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Unsaturated fat fraction from lard increases the oxidative stability of minced pork

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Abstract

Lard from pork back fat was dry fractionated based on crystallization temperature, resulting in fractions with a ratio of saturated to unsaturated fatty acids of 1.10 and 0.61. Lean minced pork was mixed with the saturated and unsaturated fat fraction and stored in modified atmosphere (80% O_2 and 20% CO_2) at 5 °C for 2, 5, 7, 9, and 12 days under light to investigate the effect on oxidative stability of lipids and proteins. The saturated fat group developed higher TBARS values and lower levels of free thiol groups during storage, indicating that the unsaturated fat fraction in minced pork promoted increased oxidative stability of both lipids and proteins. A higher content of α-tocopherol in the unsaturated fat fraction suggests that the differences in oxidative stability is causatively linked to the balance between the fatty acid composition and content of antioxidants. The TBARS values and free thiol content were negatively correlated, suggesting a relationship between lipid and protein oxidation.

Keywords

TBARS, free thiol group, α-tocopherol, dry fractionation, protein oxidation

1. Introduction

Among the oxidative reactions, lipid oxidation has been recognized as a major cause for reduced quality and acceptability of meat and meat products，resulting in off-flavor development, discoloration, texture deterioration, and loss of nutritional value, originating from a free radical chain reaction, leading to the formation of toxic compounds such as free radicals, fatty acyl hydroperoxides, and aldehydes (Min & Ahn, 2005, Fang, Zhao, Warner & Johnson, 2017). In addition, meat contains high amounts of proteins that also could be the targets for oxidation. Protein oxidation undergoes similar radical reactions to those of lipid oxidation, involving initiation, propagation, and termination stages (Schaich, 2008). These reactions lead to carbonylation of amino acid side chains, loss of free thiol groups, and further to the formation of protein cross-links and aggregation (Lund, Heinonen, Baron, & Estévez, 2011), which could negatively affect the functionality of meat proteins, and therefore result in the loss of meat quality such as tenderness (Bao & Ertbjerg, 2015) and nutritional value (Ferreira, Morcuende, Madruga, Silva, & Estévez, 2018). In meat, proteins and lipids locate closely to each other, and thus it is highly possible that oxidation reactions could be transferred from lipids to meat proteins and vice versa. However, in comparison with lipid oxidation which has been intensively studied, protein oxidation and the relationship between lipid oxidation and protein oxidation in muscle foods still remains to be understood in detail and needs further investigations.

Dry fractionation has been used to process a broad range of edible fats and oils, such as butterfat, palm oil, and lard, into fractions with different chemical compositions and physical properties (Fatouh, Singh, Koehler, Mahran, & Metwally, 2005; Rinovetz et al., 2011; Zaliha, Chong, Cheow, Norizzah, & Kellens, 2004). Fat dry fractionation based on crystallization mainly involve three stages, namely nuclei formation, growth of fat crystals, and separation of solid and liquid phases. This process separates the triglycerides with higher melting point (solid phase) and the triglycerides with lower melting point (liquid phase) by partial crystallization (Bootello, Garcés, Martínez-Force, & Salas, 2011). The solid phase is thereby

enriched with more saturated triglycerides and the liquid phase with more unsaturated triglycerides (Yanty, Marikkar, Che Man, & Long, 2011).

It is widely accepted that unsaturated fatty acids are more prone to oxidation. A large proportion of unsaturated fatty acids thus resulted in lower oxidative stability of lipids in chicken thigh muscle compared to pork and beef (Rhee, Anderson, & Sams, 1996). Accordingly, based on the assumed interactions between lipid and protein oxidation, it can be speculated that an elevated amount of unsaturated triglycerides will accelerate protein oxidation to some extent. Some studies have focused on revealing the relationships between lipid oxidation and protein oxidation by changing the fatty acid composition of muscle foods, however, contradictory results were obtained (Estévez, Ventanas, & Cava, 2007; Fuentes, Estévez, Ventanas, & Ventanas, 2014; Lund, Hviid, Claudi-Magnussen, & Skibsted, 2008). Modified atmosphere packaging (MAP) with high oxygen (70 to 80%) and carbon dioxide (20 to 30%) has become a common practice to keep the color and extend the shelf-life of retail red meat during chill storage (Lund et al., 2008; Spanos, Tørngren, Christensen, & Baron, 2016). However, high oxygen MAP also promotes oxidative processes of lipids and proteins (Bao & Ertbjerg, 2015; Kim, Huff-Lonergan, Sebranek, & Lonergan, 2010; Lund, Hviid, & Skibsted, 2007).

