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Study on lipolysis-oxidation and volatile flavour compounds of dry-cured goose with different curing salt content during production

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ABSTRACT

The effect of dry-curing salt content (4% low salt (LS), 8% high salt (HS)) on lipolysis, lipid oxidation and volatile compounds in dry-cured goose was investigated in our study. The activities of acid lipase and neutral lipase increased during dry-curing, while phospholipase reached its maximum at the end of marinating. Lipoxygenase (LOX) and thiobarbituric acid reactive substances (TBARS) values increased during dry-curing and marinating then decreased during dry-ripening. Total free fatty acids (TFFA) increased at dry-curing and dry-ripening points and decreased during marinating. Total peak area of lipids derived volatile compounds (TPALDVC) and total peak area increased during entire stages. Compared to LS, HS group has higher lipolytic and LOX activities, TBARS, TFFA, unsaturated fatty acids and TPALDVC. The higher TPALDVC in HS could be attributed to higher lipid hydrolysis and oxidation during processing.

1. Introduction

Dry-cured goose, one of Chinese traditional cured meat products has a special flavour. It was made from fresh goose meat by dry-curing, marinating and air dry-ripening. With a large of production, the goose product has covered many provinces in China. However, to our knowledge, there is still no clear explanation for its flavour composition and the mechanism of accumulation in volatile compounds. Recently, dry-cured goose is still processed in a traditional way with high curing salt content (about 8%) in China. As an important food ingredient, it has been demonstrated that sodium chloride contributes to some properties, especially the flavour of meat products (Ruusunen & Puolanne, 2005). Excessive intake of sodium chloride has been linked to hypertension and the increased risk of cardiovascular disease (MacGregor & De Wardener, 2002). How to produce the product with low sodium chloride content is a new trend in meat industry. As an alternative salt concentration, 4% is studied by many enterprises and factories now; the goose product with low content salt may be widely used in the future. However, the effect of lower curing salt content on the flavour of Chinese traditional dry-cured goose is not very clear.

Previous researches about flavour were focused on raw goose meat (Soncin, Chiesa, Cantoni, & Biondi, 2007) and other dry-cured meat products, such as Nanjing marinated duck (Liu, Xu, & Zhou, 2007), Iberian dry-cured ham (Del Pulgar, García, Reina, & Carrapiso, 2013) and dry-cured sausage (Lorenzo, Montes, Purriños, & Franco, 2012). Soncin et al. (2007) reported that compounds derived from lipid peroxidation were the predominant flavour compounds in raw duck and pork meat, while carbon disulphide and *p*-dichlorobenzene were the predominant flavour compounds in raw goose meat. The free fatty acids produced by lipolysis were the main precursors of volatile compounds; lipid oxidation was demonstrated to be directly related to the formation of meat flavour (Gianelli, Salazar, Mojica, & Friz, 2012). It is meaningful to study the process of lipolysis-oxidation in flavour field. Intensive researches have been developed on the effect of salt content on lipolysis-oxidation and volatile compounds in meat process. An obvious promoting effect on lipolysis of sodium chloride has been reported in Iberian ham (Andres, 2005). Motilva and Toldrá (1993) reported that sodium chloride above 20 g/l could activate acid lipase while inhibit neutral lipase and acid esterase in muscle. The composition of free fatty acids in many meat products during processing has been studied, such as Nanjing salted duck (Wang, Zhu, & Xu, 2009), Parma dry-cured ham

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(Vestergaard, Schivazappa, & Virgili, 2000) and Iberian dry-cured loin (Muriel, Andres, Petron, Antequera, & Ruiz, 2007). Vestergaard et al. (2000) found that the amount of free fatty acids in dry-cured ham was correlated with lipolytic activities. The pro-oxidative effect of sodium chloride has been extensively reported in meat product (Motilva & Toldrá, 1993). However, Andrés, Cava, Ventanas, Muriel, and Ruiz (2004) reported the influence of salt on lipid oxidation was limit in Iberian ham. Wang, Jin, Zhang, Ahn, and Zhang (2012) found high salt content could promote the formation of aldehydes from fatty acids. On the other hand, Corral, Salvador, and Flores (2013) reported that reducing salt in slow fermented sausages had different influences on the generation of different volatile compounds.

Most of researches were focused on the flavour of dry-cured hams or sausages. However, the effect of sodium chloride on dry-cured goose is far from being understood. The objective of the present study was, therefore, to determine the effect of sodium chloride on lipolysis, lipid oxidation and the volatile products of dry-cured goosed during processing.

2. Materials and methods

2.1. Processing of dry-cured goose and sampling

Twenty-four Eastern Zhejiang White Geese of 75 d old with living weight 3411 ± 73 g were purchased from a local processing plant. They were slaughtered in a commercial abattoir. Animals were randomly divided into two equal groups. Geese were dry-cured by two kinds of different salt contents and placed in incubator at 4 °C for 24 h. Two different salt levels were established: one group of 12 geese was dry-cured by adding 4% of salt (w/w) (low salt level (LS)), whereas another group of 12 geese was dry-cured with 8% of salt (w/w) (high salt level (HS)). After finishing the dry-cured procedure, geese were marinated in brine, which was comprised of 26% salt, 0.01% fennel, 0.02% ginger and 0.03% shallot at 4 °C for 24 h. Then, geese were air dry-ripened at 16 °C with the 68% of relative humidity for 7 d. The flowing speed of the air maintained at about 6 m per s. The central parts of biceps femoris muscles were sampled at the different processing points (raw meat, the end of dry-curing, the end of marinating, the 3 d of dry-ripening and the 7 d of dry-ripening) for enzymes, lipid oxidation and volatile compounds analysis. Samples were wrapped in aluminium foil, frozen and stored in liquid nitrogen until analysis.

