holmer, 1998; Hayakawa et al., 2000). Thus, TFA in feeds can increase their content in foods derived from non-ruminant animals, which is normally lower than in foods derived from ruminant animals (Chin et al., 1992; Aro et al., 1998; Fritsche and Steinhart, 1998).

Nowadays, there is a tendency to lower TFA content, especially in margarines and fatty spreads (Schwarz, 2000b). This is because, even though the adverse health effect of TFA is subject to controversy, its intake has been described as a risk factor for coronary heart disease (Willett et al., 1993; Kromhout et al., 1995; Aschiero et al., 1999; Oomen et al., 2001). This view is supported by

the less favourable LDL/HDL ratio (Mensink and Katan, 1990) and the greater concentration of lipoprotein(a) (Mensink et al., 1992; Kritchevsky, 2000; Noone et al., 2002) caused by TFA than by saturated fatty acids.

However, CLA, specially 9c,11t-CLA and 10t,11c-CLA, may have anticarcinogenic activity, protect against atherosclerosis and show other positive physiological effects (Pariza et al., 2001; Noone et al., 2002; Belury, 2002) though the positive effects of 10t,12c-CLA, remain controversial (Larsen, et al., 2003; Rainer et al., 2004; Tricon et al., 2004; Wahle et al., 2004).

In addition, in heated oils and fats, TFA are accompanied by several lipid oxidation products that have harmful biological effects (Esterbauer et al., 1991; Schroepfer, 2000; Cohn, 2002; Guardiola, et al., 2002). Some of these oxidation products are absorbed and then deposited in animal tissues (Chow, 1992; Schroepfer, 2000; Guardiola et al., 2002). In addition, these compounds, at high doses, affect animal growth and metabolism (Billek, 2000).

Therefore, we studied the formation of geometrical and positional fatty acid isomers in sunflower oil oxidised at various heating temperatures. By adding these heated oils and α-tocopheryl acetate and Zn supplements to broiler diets, we studied the deposition of these isomers in dark chicken meat, and whether their contents in meat can be used as markers of the addition of heated oils into the feeds.

MATERIALS AND METHODS

Preparation of Heated Oils

Two thermal treatments were carried out. The first was to heat 40 L of refined fresh sunflower oil[1] (FSO) in an indirect heating fryer at 55-60°C under agitation for 12 days. This reached a peroxide value of 90 milli-equivalents peroxide/kg oil, i.e. peroxidised sunflower oil (PSO). The second involved heating 90 L of FSO in a direct heating fryer at 190-195°C under agitation for 28 h

[1] Moyresa, Valencia, Spain, E-46024

until reaching a *p*-anisidine value of 150, i.e. very oxidised sunflower oil (VOSO). By mixing 50% of FSO with 50% of VOSO, we obtained oxidised sunflower oil (OSO). Various oxidation variables and the α-tocopherol content of these sunflower oils are shown in Table 1.

Diets and Animals

A 4 x 2 x 2 factorial design was planned and conducted in triplicate to study the influence of various dietary factors (4 types of sunflower oil, 2 levels of all-*rac*-α-tocopheryl acetate supplementation, and 2 levels of Zn supplementation) on the fatty acid composition of raw dark chicken meat.

336 female broiler chicks (Hubbard, 1-day old) received, up to 10 days of age, a typical basal diet containing 6% of animal fat. The animals were then randomly assigned to 16 dietary treatments (21 chicks per treatment, 7 birds per pen) and were fed *ad libitum* for 42 days. Dietary treatments were prepared from the experimental basal diet (Table 2) by combination of the dietary factors studied: the 4 previously described sunflower oils (FSO, PSO, OSO and VOSO), all-*rac*-α-tocopheryl acetate supplements[2] (0 and 100 mg/kg) and Zn supplements[3] (0 and 600 mg/kg as $ZnSO_4$). In addition, all dietary treatments were supplemented with 0.6 mg/kg of organic selenium derived from selenium-enriched yeast (Sel-plex™)[4]. α-Tocopherol losses caused by thermal treatments (see Table 1) were rectified by adding all-*rac*-α-tocopheryl acetate to feeds containing PSO, OSO and VOSO in order to provide the same α-tocopherol amounts as feeds prepared with FSO.

After the 4 assayed sunflower oils were prepared (described above), they were frozen in 20-L capacity drums at -20°C until feed preparation, in order to reduce further oxidation. Every 10 days, oils were thawed and the 16 dietary treatments were prepared. Finally, chickens were slaughtered

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[3] Andrés Pintaluba, S.A., Reus, Spain, E-43280
[4] Probasa, Sta. Perpetua de la Moguda, Spain, E-08130

according to commercial procedures. Legs with skin from all birds of each pen were hand-deboned, ground, vacuum-packed in high-barrier multilayer bags (approx. 20 g per bag) and immediately stored at -20°C till analysis.

Reagents and Standards

For analyses, butylated hydroxytoluene, pyrogallol, and all-*rac*-α-tocopherol were obtained from Sigma[5]. 2-Amino-2-methyl-1-propanol and *p*-anisidine were obtained from Aldrich[6]. n-Hexane was from Merck[7].

A mixture of CLA methyl esters (main isomers were *cis*-9,*trans*-11, *trans*-9,*cis*-11, *trans*-10,*cis*-12 and *cis*-10,*cis*-12; also traces of *trans*-9,*trans*-11 and other isomers were present) and standards of single fatty acid methyl esters [tetradecanoate (14:0), hexadecanoate (16:0), octadecanoate (18:0), eicosanoate (20:0), docosanoate (24:0), *cis*-9-hexadecenoate (16:1 n-7), *cis*-9-octadecenoate (18:1 n-9), *cis*-11-eicosenoate (20:1 n-9), *cis*-9,*cis*-12-octadecadienoate (18:2 n-6), all-*cis*-6,9,12-octadecatrienoate (18:3 n-6), *cis*-11,*cis*-14-eicosadienoate (20:2 n-6), all-*cis*-8,11,14-eicosatrienoate (20:3 n-6), all-*cis*-5,8,11,14-eicosatetraenoate (20:4 n-6), all-*cis*-7,10,13,16-docosatetraenoate (22:4 n-6), all-*cis*-9,12,15-octadecatrienoate (18:3 n-3), all-*cis*-5,8,11,14,17-eicosapentaenoate (20:5 n-3), all-*cis*-7,10,13,16,19-docosapentaenoate (22:5 n-3), all-*cis*-4,7,10,13,16,19-docosahexaenoate (22:6 n-3), *trans*-9-octadecenoate (*trans*-9-18:1)] were from Sigma. Methyl *cis*-11-octadecenoate (18:1 n-7) was from Fluka[8]. A mixture of the 4 geometrical isomers of LA, used as methyl esters (LA *cis/trans* isomer mixture), and a fatty acid methyl ester (FAME) mixture from menhaden fish oil were purchased from Supelco[9].

