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Lipolysis and lipid oxidation during processing of Chinese traditional smoke-cured bacon

Yechuan Huang [a,*], Hongjun Li [b], Tian Huang [a], Feng Li [a], Juan Sun [a]

[a] College of Life Science and Engineering, Southwest University of Science and Technology, Mianyang 621010, PR China
[b] College of Food Science, Southwest University, Chongqing 400715, PR China

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ABSTRACT

Lipolysis and lipid oxidation as well as the relationship between them during processing of Chinese traditional smoke-cured bacon were studied by evaluating the changes in physicochemical parameters, lipase and lipoxygenase (LOX) activities, lipid content, fatty acid composition, peroxide value (POV), and thiobarbituric acid reactive substances (TBARS). Besides phospholipids, triacylglycerols (TAG) were an important source of free fatty acids in bacon, resulting in an increase in free fatty acid content in the mid-late stage of processing, whilst phospholipids hydrolysed intensely in the early stage. Preferential lipolysis was observed for polyunsaturated fatty acids in phospholipids and for linoleic and palmitic acids in TAG. The lipolysis of TAG and phospholipids was independent and catalysed by acid lipase and phospholipase, respectively. ANOVA-partial least squares regression (APLSR) analysis showed that POV and TBARS were poorly related to LOX and closely associated with phospholipid degradation. Therefore, autoxidation may be the main cause of muscle lipid oxidation in smoke-cured bacon, which was promoted by phospholipid hydrolysis.

1. Introduction

Bacon is one of the popular traditional dry-cured meat products in China with a long history (Yu & Sun, 2005). Typical bacon processing generally takes 1–2 months, primarily starting in November and ending in December of the Chinese lunar calendar. The bacon is called 'Larou' in Chinese language. Sichuan bacon, Guangzhou bacon, and Hunan bacon are the three famous bacons in China, amongst which Sichuan bacon is prepared by salting and long-time cold-smoking and is a typical product of Chinese traditional smoke-cured bacon. Most of Sichuan areas are mountainous, with the winter temperature being 0–10 °C, which is very suitable for bacon production. Sichuan bacon is a local specialty with an attractive colour and a unique flavour and is deeply loved by consumers. In addition, it needs no refrigeration during storage or distribution and can be conveniently consumed with different practises such as steaming, stewing, and frying (Xie, Sun, & Wang, 2008).

Intramuscular lipids play an important role in the formation of the final flavour of dry-cured meat products. The first step in the conversion from lipids to flavour compounds is hydrolysis, which gives rise to a considerable increase in the free fatty acid content. Some authors have studied the lipolysis in dry-cured ham (Antequera et al., 1992; Buscailhon, Gandemer, & Monin, 1994; Martín, Córdoba, Ventanas, & Antequera, 1999; Vestergaard, Schivazappa, & Virgili, 2000; Yang, Ma, Qiao, Song, & Du, 2005), dry-cured loin (Hernández, Navarro, & Toldrá, 1999) and dry-cured duck (Xu, Xu, Zhou, Wang, & Li, 2008), and most of them found that phospholipids were the most important fractions in intramuscular lipids because free fatty acids originated mainly from phospholipids. However, Gandemer (2002) believed that the contribution of triacylglycerols (TAG) to free fatty acids could reach from 30% to 50% in the intramuscular lipids during ham processing if the TAG content in ham was high enough. Lipolysis is catalysed by lipases, such as acid lipase, neutral lipase, or phospholipase. A considerable number of researchers (Flores, Aristoy, Antequera, Barat, & Toldrá, 2012; Hernández et al., 1999; Jin et al., 2010; Motilva, Toldrá, & Flores, 1992; Motilva, Toldrá, Nieto, & Flores, 1993; Ripollés, Campagnol, Armenteros, Aristoy, & Toldrá, 2011; Vestergaard et al., 2000) have studied the changes in lipase activities during the processing of dry-cured meat products; however, the results greatly varied with different materials or processing conditions.

The oxidation of free fatty acids is the second step in the conversion from lipids to flavour compounds, which produces numerous different hydroperoxides, in conjunction with many different decomposition pathways involved, leading to a large number of volatile compounds or aroma precursors (Martín et al., 1999; Toldrá, 1998; Yang et al., 2005). The precursors can subsequently react with amino acids, Maillard reaction

* Corresponding author. Tel./fax: +86 816 6089521.
E-mail addresses: hyc2005@sina.com, 55238861@qq.com (Y. Huang).

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intermediates or other compounds to form the characteristic aroma of dry-cured meat products (Shahidi, 2001). Lipid oxidation includes autoxidation and enzymatic oxidation. A few researchers have studied the relationship between them during the processing of dry-cured meat products. Amongst them Zhou and Zhao (2007) reported that autoxidation was the main cause of muscle lipid oxidation in Jinhua ham, whereas Jin et al. (2010) found that lipoxygenase (LOX) facilitated lipid secondary oxidation in unsmoked bacon.

With regard to the relationship between lipolysis and lipid oxidation during the processing of dry-cured meat products, Coutron-Gambotti and Gandemer (1999) and Yang (2005) thought that lipolysis could promote lipid oxidation, whereas Jin et al. (2010) and Gandemer (2002) found that lipolysis was barely related to lipid oxidation.