The aims of this study were to fractionate pork back fat into fractions with different compositions and determine their effects on lipid and protein oxidation in minced pork packaged in high oxygen MAP (80% O_2, 20% CO_2) and stored under conditions mimicking retail products. In addition, the relationships between lipid and protein oxidation were also analyzed.

2. Materials and Methods

2.1 Raw materials

Pork back fat and lean meat from *longissimus thoracis et lumborum* (LTL) were obtained 24 h postmortem from a local slaughter house in Finland. In total, back fat

from four and LTL muscles from two different animals were used in sample preparation. The LTL muscles were after arrival trimmed of visible connective tissue and extracellular fat to obtain lean meat blocks (ca. 4 x 4 x 4 cm). The pH values of the muscles were 5.5 - 5.6 at 24 h postmortem. The study was conducted in two independent batches on different days. The same preparation procedures of the raw materials, lard rendering and fractionation, and sample preparation were applied to all samples. In each batch, the back fat from two animals was combined and stored at -20 °C overnight. On the day of use, minced fat and meat were obtained by mincing fat tissues and meat blocks separately through a plate with 3 mm holes in a LM-5P grinder (Koneteollisuus Oy, Klaukkala, Finland).

2.2 Lard rendering and fractionation

Lard rendering is the process to separate pork fat from fat tissues. The obtained minced fat was vacuum-packaged in polyethylene pouches using a vacuum machine (MAX, Helmut Boss Verpackungsmaschinen KG, Bad Homburg, Germany), and heated in water bath at 80 °C. Lard oil was separated from solid impurities by vacuum filtration through a piece of miracloth (EMD Millipore Corp., Billerica, MA, USA) with pore size of 22-25 µm. The lard oil was cooled at room temperature to form solid lard. The obtained lard was stored overnight in a dark cold room at 5 °C.

The lard was separated into fat fractions differing in fatty acid compositions by step-wise dry fractionation using a centrifuge (RC-5C, DuPont Co., DE, USA) (Fig. 1). The lard was melted completely into oil in a water bath at 50 °C and then cooled at room temperature to 31 °C. The oil was then centrifuged at 10,000 g for 1.5 h at 26 °C. The fats with melting point higher than 26 °C started to form fat crystals that were separated from the liquid phase during centrifugation. Two fat layers, namely solid and liquid fraction (26 °C), were obtained after the first centrifugation. The two fractions were stored overnight in the dark at 5 °C. Further fractionation was conducted to widen the differences in the fatty acid compositions of the fat fractions. After heating in a water bath at 50 °C, the solid fraction (26 °C) turned into oil and

was subsequently cooled to 35 °C at room temperature and centrifuged at 10,000 g for 1.5 h at 30 °C. The solid layer was taken as the saturated fat fraction. The liquid fraction (26 °C) was likewise heated to 50 °C, then cooled to 26 °C and centrifuged at 10,000 g for 1.5 h at 21 °C. The liquid layer was taken as the unsaturated fat fraction. Consequently, the solid fraction at 30 °C (saturated fat fraction) and liquid fat fraction at 21 °C (unsaturated fat fraction) were assumed to have the largest differences in their fatty acid compositions among the four resulting fractions (Fig. 1). Accordingly, these two fat fractions were collected and stored in the dark at 5 °C.

