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Changes in lipid oxidation levels and fatty acid composition in the meat of milk-fed lambs during refrigerated and frozen storage – Nutritional indices

Änderungen in den Lipidoxidationniveaus und der Fettsäurezusammensetzung im der Fleisch von Milch vergefüttert Lämmer während der Kühllagerung und der Gefrierkühllagerung – Ernährungs Anzeichen

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Summary

In this study changes in lipid oxidation levels and fatty acid composition of loin muscle (m. *longissimus dorsi*) intramuscular fat from milk fed lambs during i) refrigerated storage (4 °C) for 1, 3, 5 and 10 days (air packed samples) and ii) frozen storage (–18 °C) for 15, 30, 90 and 180 days (vacuum packed samples) were determined.

Lipid oxidation did not change during refrigerated storage of meat. An increase ($P<0.05$) in the levels of lipid oxidation was observed on the 180th day of frozen storage in comparison to the other test days under the same conditions. The evolution and the extent of lipid oxidation were more intense during refrigerated storage. Lipid oxidation remained at low levels during the entire storage period and for both storage conditions.

There was a statistical significant increase ($P<0.05$) in the percentage of saturated fatty acids during refrigerated storage of meat. The percentages of monounsaturated and polyunsaturated fatty acids did not change during refrigerated storage of meat. During frozen storage of meat there were no changes in the percentages of saturated, monounsaturated and polyunsaturated fatty acids.

The results showed that refrigerated and frozen storage did not have an adverse effect on the lipid oxidation levels and the fatty acid composition of meat from milk fed lambs. The nutritional indices used for the evaluation of the nutritional value of the intramuscular fat did not change during the refrigerated and frozen storage of meat.

Keywords: Fatty acid composition, lipid oxidation, milk fed lamb, lamb meat, refrigeration, freezing

Zusammenfassung

Dieses Experiment untersucht Veränderungen in der Lipidoxidation und der Fettsäurezusammensetzung in Fleisch (m. *longissimus dorsi*) von Milchlämmern während der Kühllagerung (4 °C) und der Gefrierlagerung (–18 °C). Die Fleischproben wurden nach 0, 1, 3, 5 und 10 Tagen bei gekühlter Lagerung analysiert und nach 15, 30, 90 und 180 Tagen bei Gefrierlagerung analysiert.

Die Kühllagerung beeinflusste nicht die Oxidation der Fleischproben. Eine Zunahme ($P<0.05$) des Niveaus der Lipidoxidation wurde am 180. Tag der Gefrierlagerung im Vergleich zu den anderen Testtagen unter den gleichen Bedingungen beobachtet. Die Entwicklung und das Ausmaß der Lipidoxidation waren intensiver während der gekühlten Lagerung. Die Lipidoxidation blieb auf niedrigem Niveau während des gesamten Zeitraums und für beide Lagerbedingungen.

Das Niveau der gesättigten Fettsäuren erhöhten sich ($P<0.05$) während der gekühlten Lagerung. Die Niveaus der ungesättigte Fettsäuren und der mehrfach ungesättigten Fettsäuren änderten sich nicht während der gekühlten Lagerung. Es gab keine Änderungen in den Niveaus von gesättigten Fettsäuren, einfach gesättigten Fettsäuren und mehrfach ungesättigten Fettsäuren während der Gefrierlagerung

Die Ergebnisse zeigten, dass gekühlte Lagerung und Gefrierlagerung keine nachteilige Auswirkung auf die Lipidoxidationsniveaus und Fettsäurezusammensetzung

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im der Fleisch von Milchlämmern hatte. Die Bewertung des Nährwertes des intramuskulösen Fettes änderten sich nicht während der gekühlten Lagerung und der Gefrierlagerung.

