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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.

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 (Fang, Zhao, Warner, & Johnson, 2017; Min & Ahn, 2005). 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, and Estevez, 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

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(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 × 4 × 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 6890 N 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

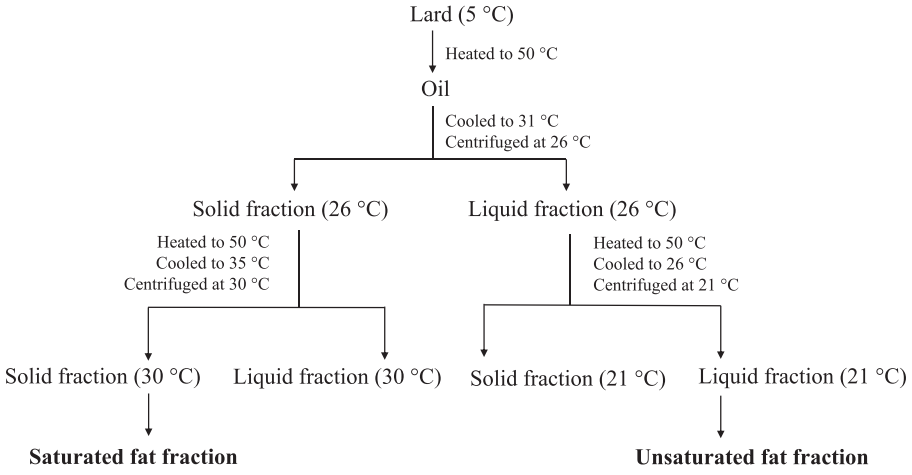

Fig. 1. Dry fractionation of lard into saturated and unsaturated fat fractions. Heating was in water bath and centrifugation at 10,000 ×*g* for 90 min.