2.3 Fatty acid composition

The fatty acid composition of the saturated and unsaturated fat fraction was determined after methylation using a modification of the procedure described by Slover and Lanza (1979). About 50 mg of the fat fraction was placed in a 10 mL Kimax tube. Then, 1 mL of NaOH (0.5 M) in methanol was added into the tube. The tube was closed tightly and heated in boiling water for 5 min. After cooling, 2 mL of 14 % (w/v) BF_3-CH_3OH was added. The tube was heated in the boiling water for another 5 min. After cooling, 5 mL of heptane and 2 mL of saturated NaCl solution was added. The tube was shaken vigorously for 1 min and allowed to stand until the mixture separated into two layers. The upper heptane layer was transferred to a test tube containing anhydrous Na_2SO_4. The test tube was shaken and allowed to stand still for 30 min before transferring the clear heptane sample into vials for gas chromatography (GC) analysis.

Profiling analysis of fatty acid methyl esters was conducted on a 6890N GC-FID gas chromatograph (Agilent, Beijing, China) equipped with an Omegawax 250 fused silica capillary column (30 m × 0.25 mm × 0.25 μm, Supelco Inc., Bellefonte, PA, USA). The initial oven temperature was held at 160 °C for 1 min, raised to 240 °C with a rate of 4 °C/min, and kept for 5 min. The injector and detector temperatures were 240 °C and 260 °C, respectively. Helium was used as carrier gas at a flow rate of 1.1 mL/min. Samples were injected at a split ratio of 1:15. Identification of fatty acid

methyl esters was carried out by comparing their retention times with those of standards (Sigma, USA). Results were expressed as percentage of total fatty acid methyl esters analyzed. The proportions of saturated (SFA), monounsaturated (MUFA), polyunsaturated (PUFA), total unsaturated (TUFA) fatty acids and the ratio of saturated fatty acids to total unsaturated fatty acids (SFA/TUFA) were calculated.

2.4 α-Tocopherol and α-tocotrienol content

α-Tocopherol and α-tocotrienol quantification of the saturated and unsaturated fat fraction was conducted using a normal-phase HPLC with fluorescence detection, following the procedures of Schwartz, Ollilainen, Piironen, and Lampi (2008) with some modifications. Around 100 mg of fat sample was weighed and dissolved in a 10 mL glass volumetric flask containing heptane. The obtained solution was then filtered through a syringe filter (GHP Acrodiscs® 13mm syringe filter, 0.2 µm membrane, Pall Corp., NY, USA) into a HPLC vial. The HPLC system was equipped with a pump (Waters 515, Waters Corp., Milford, MA, USA), an autosampler (Model 717, Waters), and a scanning fluorescence detector (Model 474, Waters). The chromatographic separation was carried out on an Inertsil 5 SI column (5 µm, 250×4.6 mm, Varian Inc., Palo Alto, CA, USA) with a silica precolumn (Guard-Pak Resolve Silica, Waters). The column temperature was maintained at 30 °C. A mobile phase of 3% 1,4-dioxane and 97% *n*-heptane (v/v) at a flow rate of 2 mL/min were used. The excitation wavelength of the fluorescence detector was 290 nm and the emission wavelength 325 nm. α-Tocopherol and α-tocotrienol compounds were further identified by comparing their retention times with those of standards and results were expressed as µg per g of fat.

2.5 Preparation and storage of minced pork

Two groups of minced meat samples with different fatty acid composition were prepared by mixing lean minced meat with the saturated and unsaturated fat fraction. The fat fractions were melted into oil in a water bath at 50 °C, and mixed with lean minced meat to obtain minced meat containing 20% of fractioned fat by weight. The

mixture was reground in the LM-5P grinder with a 3 mm plate to achieve a homogenous mince. The obtained minced meat was then divided into 25 g portions and distributed evenly on plastic trays. Day 0 samples were vacuum-packaged and stored at -60 °C before analysis.

