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# Oxidative changes of fresh loin from pig, caused by dietary conjugated linoleic acid and monounsaturated fatty acids, during refrigerated storage

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ABSTRACT

Three levels (0%, 1% and 2%) of a conjugated linoleic acid enriched oil (CLA) were combined with two levels of monounsaturated fatty acids (MUFA) (19% and 39% average) for pig feeding. Changes in instrumental colour, lipid oxidation (thiobarbituric acid reactive substances, TBARS) and volatile profile of fresh loin chops, as affected by dietary CLA, MUFA and CLA × MUFA, were studied throughout 7 days of refrigerated storage. Lightness ($L^*$) evolution was conditioned by dietary CLA, whereas changes in redness ($a^*$) and yellowness ($b^*$) were unaffected by dietary supplements. Dietary CLA at 2% led to higher TBARS values of loin chops at day 7 of refrigerated storage ($p < 0.05$), while MUFA supplementation and CLA × MUFA interaction did not affect lipid oxidation. Dietary CLA, MUFA or CLA × MUFA did not affect most volatile compounds of loin chops after 7 days of storage.



## 1. Introduction

Conjugated linoleic acid (CLA) is a collective term to describe positional and geometric isomers of linoleic acid (*cis*-9, *cis*-12 octadecadienoic acid) with conjugated double bonds. The *cis*-9, *trans*-11 and *trans*-10, *cis*-12 CLA are the major CLA isomers in nature and both have been related to positive health effects (Bhattacharya, Banu, Rahman, Causey, & Fernandes, 2006). Supplementation of swine feeding with CLA has also gained increasing attention during the last decade, as an approach for improving productive, carcass and meat quality traits and, at the same time, for obtaining meat and meat products enriched in CLA (Martin, Muriel, Antequera, Perez-Palacios, & Ruiz, 2008a; Schmid, Collomb, Sieber, & Bee, 2006).

Evidences about the involvement of CLA isomers in oxidative processes has been found. Ha, Storkson, and Pariza (1990) showed that CLA was a more effective antioxidant than α-tocopherol and almost as effective as butylated hydroxytoluene in model systems. Ip, Chin, Scimeca, and Pariza (1991) found that dietary CLA decreased the concentrations of thiobarbituric acid reactive substances (TBARS) in rat liver and mammary gland tissues. Joo, Lee, Ha, and Park (2002) also reported lower values of TBARS in loins from CLA-fed pigs. However, the antioxidant effect of CLA is unclear and controversial, since other studies have even shown a pro-oxidant effect of CLA or no implication of these fatty acids in oxidative processes (Hur, Park, & Joo, 2006). In a previous work, we found no significant effect of dietary CLA on the susceptibility to oxidation of fresh loin of pig, but liver showed a higher oxidative stability with dietary CLA (Martin et al., 2008a). Therefore, the claimed antioxidant effect of CLA needs to be more carefully examined.

The modification in the fatty acid profile caused by dietary CLA has been suggested as one of the reasons that might explain the lower levels of oxidation found by several authors in meat and meat products from CLA-fed animals. Feeding with CLA causes an increase in the ratio of saturated fatty acids (SFA) to unsaturated fatty acids (Dugan, Aalhus, & Kramer, 2004; Martin, Antequera, Gonzalez, Lopez-Bote, & Ruiz, 2007). Thus, meat from CLA-fed animals might be less susceptible to lipid oxidation as well as to the production of colour changes and volatile compounds. However, such an increase in the ratio of SFA to unsaturated fatty acids could have negative health implications from the consumer standpoint (Department of Health, 1994). Thus, including high levels of MUFA in pig diets when using dietary CLA could be a strategy for counteracting the decrease in MUFA caused by CLA.

In the present work, the effects of dietary CLA and MUFA and their interaction on lipid oxidation, changes in instrumental colour and the volatile profile of fresh loin chops during refrigerated storage was studied.

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## 2. Material and methods

### 2.1. Animals and feeding

Three levels (0%, 1% and 2%) of a commercial CLA-enriched oil supplement (CLA-60, BASF, Dortmund, Germany), containing approximately 56% of CLA isomers (28% *cis*-9, *trans*-11 and 28% *trans*-10, *cis*-12) and two levels of MUFA (low; 19% and high; 39%) were combined for pig feeding (Table 1). All diets were formulated to provide similar protein and energy levels, fulfilling the advised nutritional needs for gilts at the ages studied (National Research Council, 1998).

The experiment was conducted using 288 finishing gilts (Large white ♂ × Landrace × Large white ♀). Pigs weighing 70 kg and at about 140 days of age were randomly allotted to the six different feeding treatments in four replicates of each treatment (12 pigs per replicate). Pigs were housed in an environmentally-controlled experimental grower/finisher shed. Pigs were group-housed (12 pigs per pen) and had *ad libitum* access to feed (single space dry feeders) and water (nipple drinkers) until a final average weight of 107 kg. After fattening (53 days), pigs were slaughtered at a local slaughterhouse by electrical stunning and exsanguination.

### 2.2. Sampling

Representative samples of mixed diets were taken before the beginning of the trial in order to determine their chemical and fatty acid composition.

Eight animals from each treatment were randomly selected for sampling. Loins from slaughtered animals were taken within 10 min after bleeding and kept at 4 °C for 24 h. Loin chops (1.5 cm thick) were over-wrapped in PVC film and stored at 4 °C for 7 days. The samples were analyzed at 0, 1, 2, 4 and 7 days of storage. At each time period, a small sample of the chop (5 $cm^2$) was cut and frozen at −80 °C until analysis, while the rest of the chop was kept under refrigeration until the next sampling time.

### 2.3. Analytical methods

The analysis of the composition of the feeds was performed according to the Association of Official Analytical Chemists (AOAC, 2000): crude protein (reference 954.01), crude fat (reference 920.39), crude fibre (reference 962.09) and ash (reference 942.05). Feed analysis is shown in Table 1.