[5] Sigma, St Louis, MO
[6] Aldrich, Milwaukee, WI
[7] Merck, Darmstadt, Germany, D-64293
[8] Fluka, Buchs, Switzerland, CH-9471
[9] Supleco, Bellefonte, PA

116 *Oil Analyses*

117 To determine the α-tocopherol content, 300 mg of oil were weighed and 5 mL of absolute
118 ethanol containing 1% pyrogallol (wt/vol), 0.012% butylated hydroxytoluene (wt/vol) and 0.4%
119 anhydrous citric acid (wt/vol) was added. Then, 10 mL of 1.6 N methanolic KOH was added and
120 saponification was carried out at 70°C for 30 min. Non-saponifiable contents were then extracted
121 with petroleum ether and filtered through a 0.45 µm teflon membrane. After solvent evaporation
122 under a nitrogen stream at 30°C, the residue was redissolved in 96% ethanol. The solution was
123 separated by chromatography, using a Hewlett-Packard[10] liquid chromatograph Series 1100
124 equipped with a Rheodyne[11] 7725i model injector with a loop volume of 20 µL, a Teknokroma[12]
125 column (15 x 0.4 cm) packed with 3 µm - 80 Å Extrasil ODS2 and a pre-column (1 x 0.4 cm)
126 packed with 5 µm - 100 Å Kromasil ODS. Sample compounds were isocratically eluted with
127 methanol and detected by means of a Hewlett Packard-1046A[10] spectrofluorometric detector
128 (excitation and emission wavelengths of 288 and 330 nm, respectively). α-Tocopherol content was
129 determined by means of an experimental calibration curve using all-*rac*-α-tocopherol as external
130 standard.

131 Peroxide value was determined by the AOCS Official Method (AOCS, 1998a), as was *p*-
132 anisidine value (AOCS, 1998b). Oil-specific absorbances at 232 and 270 nm (K_{232} and K_{270}) were
133 determined as described by Grau et al. (2001). Finally, polymer content, expressed as the
134 percentage of polymerised triacylglycerols, was determined using the IUPAC Standard Method
135 (IUPAC, 1987). All analyses were conducted in triplicate.
136

[10] Hewlett-Packard, Waldbronn, Germany, D-76337
[11] Rheodyne, Cotati, CA
[12] Teknokroma, Sant Cugat del Vallés, Spain E-08190

137 *Fat Extraction for Meat and Feed analysis*

138 1 g of dark chicken meat sample or 3 g of milled feed were weighed into 32 x 210 mm tubes. 20
139 mL of chloroform/methanol (2:1, v/v) was then added and the mixture was homogenized for 40 s at
140 19,800 rpm using a Polytron PT 2000[13]. Extracts were filtered through a Whatman n°1 filter paper
141 into 50 mL screw-capped tubes and the residues were re-extracted twice with the same solvent: first
142 with 7 mL (30 s at 19,800 rpm) and then with 5 mL (10 s at 19,800 rpm). 10 mL of water was then
143 added to these tubes, which were stoppered and shaken for 30 s before being centrifuged for 20 min
144 at 500 *g*. The chloroform phase was filtered through anhydrous sodium sulphate (using a Whatman
145 n°1 filter paper), which was then washed twice with 5 mL of chloroform. The lipid extract obtained
146 was concentrated to 1 mL in a vacuum-rotatory evaporator at 35°C. The rest of the solvent was
147 removed in a light nitrogen stream, and then by the flask's being kept in a vacuum desiccator (10
148 mm Hg, overnight).
149
150 *Gas Chromatographic Analysis of FAME*

151 FAME were prepared from sunflower oils or from extracted lipid fractions after reaction with
152 sodium methoxide followed by boron trifluoride in methanol and finally extracted with n-hexane
153 (Guardiola et al., 1994). This two-step procedure (base and acid-catalyzed methylation) allows the
154 methylation of free fatty acids while the isomerisation of CLA by acid-catalyst is limited (Kramer et
155 al., 1997; Raes et al., 2002). FAME was analysed on an Agilent[10] 4890D model gas chromatograph,
156 fitted with a flame-ionization detector and split-splitless injector port, set at 300 and 270°C,
157 respectively. The split ratio was 1:30. Chromatographic separation of FAME was performed on a
158 fused-silica capillary column (60 m x 0.25 mm i.d.) coated with 0.2 µm of a stationary phase of
159 90% biscyanopropyl- plus 10% cyanopropylphenyl-polysiloxane SP-2380 model[9]. Helium, at 30
160 psi, was used as carrier gas and the oven was programmed as follows: 12 min at 170°C, then
161 increased at 1.4°C/min to 180°C and held for 0.5 min, thereafter increased at 7.3°C/min to 216°C

[13] Kinematica, Lucerne, Switzerland, CH-6014

and held for 2 min and, finally, increased at 5°C/min to 236°C and held for 6 min; sample volume injected 0.5-1 µl. All samples were injected in duplicate.

Gas Chromatography/Mass Spectrometry Analysis of DMOX Derivatives

Derivatisation of FAME by reaction with 2-amino-2-methyl-1-propanol giving the 4,4-dimethyloxazoline (DMOX) derivatives was carried out in a similar way to Fay and Richli (1991). About 10 mg FAME, dissolved in n-hexane, were placed into a microreaction vial and evaporated under nitrogen stream at room temperature. Then, 500 µl of the above-mentioned derivatising reagent was added to the vial, which was immediately closed after being flushed with argon. The reaction was conducted by heating at 180°C for 18 h. After cooling, the reaction mixture was dissolved in 5 mL of diethyl ether/hexane (1:1) and washed first with 5 mL and then with 3 mL of water (1% NaCl). Afterwards, the organic solution was dried through anhydrous sodium sulphate and then filtered and subsequently evaporated under a nitrogen stream. Finally, the residue was dissolved again in the appropriate n-hexane volume for injection.

Mass spectra were obtained with the Thermo Finnigan[14] MD800 model coupled to a Thermo Finnigan 8,000 Top gas chromatograph. The same column as in FAME analysis was used. Helium was used as carrier gas and the chromatograhic conditions were as follows: oven temperature programmed for 12 min at 180°C, increased at 1.4°C/min to 190°C and held for 1 min, then increased at 7.3°C/min to 226°C and held for 2 min and, finally, increased at 5°C/min to 245°C and held for 8 min; injector temperature 270°C; split ratio 1:40; head pressure 30 psi; sample volume injected 2 µl. The mass spectrometer was operated in full scan mode (45-450 m/z). Mass spectrometry conditions were: interphase temperature 260°C; ion source temperature 200°C; electron energy 70 eV.

