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Effects of high pressure treatment on lipolysis-oxidation and volatiles of marinated pork meat in soy sauce

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ABSTRACT

To investigate the effect of high pressure (HP) treatment (150 and 300 MPa for 15 min at 20 °C) on lipolysis-oxidation and volatile profile of marinated pork meat in soy sauce, the changes of lipase, phospholipase and lipoxygenase (LOX) activities, TBARS, free fatty acids and volatiles composition in control and HP treated samples were analyzed. Acid and neutral lipase activities and free fatty acids content decreased, while LOX activity and TBARS increased after HP treatment. Phospholipase had well stability under HP. The levels of volatile compounds from lipid oxidation and brine increased under HP and then contributed 73.16–78.25% of the typical aroma, while volatile compounds from carbohydrate fermentation, especially acetic acid, decreased with the pressure increasing. The decrease of free fatty acids during pressurization was probably attributed to the decline of lipase activity and the increase of LOX activity. These findings indicated that HP (150–300 MPa/15 min) promoted lipid oxidation and the permeation of brine, but inhibited carbohydrate fermentation.

1. Introduction

Marinated pork meat in soy sauce, a popular traditional cured meat product in China, is produced using hind leg meat or belly meat by washing, rubbing salt, marinating with brine (soy sauce and spices), and air dry-ripening. Owing to the unique flavor and attractive red ocher, it is one of four different typical sub-groups cured meat products and is popular with consumers in many south regions of China (Zeng et al., 2016). Aroma, one of the major sensory qualities of cured meat product, is closely related to its volatile composition. Zhang, Jin, Wang, and Zhang (2011) suggested that lipolysis and lipid oxidation taking place through the ripening period contributed mainly to the aroma characteristic and volatile constituent of dry-cured ham. Purrinos, Bermúdez, Franco, Carballo, and Lorenzo (2011) and Lorenzo (2014) postulated that lipolysis played an important role in dry-cured "lacón" and dry-cured foal "cecina" during processing. The free fatty acids derived from lipolysis were the major precursors of volatiles. The volatiles generated by lipid oxidation were usually responsible for the flavor characteristic of dry-cured loin (Pateiro, Franco, Carril, & Lorenzo, 2015), dry-cured Iberian ham (Andrés, Cava, Ventanas, Muriel, & Ruiz, 2004) and dry-cured foal "cecina" (Lorenzo, 2014). Enzyme activity is of the essence in dry-cured meat products for the formation of flavor substances or their precursors (Toldrá & Flores, 1998). Toldra (1998) has summarized that muscle lipases and lipoxygenase (LOX) have key contributions to lipolysis and lipid oxidation during processing of many dry-cured meat products.

High pressure (HP) treatment, a non-thermal technology, is being increasingly used in the meat industry to extend the shelf life of product and improve its quality characteristics (Grossi, Bolumar, Søltoft-Jensen, & Orlien, 2014; Marcos, Kerry, & Mullen, 2010; Wang et al., 2013). Recent studies have been reported on the effect of HP treatment on various quality properties of meat and meat products, such as lipid oxidation, fatty acid composition and volatile compounds (He et al., 2012; Kang et al., 2013; Martínez-Onandi, Rivas-Cañedo, Nuñez, & Picon, 2016; Wang et al., 2013). Yagiz et al. (2009) reported that pressure treatment at 150 MPa increased lipid oxidation in fish meat, but the oxidative stability increased after treatment at 300 MPa. However, McArdle, Marcos, Kerry, and Mullen (2010) observed that the increase of TBARS values in beef was presented at the pressure levels of 300–400 MPa. He et al. (2012) found that free fatty acid content in pork increased after HP treatments of 350 MPa and above but remained almost unchanged under lower pressures. Campus, Flores, Martinez, and Toldrá (2008) reported that pressure treatments (300, 350 and 400 MPa) had hardly any effect on the abundance of volatile compounds derived from lipid oxidation in dry-cured loins. Nevertheless, other researchers proposed that HP treatment could promote volatile formation in dry-cured ham (Rivas-Cañedo, Fernández-García, & Nuñez, 2009). Kang et al. (2013) suggested that the volatile compounds in Korean native black goat meat were affected by high pressure processing. Rivas-Cañedo, Juez-Ojeda, Nuñez, and Fernández-García