2.2. Lipases activities determination

2.2.1. Crude lipases solution extraction

Enzyme solution was extracted in liquid nitrogen frozen samples as described by Hernandez, Navarro, and Toldra (1998) with little modification. Two hundred milligrammes of frozen samples were taken from liquid nitrogen, thawed and homogenized on ice in 3 ml lyses buffer comprising of 50 mM phosphate salt (pH 7.5) and 5 mM ethylene glycol-bis-(2-aminoethylether)-N,N,N′,N′-tetraacetic acid. The homogenate was centrifuged at 12,000*g* at 4 °C for 20 min. The concentration of supernatant protein was determined using biuret method. After normalizing, the supernatant was taken as crude lipases solution for further activities determination.

2.2.2. Neutral lipase activity determination

Neutral lipase activity was measured as described by Motilva, Toldrá, and Flores (1992) with little modifications. Ten microlitres of enzyme solution were diluted with 280 μl of 0.22 M Tris/HCl buffer, pH 7.5, containing 0.05% (w/v) Triton X-100. Ten microlitres of 1.0 mM 4-methylumbelliferyl-oleate were added as substrate. After incubation at 37 °C for 30 min, the incubated samples were immediately cooled in ice-water mixture and measured within a minute. Fluorescence was measured with a 96-Well Plate Reader M200 (Tecan, Austria) and the excitation and emission wavelength were 350 and 445 nm, respectively.

2.2.3. Acid lipase activity determination

Acid lipase activity was measured as described by Motilva et al. (1992) with little modifications. Ten microlitres of enzyme solution were diluted with 280 μl reaction buffer comprising of 0.1 M disodium phosphate (pH 5.0), 0.05 M citric acid buffer, 0.05% (w/v) Triton X-100 and 0.8 mg/ml bovine serum albumin. Ten microlitres of 1.0 mM 4-methylumbelliferyl-oleate were added as substrate. The mixture was incubated at 37 °C for 30 min and stopped by adding 10 μl of 1 mol/l HCl. Fluorescence was measured with a 96-Well Plate Reader M200 (Tecan, Austria) and the excitation and emission wavelength were 350 and 445 nm, respectively.

2.2.4. Phospholipase activity determination

Phospholipase activity was assayed by the method of Motilva et al. (1992) with little modifications. Ten microlitres enzymes solution was diluted with 280 μl reaction buffer comprising of 0.1 M disodium phosphate (pH 5.0), 0.05 M citric acid buffer, 150 mM sodium fluoride, 0.05% (w/v) Triton X-100 and 0.8 mg/ml bovine serum albumin. Ten microlitres of 1.0 mM 4-methylumbelliferyl-oleate were added as substrate. The mixture was incubated at 37 °C for 30 min and stopped by adding 10 μl of 1 mol/l HCl. Fluorescence was measured with a 96-Well Plate Reader M200 (Tecan, Austria) and the excitation and emission wavelength were 350 and 445 nm, respectively.

One unit of activity (U) was defined as the amount of enzyme hydrolysing 1 μmol substrate per h at 37 °C. Neutral lipase, acid lipase and phospholipase activities were expressed as units (U) per gram protein.

2.3. Lipoxygenase (LOX) activity determination

LOX extraction was according to the method of Gata, Pinto, and Macias (1996) with little modification. Two hundred milligrammes of frozen samples were taken from liquid nitrogen, thawed and homogenized by a glass homogenizer on ice in 3 ml lyses buffer comprising of 50 mM phosphate salt (pH 7.0), 1 mM dithiothreitol and 1 mM ethylene-bis (oxyethylenenitrilo) tetraacetic acid. The homogenate was centrifuged at 12,000*g* at 4 °C for 30 min; then the supernatant was taken as crude LOX solution for further determination. The concentration of enzyme was detected by biuret method following the standard curve. The substrate solution for the assay of LOX activity was prepared as follows: 140 mg of linoleic acid were added to 5 ml of deoxygenated double-distilled water containing 180 μl Tween 20. The solution was adjusted at pH 9.0 by adding 2 mol/l NaOH until all of linoleic acid was dissolved and the pH remained stable. Finally, the mixture was diluted with distilled water to 50 ml and kept under nitrogen atmosphere. LOX activity was assayed at room temperature by measuring the increase in absorbance at 234 nm for 1 min. The reaction mixture typically contained 40 μl substrate solution, 20 μl of enzymatic solution and 380 μl of 50 mM citrate buffer (pH 5.5). The blank sample contained 400 μl of 50 mM citrate buffer (pH 5.5) and 40 μl substrate solution. After incubation at 37 °C for 1 min, absorbance values at 234 nm were read before and after incubating in a 96-Well Plate Reader M200 (Tecan, Austria). One unit of activity (U) was defined as 1 unit absorbance increased per min at 234 nm. LOX activity was expressed as units (U) per g protein.