Each tray was packaged individually in modified atmosphere packages consisting of 80% O_2 and 20% CO_2. The packaging was carried out in a Multivac D-8941 (Sepp Haggenmüller GmbH & Co., Wolferschwenden, Germany) with a D-5810 gas controller (Witt-Gasetechnik GmbH & Co KG., Witten, Germany) using PE04 packaging pouches (Flexopack S.A. Plastics Industry, Attiki, Greece) with an oxygen transmission rate of 80 $cm^3/m^2/24h/atm$ at 23 °C and 75% relative humidity. The pouches were cut into a size of 18 × 18 cm giving a headspace to meat volume ratio around 5:1 in the package. All minced meat packages were stored in a walk-in cold room at 5 °C. Tubular fluorescent lamps (Osram L 36W-76 G13 Natura, Osram, Munich, Germany) were installed to illuminate the packages for 12 h every day. The lamps were placed 20 cm above the packages with illumination intensity of approximately 2000 lx.

The two groups of meat samples were stored for 2, 5, 7, 9, 12 days. Packages with each combination of fat fraction and day of storage was prepared in duplicate. On the day of sampling, the minced meat samples were vacuum packaged and stored at -60 °C until analysis.

2.6 Lipid oxidation measurement

Lipid oxidation of minced meat samples (5 g) was evaluated by measuring the content of thiobarbituric acid-reactive substances (TBARS) as described by Bao and Ertbjerg (2015). TBARS values were expressed as mg MDA per kg of minced meat.

2.7 Protein oxidation measurement

Protein oxidation was determined by assessing the loss of free thiol content according

to the method outlined by Ellman (1959) with slight modifications based on Bao, Puolanne, and Ertbjerg (2016). Free thiol gro ups were quantified based on an L-cysteine (0.02 to 0.1 mM) standard curve. The results were expressed as nmol/mg protein.

2.8 Statistical analysis

All analyses were conducted in duplicate. The data was analyzed by IBM SPSS Statistics 24.0 software. All results were reported as mean values of the data from the two batches with standard deviations. The values for TBARS and free thiol content were analyzed by general linear model using storage time and fatty acid composition as fixed factors, and batch as a random factor. Statistical significance was evaluated by Turkey HSD test at a level of $P < 0.05$. An independent T test was used to compare the mean of each sample in the evaluation of fatty acid composition and α-tocopherol content at a significant level of $P < 0.05$.

3. Results

3.1 Fatty acid composition and α-tocopherol content of fat fractions

The step-wise dry fractionation successfully separated lard into fractions with different fatty acid compositions (Table 1). The two analyzed fractions had notable differences ($P < 0.05$) in the proportion of all the fatty acids analyzed except margaric acid (C17:0). The fatty acid profile of the saturated fat fraction was dominated by SFA (50 %), followed by MUFA (35 %). In contrast, in the unsaturated fat fraction MUFA was the most abundant fatty acids (45%), followed by SFA (36 %). PUFA constituted 11 % of the saturated and 14 % of the unsaturated fat fraction. The percentage of stearic acid (C18:0) in the saturated fraction was approximately two-fold greater ($P < 0.001$) than that of the unsaturated fraction, whereas the levels of MUFA and PUFA were lower ($P = 0.001$). As a result, the ratio of SFA to total PUFA was higher ($P < 0.001$) in the saturated fat fraction (1.10) than that in the unsaturated (0.61). In both fractions, the four fatty acids oleic acid (C18:1(n-9)), palmitic acid (C16:0), stearic acid (C18:0), and linoleic acid (C18:2(n-6)) comprised around 90% of the identified fatty acids.

The α-tocopherol and α-tocotrienol content was also affected by the fat fractionation (Table 1). The unsaturated fat fraction contained 44 % greater ($P = 0.01$) amount of α-tocopherol and 25% more ($P < 0.001$) α-tocotrienol than the saturated.