The instrumental colour (CIE $L^*$, $a^*$, $b^*$, CIE, 1976) of the chops was measured at each time point of refrigerated storage. The measurements were performed in triplicate on the surface of the chops

**Table 1**
Ingredients and chemical composition of the experimental treatments

| Ingredient (%) | Low MUFA feed | | | High MUFA feed | | |
|---|---|---|---|---|---|---|
| | 0% CLA | 1% CLA | 2% CLA | 0% CLA | 1% CLA | 2% CLA |
| Barley | 53.3 | 53.3 | 53.3 | 53.3 | 53.3 | 53.3 |
| Wheat | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Bran | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 | 8.0 |
| Soybean meal, 44% | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 | 16.0 |
| Palm oil | 1.6 | 1.1 | 0.6 | 1.0 | 0.5 | 0.0 |
| Soy olein | 0.4 | 0.4 | 0.4 | 0.0 | 0.0 | 0.0 |
| Olive olein | 0.0 | 0.0 | 0.0 | 3.0 | 3.0 | 3.0 |
| Hydrogenated stearin palm | 3.0 | 2.5 | 2.0 | 1.0 | 0.5 | 0.0 |
| CLA | 0.0 | 1.0 | 2.0 | 0.0 | 1.0 | 2.0 |
| Carbonate | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 | 1.2 |
| Phosphate | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| Salt | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 | 0.4 |
| L-Lysine 50 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 | 0.17 |
| L-Threonine | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 | 0.03 |
| Coline 75 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 |
| Vitamin and mineral premix | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| *Chemical composition (%)* | | | | | | |
| Dry matter | 89.2 | 89.6 | 89.4 | 89.3 | 89.5 | 89.6 |
| Ash | 4.9 | 5.1 | 5.0 | 5.1 | 5.6 | 5.3 |
| Crude fiber | 4.2 | 4.3 | 4.1 | 4.7 | 4.3 | 4.6 |
| Crude fat | 7.7 | 6.9 | 7.3 | 7.2 | 7.1 | 6.8 |
| Crude protein | 16.4 | 16.0 | 15.8 | 16.7 | 16.5 | 15.8 |
| Nitrogen free extractives | 62.8 | 64.1 | 64.0 | 62.4 | 62.7 | 63.8 |
| *Fatty acid composition (%)* | | | | | | |
| C14:0 | 0.8 | 0.6 | 0.5 | 0.5 | 0.3 | 0.3 |
| C16:0 | 35.3 | 30.4 | 25.6 | 25.4 | 19.7 | 15.0 |
| C16:1 | 0.1 | 0.1 | 0.1 | 0.5 | 0.4 | 0.4 |
| C18:0 | 22.8 | 20.1 | 16.6 | 11.4 | 7.6 | 4.6 |
| C18:1 $n-9$ | 18.1 | 18.0 | 18.7 | 37.8 | 37.9 | 37.8 |
| C18:2 $n-6$ | 19.9 | 20.2 | 19.8 | 20.6 | 22.2 | 22.5 |
| C18:3 $n-3$ | 1.8 | 1.7 | 1.6 | 1.8 | 2.1 | 2.1 |
| *cis*-9, *trans*-11 CLA | 0.0 | 3.9 | 8.0 | 0.0 | 4.3 | 7.9 |
| *trans*-10, *cis*-12 CLA | 0.0 | 3.7 | 7.9 | 0.0 | 4.2 | 8.1 |
| ∑SFA | 59.7 | 52.0 | 43.5 | 38.8 | 28.4 | 20.6 |
| ∑MUFA | 18.8 | 18.6 | 19.2 | 38.9 | 38.8 | 38.7 |
| ∑PUFA[a] | 21.5 | 21.9 | 21.5 | 22.4 | 24.4 | 24.7 |

CLA (conjugated linoleic acid), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), and PUFA (polyunsaturated fatty acids).
[a] Excluding CLA isomers.

using a Minolta Chromameter CR-300 (Minolta Camera Corp., Meter Division, Ramsey, NJ).

TBARS determination of loins at 0, 1, 2, 4 and 7 days of refrigerated storage was carried out using the method of Salih, Smith, Price, and Dawson (1987). Results were expressed as mg malondialdehyde (MDA) per kg of sample.

### 2.4. Volatile compounds

Volatiles compounds of the uncooked loins after 7 days of refrigerated storage were determined by headspace solid-phase microextraction (HS-SPME) (Supelco Co., Bellefonte, PA) using a fibre coated with Carboxen-poly(dimethylsiloxane)-divinylbenzene (50/30 μm thickness) (Garcia-Esteban, Ansorena, Astiasaran, Martin, & Ruiz, 2004). Muscle samples were ground with a commercial grinder after being frozen with liquid nitrogen. A portion of 0.5 g was weighed into a 4 ml screw-capped vial and 1.5 ml of saturated salt solution was added. The extractions were performed in a water bath at 40 °C for 30 min and under stirring. Before extraction, samples were equilibrated for 10 min at the same temperature used for extraction. Prior to analysis, the SPME fibre was preconditioned at 250 °C for 60 min in the GC injection port. Analyses were performed using an Agilent 6890 series gas chromatograph (Agilent, Santa Clara, CA) coupled to a mass selective detector (Agilent 5973). Analytes were separated using an HP-5 bonded-phase fused silica capillary column (Agilent, 50 m × 0.32 mm i.d., film thickness 1.05 μm), operating at 45 kPa of column head pressure, resulting in a flow of 1.3 ml/min at 40 °C. The SPME fibre was desorbed for 30 min. The injection port was in splitless mode. The temperature program was 40 °C for 10 min, raised to 200 °C at a rate of 5 °C/min and then raised to 250 °C at a rate of 20 °C/min, and maintained at this temperature for 5 min. The transfer line to the mass spectrometer was hold at 280 °C. Electron impact mass spectra were obtained at 70 eV, a multiplier voltage of 1756 V and data were collected at a rate of 1 scan/s over the *m/z* range 30–300. Volatile compounds were identified by comparing their mass spectra with those contained in the NIST/EPA/NIH and Wiley libraries and by comparison of Kovat's indices with those reported in the literature.

### 2.5. Statistical analysis

An individual loin was the experimental unit for analysis of all data. The data structure consisted of the TBARS and instrumental colour values of loin chops from pigs fed the six different treatments, measured at 0, 1, 2, 4 and 7 days of storage, as well as the volatile profiles of the loin chops at day 7 of refrigerated storage. Statistical analyses were performed by means of the general linear models procedure of the SPSS statistical software (V.15.0, SPSS Inc., Chicago, IL). The effect of the CLA and MUFA contents of the diets and their interaction on the volatile profile was evaluated by a two-way analysis of variance (ANOVA). The effect of time of storage and its respective interaction with CLA, MUFA and CLA × MUFA on TBARS and instrumental colour values were evaluated by a three-way mixed model repeated-measures test. CLA, MUFA and CLA × MUFA levels were the between-subject effects and time of storage was the within-subject effect. Differences were considered significant at $p \leqslant 0.05$. When the effect of any of the factors was significant, differences between groups were analyzed by the Tukey's post hoc test.