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(2011) found that impact of HP treatment on volatile compounds of cured meat product greatly depended on treatment conditions, especially pressure level, and on compositional features of product. Villacís, Rastogi, and Balasubramaniam (2008) reported that HP treatment at 150 MPa enhanced NaCl diffusion to reach the maximum, and HP could be a useful technique for the salting of turkey meat. No study, however, has been done to determine the influence of HP treatment on lipolysis, lipid oxidation and volatiles of marinated pork meat in soy sauce.

Therefore, the aim of this study was to evaluate the effect of HP treatment (150 and 300 MPa) on the volatile flavor (aroma) of marinated pork meat in soy sauce and whether it could promote the diffusion of brine component by investigating lipolytic enzymes and LOX activities, thiobarbituric acid reactive substances (TBARS), free fatty acids and volatiles composition.

2. Materials and methods

2.1. Preparation and pressurization of marinated pork meat in soy sauce samples

A total of 15 raw hind leg muscle pieces with an average weight of approximately 0.8–1 kg from Duroc × Landrace crossbred pigs weighing 80–100 kg were purchased from a local manufacturing plant. The experimental pigs were slaughtered in a commercial abattoir. The hind leg muscles of the carcass were sampled and cut into strips (about 6 cm × 4 cm × 20 cm) after removing subcutaneous fat and connective tissue. Then, the 15 raw strips were washed, rubbed 30 g/kg sodium chloride (w/w) and air-dried for 1 day. Thereafter, the strips were marinated in brine, which was comprised of 150 mL/kg soy sauce (v/w), 15 mL/kg white wine (v/w), 10 g/kg pepper (w/w), 50 g/kg sugar (w/w) and 4 g/kg five-spice powder (w/w)) at 4 °C for 7 days; 2–3 turnovers were given. After marinating, each strip was put in an individual polythene bag (oxygen permeability of 40–50 cm^3/m^2/day at 20 °C), covered with 30 mL brine and vacuum-packed. The vacuum-packed strips were divided into three groups. Each group consisted of 5 strips. One group was not pressurized and served as a control; the remaining two groups were treated at 150 and 300 MPa for 15 min, respectively. Treatments were carried out at 20 °C in a high-pressure equipment (Stansted Fluid Power Ltd., Harlow, England), using water as the pressure-transmitting fluid. This pressure vessel had a 2 L capacity, and the maximum operating pressure of the machine was 900 MPa. In order to minimize adiabatic heating, the increasing rate of pressure was about 100 MPa per min, releasing time was just a few seconds. Pressure treatment time excluded pressure come-up and releasing time. Pressure vessel and transmitting medium (water) were maintained at temperature 20 °C by a constant flow of water through an external jacket. After treatment, the three group samples were removed vacuum-packed bags, air-dried and ripened for 15 days according to the following temperature and humidity procedures in a temperature and humidity-controlled chamber: 40% relative humidity (RH), 15 °C under constant temperature and humidity. The ripe strips samples were cut to small cubes (about 0.5 cm × 0.5 cm × 0.5 cm), packaged with tinfoil and stored at −33 °C prior to analysis. The time of control samples and HP-treated samples held in vacuum-pack bags was the same (1 h). Each treatment was carried out in five replicates; one sample from an individual hind leg muscle equated to one replicate.

2.2. Crude lipases extraction and activities determination

Samples were thawed at 4 °C for 2 h and then minced. Two hundred milligrams of minced samples were homogenized (3 × 10 s at 10,000 rpm with ice cooling) in 3 mL of 50 mM phosphate buffer (pH 7.5), containing 5 mM ethylene glycol-bis-(2-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), using a DY89-I high speed homogenizer (Scientz co., Ningbo, China). Homogenization time was 10 s once. Interval time was 30 s. The number of homogenization was 3 times. The homogenate was centrifuged at 12,000 *g* for 20 min at 4 °C. The supernatant was filtered and used as crude lipases solution for activities determination.

