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ORIGINAL PAPER

Stability Profile of Fatty Acids in Yak (*Bos grunniens*) Kidney Fat During the Initial Stages of Autoxidation

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Abstract Previously, we have shown that the fatty acid composition of yak kidney is of reasonable value and is suitable for further development of possible commercial products. Changes in the fatty acids of yak kidney fat during the initial stages of storage have been investigated. The full period of autoxidation was determined by peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS) at 15 ± 1 °C for up to 70 days. The stability profile of the fatty acids identified by gas chromatography demonstrated that saturated fatty acids increased from 49.68 to 55.96% and that polyunsaturated fatty acids and monounsaturated fatty acids decreased from 10.73 to 6.95% and from 37.85 to 28.22%, respectively. Amounts of all the functional fatty acids except conjugated linoleic acid and linoleic acid, started to decrease after 10 days of storage. These results indicated that the initial stage of autoxidation occurred during the first 25 days of storage. It is suggested that development of potential commercial products should be accomplished within ten days, because the functional fatty acids started to decrease after this period of storage. In addition, the good correlation between PV/TBARS values and changes of individual fatty acids could be used as an indicator to monitor the changes of the functional fatty acid during the development process of yak kidney fat-related commercial products.

Keywords Yak · Kidney fat · Fatty acids · Autoxidation · Storage · Stability

Introduction

Fatty acids (FA), including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), for example functional unsaturated fatty acids (FUFA) and nutraceutical fatty acids (n-3 or n-6 PUFA), can be found in many different types of food products and extensive research on the beneficial effects of FA on human health had been conducted [1, 2]. However, if FA autoxidation occurs during processing, circulation, and preservation, it causes deterioration in taste, flavor, odor, color, texture, and appearance of food and generates toxic products such as lipid peroxides, all negatively affecting the nutritional quality and the storage life of food products and consumer acceptance [3, 4].

Yak (*Poephagus grunniens* or *Bos grunniens*) lives in extremely harsh conditions believed to be unpolluted, and yak provides a livelihood for local people. Generally yak kidney fat is simply treated as waste because of the living traditions of local inhabitants and does not contribute to the total economy of the yak. Recently, we provided the first report on the FA profile of yak kidney fat and it turned out that yak kidney fat contains several functional fatty acids and has a fatty acid composition of reasonable value [5].

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Our results indicate that yak kidney fat is suitable for further development of possible commercial products. For this purpose, in this work we determined the stability and changes of the FA composition of yak kidney fat during the autoxidation period. This analysis should be of valuable relevance for FA protection during the development of possible commercial products.

Materials and Methods

Chemicals and Reagents

Normal hexane (chromatography grade), and sodium hydroxide, sodium bisulfate, sodium chloride, trichloromethane, anhydrous sodium sulfate, and anhydrous methanol all of analytical grade were obtained from Tianjin Guangfu Chemical Research Institute (Tianjin, China). Distilled water was used throughout the analysis. Twenty fatty acid methyl ester (FAME) standards were purchased from Nu-chek Prep (Elysian, MN, USA); the purity of all the fatty acids was approximately 98%.

Animals and Region Diet

The yaks grazed on grassland at an average elevation of 3,700 m above sea level in Gannan State, Gansu province, China. They moved to summer grassland (3,700 m above sea level) in June and emigrated to winter grassland 3,000 m above sea level at the end of October.

Gannan grassland in the Qinghai-Tibetan (Qingzang) Plateau is believed to be natural grassland; it has high livestock-carrying capacity and is highly suitable for grazing. The local weather is that of typical continental monsoon climate with an average temperature of 1.7 °C and annual rainfall of 450–900 mm. The relative humidity of the region is 65% on average, and the region enjoys 30–200 frost-free days. The pasture grasses growing in the grassland mainly belong to the sedge family *Gramineae*, typically *Potentilla*, *Lactuca*, *Agrostis*, *Thermopsis*, and *Poa*.