## 3. Results and discussion

### 3.1. Instrumental colour changes

Changes in CIE $L^*$, $a^*$ and $b^*$ values during refrigerated storage are shown in Fig. 1. $L^*$ and $a^*$ values of loin chops decreased with refrigerated storage, whereas $b^*$ values increased from day 0 to day 4, with a subsequent decrease. Neither CLA nor MUFA level nor their interaction influenced the changes in $a^*$ and $b^*$ values throughout the refrigeration of chops. The changes in $L^*$ value throughout the storage were conditioned by dietary CLA [$p(t \times \text{CLA}) = 0.041$]. This significant effect was caused by the significant differences in the $L^*$ values at day 2 of refrigerated storage. Thus, the highest $L^*$ values were measured at 2% CLA (58.4, mean value, regardless of MUFA level) and the lowest $L^*$ value was registered at 1% CLA (54.1, mean value, regardless of MUFA level). No differences in $L^*$, due to dietary CLA, were observed beyond day 2 of storage.

Wiegand, Parrish, Swan, Larsen, & Baas, 2001 found higher $L^*$ values in chops from CLA-fed pigs than the control after 7 days refrigeration. However, in chicken meat with 5% dietary CLA, Du, Ahn, Nam, and Sell (2000) measured lower $L^*$ and $b^*$ values and higher $a^*$ values than the control after 7 days of storage. Hur et al. (2004) related an improved meat colour stability of beef patties containing CLA to a likely inhibition of lipid and oxymyoglobin oxidation by CLA. Nevertheless, the mechanism for the improvement of colour stability by CLA remains unclear. In the present work, no relevant effect of dietary CLA on meat colour was observed. Moreover, the obtained results reveal that the MUFA level of the diet did not interact with dietary CLA in the instrumental colour values measured. Therefore, the use of CLA in pig feeding, combined with either low or high MUFA levels, would not have any detrimental effect on the colour of fresh pork during storage. This is of interest, because colour is one of the main traits that consumers consider for the purchase of fresh meat.

### 3.2. Lipid oxidation

A significant increase in TBARS values of fresh loin chops was detected throughout refrigerated storage [$p(\text{time}) < 0.001$] (Fig. 2). This parameter was only influenced by CLA supplementation [$p(\text{time} \times \text{CLA}) = 0.029$], whereas the MUFA level of the diets and the interaction CLA × MUFA did not seem to affect lipid oxidation of the chops during refrigerated storage. Therefore, only those results concerning the effect of CLA are shown in Fig. 2.

Dietary CLA at 2% in pig diet led to higher TBARS values at 7 days of refrigerated storage (0.70 mg MDA/kg), than those of 0% CLA and 1% CLA (0.62 mg MDA/kg). Therefore, dietary CLA seems to affect the oxidative stability of refrigerated pork, but the influence of dietary CLA on oxidation seems to be independent of the MUFA level of the pig diets.

In agreement with the obtained results, a higher oxidation with dietary CLA has been also found by other authors (Hur et al., 2004). Recently, Flintoff-Dye and Omaye (2005) suggested that the effect of CLA on oxidation was dependent upon the CLA level assayed *in vitro*. In that work, CLA acted as pro-oxidant at low levels, then as antioxidant at higher doses and subsequently reverted to pro-oxidant at the highest levels assayed. In the present study, it seems that levels of CLA higher than 1% in the pig diet implied higher oxidation of refrigerated pork. Nevertheless, it is worth pointing out that times of storage less than 7 days did not affect TBARS values of loin chops from the different treatments. Moreover, differences in TBARS of loin chops at day 7 between CLA treatments were significant but not large (TBARS increased by 0.08 mg MDA/kg in the sample at 2% CLA, compared to the values at 0% and 1% CLA).

The different PUFA contents of the samples might be one of the main reasons explaining the higher TBARS values measured at 2% CLA. PUFA are considered more prone to lipid oxidation. Thus, MDA is primarily formed from the oxidation of fatty acids with three or more double bonds, with the amount of MDA increasing with increasing double bond number (Sinnhuber & Yu, 1977). The amount of fatty acids of the loin chops assayed in the present