Schlüsselwörter: Fettsäuremuster, Lipidoxidation, Milchlämmer, Lammfleisch, Kühllagerung, Gefrierlagerung

Introduction

Sheep production in the Mediterranean areas plays an important economic, environmental and sociological role (De Rancourt et al., 2006). Many people from rural and underdeveloped areas work in this sector. The production system is not intensive and the employed practices are similar to the ones followed in the organic farming which is very favourable among modern consumers. Consumption of lamb meat is rising in the European countries despite its high price in relation to meat from other species (Boutonnet, 1999). Meat from suckling lambs is a typical product in the Mediterranean countries and it reaches high prices at certain times of the year (Sañudo et al., 2000). Lipid oxidation levels and muscle fatty acid composition are important meat quality parameters concerning consumers. Lipid oxidation is one of the primary causes for deterioration of colour, texture, flavour and nutritional value of meat (Kanner, 1994; Buckley et al., 1995; Gray et al., 1996). Nowadays great attention is also given to the health risks that lipid oxidation might impose. According to Frankel (1984) lipid hydroxyperoxides and their decomposition products may cause damage to proteins, membranes and biological components affecting vital cell functions. Other workers (Liu et al., 1995; Jiménez-Colmenero et al., 2001; Łuczaj and Skrzydlewska, 2003; Ichinose et al. 2004) reported that some of the compounds formed by lipid oxidation are related to mutagenic and carcinogenic effects, and cytotoxic and genotoxic properties. In the recent years, the amount and the type of fat in sheep meat have become important issues for the consumers because they have been implicated in health related concerns. Sheep meat is characterised as having a high fat content and, being high in saturated fatty acids and low in polyunsaturated fatty acid (Enser et al., 1996). These attributes are regarded as disadvantageous for the human diet predisposing humans to obesity, cardiovascular disease, cancer and other life style/chronic diseases (Department of Health, 1994; WHO, 2003; Wood et al., 2004; Sinclair, 2007).

Despite the fact that meat from milk fed lambs is an important food product in the Mediterranean countries, the effect of storage conditions and storage duration on lipid oxidation and fatty acid composition has not been extensively studied.

This experiment aimed to study the changes in the levels of lipid oxidation and the relative changes in the fatty acid composition in terms of saturated, monounsaturated and polyunsaturated fatty acids in meat from milk fed lambs during refrigerated (4 °C) and frozen (–18 °C) storage. Changes in the nutritional profile of the fatty acid composition of the meat during refrigerated and frozen storage were also studied.

Materials and methods

Sampling procedure

Six lamb loins (m. *longissimus dorsi*) from different animals were used. Samples were purchased from a local EU-licensed abattoir. Samples were transported to the laboratory in insulated polystyrene boxes. At 24 h post slaughter loins were trimmed of all visible external/ adjacent fat and connective tissue and subsamples for each test day were prepared. Subsamples aimed for analyses during refrigerated storage were packed in high barrier film (Nylon/Binding layer/L.LDPE 70 µm thickness, oxygen permeability <15.5 cm^3 m^{-2} d^{-1} atm^{-1} at 25 °C/75 % RH) and placed in the dark at 4°C whereas subsamples aimed for analyses during frozen storage were vacuum packed using the same type of high barrier film and placed in the dark at –18 °C. Changes in lipid oxidation levels and fatty acid composition were determined on days 0, 1, 3, 5 and 10 for the refrigerated stored samples and on days 15, 30, 90 and 180 for the frozen stored samples. Samples were analysed in duplicate for lipid oxidation and fatty acid composition.

Lipid oxidation

Lipid oxidation was determined on the basis of the formation of malondialdehyde using a selective third-order derivative spectrophotometric method (Botsolou et al., 1994). Samples were blended in a domestic food processor. Subsamples were homogenised with aqueous trichloroacetic acid in the presence of hexane containing butylated hydroxytoluene and the mixture was centrifuged. The top hexane layer was discarded and an aliquot of the bottom layer was mixed with aqueous 2-thiobarbituric acid. The mixture was incubated for 30 min at 70 °C. After incubation the mixture was cooled in a cold water bath and the absorbance was measured at 521.5 nm against the blank sample using a Shimadzu model UV-1601 (Tokyo, Japan) spectrophotometer. The concentration of malondialdehyde in the analysed samples was calculated on the basis of the height of the third order derivative peak at 521.5 nm by referring to slope and intercept data of the computed least squares fit of a standard calibration curve prepared using 1,1,3,3-tetraethoxypropane. Lipid oxidation is expressed as nanograms of malondialdehyde (MDA) per gram of muscle.