[14] Thermo Finnigan, San Jose, CA

Identification and Quantification of Fatty Acids

Fatty acids were identified by comparison of the relative retention times with FAME standards and by addition of these standards to the samples (co-chromatography). Fatty acids were also confirmed by gas chromatography/ mass spectrometry through DMOX derivatives. The area of each FAME peak was integrated by using ChemStation software, and total peak area was used to calculate the relative fatty acid composition.

Determination of Crude Fat Content

Fat content of the raw meat from all the experimental treatments was measured by the AOAC Official Method 991.36 (AOAC, 2000).

Statistical Analyses

ANOVA (n = 16) was used to study the effect of thermal treatments on sunflower oil and feed fatty acid composition. Multifactorial ANOVA (n = 48) determined whether the dietary factors studied had any significant effects on the fatty acid composition of dark chicken meat, BW, feed conversion ratio, and chicken meat crude fat content. Interactions between factors higher than an order of two were ignored. Means and least-squares means for the main factors with a significant effect were separated by Duncan's test. A discriminant analysis, in which dark chicken meat fatty acid contents were taken as the discriminant variables and the four sunflower oils as the grouping variable, was performed. In addition, we studied the discriminating power of the variables involved in the former analysis by means of Wilks' lambdas (Wilks' lambda varies from 0 to 1; the smaller the lambda for a variable, the more that variable contributes to the discriminant function). A linear regression analysis of dark chicken meat fatty acid composition *versus* their feed content was also performed. In all cases, $P \leq 0.05$ was considered significant.

RESULTS

Identification of FAME

Identification was mainly achieved by FAME standards, and confirmed in oil and meat samples by means of DMOX derivatives analysed by mass spectrometry.

Although *trans*-9-octadecenoic acid was tentatively identified by the corresponding FAME standard, this peak was found by DMOX derivative mass spectra to be a group of positional *trans*-18:1 isomers. In addition, FAME of the *cis*-9,*trans*-12- and *trans*-9,*cis*-12-LA isomers were well resolved when an LA isomer mixture was analysed. However, there was poor resolution between these isomers when oil, feed and meat were analysed, which made it necessary to take them together as *monotrans*-LA, while the *trans*-9,*trans*-12-LA (*ditrans*-LA) was well resolved. The identity of these isomers was confirmed by mass spectrometry of the DMOX derivatives.

Through the CLA standard, 3 possible CLA peaks' were also found in samples with the DMOX mass spectra characteristic of a fatty acid structure of 18 carbon atoms and two double bonds (m/z 333). The first eluted peak showed 3 intense typical diagnostic fragment ions (m/z 182, 262, 276) and characteristic losses of 12 amu (196, 208 and 222, 234), which led to identification of the compound as a CLA with the double bonds located at carbons 9 and 11. The second eluted peak showed 3 intense typical diagnostic fragment ions (m/z 210, 290, 304) and characteristic losses of 12 amu (210, 222 and 236, 248), which identified the compound as a CLA with the double bonds located at carbons 10 and 12. Hence, the analysis of the CLA standard and the previous literature (Kramer et al., 2001, 2002; Roach et al., 2002) led to identification of the first peak as *cis*-9,*trans*-11-octadecadienoic acid (9c,11t-CLA) and the second peak as *trans*-10,*cis*-12-octadecadienoic acid (10t,12c-CLA). However, the third eluted peak was identified as a mixture of different isomers. This mixture, in accordance with bibliographical data (Kramer et al., 2001, 2002; Roach et al., 2002; Juanéda et al., 2003), was identified as *ditrans*-CLA.

Moreover, traces of 13-docosenoic (probably *cis*-13) and 15-tetracosenoic (probably *cis*-15) were found to elute together with 20:4 n-6 and 22:4 n-6, respectively.

Two more peaks with no suitable standards were identified. The first was identified by means of DMOX derivative mass spectra as a hexadecenoic acid (m/z 307) with 3 intense diagnostic fragment ions (m/z 180, 208, 264) and characteristic losses of 12 amu (168, 180), which led to its identification as 7-hexadecenoic (16:1 n-9), probably the *cis* isomer according to the literature (Adlof and Emken, 1986). The second peak was identified as all-*cis*-4,7,10,13,16-docosapentaenoic acid (22:5 n-6) on the basis of bibliographical data (Guardiola et al., 1994; Simopoulos and Salem, 1992), because there was no suitable standard and DMOX mass spectra did not show typical ion fragments for any penta or hexaenoic fatty acids. This may be due to the low amounts present for these fatty acids and the harsh conditions for DMOX derivatisation.

Oil Fatty Acid Composition

Thermal treatments led to significant differences in sunflower oil fatty acid composition. The formation of low molecular weight compounds and other fatty acid oxidation compounds (Table 1) will explain both total n-6 PUFA and total n-3 PUFA losses in heated oils.

As PUFA decreased on heating, this explains the total SFA relative increase recorded in all heated oils (Table 3). A similar trend was observed for MUFA, although the differences between treatments were not as marked as for SFA. Despite this, total MUFA and oleic acid in the VOSO were significantly higher than in the other oils.

Only oils heated at temperatures above 180-190°C (OSO and VOSO) had a higher content of *trans*-18:1 than FSO or PSO (Table 3). Likewise, geometrical isomers of LA (*mono*- and *ditrans*-LA) were higher in oils heated at high temperatures.

As for positional isomers, 9c,11t-CLA and *ditrans*-CLA content were higher as a consequence of heating at high temperatures whereas the content in 10t,12c-CLA was not affected. Moreover,

9c,11t-CLA, *ditrans*-CLA and the sum of positional isomers showed significant differences between OSO and VOSO treatments.

Feed Fatty Acid Composition

Lipid fractions from feeds, containing 6% of each sunflower oil, had more linolenic acid and total SFA and moderately lower total MUFA and total n-6 PUFA than their corresponding sunflower oils (Table 3). However, as feed fatty acid composition greatly reflects sunflower oil composition, VOSO feed had less LA and increased total SFA and total MUFA than FSO feed. However, no significant differences between feeds were observed for linolenic acid because this fatty acid came mainly from feed ingredients other than sunflower oil. This finding confirms that the differences for fatty acids between feeds were less clear than for oils, due to dilution of sunflower oils in the basal feed lipid fraction.

For the content of TFA in feeds containing OSO and VOSO, compared with TFA content in oils, a dilution effect, which indicates that *trans* isomers were slightly present in the basal diet, was also found.

