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Changes in lipid composition, fatty acid profile and lipid oxidative stability during Cantonese sausage processing

Chaoying Qiu [a], Mouming Zhao [a,b], Weizheng Sun [a,*], Feibai Zhou [a], Chun Cui [a]

[a] College of Light Industry and Food Sciences, South China University of Technology, Guangzhou 510640, China
[b] State Key Laboratory of Pulp and Paper Engineering, South China University of Technology, Guangzhou 510640, China

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ABSTRACT

Lipid composition, fatty acid profile and lipid oxidative stability were evaluated during Cantonese sausage processing. Free fatty acids increased with concomitant decrease of phospholipids. Total content of free fatty acids at 72 h in muscle and adipose tissue was 7.341 mg/g and 3.067 mg/g, respectively. Total amount of saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA, and PUFA) in neutral lipid exhibited a little change during processing, while the proportion of PUFA significantly decreased in the PL fraction. The main triacylglycerols were POO + SLO + OOO, PSO (P = palmitic acid, O = oleic acid, L = linoleic acid, S = stearic acid), and a preferential hydrolysis of palmitic, oleic and linoleic acid was observed. Phosphatidylcholines (PC) and phosphatidylethanolamines (PE) were the main components of phospholipids and PE exhibited the most significant degradation during processing. Thiobarbituric acid values (TBARS) increased while peroxide values and hexanal contents varied during processing.

1. Introduction

Cantonese sausage is a traditional semi-dry cured meat product in China. It is characterized by a strong flavor intensity (Sun, Cui, Zhao, Zhao, & Yang, 2011). 30% lipids are added to Cantonese sausage, which play an important role in flavor development and thermal treatment results in typical lipolytic processes. During processing, lipids undergo lipolysis controlled by both lipases and phospholipases, releasing free fatty acids. Lipids are also subjected to oxidation, giving rise to numerous volatile compounds which contribute to the sensory traits (Coutron-Gambotti & Gandemer, 1999; Gandemer, 2002).

Lipid composition in adipose tissue and muscle has great impacts on the functionality, oxidative stability and flavor of meat products (Gandemer, 2002; Visessanguan, Benjakul, Riebroy, Yarchai, & Tapingkae, 2006). Both glycerides and phospholipids contribute to FFA generation and phospholipids are the main substrates for lipolysis (Buscailhon, Gandemer, & Monin, 1994), while the high content of glycerides also provide a significant amount of FFAs in muscles (Alasnier, David-Briand, & Gandemer, 2000). The relationship between lipolysis and lipid oxidation is not clear. It was generally considered that lipolysis promotes oxidation, but recently several authors reported that parameters promoting lipolysis have no effect on volatiles arising from lipid oxidation (Fernández, de la Hoz, Díaz, Cambero, & Ordoñez, 1995; Gandemer, 2002). Several workers have investigated the fatty acid composition of the three fractions (glycerides, phospholipids and free fatty acids) of intramuscular fat during the processing of Iberian dry-cured ham and raw ham (Cava et al., 1997; Coutron-Gambotti & Gandemer, 1999; Martín, Córdoba, Ventanas, & Antequera, 1999; Yang et al., 2010). The differences in ripening condition and materials used in Cantonese sausage, might cause lipids to undergo lipolytic or oxidation patterns during processing, which differ from other dry-cured meats or fermented sausages that have been widely studied (Coutron-Gambotti & Gandemer, 1999; Martín et al., 1999). The objective of the present study was therefore to profile the fatty acid composition of the three lipid fractions (neutral lipid, phospholipids and free fatty acids) and the composition of the triacylglycerols and phospholipids from the muscle and adipose tissue of Cantonese sausage during processing. Lipid oxidation was also evaluated.

2. Material and methods

2.1. Samples

Lean pork and pork back fat were obtained from a local commercial abattoir (Zhongshan, China) and the pigs were slaughtered at about 6 months of age following the operating procedures of pig-slaughtering GB/T 17236-2008 (Chinese National Standards). Cantonese sausage was prepared in Jinrong Meat Products Co. Ltd. (Zhongshan, China) according to the following formulation: lean pork (70 g) and back fat (30 g), salt (3.5 g), sugar (12 g), Chinese liquor (4 g), sodium nitrite (0.02 g), and water (20 g). Lean pork was ground through a 5 mm plate and back fat was diced into 6 mm cubes. These raw materials were mixed together using a miniature mixer (CH-10, Zhongnan Pharmaceutical Machinery Factory, Changsha, Hunan, China) and stuffed into

* Corresponding author. Tel./fax: +86 20 87113914.
E-mail address: fewzhsun@scut.edu.cn (W. Sun).