Acid lipase activity was performed as described by Motilva, Toldrá, and Flores (1992) with slight modifications. Ten microlitres of enzyme extract was diluted with 280 μL of 0.1 M disodium phosphate/0.05 M citric acid buffer (pH 5.0), containing 0.05% (w/v) Triton X-100 and 0.8 mg/mL bovine serum albumin (BSA). To the mixture, 10 μL of 1.0 mM 4-methylumbelliferyl-oleate was added as substrate. The mixture was incubated for 30 min at 37 °C and stopped with 10 μL of 1 M HCl. The fluorescence was monitored at λ_{ex} = 350 nm and λ_{em} = 445 nm for measuring the released 4-methylumbelliferone using a 96-Well Plate Reader M200 (Tecan, Austria).

Neutral lipase activity was also determined following the method of Motilva et al. (1992) with slight modifications. Ten microlitres of enzyme extract was diluted with 280 μL of 0.22 M Tris/HCl buffer (pH 7.5), containing 0.05% (w/v) Triton X-100. To the mixture, 10 μL of 1.0 mM 4-methylumbelliferyl-oleate was added as substrate. After incubation for 30 min at 37 °C, the incubated samples were instantly cooled in ice-water mixture and determined within a minute. The wavelengths were λ_{ex} = 350 nm and λ_{em} = 445 nm.

Phospholipase activity was measured by the method of Motilva et al. (1992) with slight modifications. Ten microlitres of enzyme extract was diluted with 280 μL of 0.1 M disodium phosphate/0.05 M citric acid buffer (pH 5.0), containing 150 mM sodium fluorid, 0.05% (w/v) Triton X-100 and 0.8 mg/mL BSA. To the mixture, 10 μL of 1.0 mM 4-methylumbelliferyl-oleate was added as substrate. After incubation for 30 min at 37 °C, the reaction was stopped with 10 μL of 1 M HCl. The fluorescence was determined at λ_{ex} = 350 nm and λ_{em} = 445 nm.

Activities of acid lipase, neutral lipase and phospholipase were calculated following the standard curve. One unit (U) of activity was defined as the amount of enzyme hydrolyzing 1 μmol substrate per hour at 37 °C. Activities were expressed as units (U) per g protein.

2.3. Lipoxygenase (LOX) activity determination

LOX extraction was performed by the method of Gata, Pinto, and Macias (1996) with some modifications. Samples were thawed at 4 °C and then minced. Two hundred milligrams of minced samples were homogenized (3 × 10 s at 10,000 rpm with ice cooling) in 3 mL of 50 mM phosphate buffer (pH 7.0), containing 1 mM dithiothreitol (DTT) and 1 mM ethylenediaminetetra acetic acid (EDTA) with a DY89-I high speed homogenizer (Scientz co., Ningbo, China). The homogenate was centrifuged at 12,000 *g* for 30 min at 4 °C. The supernatant was filtered and used as crude LOX solution for activity determination. Enzyme concentration was determined by biuret method according to the standard curve.

The linoleic acid substrate was prepared as follows: 140 mg of linoleic acid was dissolved in 5 mL of deionized water containing 180 μL Tween 20. The solution was regulated and kept at pH 9.0 by adding 2 M NaOH. Thereafter, the mixture was diluted to 50 mL with deionized water and kept under nitrogen conditions. LOX activity was measured with a 96-Well Plate Reader M200 (Tecan, Austria) at room temperature by determining the increase of absorbance at 234 nm for 1 min. The reaction medium contained 40 μL of linoleic acid substrate, 20 μL of enzymatic solution, and 380 μL of 50 mM citrate buffer (pH 5.5). The blank sample contained 40 μL of linoleic acid substrate and 400 μL of citrate buffer. One unit (U) of LOX activity was defined as 1 unit absorbance increased per minute and per g protein at 234 nm.

2.4. Lipid oxidation analysis

The level of lipid oxidation was assayed by determining thiobarbituric acid reactive substances (TBARS) values. The TBARS values were performed by the method of our previous study (Wang et al., 2016) and expressed as mg of malondialdehyde (MDA) per kg of muscle.