This meant that the contents of 9c,11t- and 10t,12c-CLA did not differ between feeds containing OSO and VOSO (Table 3). However, for other *trans* isomers, there were significant differences between feeds containing OSO and VOSO. Thus, these results led to significant differences between OSO and VOSO feeds for total LA isomers, total CLA isomers, total octadecadienoic (18:2) acid isomers and total isomers.

Bird Performance and Meat Crude Fat Content

Final BW, feed intake, feed conversion, and mortality were not affected by dietary treatments after 41 d of breeding (Table 4).

In addition, dietary sunflower source, and Zn and α-TA supplements did not affect dark chicken meat crude fat content (Table 4).

Meat Fatty Acid Composition

Total SFA, MUFA and PUFA were not affected by dietary oil source in dark chicken meat (Table 5). However, a significant increase in 20:0 was observed in dark meat coming from chickens fed OSO and VOSO diets, which seems to indicate that this fatty acid was largely influenced by the diet. Although, the slight differences between the fatty acid compositions of assayed feeds did not lead to other significant differences in meat fatty acid composition, apart from the differences observed for isomerised fatty acids (Table 5).

In relation to the *trans*-18:1 isomers, the higher relative content of these fatty acids in dark chicken meat (Table 5) than in feeds (Table 3) seems to indicate that they are incorporated into dark chicken meat more easily than other fatty acids.

Monotrans-LA and *ditrans*-LA of dark chicken meat were also affected by the sunflower oil source added to the feed because they reflected, to some extent, the differences observed in the diets.

In relation to positional isomers, as dietary treatments showed no differences between 9c,11t-CLA and 10t,12c-CLA contents, they did not affect the content of these fatty acids in dark chicken meat. However, 9c,11t-CLA was much higher incorporated in dark chicken meat than 10t,12c-CLA (Table 5).

On the other hand, *ditrans*-CLA showed significant differences between dietary sunflower oil sources, indicating the effect of feed fatty acid composition. However, this group of isomers were incorporated into dark meat less than 9c,11t-CLA and more than 10t,12c-CLA.

α-Tocopheryl acetate supplementation in diet brings about an increase in 20:3 n-6, 22:4 n-6 and 22:5 n-3 and a decrease in 20:1 n-9 (Table 5).

Zn supplementation increased the *trans*-18:1 content (Table 5). In addition, an interaction between Zn supplementation and sunflower oil source influenced the *trans*-18:1 content.

Canonical discriminant analysis revealed that 3 discriminant functions (Wilks' lambda = 0.037; $P < 0.001$) including various fatty acids (*ditrans*-CLA, 20:2 n-6, 22:5 n-3 and 9c,11t-CLA) distinguish correctly both OSO and VOSO from the other oils in dark chicken meat fed different sunflower oils (Figure 1). As can be seen, chickens fed FSO and PSO treatments were not distinguished clearly. Isomers had the main responsibility for discriminating, because the cumulative proportion of explained variance in function 1 was 98.7%. In fact, discriminant analysis predicted correctly 58.3% of chickens fed FSO and 83.3% of chickens fed PSO, whereas 100% correct allocation to their groups was achieved for chickens fed with oxidised oils (OSO and VOSO).

Moreover, regarding individual Wilks' lambda-obtained values (lambda varies from 0 to 1, with 0 meaning that group averages differ), it can be confirmed that *ditrans*-CLA content was the main factor responsible for predicting group memberships (Table 6). In this table, each fatty acid or sum of fatty acids was ranked by its Wilks' lambda values. As can be seen, sums of fatty acid isomers also had low lambda values, though they were not as low as for the *ditrans*-CLA lambda value. Moreover, the discriminant analysis run again with only *ditrans*-CLA distinguished fairly well dark chicken meats to whose feeds heated (above 190°C) sunflower oils (OSO and VOSO) were added (Table 6). Addition of the total 18:2 isomer content to the discriminant analysis did not improve its ability to distinguish groups. It was only improved after the addition of the 9c,11t-CLA isomer. Finally, the addition of the 20:2 n-6 to the discriminant analysis led to the previously reported values.

Since fatty acid isomers were able to predict groups, we also ran the regression analysis of these isomers in chicken meat and its feed content (Table 7). The best linear regression analyses were for *ditrans*-CLA dark chicken meat content *versus ditrans*-CLA feed content ($R^2 = 0.876$) and *versus* total CLA feed content ($R^2 = 0.878$). However, regression analysis for chicken meat content of 9c,11t-CLA *versus* its feed content was not significant and showed a very low R^2.

DISCUSSION

Oil Fatty Acid Composition

As a result of the thermal treatments, PUFA losses occurred and caused a relative increase of SFA and MUFA. This has been previously reported in heated linseed oil (Wolff, 1993) and sunflower oil (Juanéda et al., 2003).

TFA are formed in edible oils during refining processes (Cmolík and Pokorný, 2000; Cmolik, et al., 2000; Schwarz, 2000a) and also during partial hydrogenation (Ackman and Mag, 1998; Jung et al., 2002; Ledoux et al., 2000). These isomers are mainly *monotrans* geometrical and positional isomers (Ledoux et al., 2000; Precht and Molkentin, 2000; Wolff et al., 1998)

However, thermal treatments above 180°C also led to TFA formation (Martin et al., 1998; Wolff, 1993, Juanéda et al., 2003). This explains the low content in isomerised fatty acids found in FSO and why their content did not increase in PSO whereas it did in oxidised oils (OSO and VOSO).

Interesting results were the significant increase in *trans*-18:1 observed in OSO and VOSO, because monoenoic fatty acids are very resistant to *trans* isomerisation (Schwarz, 2000a). However, Sébédio et al. (1988) also reported the presence of *cis* and *trans* monoenes after heating sunflower oil above 200°C. Similar results have been reported for rapeseed and soybean oil (Grandgirard et al., 1984).

On geometrical isomerisation, Juanéda et al. (2003) did not find an increase in *monotrans*-LA in sunflower oil heated at 180°C, whereas it did increase a lot at 220°C. Similar results were described by Sébédio et al. (1996b) in deep-fat frying of French fries in peanut or soybean oil heated at 220°C. Therefore, confirming these authors' findings, the present results show that LA isomerisation occurs when sunflower oil is heated at temperatures above 190°C for 28 h.

It is also worth commenting on the increase in *ditrans*-LA isomers in oxidised sunflower oils (Table 3), because *ditrans* isomers were only found in linolenic acid heated above 200°C (Grandgirard et al., 1984). Further, even after 30 frying operations at 220°C, Sébédio et al. (1996b)

found no *ditrans*-LA in oils used in deep fat frying of potatoes. Wolff (1993), heating linseed oil at different times and temperatures, only found *ditrans*-LA after 8 h at 245°C, and Martin et al. (1998) found it above 220°C after 30 h of heating. However, Sébédio et al. (1988) found *ditrans*-LA after heating sunflower oil at 200°C for 48 h in a commercial fryer and also in used frying oil.