As far as Chinese traditional smoke-cured bacon is concerned, a few authors (Xie et al., 2008; Yu & Sun, 2005; Yu, Sun, Tian, & Qu, 2008) have reported the volatile flavour compounds in it; however, to the best of our knowledge there is no information available on the lipolysis or lipid oxidation during the processing of it. The processing conditions of Chinese traditional smoke-cured bacon greatly differ from those of dry-cured ham, unsmoked bacon, or other dry-cured meat products. In addition, the materials used for bacon processing in China usually have high intramuscular lipid content. These differences would affect the activities of lipase and LOX in bacon, and further the extent and pathway of lipid breakdown and oxidation, forming the unique flavour of smoke-cured bacon. Therefore, the objective of this study was to investigate the changes in physiochemical parameters (moisture content, salt content, and pH value), lipolysis (lipid content, fatty acid composition, lipase activities), and lipid oxidation [LOX activity, peroxide value (POV), and thiobarbituric acid reactive substances (TBARS) value] indicators during the processing of Sichuan bacon, with particular interest in the relationship between lipolysis and lipid oxidation.

2. Materials and methods

2.1. Processing and sampling of bacon

A total of 30 green belly muscle pieces with an average weight of approximately 800–1000 g from Rongchang (RC) pigs provided by Animal Sciences Academy of Chongqing City were used. RC pig is a Chinese indigenous breed known as 'China Treasure Pig', and has high intramuscular lipid content (Huang, Li, He, Wang, & Qin, 2010). The experimental pigs were bred under the same production system, and slaughtered at their market weight in a local commercial slaughterhouse. The facilities of the slaughterhouse met the requirements of the Institute of Animal Care and Use Committee. 24 h post-mortem, the belly muscles of the carcass were sampled and cut into pieces. Bacon was processed according to the traditional procedures of Sichuan bacon production. First, the green bacons were salted for 3 days with 4% dry salt (g/100 g green bacon) at 0–4 °C, during which 1–2 turnovers were given. After curing, the pieces were washed to remove the excess salt on the surface and then drained for a day at 0–4 °C. Finally, the pieces were intermittently cold-smoked using raw firewood for 30 days until the meat was dried. Smoking was performed in the daytime, with the temperature ranging from 25 to 30 °C, and stopped in the night. The smoking time was approximately 12 h every day. The relative humidity during the processing depended on the climatic conditions.

Six bacon pieces were sampled at each of the five different processing points: green bacon (sample A), end of salting (sample B), and at 10 (sample C), 20 (sample D), and 30 (sample E) days of smoking. Approximately 50 g of samples were sampled from the central fraction of each piece. Immediately after sampling, all the samples were vacuum packed and stored at −80 °C for further analyses.

2.2. Intramuscular lipid content and fatty acid composition analysis

Intramuscular lipids were extracted according to the method of Folch, Lees, and Stanley (1957). The total lipid content was measured by weighing after solvent evaporation. Fractions of intramuscular phospholipids and neutral lipids were prepared with silica cartridges (Sep-Pack, Waters, Milford, MA, USA) using the method of Juaneda and Rocquelin (1985). Neutral lipids were quantified by weighing and phospholipids were quantified by phosphorous determination (Bartlett, 1959). Free fatty acids were purified from neutral lipids using an anionic exchange resin (Amberlyst A26, Sigma Aldrich, St. Louis, MO, USA) as described by Gandemer, Morvan-mahi, Meynier, and Leperq (1991). Free fatty acids were quantified by gas chromatography of their methyl esters using methyl heptadecanoate (Sigma, St. Louis, MO, USA) as the internal standard. TAG were defined as neutral lipids minus free fatty acids (Buscailhon et al., 1994). The total lipids, phospholipids, TGA and free fatty acids were methylated with boron fluoride-methanol (Sigma Aldrich, Buchs, Switzerland) according to the method of Morrison and Smith (1964). The fatty acid methyl esters were analysed using a QP-2010 Gas Chromatograph (Shimadzu, Kyoto, Japan) equipped with a flame ionisation detector and a split injector. One microliter of fatty acid methyl esters was injected in split mode (10:1) onto a Rtx-Wax capillary column (Restek, Bellefonte, PA, USA; 30 m × 0.25 mm id × 0.25 μm film thickness). The temperature of the column was programmed as follows: 3 min at 160 °C, increments of 10 °C/min to 200 °C and maintained at 200 °C for 2 min, then increments of 2 °C/min to 230 °C and maintained at 230 °C for 10 min. The temperature of the injector and the detector was 250 and 280 °C, respectively. The flow rate of the carrier gas (N_2) was 1.5 mL/min. Identification of fatty acids was performed by comparison of the retention times with those of standards (Supelco, Bellefonte, PA, USA). The results are expressed as the percent of the total fatty acid methyl esters present.

2.3. Physiochemical analysis

The moisture content was determined according to ISO 1442:1997(E); the salt content was evaluated as chloride and was assayed according to ISO 1841-1:1996(E). The results of moisture and salt content were both expressed as g per 100 g muscle. For pH determination, 10 g of sample and 10 mL of distilled water were mixed with a FSH-2A homogenizer (Meixiang Instrument Co., Ltd., Shanghai, China) at 15,000 rpm for 30 s at 20 °C, and then pH was determined according to ISO 2917:1999(E).

2.4. Lipases extraction and activities assay

Samples were thawed and minced after removing visible fat and connective tissue. Five grams of minced samples were homogenised (4 × 10 s at 25,000 rpm with ice cooling) in 25 mL of 50 mmol/L phosphate buffer, pH 7.5, containing 5 mmol/L of ethylene glycol-bis(2-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (EGTA) by using a Polytron homogenizer (Kinematica, Luzern, Switzerland). The homogenate was stirred for 30 min and then centrifuged at 10,000*g* for 20 min at 4 °C, following which the supernatants were filtered through glass wool and collected for enzyme assays.

Acid lipase activity was determined according to the method of Vestergaard et al. (2000) with some minor modifications. A portion of muscle extract (0.1 mL) was diluted with 2.8 mL of 0.1 mol/L