Fatty acid composition

Tissue fat was extracted with petroleum ether in an automated Soxhlet extraction system (Soxtec 2050, Foss, Tecator, Denmark). The fatty acid methyl esters were prepared with boron trifluoride in methanol solution. An appropriate quantity of the extracted fat was saponified by the addition of NaOH in methanol followed by heating at 100 ºC for 15 minutes. Fatty acid methyl ethers were prepared by incuba-

tion at 100 ºC for 5 minutes in boron trifluoride methanol reagent. The produced fatty acid methyl esters were extracted by the addition of hexane after vigorous agitation and washing with a saturated solution of potassium hydroxide (Christie, 2003). The fatty acid methyl esters were analyzed using a HP 5890 (Hewlett-Packard) gas chromatograph equipped with flame ionisation detector and a DB-23 (60 m x 0.25 mm x 0.25 µm) column (J & W Scientific, Inc., Folsom, California, USA). GC (Hewlett-Packard, model 5890) conditions were: carrier gas He; packed mode injection; injector and flame ionisation detector temperatures 250 °C and 280 °C respectively; initial oven temperature 50 °C for 1 min, increased at 25 °C per min to 175 °C, increased at 4 °C per min to 230 °C, held at 230 °C for 5 min.

A 37 component mixture (Supelco, Bellefonte, Pennsylvania, USA) of fatty acids methyl esters (FAME) was used as a reference standard. The mixture was purchased as a 100-mg neat mixture, containing C4–C24 FAMEs (2–4 % relative concentration). Fatty acids methyl esters were identified by comparing their retention times with the retention times of the FAME mixture. Fatty acids were quantified by peak area measurement and the results are expressed as percent (%) of the total fatty acids present in the sample. The reported fatty acids represent over 90 % of the total fatty acids present in the tissues.

Statistical analysis

One-way analysis of variance (ANOVA) was used to determine differences in lipid oxidation levels and fatty acid composition between the different storage periods within the same storage condition. Initial values (storage day 0) were included in the analyses of both storage conditions. Post-hoc analysis was undertaken using Tukey's test at a 5 % level of significance. Statistical software package SPSS version 13.0 (2004) for Windows (SPSS, Chicago, IL, USA) was used.

Results and discussion

Lipid oxidation

Lipid oxidation during refrigerated and frozen storage is presented in Figure 1. During refrigerated storage there were intense but not statistical significant changes in the levels of lipid oxidation. Lipid oxidation levels increased during the storage period as expected, in overall. The highest MDA values were observed on storage day 5. On storage day 10 there was a decrease by 48 % in the MDA values in comparison to the MDA values observed on storage day 5. The decrease in the concentration of MDA may be attributed to the decomposition of MDA by bacteria, such as *Pseudomonas* and *Enterobacteriaceae,* which possess the ability to selectively attack and utilise carbonyl compounds, including MDA (Smith and Alford, 1968; Moerck and Ball, 1974). The reduction of the MDA value may also be related to further oxidation of MDA to other organic products of lipid oxidation such as alcohols and acids which do not react with the thiobarbituric acid (Almandos et al., 1986; Fernández et al., 1997). Rhee et al (1997) also reported that the high populations of microorganisms observed at prolonged storage of air packed beef meat (longer than 8 days at 4 °C) might have utilised high amounts of oxygen causing a decrease on oxygen availability for lipid oxidation. Regarding lipid oxidation during frozen storage, there was a significant increase ($P<0.05$) in the MDA levels on storage day 180 in relation to the concentration of MDA observed during the first 90 days of storage under the same conditions. The concentrations of MDA in the frozen stored samples were lower due to vacuum packaging that limited lipid oxidation. Lipid oxidation increased as the length of frozen storage was extended. Frozen storage in combination with vacuum packaging is very effective in controlling lipid oxidation. According to Buckley et al. (1995) when