Samples Disposal and Storage Conditions

The yak kidney fat samples were selected randomly from 36 healthy yaks (3 to 5 years old) and were mixed at −4 °C in an icebox. The mixture (1,400 g) was divided into 14 equal aliquots (100 g aliquot^{-1}) and stored for up to 70 days in open containers at 15 ± 1 °C controlled with a precision oven simulating the local weather conditions during the time of slaughter. Every five days one aliquot of the sample (100 g) was taken out and divided further into 15, 15, and 60 g for PV, TBARS and FA analyses, respectively. Each analysis was performed in triplicate.

Peroxide Value

Peroxide value (PV) was measured in accordance with published procedures [6, 7]. Fat (5 g) was dissolved in 30 mL 3:2 (*v*/*v*) acetic acid–chloroform then 0.5 mL saturated KI (freshly prepared) was added. After 1 min standing in the dark, 30 mL distilled H_2O was added and the mixture was titrated with 0.1 N $Na_2S_2O_3$ until the color turned to pale yellow. Starch solution was then added and titration was completed to obtain a colorless mixture. 0.1 N $Na_2S_2O_3$ was used as a blank reference and PV was calculated by use of the equation:

$$\mathrm{PV} = \frac{(\text{sample} - \text{blank}) \times \text{normality of } Na_2S_2O_3 \times 1,000}{\text{weight of sample}}$$

Thiobarbituric Acid-Reactive Substances

Oxidative rancidity of fat was measured by a 2-thiobarbituric acid reactive substances (TBARS) assay of malondialdehyde (MDA) as described by Racanicci et al. [8]. TCA solution (7.5% trichloroacetic acid, 0.1% of EDTA, and 0.1% of propylgallate; 15 mL) was added to 5 g sample and mixed, then centrifuged at 13,500 rpm for 45 s (model 3740, Kubota, Japan) and filtered. Filtered sample (5 mL) was mixed with 5 mL 0.02 M TBA (2-thiobarbituric acid), and the reaction mixture was placed in a water bath at 90 °C for 40 min. Absorbance was measured at 532 and 600 nm using a UV–VIS spectrophotometer (Shimadzu, Japan; model UV-2450) and the differences (A532 nm − A600 nm) were used to correct the absorbance for turbidity. The results were reported as mg MDA/kg sample.

Fatty Acid Methyl Esters Preparation and GC Analysis

Fatty acid methyl esters (FAME) preparation procedure and GC analysis were as described previously [5] and individual FA was identified by comparing retention times with those of FAME standards. Quantification was performed by comparing peak area with that of the internal standard C15:0.

Statistical Analysis

Lipid autoxidation was evaluated periodically. The means were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test, and the statistical significance was given as $P < 0.05$. The Statistical Package for Social Sciences (SPSS, version 13.0; SPSS, Chicago, IL, USA) was used to perform cluster

analysis based on Spearman correlations. The data were entered, verified, and analyzed using SPSS.

Results and Discussion

PV and TBARS Value

In this work, PV and TBARS values were selected to obtain an overview of the autoxidation process of the yak kidney fat.

Peroxide value, the concentration of peroxides and hydroperoxides per kg of fat, is one of the most widely used tests to assess the extent to which spoilage has advanced. As shown in Fig. 1, the PV of the yak kidney fat during storage at 15 ± 1 °C increased continuously up to 35 days representing the earlier stage of lipid oxidation. The flat portion of the curve (1–15 days) prior to the rapid rise (15–35 days) in peroxide concentration is an indication of the induction period (i.e., stability) during the earlier stage. In contrast, after reaching the maximum values (14.9 ± 1.2) after prolonged storage (35 days), PV was observed to decline which would suggest that the later stage of lipid degradation started after 35 days of storage [9].