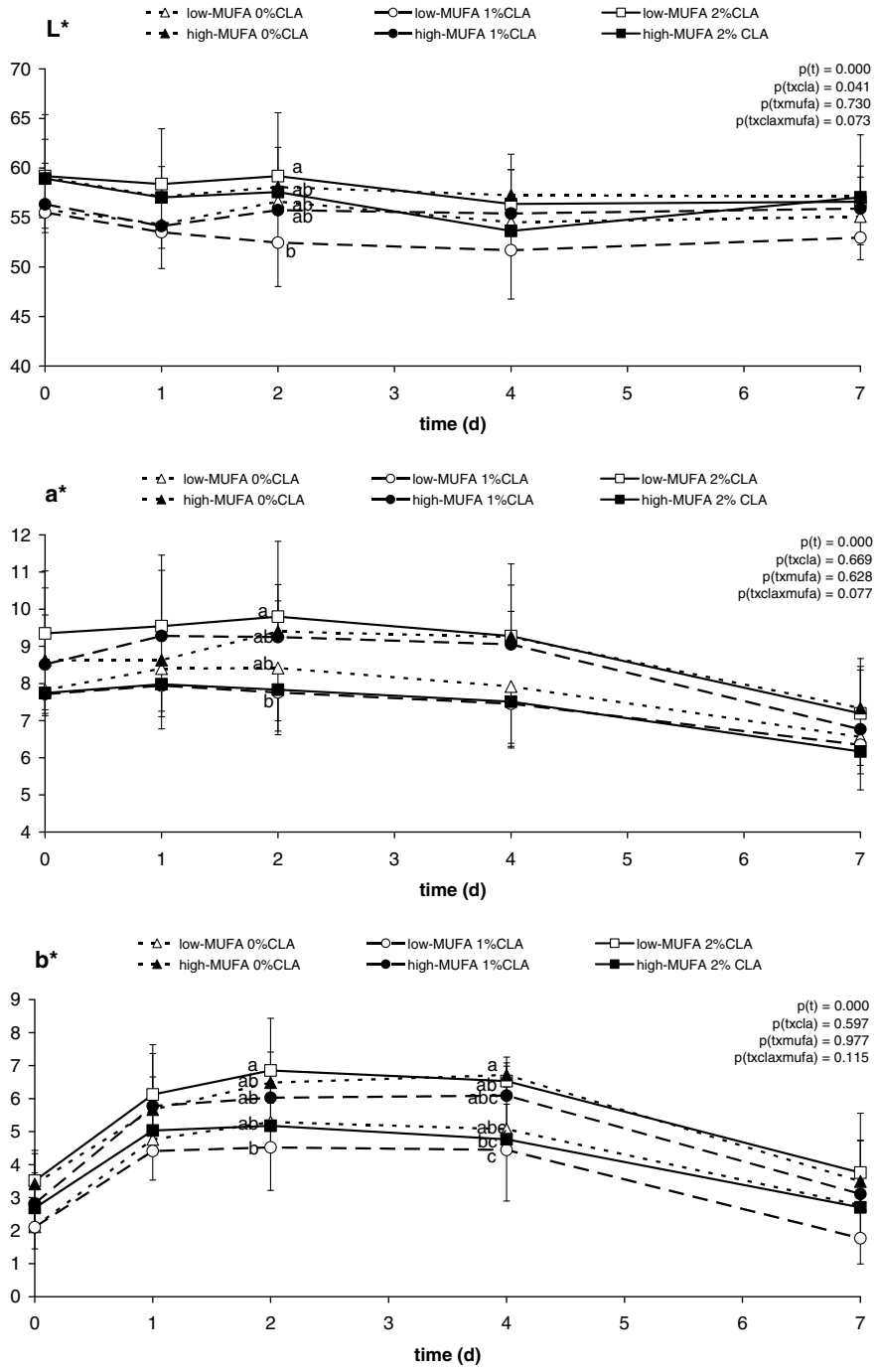


**Fig. 1.** Instrumental colour ($L^*$, $a^*$, $b^*$) of fresh loin during refrigerated storage as affected by dietary CLA and MUFA.

work have been previously quantified and will be published elsewhere. Thus, at day 0 of refrigerated storage, both neutral lipids and polar lipids showed a higher PUFA content (excluding CLA isomers) at 2% CLA, compared to that at 0% CLA. Therefore, the higher PUFA content of pork lipids at the beginning of the assay for 2% CLA diets would be in agreement with the higher formation of MDA at 2% CLA after 7 days of refrigeration of the loin chops.

Besides the contribution of the oxidation of PUFA to the MDA values, the oxidation of CLA isomers might also influence the obtained TBARS results. Several studies have shown higher oxidation rates of CLA isomers compared to the non-conjugated isomers of linoleic acid (Chen, Chan, Kwan, & Zhang, 1997; Livisay, Zhou, Ip, & Decker, 2000; van den Berg, Cook, & Tribble, 1995). In addition, Yang, Leung, Huang, and Chen (2000) stated that CLA as free fatty acid is extremely unstable in air and it becomes more stable if it is incorporated into triacylglycerols. The present work showed increasing levels of CLA isomers as free fatty acid with increasing levels of dietary CLA at day 7 of refrigerated storage (data published elsewhere). Thus, if CLA isomers were more unstable as free fatty acids, they might contribute to the highest MDA content found at 2% of dietary CLA at day 7 of refrigerated storage. Nevertheless, the higher or lower oxidative stability of CLA isomers remains unclear, since several studies have stated a higher stability of CLA isomers than other PUFA, and even higher than the non-conjugated isomers of linoleic acid (Du et al., 2000; Shantha, Crum, & Decker, 1994).

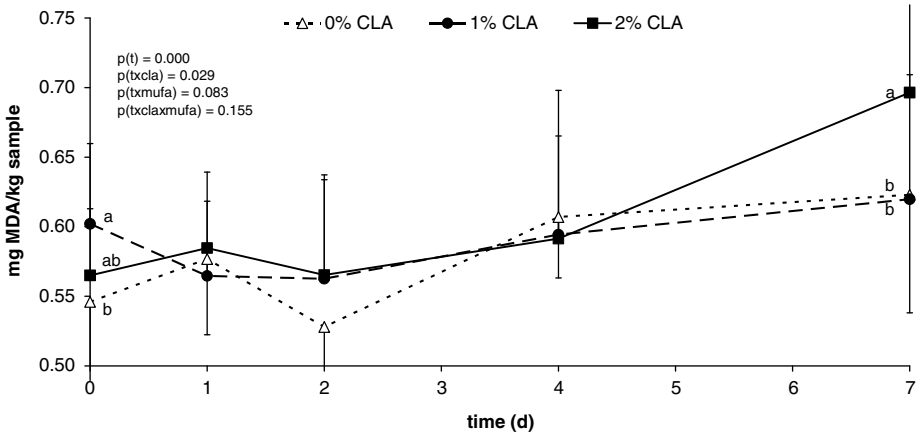


**Fig. 2.** TBARS values (mg MDA/kg sample) of fresh loin as affected by dietary CLA during refrigerated storage.

### 3.3. Volatile profile

The volatile profile of refrigerated loin chops at day 7 of storage is shown in Table 2. Ketones (8 compounds, 45.1% of total area counts), aldehydes (9 compounds, 15.9% of total area counts), alcohols (7 compounds, 15.9% of total area counts) and esters (3 compounds, 14.4% of total area counts) were the major groups of volatile compounds. Aliphatic hydrocarbons (8 compounds, 5.3% of total area counts), aromatic hydrocarbons (5 compounds, 2.2% of total area counts), acids (1 compound, 0.4% of total area counts) and other minor volatiles were also detected.

No volatiles exclusive to a dietary treatment were found, i.e., dietary CLA, MUFA or the interaction CLA × MUFA do not seem to cause qualitative differences in the volatile profile of refrigerated pork loin. Moreover, neither CLA nor CLA × MUFA generated quantitative differences in most detected volatile compounds. MUFA supplementation caused a lower content (area counts) in total volatile compounds in refrigerated loins ($p = 0.002$).

Du, Nam, Hur, Ismail, and Ahn (2002) and Patorelli et al. (2005) did not find relevant effects of dietary CLA on the volatile compounds detected in loins from CLA-fed pigs, or in breast fillets from CLA-fed broilers, respectively.