3.2 Lipid oxidation development

The initial TBARS values of minced pork mixed with the fat fractions were around 0.14 mg MDA/kg meat, and did not differ between groups on day 0 and day 2 of storage (Fig. 2). The TBARS values of the saturated group were higher than those of the unsaturated group from day 5 to the end of the storage period ($P < 0.01$). Accordingly, the increase of TBARS was about 50% higher in minced meat from the more saturated group (ΔTBARS-saturated: 3.6 mg MDA/kg meat) than in minced meat from the unsaturated group (ΔTBARS-unsaturated: 2.4 mg MDA/kg meat).

3.3 Protein oxidation development

Loss of free thiol groups appear to be a good marker for protein oxidation in pork (Bao & Ertbjerg, 2015). In the current study, storage time considerably affected the free thiol content of minced pork ($P < 0.01$), decreasing from 63 to 26 nmol/mg protein (58% loss) in the saturated fat group and from 62 to 40 nmol/mg protein (35% loss) in the unsaturated fat group during 12 days of storage (Fig. 3). From day 7 of storage the free thiol content of the group with the unsaturated fat fraction decreased more slowly than that with the saturated fat. As a consequence, the unsaturated group contained a larger amount of free thiols than the saturated group from day 7 of storage ($P < 0.001$), indicating less protein oxidation in the unsaturated fat group at the longer storage times.

4. Discussion

4.1 Fatty acid composition and α-tocopherol content as influenced by dry fractionation

By dry fractionation of lard based on crystallization temperatures of the fatty acids we

here obtained a fat fraction enriched in saturated and another enriched in mono- and polyunsaturated fatty acids similar to what was obtained in other studies (Riemenschneider, Luddy, Swain, and Ault, 1946; Wang and Lin, 1995). Also the vitamin E distribution was greatly influenced by fat fractionation, as the levels of α-tocopherol and α-tocotrienol in the unsaturated fraction was higher than those in the saturated fraction. Therefore, α-tocopherol concentrated in the liquid fraction during centrifugation. In agreement, Fatouh et al. (2005) found that an increase in the melting point of fat fractions was followed by a decrease of the vitamin E content. It is reasonable that as an antioxidant, α-tocopherol locate at where the oxidation deterioration is more likely to happen. The biological function of vitamin E appears to be related to stabilization of PUFA especially in the lipid bilayer and its localization is influenced by interaction with the phospholipid glycerol backbone (Raederstorff, Wyss, Calder, Weber, & Eggersdorfer, 2015). However, α-tocopherol is a lipid-soluble vitamin and can also be stored in body fat reserves. Together with a relative low melting point of 2-4 °C, this may explain why α-tocopherol is distributed more abundantly in the unsaturated fat fraction from lard in the present experiment. However, the mechanism of α-tocopherol distribution during dry fractionation of fat is not well understood, and to our knowledge, the distribution of tocopherols in pork back fat after dry fractionation has not been studied before.

4.2 Effects of fat fractions on lipid and protein oxidation

As expected, the TBARS values increased after 2 days of storage (Fig. 2), indicating a development of oxidative deterioration which occurred in both groups. The TBARS values on day 1 (0.09 mg MDA/kg meat) in the study conducted by Lorenzo, Sineiro, Amado, and Franco (2014) were close to those of day 0 samples in our study, suggesting that the fat fractionation did not induce high oxidative stress on the lipids that were mixed into the minced meat. However, at the end of storage, the TBARS values we observed (saturated group: 3.71 MDA/kg meat; unsaturated group: 2.53 MDA/kg meat) were much higher than the values reported by those authors (0.63 MDA/kg meat), possibly related to differences in factors such as light intensity and fat

content. An increase in the proportion and unsaturation degree of unsaturated fatty acids has been reported to be related to a higher oxidative instability of muscle foods (Ahn, Lutz, & Sim, 1996; Choi et al., 2010; Rhee et al., 1996). On the contrary, we found that the unsaturated group exhibited a higher stability against lipid oxidation than the more saturated group (Fig. 2). Similar results were also observed in meat products made with plant oil, which were explained by the assumption that a large amount of antioxidants, such as α-tocopherol and phenolic compounds in the plant oil inhibited TBARS formation (Fuentes et al., 2014; Rodríguez-Carpena, Morcuende, and Estévez, 2012).