The data presented here also indicate that although high PV are a definite indication of a rancid fat, moderate values may be the result of depletion of peroxides after reaching high concentrations (Fig. 1). Thus when handling and testing samples by use of PV, care must be taken because PV are not static. Furthermore, the unstable property of KI used for PV analysis may give rise to an inaccurate data. Therefore we also performed the TBARS test, which is also widely accepted as evidence of fat oxidation, because of its distinct benefit of simultaneously measuring several potential lipid oxidation products, thereby providing a comprehensive view of overall lipid oxidation. As shown in Fig. 1, TBARS value increased during the entire period of storage at 15 ± 1 °C, indicating that lipid oxidation occurred from the outset of storage. In agreement with PV, the TBARS value increased only slightly within the first 15 days (induction period); after a moderate increase (15–25 days) ($P < 0.01$), the TBARS increased significantly up to 45 days which suggests that lipid oxidation mainly occurs during this period. The TBARS value between 45 and 50 days ($P > 0.05$) storage did not change, and this could be regarded as a critical point between the metaphase and terminal stages of autoxidation.

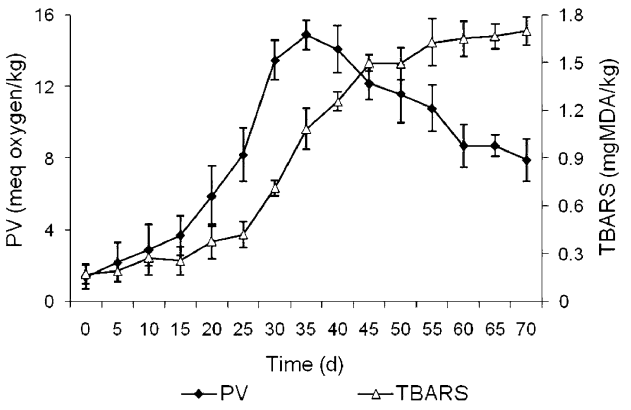

Fig. 1 PV and TBARS values during 0–70 days of storage at 15 ± 1 °C

Together, it can be concluded that the first 25 days storage is the initial stage of autoxidation and the propagation stage occurs between 25 and 45 days storage for yak kidney fat under 15 ± 1 °C storage conditions. The increase of TBARS values after 45 days storage is also in agreement with the decrease of PV (Fig. 1). This indicates that the hydroperoxides produced are probably unstable and highly susceptible to further changes that may form secondary oxidation products in the later stages of lipid oxidation [10–12].

Changes of the FA Content of Yak Kidney Fat During the Initial Stage of Autoxidation

For the development of potential commercial products, it is also necessary to know how individual FA change in order to develop respective strategies to protect the more easily affected FAs. For this purpose, we evaluated the changes of each identified FA in yak kidney fat [5] after different storage times within the initial autoxidation stage (0–25 day); the results were shown in Table 1.

Change of SFA, MUFA and PUFA

As shown in Table 1, the amount of SFA substantially increased from 49.68 to 55.96% because of saturation of MUFA and PUFA, which decreased from 37.85 to 28.22% and from 10.73 to 6.95%, respectively. The increase of SFA was mainly because of the increase of C8:0, C10:0, and C14:0, and no significant variations (0.8%) were observed for C12:0, C16:0, C18:0, and C20:0. This observation may be because the degradation products of long-chain unsaturated fatty acids are aldehydes, ethers, and short-chain fatty acids such as C8:0, C10:0, and C14:0, and the free aldehydes, aliphatic carboxylic acids, and alcoholic aldehydes may also aggregate together to form additional short-chain fatty acids.

Among the two identified MUFA in yak kidney fat, C18:1 decreased significantly ($P < 0.01$) with a value of 8.75% after 25 days of storage. In contrast, the decrease of C16:1 was not prominent (0.85%). PUFA, C20:4 and C22:6 decreased ~1.3%, whereas all the others decreased only slightly (≤0.7%). It is obvious that the percentage of UFA decreased with storage time in this analysis.