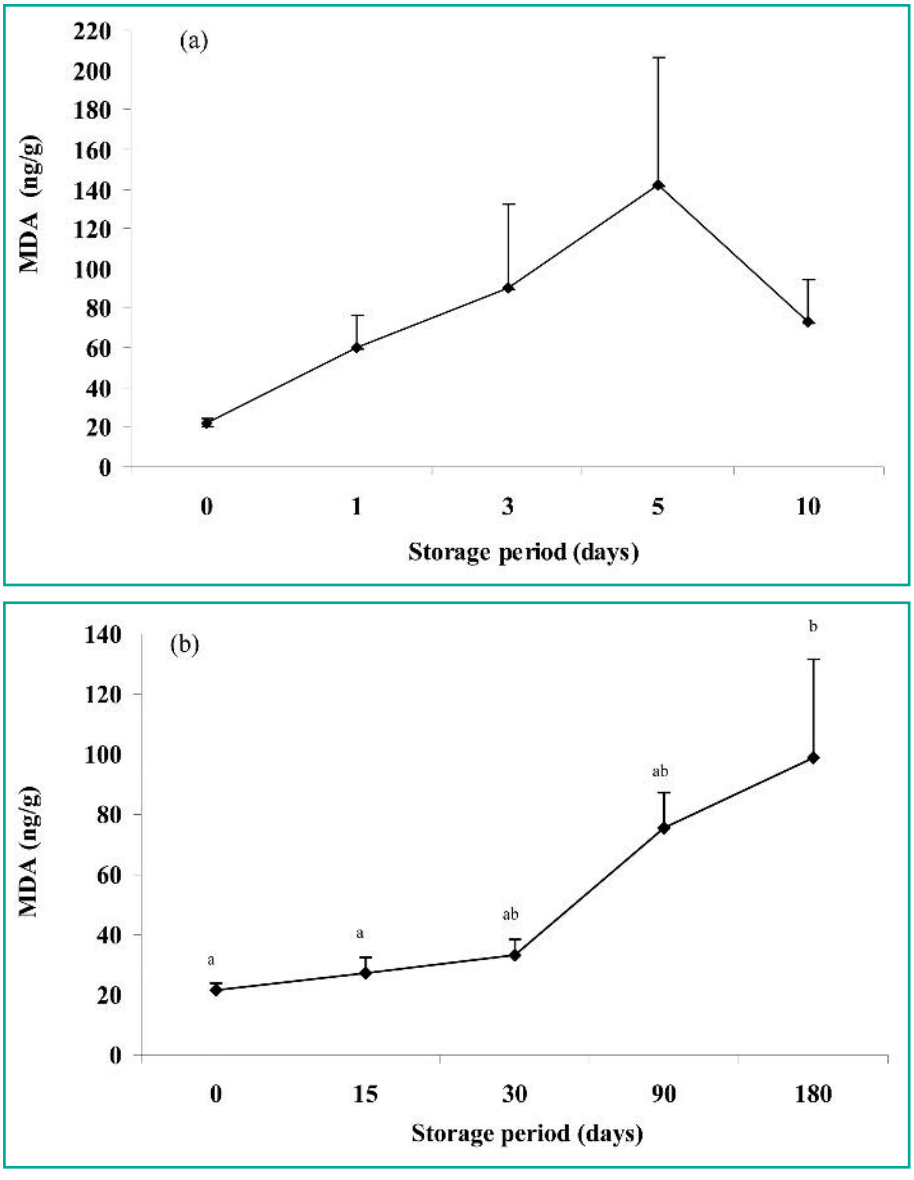

FIGURE 1: *Changes in the levels of malondialdehyde (MDA) in lamb m. longissimus dorsi samples (n=6) during storage at 4 °C (a) and at –18 °C (b). Means with different letters (a and b) are significantly different (P<0.05).*

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muscle foods are stressed as in frozen storage cells are injured favouring oxidation processes. In addition, vacuum packaging does not remove oxygen completely. The low levels of residual oxygen could be enough to cause lipid oxidation (Smiddy et al., 2002). Cifuni et al. (2000) reported marginally lower MDA concentrations in frozen stored (–20 °C for 180 days) legs from Apulian lambs slaughtered at 45 days of age in comparison to our study.