These controversial results for *ditrans*-LA amounts could be explained by a number of factors. For example, the manner and time of heating (Grandgirard et al., 1984; Wolff, 1993), the composition and their arrangement on the triacylglycerols (Martin et al., 1998) all affect *trans* formation.

On CLA content, different authors described CLA formation after heating sunflower oil above 180°C (Sébédio et al., 1988; Juanéda et al., 2003). This was confirmed by our results for the OSO and VOSO oils.

Unlike LA geometrical isomers, positional isomers of both oxidised oils (OSO and VOSO) had greater *ditrans*-CLA than *monotrans*-CLA. The ratio for *ditrans*-CLA/(9c,11t- and 10t,12c-CLA) in VOSO treatment was close to 2 which is similar to that reported by Juanéda et al. (2003) when sunflower oil was heated at 180°C [sum of *ditrans*-CLA/(9c,11t- and 10t,12c-CLA) = 1.9]. These former authors also reported that the relative proportions of positional isomers are temperature-dependent, with the relative *ditrans* content higher at high temperatures.

In relation to this, Juanéda et al. (2003) reported that at temperatures of 180°C the main *monotrans* positional isomers found were the 9c,11t-CLA and the 10t,12c-CLA, and the main *ditrans* isomers were the 9t,11t- and 10t,12t-CLA. In addition, these authors did not detect the presence of 8c,10t- or 11t,13c-CLA isomers in heated oils containing LA. These results confirm our results, though we only identified the 9c,11t-CLA and the 10t,12c-CLA and a peak where some *ditrans*-CLA were present, whereas other isomers were not detected.

Therefore, the reported results seemed to confirm the presence of different geometrical and positional TFA due to isomerisation at temperatures above 190°C. However, while some TFA such as *ditrans*-CLA are mainly formed during heating at high temperatures (Sébédio et al., 1988; Juanéda et al., 2003), others like 9c,11t-CLA are mainly found in ruminant animal fats (Chin et al., 1992).

Meat Fatty Acid Composition

Different authors reported decreased PUFA content in chicken meats from animals fed different vegetable oils oxidized at low temperatures (30°C) or high temperatures (140°C) (Jensen et al., 1997; Sheehy et al., 1993) whereas others reported no changes in chicken meat fatty acid composition from those animals fed on oxidized sunflower oil heated for 11h at 120°C (Sheehy et al., 1994). These controversial results can be mainly explained by the extent of PUFA losses in these oxidized oils added to the feeds.

In relation to the amounts of geometrical isomers of LA and *trans*-18:1 in dark chicken meat from animals fed FSO, they were much lower than those previously described in chicken meat (Aro et al., 1998) and turkey meat (Wong and Sampugna, 1993). Aro et al. (1998), who studied various meat products from several European countries, found that chicken and turkey meats had slightly more variable proportions of TFA than other meats. In this study, total *trans* content (CLA were not included) ranged from 0.24 to 1.7%. This great variability was attributed to feed composition differences. Actually, the incorporation of TFA into most tissues is roughly in proportion to their abundance in the diet of swine (Royce et al., 1983, Pettersen and Opstevedt, 1992) and rats (Bysted et al., 1998).

Consistent with this, Emken (1995) reported in a review that TFA are subject to the same metabolic control mechanisms that regulate the metabolism of saturated and *cis* unsaturated fatty acids. However, whereas adipose tissue seemed not to incorporate or exclude selectively any specific fatty acid, other more specific tissues like brain have a low and relatively constant TFA content (Holmer, 1998; Emken, 1995).

In addition, some *trans*-18:1 isomers are hindered from incorporation into various tissue lipid classes, whereas other *trans*-18:1 are preferred (Holmer, 1998; Bysted et al., 1998). The higher

relative content of these fatty acids in dark chicken meat (Table 5) than in feeds (Table 3) seems to indicate that *trans*-18:1 are incorporated into dark chicken meat more easily than other fatty acids such as oleic acid (Table 5).

In relation to *monotrans*-LA and *ditrans*-LA content in dark chicken meat, the LA/*ditrans*-LA isomer ratios in chicken meats were fairly different from those in the feeds, whereas the LA/*monotrans*-LA isomer ratios were similar to those in the diets, indicating that *monotrans*-LA isomers behave similarly to LA, which confirms previously reported results (Sébedio and Chardigny, 1998). In addition, the LA/*ditrans*-LA ratios in feeds and meats suggest that the incorporation rate in chicken meat is higher for *ditrans*-LA than for LA.

As for meat positional isomers, Chin et al. (1992) also detected CLA in commercial chicken meat samples (0.09% CLA), in which 9c,11t-CLA was about 84% of total CLA. The reported content was higher than the levels we found in meat from animals fed on FSO (Table 3).

CLA, present in all dietary treatments (Table 3), were absorbed and deposited, in line with previously reported results, in chicken meat (Szymczyk et al., 2001; Simon et al., 2000; Badinga et al., 2003) and eggs (Raes et al., 2001; Ahn et al., 1999; Du et al., 1999). However, as dietary treatments showed no differences between 9c,11t-CLA and 10t,12c-CLA contents, they did not affect the content of these fatty acids in dark chicken meat.

Nevertheless, the ratios for these CLA isomers in feeds and meats seem to indicate that they were incorporated into dark meat at different rates. Thus, 9c,11t-CLA seemed to be more easily incorporated in dark chicken meat than 10t,12c-CLA (Table 5). These results corroborate previous findings (Szymczyk et al., 2001; Simon et al., 2000; Badinga et al., 2003). However, it is not completely understood whether these results are due to an increased incorporation of 9c,11t-CLA or to a higher enzymatic modification (elongation, desaturation or β-oxidation) of 10t,12c-CLA (Evans et al., 2002; Sébédio et al., 1997; Sergiel et al., 2001). In addition, the likely biosynthesis of 9c,11t-CLA *via* the action of Δ^9 desaturase on *trans*-11-18:1, described in humans (Adlof et al., 2000; Turpeinen et al., 2002) and in other animals (Pariza et al., 2001; Palmquist and Santora, 1999; Gläser et al., 2000; Thompson and Christie, 1991), should be taken into account.

On the other hand, *ditrans*-CLA showed significant differences between dietary sunflower oil sources, indicating the effect of feed fatty acid composition. Likewise, Yang et al. (2002) reported that *ditrans*-CLA were preferentially incorporated into rat liver rather than other CLA and that there was also discrimination between *monotrans* isomers. Nevertheless, these authors also reported that rat milk CLA isomeric distribution reflected the distribution in the diet. In addition, these results for rat liver isomer composition seemed to confirm those reported for hepatic fatty acid composition in chickens fed a CLA mixture (Badinga et al., 2003).