Unbranched aldehydes are considered the most important breakdown products of lipid oxidation in meat and meat products (Frankel, 1985). In the present work, most detected aldehydes were unbranched aldehydes, whereas branched aldehydes were lower, both qualitatively and quantitatively. Although not significant, the content of all unbranched aldehydes seemed to increase with dietary CLA (from 9573 area counts at 0% CLA to 14,100 area counts at 2% CLA). This was mainly due to the content of the major unbranched aldehyde, hexanal. Nevertheless, only the increase in the content of heptanal was especially influenced by the level of dietary CLA ($p = 0.058$).

Most unbranched aldehydes derive from lipid oxidation of unsaturated fatty acids. Each unsaturated fatty acid produces specific hydroperoxides that in turn decompose to specific aldehydes (Frankel, 1985). Thus, heptanal might mainly result from the oxidation of oleic acid (C18:1 $n-9$) (Ladikos & Lougovois, 1990). Therefore, the higher the C18:1 $n-9$ content the higher the expected level of heptanal. However, this relationship was not observed in the studied loins, which showed a decrease in the proportion of C18:1 $n-9$ with dietary CLA (Martin, Muriel, Gonzalez, Viguera, & Ruiz, 2008b). Therefore, it seems that heptanal could have a different origin to that of the oxidation of C18:1 $n-9$. The formation of heptanal through the oxidation of CLA isomers should not be discarded. In preliminary model systems, in which CLA isomers were autoxidised we have detected high levels of heptanal.

Hexanal might mainly result from the oxidation of linoleic acid (C18:2 $n-6$) (Ladikos & Lougovois, 1990). In these samples, a significant increase in the proportion of non-conjugated C18:2 $n-6$ was observed in the polar lipid fraction, with increased dietary CLA (Martin et al., 2008a). The PUFA from polar lipids (mainly phospholipids) are more susceptible to oxidation, due to their location in membranes close to haem pigments and oxidant systems (Gray & Pearson, 1987). Thus, the higher level of non-conjugated C18:2 in polar lipids of loin chops with dietary CLA could explain the increase in hexanal. Moreover, the higher content in conjugated isomers of C18:2 (*cis*-9, *trans*-11 CLA and *trans*-10, *cis*-12 CLA) with dietary CLA, both in polar lipids and neutral lipids of loin chops (Martin et al., 2008a), might also cause an increase in hexanal, due to the oxidation of these CLA isomers.

As it was reviewed by Imafidon and Spanier (1994), enrichment of pig diets in unsaturated fatty acids, especially in C18:2 $n-6$, seems to result in higher concentrations of aldehydes in lean meat, suggesting an increased trend to oxidation of these meats. Larick, Turner, Schoenherr, Coffey, and Pilkington (1992) observed an increase in the aldehydes derived from oxidation of C18:2 $n-6$ in the volatile compounds of meat from pigs fed increasing levels of C18:2 $n-6$. Aldehydes have also been the largest detected group of volatile compounds in meat products from CLA-fed animals (Du et al., 2000; Patorelli et al., 2005).

Due to the origin of aldehydes from the oxidative degradation of fatty acids, the analysis of aldehydes, especially the hexanal content, has been considered as an indicator of the rate and extent of the oxidation process (Ross & Smith, 2006). Thus, hexanal content in most cases is correlated with the results of TBARS tests. In the present work, as was previously reported, the highest TBARS values were measured at 2% CLA in loin at day 7 of storage. In agreement with this, the hexanal proportion, with respect to the total area counts was 9.6% for 0% CLA diets, 9.4% for 1% CLA diets and 13.9% for 2% CLA diets.

Total aldehydes content was also affected by the MUFA level of the diets ($p = 0.049$). Thus, regardless of the dietary CLA level, MUFA supplementation led to a lower content in total aldehydes. This lower amount of aldehydes with MUFA enrichment of the pig diet was mainly due to the lower content in the major aldehyde, hexanal ($p = 0.075$). As previously explained, the higher susceptibility to oxidation of PUFA from phospholipids is one of the main origins of aldehydes. The assayed loin chops from pigs fed with MUFA-enriched diets showed lower PUFA content in the polar lipid fraction (Martin et al., 2008a). This lower PUFA content was in agreement with the lower aldehyde concentration in loin chops for high MUFA diets.

**Table 2**
Volatile profile (total area counts × $10^4$) of refrigerated loin from pigs fed different levels of CLA and MUFA