Free thiol groups are sensitive to oxidation, and are believed to be a major target for oxidative damage of proteins (Frederiksen, Lund, Andersen, & Skibsted, 2008). Hence, the loss of free thiol groups has been used as a quantitative assessment method for protein oxidation in muscle foods, such as chicken meat (Soyer et al., 2010), horse mackerel mince (Eymard, Baron, & Jacobsen, 2009) and beef patties (Bao et al., 2016). In agreement, we detected a loss of free thiols in minced pork during storage. Lund et al. (2008) observed that the free thiol content decreased around 37% in pork patties containing 13% fat after 7 days of storage at 4 °C in darkness. In our study the loss of free thiols was up to 60% in the saturated group after 12 days storage, probably due to a combination of light and oxygen creating reactive oxygen species and the relative high fat content. The fat fractions thus had an impact on oxidative deterioration of proteins during prolonged storage, demonstrating that lipid oxidation products are able to react with proteins causing protein oxidation. In agreement with the results on lipid oxidation, the unsaturated fat group of minced pork showed less protein oxidation than the saturated fat. This likely resulted from the different α-tocopherol levels of the pork patties after mixing with the fat fractions.

α-Tocopherol as a free radical scavenger has been reported to have a protective effect against lipid and protein oxidation in tissues and meat products under various experimental conditions (Estévez, Kylli, Puolanne, Kivikari, & Heinonen, 2008; Haak,

Raes, & De Smet, 2009; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004; Pfalzgraf, Frigg, & Steinhart, 1995). The unsaturated fat fraction and consequently also the minced meat with this fraction contained a higher level of α-tocopherol than the saturated in the current study. It is likely that the oxidative stability of not only the lipids but also the proteins was determined by the degree of saturation of the lipids and the presence of antioxidants such as vitamin E. However, the level of antioxidants including α-tocopherol appeared to have a relatively larger effect on the susceptibility to oxidation, as the less saturated minced pork displayed a higher oxidative stability than the more saturated group, despite of their higher unsaturation degree in fatty acid composition.

A negative correlation was observed between TBARS numbers and the amount of thiol groups ($r = -0.92$, $P < 0.001$), demonstrating a relationship between lipid and protein oxidation. Similar correlations between lipid oxidation products and protein oxidation products were also found in fresh meat and meat products (Estévez et al., 2007; Ventanas, Estevez, Tejeda, and Ruiz, 2006). Conversely, in a study by Lund et al. (2008), a higher TBARS level did not lead to a higher level of protein oxidation. These authors speculated that the lipid oxidation occurred faster than protein oxidation, and increased protein oxidation was not observed during 7 days of storage. This is supported by our results as the free thiol content of the two groups differed only after 7 days of storage, whereas significant differences were detected in TBARS values from day 5 of storage. On the other hand, this phenomenon could also result from the possibility that α-tocopherol was more effective to inhibit lipid oxidation than protein oxidation in the present study. In agreement, Haak et al. (2009) found that tocopherol strongly suppressed lipid oxidation, whereas inhibitive effects on protein oxidation only occurred at the end of the display period. Free radicals may therefore first attack phospholipids, whereas protein oxidation can be induced by lipid oxidation products not trapped by α-tocopherol.

5. Conclusion

Saturated and unsaturated fat fractions were obtained through lard rendering and dry fractionation and differed notably in fatty acid composition and α-tocopherol content. Minced pork patties containing more unsaturated fat showed greater stability against both lipid and protein oxidation during storage. The data suggests that in meat products with added fat, an increasing proportion of unsaturated relative to saturated fatty acids may increase the oxidative stability due to the level of antioxidants such as α-tocopherol.

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Figure legends

Fig. 1. Dry fractionation of lard into saturated and unsaturated fat fractions. Heating was in water bath and centrifugation at 10,000 x g for 90 min.