In relation to the studied dietary factors, the α-tocopheryl acetate supplementation in diet brings about an increase in PUFA which agreed with previously reported works (Cherian et al., 1996; Ajuyah et al., 1993; Surai and Sparks, 2000). Cherian et al. (1996) suggested that these PUFA were protected from oxidation by tocopherol supplementation.

On the other hand, the increased *trans*-18:1 content as a result of Zn supplementation is difficult to explain, even more so when the interaction between Zn supplementation and sunflower oil source in the diet is taken into account. It is not completely understood why Zn supplementation decreases *trans*-18:1 content in dark meat coming from chickens fed PSO diet, while the *trans*-18:1 content is increased by Zn supplementation in meat from chickens fed FSO, OSO or VOSO diets. Thus, further studies are required to confirm the effect of Zn supplementation and its interaction on *trans*-18:1 content in dark chicken meat.

Therefore, some TFA isomers seemed to be more easily incorporated than others and, in spite of the reported effects and interactions caused by Zn and tocopherol supplements, the discriminant analysis showed that the content of *ditrans*-CLA and total CLA isomers are suitable markers, even at trace levels, of the addition of heated (above 190°C) sunflower oil to feeds.

However, isomers such as *trans*-18:1 and 10t,12c-CLA were not suitable for predicting group memberships of chicken meats to which different dietary sunflower oils were added (Table 6).

Some TFA and the significantly increased content of 20:0 as a result of heating also did not increase the correctly predicted group membership in the discriminant analysis (Table 6). This fact can be explained by the strong correlations observed between these fatty acids and *ditrans*-CLA (data not shown).

Therefore, the relevance of *ditrans*-CLA as possible markers is due to their being mainly formed during fat and oil heating at high temperatures, while other potential markers, like 9c,11t-CLA, have other important origins like biohydrogenation, partial hydrogenation and refining. Nevertheless, 9c,11t- and 10t,12c-CLA were not completely necessary to distinguish dark meats from chickens fed these heated oils.

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TABLE 1. Oxidation Variables and α-Tocopherol Content of the Sunflower Oils Added to the Basal Diet.

Sunflower Oil	PV[a]	pAV[1]	K^2_{232}	K^3_{270}	Polymers[4] [%]	α-tocopherol[5] [mg/l]
Fresh	2.1	6.4	3.58	1.74	0.7	683
Peroxidised	91.9	8.5	13.66	1.78	0.7	480
Oxidised	7.0	83.1	10.28	3.25	4.9	655
Very oxidised	17.2	153.0	17.67	4.82	9.4	619

[1] PV: Peroxide value in millieq. peroxide/kg. AOCS Official Method (Cd 8-53).

[2] pAV: *p*-Anisidine value. AOCS Official Method (Cd 8-90).

[3] K_{232} and K_{270} are the oil-specific absorbances at 232 and 270 nm (Grau *et al.*, 2001).

[4] Polymers expressed as percentage of polymerised triacylglycerols. IUPAC standard method 2.508.

[5] Tocopherol content analysis performed as described in Material and Methods section.

792 **TABLE 2. Ingredients and Composition of the Basal Diet Fed from 11 to 42 Days of Age.**

Ingredients	Percentage	Composition[1]	Percentage
Wheat	56.61	Dry matter	90.1
Soybean meal	24.69	Crude protein	20.9
Sunflower oil[2]	6.00	Crude fat	8.2
Sunflower meal	5.03	Crude fiber	3.6
Soy grass	3.14	Ash	6.1
Calcium carbonate	1.69		
Monocalcium phosphate	1.23		
Mineral-vitamin premix[3]	0.50		
L-Lysine	0.38		
DL-Methionine	0.26		
Salt	0.20		
Sodium bicarbonate	0.14		
Enzymes	0.12		
Choline chloride	0.03		

793 [1] Metabolizable energy 3,000 cal/g. Results shown are estimated values.

794 [2] Four sources of sunflower oil were added to the feeds depending on the dietary treatments.

795 [3] Include all-*rac*-α-tocopheryl acetate (30 mg/kg of feed) and Se-enriched yeast (0.6 mg Se/kg of feed).

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797 **TABLE 3. Fatty Acid Composition (Expressed as Area Normalization in %) of the**

798 **Experimental Oils and Feeds.**

Fatty acid	Sunflower oils				Feeds with added sunflower oils			
	FSO	PSO	OSO	VOSO	FSO	PSO	OSO	VOSO
14:0	0.068a	0.070b	0.070b	0.073c	0.114	0.118	0.111	0.114
16:0	6.148a	6.256b	6.296b	6.503c	9.114a	9.309bc	9.204ab	9.379c
18:0	3.982a	4.075b	4.087c	4.206d	3.920a	3.961b	3.952ab	4.019c
20:0	0.280a	0.286b	0.289c	0.296d	0.287a	0.291ab	0.292b	0.297c
22:0	0.714a	0.729b	0.737c	0.764d	0.564a	0.567a	0.576b	0.585c
24:0	0.229a	0.230ab	0.232b	0.238c	0.197b	0.186a	0.200b	0.200b
Total SFA	11.422a	11.646b	11.710c	12.080d	14.196a	14.431b	14.334b	14.593c
16:1 n-9	0.020	0.022	0.020	0.019	0.036a	0.039b	0.039b	0.040b
16:1 n-7	0.087a	0.089ab	0.089ab	0.095b	0.154	0.158	0.149	0.153
18:1 n-9	26.673a	27.152b	27.174b	27.719c	24.276a	24.470bc	24.395ab	24.596c
18:1 n-7	0.549	0.529	0.544	0.558	0.748	0.758	0.786	0.785
20:1 n-9	0.156a	0.158bc	0.157ab	0.159c	0.237	0.245	0.239	0.241
Total MUFA	27.486a	27.950b	27.985b	28.551c	25.452a	25.670b	25.607b	25.815c
18:2 n-6	60.379a	59.710b	59.463c	58.400d	57.254a	56.777b	56.838b	56.244c
18:3 n-6								
20:2 n-6								
20:3 n-6								
20:4 n-6					0.028	0.030	0.028	0.031
22:4 n-6								
22:5 n-6								
Total n-6 PUFA	60.379a	59.710b	59.463c	58.400d	57.282a	56.807b	56.866b	56.275c
18:3 n-3	0.171a	0.164b	0.160c	0.148d	2.660	2.693	2.679	2.740
20:5 n-3								
22:5 n-3								
22:6 n-3					0.022	0.024	0.019	0.020
Total n-3 PUFA	0.171a	0.164b	0.160c	0.148d	2.683	2.716	2.698	2.760
Total PUFA	60.550a	59.874b	59.623c	58.548d	60.025a	59.523b	59.564b	59.035c
Trans-18:1	0.011a	0.011a	0.042b	0.066c	0.034a	0.033a	0.047b	0.057c
Ditrans-LA	0.021a	0.021a	0.048b	0.071c	0.005a	0.000a	0.038b	0.051c
Monotrans-LA	0.404ab	0.382a	0.428b	0.471c	0.247b	0.238a	0.276c	0.285d
Total LA isomers	0.425a	0.404a	0.476b	0.543c	0.252b	0.238a	0.313c	0.337d
9c,11t-CLA	0.025a	0.032a	0.036b	0.041c	0.027	0.032	0.034	0.039
10t,12c-CLA	0.019	0.022	0.026	0.028	0.018	0.018	0.022	0.024
Ditrans-CLA	0.062a	0.061a	0.102b	0.144c	0.057a	0.058a	0.078b	0.100c
Total CLA	0.106a	0.115a	0.164b	0.213c	0.102a	0.108a	0.134b	0.164c
Total 18:2 isomers	0.532a	0.519a	0.640b	0.756c	0.354b	0.346a	0.447c	0.500d
Total isomers	0.543a	0.530a	0.682b	0.822c	0.388b	0.379a	0.494c	0.558d