Lipid oxidation was far below the reported threshold values for the detection of rancidity by taste panellists. Camo et al. (2008) reported that TBA values higher than 2 mg MDA/kg muscle are required for the detection of oxidised flavours by trained panellists in lamb meat. Lipid oxidation within the same animal species, though, are influenced by a number of factors such as animal diet, dietary fat content and type, dietary antioxidants, type of muscle, animal age, meat condition i. e. raw, cooked or processed, type of packaging, storage temperature and storage duration, the type of taste panel used for the sensory evaluation of meat i. e. trained or untrained and the method used for the determination of the lipid oxidation levels. Additionally, detection of lipid oxidation is not related to acceptability of the meat by the panellists and the relevant TBA numbers should not be considered as a general reference number (Melton, 1983; Boles and Parish, 1990; Fernández et al., 1997).

The low concentrations of MDA during refrigerated and frozen storage were expected considering the fatty acid composition and particularly the high levels of saturated fatty acids and the low levels of the easily peroxidable polyunsaturated fatty acids (Tab. 1) that are the principal factors affecting the rate and the extent of lipid oxidation (Kanner 1994; Gray et al., 1996).

Fatty acid composition

Fatty acid composition of the *longissimus dorsi* muscle on storage day 0 (control) is shown in Table 1. Saturated fatty acids consisted mainly of palmitic acid (C16:0) and stearic acid (C18:0) and monounsaturated fatty acids consisted mainly of oleic acid (C18:1 n-9). Regarding polyunsaturated fatty acids the only fatty acid identified at detectable levels was linoleic acid (C18:2 n-6). Arsenos et al. (2006) reported similar fatty acid composition in meat from suckling lambs of indigenous dairy Greek breeds of sheep slaughtered at 42 days of age. In this study the percentage of saturated fatty acids was 5 % than the average levels reported by Arsenos et al. (2006). The relatively high proportion of myristic acid (C14:0) as well as the presence of the odd chain fatty acids decapentanoic (C15:0) and heptadecanoic (C17:0) in low concentration levels are attributed to the milk based diet the lambs were fed on and, to the partially functional rumen of young unweaned small ruminants (Rojas et al., 1994, Sañudo et al., 1998). Refrigerated storage did not affect the levels of monounsaturated and polyunsaturated fatty acids whereas there was an increase ($P<0.05$) in the levels of saturated fatty acids (Figure 2). On storage day 10, the levels of the saturated fatty acids increased ($P<0.05$) by 5.7 %, in comparison to the levels observed on day 0, due to an increase ($P<0.05$) in the levels of stearic acid. Some workers (Morcuende et al., 2003; Álvarez et al., 2009) reported also changes in the fatty acid composition of meat during storage due to hydrolytic and oxidative processes. Frozen storage had no impact on the levels of saturated, monounsaturated and polyunsaturated fatty acids (Fig. 2) but there were significant fluctuations ($P<0.05$) in the levels of decapentanoic, palmitic and stearic acids. There is no information reported in the literature regarding changes in the levels of the different fatty acid classes during frozen storage of lamb meat.

TABLE 1: *Fatty acid composition and nutritional value of lamb m. longissimus dorsi samples on storage day 0 (control samples) (n=6).*

	Percent (%) of the total fatty acids present in the sample
Fatty acid	
C 14:0 Myristic	8.09
C 15:0 Decapentanoic	0.37
C 16:0 Palmitic	28.00
C 16:1 n-7 Palmitoleic	2.15
C 17:0 Heptadecanoic	0.96
C 18:0 Stearic	13.88
C 18:1 n-9 Oleic	36.95
C 18:2 n-6 Linoleic	4.45
Nutritional indices	
Fat content (g/100g muscle)	2.30
PUFA:SFA	0.09
(C18:0 + C18:1):C16:0	1.82
∑ Desirable Fatty acids (DFA) (%)	57.43