Fig. 2. Thiobarbituric acid–reactive substances (TBARS) of minced pork with more saturated or unsaturated fatty acid composition stored in modified atmosphere (80% O_2, 20% CO_2) at 5 ^{o}C. Means with standard deviation (n=8) are shown.

Fig. 3. Free thiol groups of minced pork with with more saturated or unsaturated fatty acid composition stored in modified atmosphere (80% O_2, 20% CO_2) at 5 ^{o}C. Means with standard deviation (n=8) are shown.

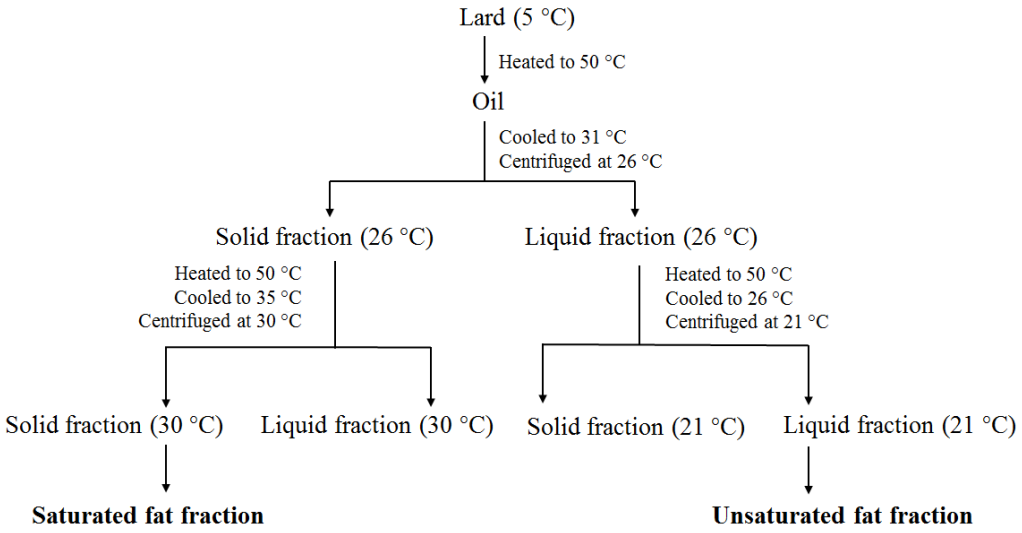

Figure 1

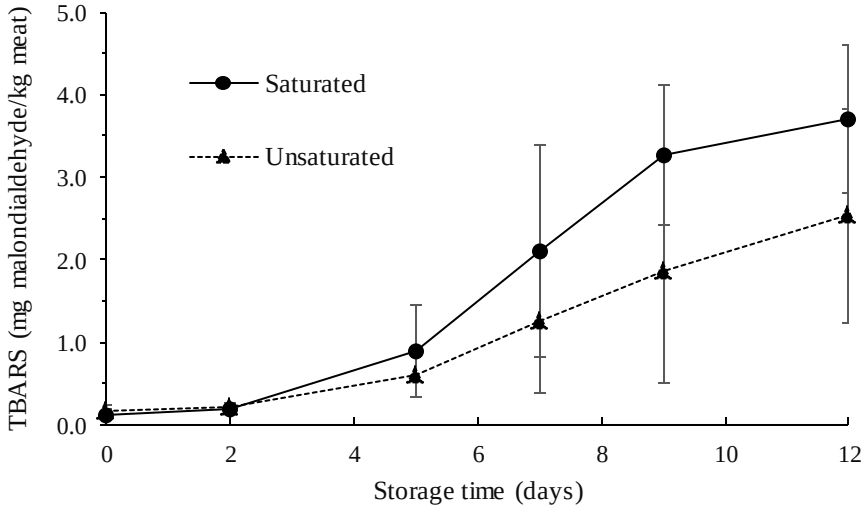

Figure 2

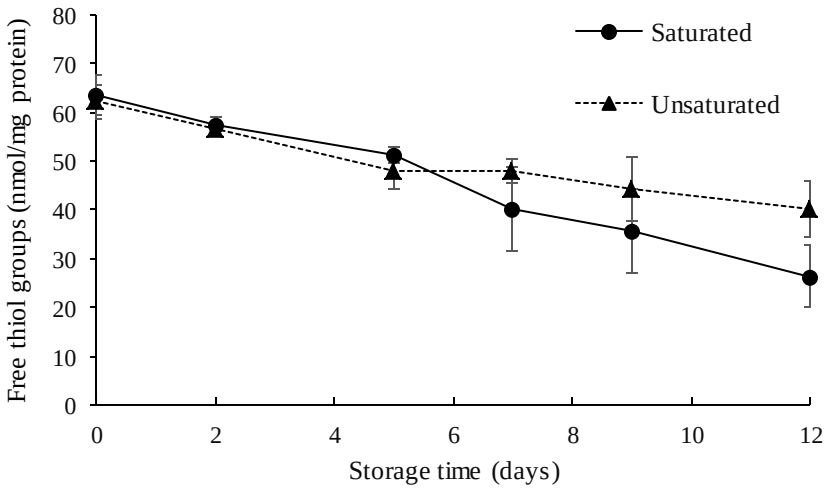

Figure 3

Table 1 Fatty acid composition[a] and α-tocopherol and α-tocotrienol content[b] (means ± SD) of lard fractionated into saturated and unsaturated fat fractions (n=4).

	Saturated	Unsaturated	SEM	*P* value
Fatty acid				
C12:0	0.06 ± 0.01	0.08 ± 0.01	0.01	0.018
C14:0	1.17 ± 0.09	1.49 ± 0.11	0.07	0.005
C16:0	26.86 ± 0.48	23.29 ± 0.40	0.69	0.000
C16:1	1.59 ± 0.03	2.20 ± 0.04	0.12	0.000
C17:0	0.39 ± 0.15	0.33 ± 0.14	0.05	0.641
C18:0	21.57 ± 0.66	10.88 ± 0.28	2.03	0.000
C18:1(n-9)	32.78 ± 0.95	41.64 ± 0.89	1.07	0.000
C18:2(n-6)	9.86 ± 0.65	12.82 ± 0.82	0.61	0.001
C18:3(n-3)	0.87 ± 0.05	1.15 ± 0.10	0.06	0.005