Table 1 Changes of the fatty acids of yak kidney fat during 0–25 days of storage at 15 ± 1 °C

Fatty Acid	Time (days) (g per 100 g fat)					
	0	5	10	15	20	25
SFA						
C8:0 (octanoic acid)	1.92 ± 0.24	2.03 ± 0.42	2.35 ± 0.33	2.54 ± 0.39	3.05 ± 0.11	3.58 ± 0.53
C10:0 (capric acid)	1.77 ± 0.52	1.82 ± 0.35	1.91 ± 0.21	2.03 ± 0.25	2.36 ± 0.42	2.87 ± 0.36
C12:0 (lauric acid)	1.15 ± 0.22	1.23 ± 0.43	1.24 ± 0.14	1.35 ± 0.29	1.36 ± 0.32	1.47 ± 0.52
C14:0 (myristic acid)	3.20 ± 0.26	3.28 ± 1.02	3.45 ± 0.50	3.78 ± 0.62	4.32 ± 0.36	4.97 ± 0.43
C18:0 (stearic acid)	20.02 ± 2.45	20.00 ± 0.77	20.46 ± 2.84	20.68 ± 3.15	21.19 ± 1.25	20.83 ± 1.10
C20:0 (arachidic acid)	3.22 ± 0.42	3.31 ± 0.23	3.44 ± 0.28	3.58 ± 0.73	3.65 ± 0.47	3.79 ± 0.63
ΣSFA	49.68 ± 5.22	50.22 ± 5.44	51.52 ± 6.21	52.65 ± 5.57	54.14 ± 3.25	55.96 ± 4.26
MUFA						
C16:1 (palmitoleic acid)	4.55 ± 1.02	4.51 ± 0.78	4.26 ± 2.54	3.96 ± 1.72	3.79 ± 0.84	3.70 ± 0.63
C18:1 (oleic acid)	33.30 ± 2.73	32.82 ± 3.25	30.01 ± 1.57	29.10 ± 2.85	26.91 ± 0.85	24.52 ± 3.22
ΣMUFA	37.85 ± 3.75	37.33 ± 4.03	34.27 ± 4.11	33.06 ± 4.57	30.70 ± 1.69	28.22 ± 3.85
PUFA						
*c*9*t*11C18:2 (conjugated linoleic acid, CLA)	2.94 ± 0.12	2.92 ± 0.31	2.99 ± 0.26	2.96 ± 0.22	2.89 ± 0.17	3.02 ± 0.28
18:2(n-6) (linoleic acid, LA)	1.98 ± 0.18	2.08 ± 0.41	2.02 ± 0.32	2.13 ± 0.35	2.00 ± 0.24	1.66 ± 0.28
C18:3(n-3) (alpha linolenic acid, ALA)	0.37 ± 0.11	0.34 ± 0.20	0.38 ± 0.08	0.31 ± 0.15	0.22 ± 0.07	0.15 ± 0.04
C20:4(n-6) (arachidonic acid, AA or ARA)	2.86 ± 0.26	2.96 ± 0.45	2.80 ± 0.26	2.73 ± 0.30	2.31 ± 0.34	1.55 ± 0.24
C20:5(n-3) (eicosapentaenoic acid, EPA)	1.11 ± 0.22	1.07 ± 0.32	1.09 ± 0.24	0.85 ± 0.09	0.73 ± 0.23	0.42 ± 0.13
C22:6(n-3) (docosahexaenoic acid, DHA)	1.47 ± 0.36	1.43 ± 0.28	1.32 ± 0.22	0.93 ± 0.15	0.51 ± 0.19	0.15 ± 0.08
ΣPUFA	10.73 ± 1.25	10.80 ± 1.97	10.60 ± 1.38	9.91 ± 1.26	8.66 ± 1.24	6.95 ± 1.05

Data are expressed as mean ± standard deviation of three replicates

c9t11C18:2, *cis*-9*trans*-11CLA, *SFA* saturated fatty acids, *MUFA* monounsaturated fatty acids, *PUFA* polyunsaturated fatty acids

The health effects of both MUFA and PUFA are well known [13]. It has been reported that the ratio of PUFA and MUFA to SFA in the diet has an effect on plasma and liver lipid concentrations. The ideal situation to keep low plasma and liver cholesterol is:

1 low MUFA/SFA ratio;
2 the ratio of PUFA/SFA between 0.4 and 1.0;
3 high PUFA/MUFA ratio; and
4 PUFA + MUFA/SFA ratio not to exceed 2 [2, 14].