799 Values given in this Table for sunflower oils and feeds are means (n = 16). Values in the same row with different letters
800 differ significantly ($P \leq 0.05$). FSO = fresh sunflower oil, PSO = peroxidised sunflower oil, OSO = oxidised sunflower
801 oil, VOSO = very oxidised sunflower oil. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA =
802 polyunsaturated fatty acids; LA = linoleic acid; CLA = conjugated linoleic acid.

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TABLE 4. Effect of Dietary Factors on BW, Feed Conversion Ratio and Crude Fat Content of Dark Meat of Chickens Fed 41 d Different Dietary Treatments.

Studied factor	BW (kg)	Feed Conversion Ratio	Fat (g)
Sunflower oil			
FSO	1.91	1.96	14.7
PSO	1.89	1.97	15.2
OSO	1.92	1.97	15.7
VOSO	1.89	2.02	15.4
SEM	0.025	0.022	0.47
α-tocopheryl acetate supplementation (mg/kg)			
0	1.92	1.98	15.5
100	1.89	1.98	15.0
SEM	0.018	0.015	0.33
Zn supplementation (mg/kg)			
0	1.91	2.00	15.5
600	1.90	1.96	15.0
SEM	0.018	0.015	0.33

FSO = chicken fed fresh sunflower oil diet, PSO = chicken fed peroxidised sunflower oil diet, OSO = chicken fed oxidised sunflower oil diet, VOSO = chicken fed very oxidised sunflower oil diet.

TABLE 5. Effect of Dietary Factors on the Fatty Acid Composition (expressed as Area Normalization in %) of Dark Chicken Meat.

Fatty acid	Dark chicken meat							
	Sunflower oil added				Tocopherol added [mg/kg]		Zn added [mg/kg]	
	FSO	PSO	OSO	VOSO	0	100	0	600
14:0	0.370	0.373	0.369	0.364	0.368	0.370	0.370	0.368
16:0	16.135	16.259	16.446	16.273	16.282	16.274	16.406	16.150
18:0	5.729	5.625	5.710	5.598	5.650	5.681	5.644	5.687
20:0	0.206ab	0.201a	0.224b	0.226b	0.214	0.215	0.211	0.218
22:0								
24:0								
Total SFA	22.441	22.457	22.749	22.460	22.407	22.433	22.528	22.312
16:1 n-9	0.413	0.429	0.400	0.402	0.409	0.413	0.415	0.407
16:1 n-7	2.371	2.527	2.586	2.713	2.563	2.535	2.597	2.502
18:1 n-9	29.930	30.477	30.581	30.794	30.605	30.286	30.648	30.244
18:1 n-7	1.110	1.124	1.088	1.124	1.106	1.118	1.087	1.136
20:1 n-9	0.259	0.263	0.259	0.262	0.264a	0.258b	0.262	0.260
Total MUFA	34.084	34.820	34.914	35.295	34.947	34.610	35.008	34.548
18:2 n-6	39.294	38.613	38.292	38.011	38.458	38.647	38.243	38.862
18:3 n-6	0.109	0.109	0.109	0.106	0.108	0.108	0.108	0.108
20:2 n-6	0.315	0.309	0.295	0.303	0.304	0.307	0.304	0.307
20:3 n-6	0.238	0.247	0.242	0.256	0.240a	0.252b	0.245	0.247
20:4 n-6	0.889	0.882	0.820	0.891	0.845	0.895	0.861	0.880
22:4 n-6	0.257	0.249	0.227	0.244	0.235a	0.254b	0.241	0.247
22:5 n-6	0.063	0.063	0.058	0.065	0.060	0.065	0.061	0.063
Total n-6 PUFA	41.165	40.469	40.043	39.878	40.356	40.641	40.173	40.824
18:3 n-3	1.784	1.753	1.768	1.787	1.768	1.778	1.758	1.788
20:5 n-3	0.035	0.031	0.035	0.037	0.033	0.036	0.035	0.034
22:5 n-3	0.096	0.093	0.084	0.093	0.087a	0.096b	0.091	0.092
22:6 n-3	0.067	0.062	0.051	0.060	0.057	0.063	0.062	0.058
Total n-3 PUFA	1.983	1.937	1.938	1.976	1.945	1.973	1.946	1.972
Total PUFA	43.148	42.407	41.981	41.854	42.301	42.614	42.119	42.795
Trans-18:1	0.116	0.114	0.122	0.127	0.118	0.122	0.110a	0.129b
Ditrans-LA	0.046a	0.045a	0.051a	0.066b	0.052	0.052	0.053	0.051
Monotrans-LA	0.187a	0.182a	0.201b	0.207b	0.193	0.196	0.196	0.193
Total LA isomers	0.233ab	0.227a	0.253b	0.272c	0.245	0.243	0.244	0.244
9c,11t-CLA	0.050	0.045	0.048	0.051	0.049	0.048	0.048	0.049
10t,12c-CLA	0.010	0.007	0.006	0.008	0.007	0.009	0.009	0.006
Ditrans-CLA	0.034a	0.036a	0.047b	0.060c	0.045	0.037	0.044	0.044
Total CLA	0.094a	0.086a	0.101a	0.119b	0.100	0.101	0.102	0.100
Total 18:2 isomers	0.327a	0.316a	0.354b	0.391c	0.345	0.349	0.350	0.344
Total isomers	0.444a	0.430a	0.475b	0.519c	0.463	0.470	0.461	0.473

Values given are least-squares means obtained from MANOVA (n = 48). Values in the same row with different letters differ significantly ($P \leq 0.05$). FSO = fresh sunflower oil, PSO = peroxidised sunflower oil, OSO = oxidised sunflower oil, VOSO = very oxidised sunflower oil. SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids; LA = linoleic acid; CLA = conjugated linoleic acid.