C20:0	0.34 ± 0.04	ND	0.06	0.001
C20:1	0.67 ± 0.02	0.89 ± 0.03	0.04	0.000
C20:4(n-6)	0.21 ± 0.03	0.30 ± 0.05	0.02	0.024
SFA	50.36 ± 1.39	36.08 ± 0.94	2.73	0.000
MUFA	35.04 ± 0.93	44.74 ± 0.82	1.86	0.000
PUFA	10.94 ± 0.65	14.26 ± 0.77	0.67	0.001
TUFA	45.98 ± 1.57	59.00 ± 1.59	2.52	0.000
SFA/TUFA	1.10 ± 0.07	0.61 ± 0.03	0.09	0.000
Others[c]	3.66 ± 0.27	4.92 ± 0.65	0.29	0.024
α-Tocopherol	8.83 ± 1.30	12.73 ± 1.69	0.89	0.011
α-Tocotrienol	2.82 ± 0.16	3.53 ± 0.14	0.14	0.001

SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; TUFA: total unsaturated fatty acids; ND: not detected; SEM: standard error of mean.
[a]Results are expressed as percentage of total fatty acids analyzed.
[b]μg/g fat.
[c]Others are fatty acids that could not be identified.

Highlights:

- Lard from pork back fat was dry fractionated based on crystallization temperature
- Fractions obtained: Ratio of saturated to unsaturated fatty acids of 1.10 and 0.61
- Lean minced pork was mixed with saturated and unsaturated fat fraction and stored
- Saturated fat induced higher TBARS values and lower levels of free thiol groups
- Unsaturated fat fraction enhanced the oxidative stability of lipid and protein