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casings with a diameter of 37 mm and oven-dried for 3 h at 50 °C, followed by a reduction of temperature (to 42 °C to 45 °C), then oven-dried for 69 h. Samples were taken at 0th, 6th, 18th, 36th, 54th and 72nd h and kept at −18 °C for further analyses (samples were analyzed within one week).

2.2. Lipid analysis

2.2.1. Lipid extraction

Muscle and adipose tissue (back fat) from Cantonese sausage were carefully separated and minced in a blender. Lipids were extracted from samples with chloroform:methanol (2:1, v/v) according to Folch, Lees, and Sloane-Stanley (1957). The extracts were dried under vacuum on a rotary evaporator and finished with a nitrogen flow.

2.2.2. Lipids' fractionation and analysis of fatty acid composition by GC–MS

Total lipids were fractionated into neutral lipid, free fatty acids and phospholipids on NH_2-aminopropyl minicolumns (55 μm, 70A, 500 mg/6 mL, Phenomenex, USA) as described by García, Gibert, and Díaz (1994). Free fatty acids were quantified by GC–MS. The amounts of neutral lipid and phospholipids were gravimetrically determined and the results were expressed as percent of total weight. Fatty acid composition of each fraction was determined by gas liquid chromatography of methyl esters prepared according to Xu, Xu, Zhou, Wang, and Li (2008). Free fatty acid content was quantified using heptadecanoic acid as internal standard. The GC–MS system consisted of a Trace Ultra GC (ThermoFinnigan, San Jose, CA, USA), a Trisplus automated sampler and a quadrupole DSQ II MS. GC was performed on a TR-5MS capillary column (30 m × 0.2 mm, 0.25 μm, Thermo, USA). Sample volumes of 1.0 μL were injected with split injection (100:1). Helium was used as the carrier gas at a flow rate of 1.0 mL/min. Injection temperature was 250 °C and the ion source was set at 230 °C. The oven temperature program was as follows: holding time 1 min at 40 °C, and then an increase at 10 °C/min to 150 °C (holding 2 min); an increase of 10 °C/min to 220 °C without holding, and finally an increase of 5 °C/min to 280 °C (holding 3 min). Mass spectra were recorded at three scans per second with a scanning range of 50 to 500 m/z. Chromatograms and mass spectra were evaluated using the Xcalibur™ software bundle version 2.0 (ThermoFinnigan, San Jose, CA, USA). The relative percentages of fatty acids were determined by their peak areas (Gandemer, 2002).

2.2.3. Triacylglycerols' composition identification

The compositions of final samples TAG were determined by reversed-phase high-performance liquid chromatography (RP-HPLC) according to Liu et al. (2012). The chromatographic apparatus consisted of a Waters P600 pump with a quaternary gradient system (Waters, Massachusetts, USA), and a 3300 evaporative light-scattering detector (ELSD, Alltech, USA) with an atmosphere compression pump (Tianjing, China). The chromatographic separation was carried out with a Purospher® 149 STAR RP-18e column (250 × 4.6 mm i.d., particle size 5 μm, Merck, Darmstadt, Germany). Gradient elution was achieved by mobile phases A (acetonitrile:acetic acid = 99.95:0.05, v/v), and B (dichloromethane). The gradient was operated as follows: 0–4 min 100% A; 4–12 min 90% A and 10% B; 12–15 min 70% A and 30% B; 15–19 min 20% A and 80% B; 19–31 min 80% A and 20% B; 31–36 min 90% A and 10% B; 36–39 min 100% A; 39–42 min 100% A. Injection volumes of 10 μL and elution flow-rates of 0.6 mL/min were used in all experiments. The effluent was monitored by the ELSD at an evaporator temperature of 40 °C and flow-rate of the atmosphere set at 1.5 L/min. The components of the samples were identified by HPLC/MS, and the conditions were as described by Zhong et al. (2009).