| | LRI[B] | Reliability[C] | CLA level[A] | | | MUFA level | | SEM | CLA | MUFA | CLA × MUFA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | 0% | 1% | 2% | Low | High | | | | |
| *Acids* | | | | | | | | | | | |
| 3-Methyl butanoic acid | 839 | A | 262 | 381 | 293 | 382 | 241 | 32.0 | 0.273 | 0.024 | 0.259 |
| Total acids | | | 262 | 381 | 293 | 382 | 241 | 32.0 | 0.273 | 0.024 | 0.259 |
| *Alcohols* | | | | | | | | | | | |
| Ethanol | 502 | B | 3294 | 4275 | 4011 | 3715 | 4005 | 769.7 | 0.876 | 0.858 | 0.309 |
| 1-Propanol | 559 | A | 1589 | 1534 | 1335 | 1298 | 1673 | 102.0 | 0.549 | 0.074 | 0.387 |
| 3-Methyl-1-butanol | 739 | A | 3808 | 6454 | 5094 | 5311 | 4926 | 699.1 | 0.358 | 0.796 | 0.672 |
| 2-Methyl-1-butanol | 743 | A | 672 | 1701 | 1257 | 1354 | 1066 | 150.5 | 0.032 | 0.340 | 0.250 |
| 2-Octanol | 947 | B | 287 | 387 | 262 | 361 | 263 | 41.3 | 0.494 | 0.275 | 0.901 |
| 2,6-Dimethyl-4-heptanol | 953 | C | 841 | 555 | 550 | 855 | 443 | 112.0 | 0.434 | 0.066 | 0.186 |
| 2,6-Bis(1,1-dimethylethyl)-4-methylphenol | 1535 | A | 135 | 113 | 156 | 156 | 113 | 26.5 | 0.822 | 0.453 | 0.278 |
| Total alcohols | | | 10,626 | 15,019 | 12,665 | 13,050 | 12,489 | 1191.0 | 0.308 | 0.900 | 0.326 |
| *Aldehydes* | | | | | | | | | | | |
| 3-Methylbutanal | 646 | A | 770 | 787 | 696 | 912 | 591 | 113.8 | 0.946 | 0.187 | 0.483 |
| 2-Methylbutanal | 656 | A | 430 | 321 | 325 | 374 | 344 | 33.1 | 0.345 | 0.643 | 0.115 |
| Pentanal | 697 | A | 504 | 539 | 728 | 774 | 407 | 104.8 | 0.643 | 0.106 | 0.731 |
| Hexanal | 801 | A | 7513 | 7936 | 10,923 | 11,114 | 6466 | 1251.8 | 0.478 | 0.075 | 0.705 |
| Heptanal | 903 | A | 301 | 385 | 669 | 545 | 359 | 68.1 | 0.058 | 0.153 | 0.467 |
| Benzaldehyde | 969 | A | 182 | 188 | 305 | 368 | 81.8 | 56.8 | 0.645 | 0.116 | 0.347 |
| Octanal | 1004 | A | 450 | 570 | 544 | 624 | 419 | 71.6 | 0.762 | 0.154 | 0.141 |
| Nonanal | 1106 | A | 906 | 928 | 1237 | 1224 | 823 | 126.4 | 0.488 | 0.125 | 0.444 |
| Dimethyl acetal olealdehyde | 1613 | C | 79.6 | 78.7 | 97.3 | 94.7 | 75.8 | 9.4 | 0.764 | 0.451 | 0.536 |
| Total aldehydes | | | 11,136 | 11,733 | 15,524 | 16,030 | 9567 | 1628.4 | 0.455 | 0.049 | 0.560 |
| *Aliphatic hydrocarbons* | | | | | | | | | | | |
| 2-Methylpentane | 561 | A | 759 | 696 | 703 | 632 | 807 | 51.8 | 0.868 | 0.118 | 0.872 |
| 3-Methylpentane | 576 | A | 818 | 736 | 790 | 733 | 829 | 51.0 | 0.812 | 0.364 | 0.241 |
| Heptane | 700 | A | 730 | 631 | 761 | 620 | 795 | 76.0 | 0.776 | 0.257 | 0.136 |
| 2-Methylheptane | 769 | A | 301 | 197 | 557 | 176 | 527 | 48.5 | 0.001 | 0.001 | 0.001 |
| 3-Methylheptane | 777 | B | 338 | 295 | 891 | 243 | 773 | 79.1 | 0.002 | 0.001 | 0.002 |
| Decane | 1000 | A | 651 | 732 | 608 | 730 | 597 | 87.2 | 0.872 | 0.534 | 0.483 |
| Undecane | 1100 | A | 185 | 281 | 362 | 306 | 246 | 42.1 | 0.262 | 0.495 | 0.604 |
| Pentadecane | 1500 | A | 79.5 | 68.0 | 721 | 507 | 72.0 | 179.3 | 0.187 | 0.173 | 0.135 |
| Total aliphatic hydrocarbons | | | 3862 | 3636 | 5393 | 3947 | 4646 | 270.6 | 0.968 | 0.724 | 0.367 |
| *Aromatic hydrocarbons* | | | | | | | | | | | |
| Methylcyclopentane | 622 | A | 638 | 459 | 608 | 536 | 601 | 74.3 | 0.569 | 0.652 | 0.065 |
| Methylbenzene | 772 | A | 633 | 605 | 720 | 603 | 703 | 44.1 | 0.549 | 0.273 | 0.348 |
| Ethylbenzene | 869 | A | 146 | 162 | 169 | 166 | 152 | 11.6 | 0.712 | 0.567 | 0.236 |
| 1,3-Dimethylbenzene | 876 | A | 223 | 230 | 275 | 235 | 251 | 14.1 | 0.298 | 0.582 | 0.711 |
| 1,2-Dimethylbenzene | 899 | A | 138 | 131 | 128 | 135 | 129 | 10.9 | 0.921 | 0.779 | 0.057 |
| Total aromatic hydrocarbons | | | 1778 | 1587 | 1900 | 1675 | 1836 | 109.9 | 0.432 | 0.345 | 0.345 |
| *Esters* | | | | | | | | | | | |
| Acetic acid ethyl ester | 611 | A | 15855 | 5048 | 6662 | 13383 | 4994 | 2717.0 | 0.161 | 0.095 | 0.071 |
| Acetic acid propyl ester | 718 | A | 1572 | 1194 | 1423 | 1508 | 1284 | 117.8 | 0.418 | 0.336 | 0.109 |
| Propanoic acid propyl ester | 813 | A | 1264 | 794 | 1002 | 1016 | 1024 | 110.9 | 0.251 | 0.970 | 0.376 |
| Total esters | | | 18,691 | 7036 | 9087 | 15,907 | 7302 | 2787.0 | 0.140 | 0.089 | 0.066 |
| *Ketones* | | | | | | | | | | | |
| 2-Propanone | 514 | A | 2009 | 1563 | 2082 | 2030 | 1739 | 134.9 | 0.245 | 0.276 | 0.182 |
| 2,3-Butanedione | 592 | A | 5833 | 9192 | 6642 | 8548 | 5896 | 820.7 | 0.241 | 0.115 | 1.000 |
| 2-Pentanone | 685 | A | 196 | 167 | 158 | 180 | 168 | 11.3 | 0.399 | 0.620 | 0.283 |
| 3-Hydroxy-2-butanone | 709 | A | 22,258 | 32,387 | 22,545 | 31,551 | 19,908 | 3125.2 | 0.336 | 0.069 | 0.887 |
| 2-Heptanone | 892 | A | 154 | 138 | 135 | 137 | 147 | 8.8 | 0.699 | 0.603 | 0.313 |
| 2,3-Octanedione | 984 | A | 429 | 629 | 716 | 857 | 326 | 114.3 | 0.526 | 0.020 | 0.352 |
| 6-Methyl-5-hepten-2-one | 988 | A | 313 | 389 | 367 | 427 | 286 | 32.3 | 0.542 | 0.020 | 0.068 |
| 2-Nonanone | 1094 | A | 144 | 135 | 280 | 236 | 136 | 36.0 | 0.198 | 0.174 | 0.516 |
| Total ketones | | | 31,336 | 44,600 | 32,925 | 43,966 | 28,606 | 3962.9 | 0.333 | 0.058 | 0.875 |
| *Others* | | | | | | | | | | | |
| Carbon disulfide | 549 | A | 911[a] | 524[ab] | 446[b] | 639 | 615 | 82.0 | 0.040 | 0.879 | 0.261 |
| Limonene | 1037 | A | 65.6 | 91.2 | 121.9 | 87.5 | 98.4 | 11.7 | 0.243 | 0.667 | 0.676 |
| Total volatiles | | | 78,668 | 84,607 | 78,355 | 95,684 | 65,400 | 14027.0 | 0.707 | 0.002 | 0.435 |

[A] Different lower-case letters within the same row differed significantly ($p \leqslant 0.05$).
[B] Linear retention index.
[C] Reliability of identification: (A) mass spectrum and KI in agreement with the literature; (B) mass spectrum consistent with spectrum in NS and Wiley libraries; and (C) tentative identification by mass spectrum.