If development of yak kidney fat into potential food-related products is intended, the above four characteristics would also have to be considered. As shown in Fig. 2, the ratio of MUFA/SFA and UFA (PUFA + MUFA)/SFA decreased whereas the ratio of PUFA/MUFA first increased then decreased with storage time. Most importantly, the ratio did not exceed 2 (≤0.98). These data indicate that it is still possible to maintain a reasonable ratio of saturated to unsaturated FAs in yak kidney fat during the initial stage of autoxidation.

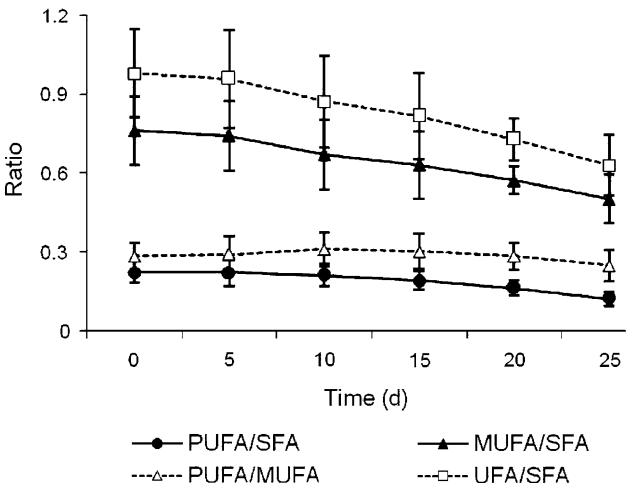

Fig. 2 Changes of MUFA/SFA, PUFA/SFA during 0–25 days of storage

Changes of n-3, n-6 PUFA and the Ratio of n-6/n-3 PUFA

Among the dietary factors implicated in the development of diseases such as coronary heart diseases, n-6 PUFA and n-3 PUFA content and low-dietary n-6/n-3 ratio are well recorded [15–17]. Thus it is interesting in our analysis to point out the changes of these important functional PUFA separately. As shown in Table 1, n-3 and n-6 PUFA were reduced to 2.23 and 1.63%, respectively. The decrease of

n-3 PUFA was more prominent and mainly contributed by the decrease of C20:5n-3 and C22:6n-3 after ten days of storage (0.69 and 1.32%, respectively, $P < 0.01$). The n-6 PUFA, C18:2 started to decrease slightly after 20 days of storage, thus the decrease of n-6 PUFA might be mainly because of the decrease of C20:4 (1.31%, $P < 0.01$) within the initial 25 days of storage.

Both C20:4 and C20:5 contain 20 carbon atoms, but with different numbers of double bonds. Because C20:5 contains one more double bond, one would speculate that the amount of C20:5 would decrease more quickly than that of C20:4, because C20:5 would be more easily prone to oxidation [18]. Furthermore, yak kidney fat contains less C20:5 than C20:4 (Table 1), thus the decrease of C20:5 would be more prominent. To our surprise, however, the decrease of C20:4 was more ($P < 0.05$) than that of C20:5 after 10 days of storage (Table 1). Although further elucidations of mechanism are required to explain this observation, one possible explanation could be that generation of free radicals from the double-bonds occurs simultaneously and randomly regardless of the numbers of double bonds existing in one molecule. If this hypothesis is true, it is interesting to speculate that maybe it is possible to use inexpensive and more abundant sources of less-unsaturated PUFA (for example C20:4) to protect those important more-unsaturated PUFA (for example C20:5) from oxidation for some purposes in the food industry.