2.2.4. Phospholipids' identification

Phospholipids' composition was analyzed by HPLC. The chromatographic apparatus consisted of a waters P600 pump with a quaternary gradient system (Waters, Massachusetts, USA). Samples were filtered through a 0.45 μm nylon membrane filter to remove impurities, and 20 μL samples were separated through a LiChrosorb Si-60 5 μm column (250 × 4.6 mm i.d., Grace Division, IL, USA) by HPLC-ELSD. A gradient of solvent A [$CHCl_3$/MeOH/NH_4OH (30%) 80:19.5:0.5, v/v/v], and solvent B [$CHCl_3$/MeOH/H_2O/NH_4OH (30%) 60:34:5.5:0.5, v/v/v] was used. The gradient which started at 100% of A, decreased to 0% A (100% B) in 10 min, and was then held for 15 min; and then reverted back 100% A in 5 min. The flow rate was 1.0 mL/min and the injection loop was 5 μL. A 3300 ELSD detector (Alltech, USA) with an atmosphere compression pump (Tianjing, China) was used with an evaporator temperature of 40 °C and flow-rate of the atmosphere set at 1.5 L/min.

2.2.5. Lipid oxidation measurement

The formation of primary products of lipid oxidation (peroxides) was evaluated on an aliquot of the fat extract according to the method of Low and Ng (1987). The POV was defined as the oxidized potassium iodide content, expressed as meq of hyperoxide per kg of lipid. Thiobarbituric acid values (TBARS) were measured following the extraction method described by Sun et al. (2009). TBARS were expressed as mg of malonaldehyde per kilogram of tissue (mg/kg tissue). Hexanal was extracted using a solid-phase microextraction (SPME) device (Supelco, Bellefone, PA, USA) according to Sun, Zhao, Zhao, Zhao, and Yang (2010), with 75 μm of carboxen/polydimethylsiloxane (CAR/PDMS) fiber. The compounds were separated in a TR-5MS capillary column (30 m × 0.2 mm, 0.25 μm, Thermo, USA). Hexanal was identified by comparing its mass spectra and retention time with that of a standard (Sigma, St. Louis, USA) analyzed under the same conditions.

2.2.6. Statistical analyses

The statistical package SPSS 11.5 (SPSS Inc., Chicago, IL) for one-way ANOVA was used. The Student–Newman–Keuls test was used for comparison of mean values among samples at different times, and to identify significant differences ($P<0.05$) among samples. All the data were expressed as mean ± standard deviation of triplicate determinations.

3. Results and discussion

3.1. Changes in lipid composition

Neutral lipid accounted for 74.66% of the total lipid in muscle and 95.90% in adipose tissue at 0 h (Table 1), followed by phospholipids (PL) and a trace amount of free fatty acids (FFAs). In muscle, the amount of phospholipids and FFAs was negatively correlated ($R = -0.787$, $P<0.01$), suggesting that the hydrolysis of phospholipids mainly took place during processing. In adipose tissue, the correlation of FFAs and PL content was not significant ($P>0.05$). The proportion of phospholipids is 4.00% at 0 h, a little higher than that of Corsican pig (Buscailhon et al., 1994; Gandemer, 2002).

The quantity of FFAs is rather less compared to other dry-fermented sausages or dry-salted hams (Coutron-Gambotti & Gandemer, 1999; Hernandez, Navarro, & Toldra, 1999), which may be accounted for by the short ripening time employed in Cantonese sausage production which caused limited lipolysis. However, this value is similar to that reported by Visessanguan et al. (2006) on Nham with 84 h of fermentation. The increase of FFAs proved that lipolysis occurred during processing though the activity of lipolytic enzymes might reduce as drying progresses due to lower water activity at the later stages of Cantonese sausage production. Intense lipolysis can only be expected when ripening/drying time conditions are mild which allows prolonged enzyme action (Toldrá, 2006).

3.2. Changes of triacylglycerol composition in Cantonese sausage

Changes in individual triacylglycerol proportion during processing are listed in Table 2. POO + SLO + OOO and PSO were the main