815 **TABLE 6. Wilks' Lambda and Discriminant Analysis Results for Dark Chicken Meat Fatty**

816 **Acids.**

			Correctly predicted group memberships [cumulative %]			
	Wilks' lambda	*P*	FSO	PSO	OSO	VOSO
Ditrans-CLA	0.109	<0.001	58.3	50.0	83.3	100.0
Total 18:2 isomers	0.447	<0.001	58.3	50.0	83.3	100.0
Ditrans-LA	0.467	<0.001	58.3	50.0	83.3	100.0
Total LA isomers	0.491	<0.001	58.3	50.0	83.3	100.0
Total isomers	0.515	<0.001	58.3	50.0	83.3	100.0
Total CLA	0.611	<0.001	58.3	50.0	83.3	100.0
Monotrans LA	0.645	<0.001	58.3	50.0	83.3	100.0
20:0	0.834	0.044	58.3	50.0	83.3	100.0
16:1 n-7	0.836	0.046	58.3	50.0	83.3	100.0
22:6 n-3	0.846	0.059	58.3	50.0	83.3	100.0
22:5 n-3	0.868	0.098	58.3	50.0	83.3	100.0
20:5 n-3	0.880	0.127	58.3	50.0	83.3	100.0
22:4 n-6	0.881	0.131	58.3	50.0	83.3	100.0
16:1 n-9	0.886	0.144	58.3	50.0	83.3	100.0
18:1 n-9	0.887	0.150	58.3	50.0	83.3	100.0
9c,11t-CLA	0.891	0.162	58.3	83.3	91.7	100.0
18:2 n-6	0.893	0.170	58.3	83.3	91.7	100.0
20:3 n-6	0.905	0.219	58.3	83.3	91.7	100.0
20:2 n-6	0.912	0.252	58.3	83.3	100.0	100.0
20:4 n-6	0.925	0.325	58.3	83.3	100.0	100.0
22:5 n-6	0.940	0.432	58.3	83.3	100.0	100.0
18:3 n-3	0.948	0.498	58.3	83.3	100.0	100.0
Trans-18:1	0.949	0.507	58.3	83.3	100.0	100.0
18:0	0.959	0.599	58.3	83.3	100.0	100.0
14:0	0.966	0.670	58.3	83.3	100.0	100.0
16:0	0.970	0.718	58.3	83.3	100.0	100.0
18:3 n-6	0.970	0.721	58.3	83.3	100.0	100.0
10t,12c-CLA	0.976	0.784	58.3	83.3	100.0	100.0
18:1 n-7	0.979	0.815	58.3	83.3	100.0	100.0
20:1 n-9	0.986	0.890	58.3	83.3	100.0	100.0

817 FSO = chicken fed fresh sunflower oil diet, PSO = chicken fed peroxidised sunflower oil diet, OSO = chicken fed

818 oxidised sunflower oil diet, VOSO = chicken fed very oxidised sunflower oil diet, CLA = conjugated linoleic acid, LA=

819 linoleic acid.

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820 **TABLE 7. Regression Equations of Selected Dark Chicken Meat Fatty Acid Contents *Versus***

821 **Selected Feed Fatty Acid Contents.**

Chicken meat fatty acid content (y)	Feed fatty acid content (x)	Equation	R^2
ditrans-CLA	*ditrans*-CLA	y = 0.003 + 0.567x	0.876
ditrans-CLA	total CLA	y = -0.008 + 0.411x	0.878
total 18:2 isomers	total 18:2 isomers	y = 0.094 + 0.652x	0.518
ditrans-LA	*ditrans*-CLA	y = 0.020 + 0.442x	0.480
total isomers	total isomers	y = 0.233 + 0.287x	0.446
ditrans-LA	*ditrans*-LA	y = 0.044 + 0.348x	0.438
9c,12t-CLA	9c,12t-CLA	y = 0.044 + 0.152x	0.008

822 CLA = conjugated linoleic acid, LA= linoleic acid.

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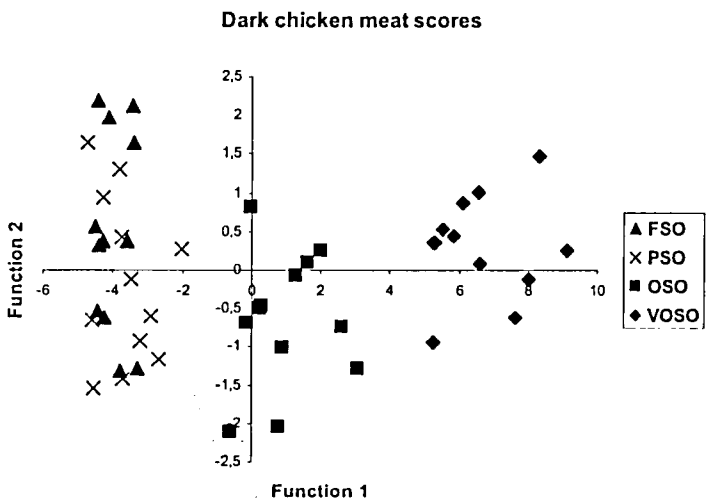

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824 **Figure 1.** Bou et al.

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826 **FIGURE CAPTIONS**

827

828 **FIGURE 1.** Plot scores of the first two canonical functions in dark chicken meat samples.
829 Canonical discriminant functions are: function 1 = -16.17 + 83.78x(cis-9,trans-12-octadecadienoic
830 acid) + 403.28x(ditrans-conjugated linoleic acid) – 34x(20:2 n-6) + 56.3x(22:5 n-3); function 2 = -
831 14.36 + 70.75x(cis-9,trans-12-octadecadienoic acid) + 4.77x(ditrans-conjugated linoleic acid) –
832 16.95x(20:2 n-6) + 60.62x(22:5 n-3); function 3 = -1.30 + 120.73x(cis-9,trans-12-octadecadienoic
833 acid) – 22.95x(ditrans-conjugated linoleic acid) – 2.06x(20:2 n-6) – 31.98x(22:5 n-3). These
834 functions distinguish dark chicken meat fed very oxidised sunflower oil (VOSO) and oxidised
835 sunflower oil (OSO) from other sunflower oils (FSO = fresh and PSO = peroxidised).