Considerable attention has been devoted to conjugated linoleic acid (CLA) one of a group of dietary FA isomers, because of its anticarcinogenic, antidiabetic, antiatherogenic, and immunomodulatory properties [19, 20]. CLA is a major dietary and biologically active isomer [21]. The PUFA identified in yak kidney fat contain 27.4% *c*9*t*11C18:2 (Table 1) [5]. In contrast to reduction of most of the PUFA identified, the *c*9*t*11C18:2 content remained nearly unchanged during the initial stage of oxidation. Considering the decrease of PUFA, the percentage of *c*9*t*11C18:2 in the PUFA increased significantly after 25 days of storage (43.5%). Obviously the storage conditions in this work have little effect on *c*9*t*11 CLA ($P > 0.05$), which is in agreement with the results of Chaijan et al. [22] and Liu et al. [23]. This finding is surprising at first glance—because CLA contains a conjugated double bond it should be easier to oxidize or isomerize [24, 25], thus its level would be expected to be reduced. Although the reason the amount of CLA remains constant in this study is unclear, different authors have reported controversial results regarding CLA content under different storage conditions [23, 26]. Linoleic acid (C18:2) has been found to have low oxidative stability [27], yet the C18:2 (LA) content remained nearly unchanged except after 20 days of storage.

The ratio of n-6/n-3 was practically constant within the first 10 days storage (Table 1), but the ratio increased significantly from 1.73 to 4.46 after 10 days of storage. It has been reported that a high ratio of n-6/n-3 PUFA may promote many kinds of pathogenesis, including cardiovascular disease and cancer [28]. The recommended ratio of n-6/n-3 PUFA should not exceed 4 and a lower number is expected [23, 29]. Thus the development process of potential commercial products from yak kidney fat should not exceed 20 days in order to maintain a reasonable ratio of n-6/n-3 PUFA (Table 1).

Correlations Between the TBARS and PV Values and the UFA Content

During the commercial product development process, it would be more convenient to monitor the stability profile of FAs identified in yak kidney fat by use of the PV and TBARS values as they can be more easily checked. For this purpose, we compared the PV and TBARS values with the stability of the identified FAs. Interestingly, the PV and TBARS values correlate (negatively) well with the stability of most MUFA and PUFA (Table 2). When PV and TBARS values increased, the amounts of C16:1, C18:1, ALA, ARA, EPA, and DHA decreased. Although the changes of CLA and LA were not well indicated by the PV and TBARS value, it was still possible to obtain an approximate indication of the changes of the FA in yak kidney fat during the commercial product development process.

Table 2 Spearman correlation coefficients for TBARS and PV values of unsaturated fatty acids in yak kidney fat during 0–25 days of storage at 15 ± 1 °C

Oxidation	Fatty acid										
Index	C16:1	C18:1	ALA	CLA	LA	ARA	EPA	DHA	SFA	MUFA	PUFA
PV	−0.927**	−0.975**	−0.966**	0.352	−0.746	−0.961**	−0.976**	−0.986**	0.987**	−0.973**	−0.985**
TBARS	−0.932**	−0.981**	−0.891*	0.314	−0.679	−0.905*	−0.899*	−0.948**	0.971**	−0.978**	−0.926*

* $P < 0.05$; ** $P < 0.01$

LA linoleic acid, *ALA* alpha linolenic acid, *CLA* conjugated linoleic acid, *ARA* arachidonic acid, *EPA* eicosapentaenoic acid, *DHA* docosahexaenoic acid

SFA = C8:0 + C10:0 + C12:0 + C14:0 + C16:0 + C18:0 + C20:0. PUFA = c9t11C18:2 + C18:2 + C18:3 + C20:4 + C20:5 + C22:6. MUFA = C16:1 + C18:1

Conclusion

To provide further valuable information for the development of potential yak kidney fat-related commercial products, we assessed the stability profile of the FAs in fat under different storage time conditions. Our results indicate that the initial stage of autoxidation of yak kidney fat, as indicated by PV and TBARS value, occurred during the first 25 days storage. The decrease of n-3PUFA and n-6PUFA correlates well with the increase of PV. This indicates that free radicals generated from the double-bonds of n-3PUFA and n-6PUFA lead to formation of hydroperoxides, which may be further converted into secondary oxidation products, especially aldehydes, in the later stages of lipid oxidation, leading to increased PV.