Besides aldehydes, ketones were the most abundant group of detected volatile carbonyls of refrigerated loin. Ketones can be produced both by lipid autooxidation and by microbiological metabolism. The MUFA level of the diet tended to affect the total content of ketones from stored loin ($p = 0.058$) by causing a lower content in total ketones in loins from high MUFA treatments, similarly to aldehydes. This decrease in total ketones was mainly due to the significant decrease in the content of 2,3-octanedione and 6-methyl-5-hepten-2-one ($p = 0.020$ for both compounds) with MUFA supplementation. It is important to point out that 3-hydroxy-2-butanone, was the major volatile compound found in the volatile fraction of the loins after 7 days of refrigeration, regardless of the assayed treatments (32.0% average value of the six treatments). This ketone has been mainly related to the fermentation of sugars by lactic acid bacteria (Ordonez, Hierro, Bruna, & de la Hoz, 1999).

Unbranched alcohols are also derived from lipid oxidation due to the reduction of aldehydes to their corresponding alcohols (Shahidi, 1994), whereas branched alcohols may derive from Maillard reactions. In the present work, no changes were observed in those alcohols likely to be derived from lipid oxidation (1-propanol, 2-octanol). On the contrary, a significant increase in 2-methyl-1-butanol ($p = 0.032$) and a trend to higher levels of 3-methyl-1-butanol contents with dietary CLA were found. This was in agreement with the simultaneous decreasing trend in the contents of the equivalent branched aldehydes (2-methylbutanal and 3-methylbutanal) with CLA supplementation.

As far as the aliphatic hydrocarbons are concerned, the content of 2-methylheptane and 3-methylheptane seemed to decrease at 1% CLA and increase at 2% CLA. The content of these two alkanes was also affected by the MUFA level of the diets. Thus, regardless of CLA supplementation, MUFA enrichment of the pig diets caused an increase in the content of 2-methylheptane and 3-methylheptane. Furthermore, a significant effect of CLA $\times$ MUFA interaction on the content of these two volatile compounds was obtained.

Regarding minor volatile compounds, some significant differences were detected. Total level of volatile acids was lower for high MUFA treatments ($p = 0.017$), mainly due to the significant lower content of 3-methylbutanoic acid. Carbon disulfide content decreased with dietary CLA. Carbon disulfide has been related to cabbage odour, and has a low odour threshold (Insausti, Beriain, & Gorraiz, 2002); its origin could be from proteolytic bacterial enzymes (Kontou, Tsipi, & Tzia, 2004). Limonene was also detected in the loin chops. The major source of this terpene is related to the use of spices in the preparation of meat products. Nevertheless, this compound has been also found in meat, due to its presence in animal feeds (Ruiz, Ventanas, Cava, Andres, & Garcia, 1996).

## 4. Conclusions

The present study shows that CLA supplementation at doses lower than 1% in pig diets does not seem to affect lipid oxidation of refrigerated loin. In addition, the obtained results reveal that the effect of dietary CLA in oxidative processes seems to be independent of the MUFA level of swine feed. This lack of effect of interaction between CLA and MUFA supplementations on the oxidative quality of loin was found both in colour changes and in lipid oxidation and volatile profile.

The obtained results in the present work are of interest, since the drawback of the increase of the ratio of SFA to unsaturated fatty acids of pig tissues, caused by CLA supplementation, could be counteracted by including high levels of MUFA in the swine diets, without detriment to the oxidative stability of meat products. Nevertheless, further investigations are necessary, in order to clarify the role of dietary CLA isomers in oxidative processes.

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## References

Association of Official Analytical Chemist (2000). *Official methods of analysis* (17th ed.). Gaithersburg, Maryland: Association of Official Analytical Chemists.

Bhattacharya, A., Banu, J., Rahman, M., Causey, J., & Fernandes, G. (2006). Biological effects of conjugated linoleic acids in health and disease. *Journal of Nutritional Biochemistry, 17*, 789–810.

Chen, Z. Y., Chan, P. T., Kwan, K. Y., & Zhang, A. (1997). Reassessment of the antioxidant activity of conjugated linoleic acid. *Journal of the American Oil Chemical Society, 74*, 749–753.

Department of Health (1994). *Nutritional aspects of cardiovascular disease*. Report on Health and Social Subjects No. 46. London: HMSO.

Du, M., Ahn, D. U., Nam, K. C., & Sell, J. L. (2000). Volatile profiles and lipid oxidation of irradiated raw chicken meat patties from laying hens fed with diets containing conjugated linoleic acid. *Meat Science, 56*, 387–395.

Du, M., Nam, K. C., Hur, S. J., Ismail, H., & Ahn, D. U. (2002). Effect of dietary conjugated linoleic acid, irradiation, and packaging conditions on the quality characteristics of raw broiler breast fillets. *Meat Science, 60*, 9–15.

Dugan, M., Aalhus, J., & Kramer, J. (2004). Conjugated linoleic acid pork research. *American Journal of Clinical Nutrition, 79*, 1212S–1216S.

Flintoff-Dye, N. L., & Omaye, S. T. (2005). Antioxidant effects of conjugated linoleic acid isomers in isolated human low-density lipoproteins. *Nutrition Research, 25*, 1–12.

Frankel, E. N. (1985). Chemistry of autoxidation. Mechanism, products and flavor significance. In D. B. Mi & D. B. Min (Eds.), *Flavor chemistry of fats and oils*. Champaign: American Oil Chemists Society. p. 1.

Garcia-Esteban, M., Ansorena, D., Astiasaran, I., Martin, D., & Ruiz, J. (2004). Comparison of simultaneous distillation extraction (SDE) and solid-phase microextraction (SPME) for the analysis of volatile compounds in dry-cured ham. *Journal of the Science of Food and Agriculture, 84*, 1364–1370.