SFA saturated fatty acids; PUFA polyunsaturated fatty acids;
∑ Desirable Fatty acids (DFA) = C 16:1 n-7 + C 18:1 n-9 + C 18:2 n-6 + C 18:0

Changes in the fatty acid profile during refrigerated and frozen storage may be attributed to the activities of lipolytic enzymes whose stability is affected by both storage temperature (Rhee et al., 1996; Min et al., 2008) and duration (Hernández et al., 1999). Variations of the fat content within the same muscle, as it has been reported by Cameron and Enser (1991) and Lawrie and Ledward (2006) for pork m. *longissimus dorsi,* might also affect the fatty acid composition during storage. It is also noted that changes in the fatty acid composition reflect also individual variations in the animals based in the diets of the ewes. In the present study, samples were randomly collected from the abattoirs and thus there is no available information about the breed and the diet of the ewes.

Nutritional indices

The nutritional value of meat can be evaluated using a number of indices. According to the nutritional guidelines of the Department of Health (1994) the three most important indices are total fat content, the polyunsaturated: saturated (PUFA: SFA) ratio and the n-6: n-3 ratio. As shown in Table 1, the fat content was far below 5 %, a value generally considered to characterize low-fat foods (Enser et al., 1996; Sinclair, 2007). The recommended range for PUFA: SFA ratio is 0.45–1. In the present study, the PUFA: SFA ratio fell well short and outside the suggested range. The PUFA: SFA ratio in ruminant meat is usually around 0.1 (Wood et al. 2004). The low PUFA: SFA ratio in the lean meat can be corrected by the consumption of other food products since the recommended values refer to the diet as whole.

Other nutritional indices such as the concentrations of Desirable Fatty Acids (DFA) and the ratio (C18:0 + C18:1): C16:0 are also used for the evaluation of the effects of meat