During food storage, FA may undergo many kinds of reaction, for example oxidation, hydrolysis, polymerization, and cyclization, and many toxic compounds can be formed, reducing food safety and quality. Interestingly, oxidation and the consequent formation of lipid hydroperoxides are found to be related to all of these reactions [30]. Different environmental conditions, for example metal ion species and content, temperature, irradiation, packaging methods, etc. [26, 31–34], all have a significant effect on the autoxidation process of fat. Although further analyses are required to determine the stability of yak kidney fat under these storage conditions, the data presented here indicate that the development of potential commercial products from yak kidney fat must be done within the first 10 days of storage, because the FA, especially the functional essential FAs (e.g. DHA), seem to be stable during this period (Table 1).

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References

1. Spiller GA (2006) Handbook of lipids in human nutrition. CRC Press, Boca Raton
2. Wood JD, Enser M, Fisher AV, Nute GR, Sheard PR, Richardson RI, Hughes SI, Whittington FM (2008) Fat deposition, fatty acid composition and meat quality: a review. Meat Sci 78:343–358
3. Jenschke BE, Hodgen JM, Meisinger JL, Hamling AE, Moss DA, Lundesjö Ahnström M, Eskridge KM, Calkins CR (2007) Unsaturated fatty acids and sodium affect the liver-like off-flavor in cooked beef. J Anim Sci 85:3072–3078
4. Kouba M, Benatmane F, Blochet JE, Mourot J (2008) Effect of a linseed diet on lipid oxidation fatty acid composition of muscle perirenal fat and raw and cooked rabbit meat. Meat Sci 80:829–834
5. Wang Q, Wu J, Zhang S, Zhang Y, Zhang H, Fan E (2009) GC analysis of the fatty acid composition of yak kidney. Chromatographia 69:139–143
6. American Oil Chemists' Society (1998) Official methods and recommended practices of the American Oil Chemists' Society, 5th edn. AOCS Press, Champaign
7. Valsta LM, Tapanainen H, Männistö S (2005) Meat fats in nutrition. Meat Sci 70:525–530
8. Racanicci AMC, Danielsen B, Skibsted LH (2008) Mate (*Ilex paraguariensis*) as a source of water extractable antioxidant for use in chicken meat. Eur Food Res Technol 227:255–260
9. Nawar WW (1996) Lipids. In: Fennema OR (ed) Food chemistry. Plenum Press, New York, p 276
10. Orthoefer FT, Cooper DS (1996) Evaluation of used frying oil. In: Perkin EG, Erickson MD (eds) Deep frying chemistry, nutrition and practical applications. American Chemical Society Press, Washington, pp 285–310
11. Kiokias S, Gordon MH (2003) Antioxidant properties of annatto carotenoids. Food Chem 83:523–529
12. Danowska-oziewicz M, Karpinska-tymoszczyk M (2005) Quality changes in selected frying fats during heating in a model system. J Food Lipids 12:159–168
13. Finley JW, Shahidi F (2001) The chemistry, processing and health Benefits of highly unsaturated fatty acids: an overview. In: John WJ, Shahidi F (eds) Omega-3 fatty acids chemistry nutrition and health effects. American Chemical Society Press, Washington, pp 258–279
14. Chang NW, Huang PC (1998) Effects of the ratio of polyunsaturated and monounsaturated fatty acid to saturated fatty acid on rat plasma and liver lipid concentrations. Lipids 33:481–487
15. Ulbright TLV, Southgate DAT (1991) Coronary heart disease: seven dietary factors. Lancet 338:985–992
16. Riediger ND, Ree M, Cadieux A, Suh M, Moghadasian M (2007) Low dietary n-6/n-3 fatty acid ratio reduce cardiovascular risks in mice regardless of the origin of n-3 fatty acids. FASEB J 21:847.13
17. Riediger ND, Othman R, Fitz E, Pierce GN, Suh M, Moghadasian MH (2008) Low n-6:n-3 fatty acid ratio, with fish- or flaxseed oil, in a high fat diet improves plasma lipids and beneficially alters tissue fatty acid composition in mice. Eur J Nutr 47:153–160
18. Hammer CT, Wills ED (1979) The effect of ionizing radiation on the fatty acid composition of natural fats and on lipid peroxide formation. Int J Radiat Biol 35:323–332
19. Belury MA (2002) Dietary conjugated linoleic acid and health: physiological effects and mechanisms of action. Annu Rev Nutr 22:505–531
20. Yun HS, Do SH, Jeong WI, Yang HJ, Yuan DW, Hong IH, Lee HR, Lee IS, Kim YK, Choi MS, Kim HA, Jeong KS (2008) Cytotoxic effects of the conjugated linoleic acid isomers t10c12, c9t11-CLA and mixed form on rat hepatic stellate cells and CCl4-induced hepatic fibrosis. J Nutr Biochem 19:175–183
21. Chin SF, Liu W, Storkson JM, Ha YL, Pariza MW (1992) Dietary sources of conjugated dienoic isomers of linoleic acid, a newly recognized class of anticarcinogens. J Food Compos Anal 5:185–197
22. Chaijan M, Benjakul S, Visessanguan W, Faustman C (2006) Changes of lipids in sardine (*Sardinella gibbosa*) muscle during iced storage. Food Chem 99:83–91
23. Liu SJ, Bu DP, Wang JQ (2007) Effects of processing temperature and storage time on fatty acid in rich-conjugated linoleic acid milk. Trans Chin Soc Agric Eng 23:266–269
24. Ha YL, Starnson J, Pariza MW (1990) Inhibition of benzo(*a*)-pyrene-induced mouse forestomach neoplasia by conjugated dienoic derivatives of linoleic acid. Cancer Res 50:1097–1101
25. Liu MY, Ren FZ (2006) Study on the oxidative stability of conjugated linoleic acid esters. Food Sci 27:37–40