Gray, J. I., & Pearson, A. M. (1987). Rancidity and warmed-over flavour. In A. M. Pearson & T. R. Dutson (Eds.), *Advances in meat research*. New York: Van Nostrand Reinhold Company. pp. 221–269.

Ha, Y. L., Storkson, J., & Pariza, M. W. (1990). Inhibition of benzo[*a*]pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. *Cancer Research, 50*, 1097–1101.

Hur, S., Park, G., & Joo, S. (2006). Biological activities of conjugated linoleic acid (CLA) and effects of CLA on animal products. *Livestock Science, 110*, 221–229.

Hur, S. J., Ye, B. W., Lee, J. L., Ha, Y. L., Park, G. B., & Joo, S. T. (2004). Effects of conjugated linoleic acid on color and lipid oxidation of beef patties during cold storage. *Meat Science, 66*, 771–775.

Imafidon, G. I., & Spanier, A. M. (1994). Unraveling the secret of meat flavors. *Trends in Food Science and Technology, 5*, 315–321.

Insausti, K., Beriain, M. J., & Gorraiz, C. (2002). Volatile compounds of raw beef from 5 local Spanish cattle breeds stored under modified atmosphere. *Journal of Food Science, 67*, 1580–1589.

Ip, C., Chin, S. F., Scimeca, J. A., & Pariza, M. W. (1991). Mammary cancer prevention by conjugated dienoic derivative of linoleic acid. *Cancer Research, 51*, 6118–6124.

Joo, S. T., Lee, J. J., Ha, Y. L., & Park, G. B. (2002). Effect of dietary conjugated linoleic acid composition, lipid oxidation, color and water-holding capacity of pork loin. *Journal of Animal Science, 801*, 108–112.

Kontou, S., Tsipi, D., & Tzia, C. (2004). Kinetics of Maneb degradation during thermal treatment of tomatoes. *Journal of Agricultural and Food Chemistry, 52*, 1212–1219.

Ladikos, D., & Lougovois, V. (1990). Lipid oxidation in muscle food: A review. *Food Chemistry, 35*, 295–314.

Larick, D. K., Turner, B. E., Schoenherr, W. D., Coffey, M. T., & Pilkington, D. H. (1992). Volatile compound content and fatty acid composition of pork as influenced by linoleic acid content of the diet. *Journal of Animal Science, 70*, 1397–1403.

Livisay, S., Zhou, S., Ip, C., & Decker, E. (2000). Impact of dietary conjugated linoleic acid on the oxidative stability of rat liver microsomes and skeletal muscle homogenates. *Journal of Agricultural and Food Chemistry, 48*, 4162–4167.

Martin, D., Antequera, T., Gonzalez, E., Lopez-Bote, C., & Ruiz, J. (2007). Changes in the fatty acid profile of the subcutaneous fat of swine throughout fattening as affected by dietary conjugated linoleic acid and monounsaturated fatty acids. *Journal of Agricultural and Food Chemistry, 55*, 10820–10826.

Martin, D., Muriel, E., Antequera, T., Perez-Palacios, T., & Ruiz, J. (2008a). Fatty acid composition and oxidative susceptibility of fresh loin and liver from pigs fed conjugated linoleic acid in combination with monounsaturated fatty acids. *Food Chemistry, 108*, 86–96.

Martin, D., Muriel, E., Gonzalez, E., Viguera, J., Ruiz, J. (2008b). Effect of dietary conjugated linoleic acid and monounsaturated fatty acids on productive,

carcass and meat quality traits of pigs. *Livestock Science*, in press. doi:10.1016/j.livsci.2007.12.005.
National Research Council (1998). *Nutrient requirements of swine* (10th ed.). Washington, DC: National Academy Press.
Ordonez, J. A., Hierro, E. M., Bruna, J. M., & de la Hoz, L. (1999). Changes in the components of dry-fermented sausages during ripening. *Critical Reviews in Food Science and Nutrition, 39*, 329–367.
Patorelli, G., Moretti, V., Macchioni, P., Lo Fiego, D., Santoro, P., Panseri, S., et al. (2005). Influence of dietary conjugated linoleic acid on the fatty acid composition and volatile compounds profile of heavy pig loin muscle. *Journal of the Science of Food and Agriculture, 85*, 2227–2234.
Ross, C. F., & Smith, D. M. (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. *Comprehensive Reviews in Food Science and Food Safety, 5*, 18–25.
Ruiz, J., Ventanas, J., Cava, R., Andres, A., & Garcia, C. (1996). Volatile compounds of dry-cured Iberian ham as affected by the length of the curing process. *Meat Science, 52*, 19–27.
Salih, A. M., Smith, D. M., Price, J. F., & Dawson, L. E. (1987). Modified extraction 2-thiobarbituric acid method for measuring lipid oxidation in poultry. *Poultry Science, 66*, 1483–1489.
Schmid, A., Collomb, M., Sieber, R., & Bee, G. (2006). Conjugated linoleic acid in meat and meat products: A review. *Meat Science, 73*, 29–41.
Shahidi, F. (1994). Flavor of meat and meat products – An overview. In F. Shahidi (Ed.), *Flavor of Meat and Meat Products* (pp. 1). London: Blackie Academic and Professional.
Shantha, N. C., Crum, A. D., & Decker, E. A. (1994). Evaluation of conjugated linoleic acid concentrations in cooked beef. *Journal of Agricultural and Food Chemistry, 42*, 1757–1760.
Sinnhuber, R. O., & Yu, T. C. (1977). Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. *Journal of Japanese Society of Fishery Science, 26*, 259–267.
van den Berg, J. I. M., Cook, N. E., & Tribble, D. L. (1995). Reinvestigation of the antioxidant properties of conjugated linolei acid. *Lipids, 30*, 599–605.
Wiegand, B. R., Parrish, F. C., Swan, J. E., Larsen, S. T., & Baas, T. J. (2001). Conjugated linoleic acid improves feed efficiency, decreases subcutaneous fat, and improves certain aspects of meat quality in stress-genotype pigs. *Journal of Animal Science, 79*, 2187–2195.
Yang, L., Leung, L. K., Huang, Y., & Chen, Z. Y. (2000). Oxidative stability of conjugated linoleic acid isomers. *Journal of Agricultural and Food Chemistry, 48*, 3072–3076.