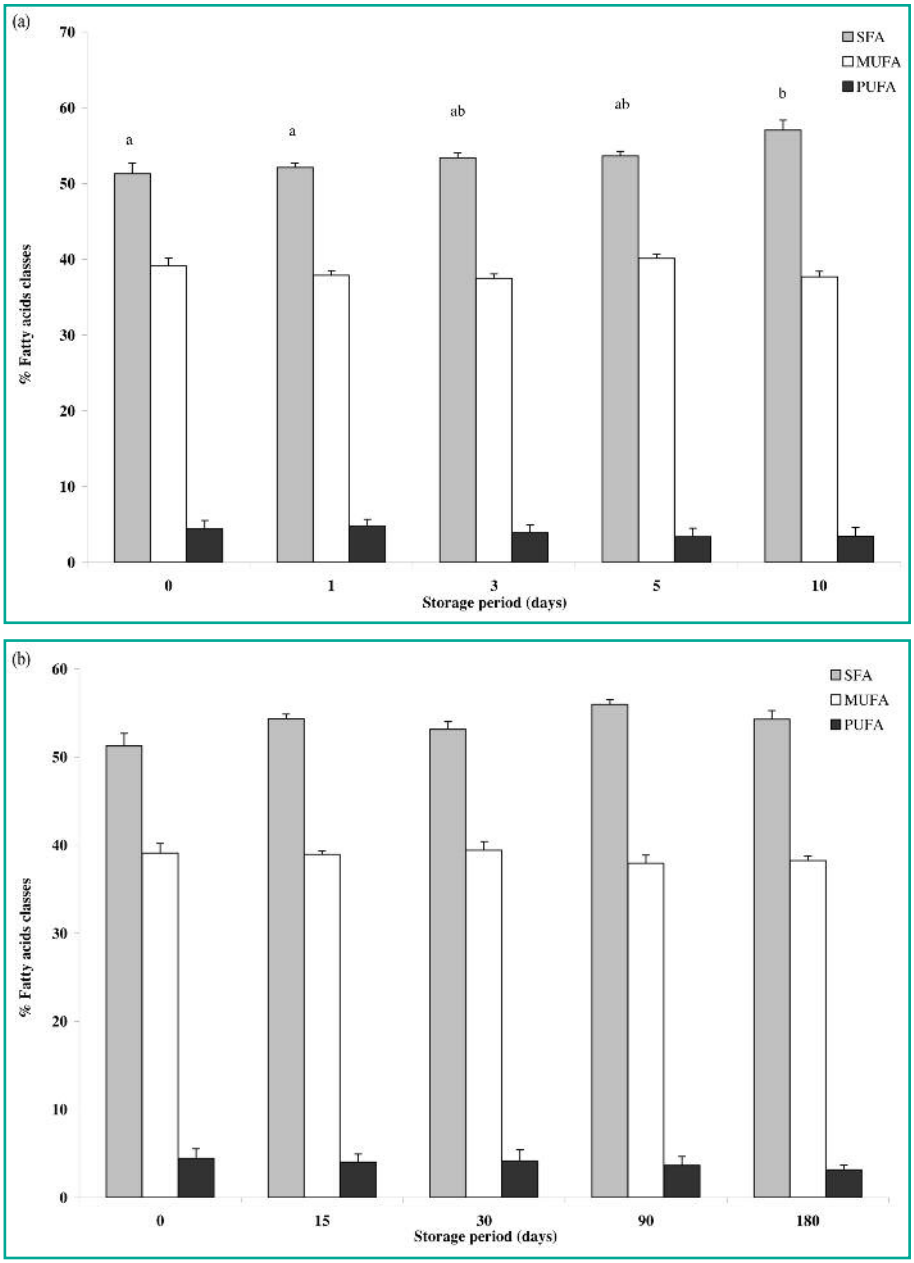

FIGURE 2: *Changes in the percentages of lipid classes during storage at 4 °C (a) and at –18 °C (b) of lamb longissimus dorsi samples (n=6) (SFA saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids). Means with different letters (a and b) within the same lipid class are significantly different (P<0.05).*

lipids on human health. In detail, Rhee (1992) classified types of meat according to their concentrations of DFA and undesirable fatty acids. DFA are the fatty acids considered to have either neutral or cholesterol-lowering effects and undesirable fatty acids are those considered as potentially cholesterol-raising. The DFA correspond to the sum of all unsaturated fatty acids and C18:0. In the present study, the average concentrations of DFA during refrigerated and frozen storage were approximately 58 % and 55 % respectively (data not shown). According to Banskalieva et al. (2000) the DFA concentrations reported in the literature lie in the range of 65–71 % for lamb and sheep meat. The same workers suggested the (C18:0 + C18:1): C16:0 ratio as a better index to describe the possible health effects of meat fatty acids taking into consideration the suggestions of Bonanome and Grundy (1988) that only C16:0 increases blood cholesterol, whereas C18:0 has no effect and C18:1 decreases blood cholesterol content. These fatty acids represent the majority of fatty acids in meat (Valsta et al., 2005). In our study the average (C18:0 + C18:1): C16:0 ratio was higher (1.73) during refrigerated storage in comparison to the average ratio (1.59) observed during frozen storage (data not shown). However, both values were quite lower than those reported (2.13–2.75) in the literature for lamb and sheep meat (Banskalieva et al., 2000).

Conclusions

The concentration of MDA was low during refrigerated storage for 10 days and frozen storage for 180 days. However, the lower MDA concentration on storage day 10, as compared with the MDA concentration on storage day 5, raise questions regarding the microbiological safety of meat from milk fed lambs stored at 4 °C for longer than 5 days. To reach firmer conclusions, a new study with a greater number of samples and a parallel evaluation of the microbiological quality of the meat is needed.

Frozen storage of meat from milk fed lambs does not affect the fatty acid composition of the meat. The nutritional indices regarding healthy fat intake were also not affected during refrigerated and frozen storage of meat. The nutritional quality of the meat from suckling lambs was good and in general agreement to the nutritional guidelines regarding fat consumption.

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