26. Alfaia CMM, Ribeiro PJLC, Trigo MJP, Alfaia AJI, Castro MLF, Fontes CMGA, Bessa RJB, Prates JAM (2007) Irradiation effect on fatty acid composition and conjugated linoleic acid isomers in frozen lamb meat. Meat Sci 77:689–695
27. Kamal-eldin A, Andersson R (1997) A multivariate study of the correlation between tocopherol content and fatty acid composition in vegetable oils. J Am Oil Chem Soc 74:375–380
28. Simopoulos AP (2004) Omega-6/omega-3 essential fatty acid ratio and chronic diseases. Food Res Int 20:77–90
29. Chen Y-C, Nguyen J, Semmens K, Beamer S, Jaczynski J (2008) Chemical changes in omega-3-enhanced farmed rainbow trout (*Oncorhynchus mykiss*) fillets during abusive-temperature storage. Food Control 19:599–608
30. Gotoh N, Wada S (2006) The importance of peroxide value in assessing food quality and food safety. J Am Oil Chem Soc 83:473–474
31. Eboh L, Mepba HD, Ekpo MB (2006) Heavy metal contaminants and processing effects on the composition, storage stability and fatty acid profiles of five common commercially available fish species in Oron Local Government, Nigeria. Food Chem 97:490–497
32. Chen GQ, Jiang Y, Chen F (2008) Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change. Food Chem 109:88–94
33. Chen YJ, Zhou GH, Zhu XD, Xu XL, Tang XY, Gao F (2007) Effect of low dose gamma irradiation on beef quality and fatty acid composition of beef intramuscular lipid. Meat Sci 75:423–431
34. Bosset JO, Gallmann PU, Sieber R (1994) Influence of light transmittance of packaging materials on the shelf life of milk and dairy products: a review. In: Mathlouthi M (ed) Food packaging and preservation. Plenum Press, New York, pp 222–268