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Effects of high pressure in combination with thermal treatment on lipid hydrolysis and oxidation in pork

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ABSTRACT

For investigating the effects of high pressure in combination with thermal (HP-T) treatment on lipid hydrolysis and oxidation in pork, minced pork was treated with combinations of different pressures (200, 400, and 600 MPa) and temperatures (20 and 50 °C); the content and fatty acid profiles of intramuscular lipids, peroxide value (POV), thiobarbituric acid reactive substances (TBARS), and lipoxygenase (LOX) activity were measured. Treatment of samples at pressures of 400 MPa or above significantly enhanced the uneven hydrolysis of phospholipids, resulting in more polyunsaturated fatty acids (PUFA) in degraded phospholipids or generated free fatty acids (FFA); treatment temperature had a synergistic effect on phospholipid hydrolysis. Both POV and TBARS values increased with the intensity of treatment conditions until they reached the highest value, and then gradually decreased; neither corresponded closely with the changes in LOX activity. ANOVA-partial least squares regression (APLSR) analysis indicated that phospholipid hydrolysis positively correlated with TBARS but weakly correlated with POV. Thus, phospholipid hydrolysis could enhance lipid secondary oxidation, which mainly arising from auto-oxidation.

1. Introduction

Although the application of high pressure (HP) in food processing began more than 100 years ago, this technology did not become far advanced owing to the limitations of instrument and technology at that time. In the 1990s, European countries, the United States, and Japan began to invest large amounts of human and financial resources in research on HP technology, thereafter this technology became significantly important in food industry (Ma, 2004). HP treatment could effectively prolong the shelf life of foods by inhibiting or inactivating harmful bacteria and enzymes at low or normal temperatures. Compared with conventional heat treatment, HP treatment causes much less damages to food nutrition and sensory qualities and maximally preserves color, fragrance, taste, and nutritional components (Cheftel & Culioli, 1997; Torres & Velazquez, 2005). However, HP treatment can also lead to some unfavorable effects on foods, such as acceleration of food lipid oxidation under certain pressures, leading to deterioration in quality of meat-related foods (Ma, Ledward, Zamri, Frazier, & Zhou, 2007).

Most previous studies and applications of HP technology to meat products have been conducted at ambient temperature. However, some pressure-resistant spore bacteria are still stable even at pressure up to 1000 MPa, whereas HP in combination with low temperature (50–60 °C) thermal treatment shows good effects on inhibition of these bacteria. Therefore, most recent applications of HP technology to meat products have been conducted with a combination of HP and thermal treatment (Ma, 2004). Several groups of investigators have studied the role of HP-T treatment on lipid oxidation in beef (Ma et al., 2007; McArdle, Marcos, Kerry, & Mullen, 2010, 2011), lamb (McArdle, Marcos, Mullen, & Kerry, 2013), chicken(Ma et al., 2007), and fish (Mckenna, Nanke, & Olson, 2003), respectively; however, to the best of our knowledge, there has been no study on the effect of HP-T treatment on lipid oxidation in pork. Some researchers have found that HP-T treatment caused no marked change in intramuscular total fatty acid profile in meat (McArdle et al., 2010, 2011, 2013). However, intramuscular lipids are composed of phospholipids, triglycerides, and free fatty acids (FFA), an absence of change in intramuscular total fatty acid composition

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under HP-T treatment does not mean no change in the fatty acid composition of phospholipids, triglycerides, and FFA too. Thus, studies evaluating the effects of HP-T on fatty acid profiles not only of total lipids but also of triglyceride, phospholipid, and FFA in meat are required.

Investigators have studied the causes of lipid oxidation in muscles subjected to pressure. However, the results of these studies were inconsistent and suggested that lipid oxidation in muscles may be associated with the cooperative actions of denatured proteins (Tanaka, Xueyi, Nagashima, & Taguchi, 1991), release of metal ions (Beltran, Pla, Yuste, & Mor-Mur, 2004), changes in enzymatic activities (Huang, He, Li, Li, & Wu, 2012), or damages to cell membranes (Orlien, Hansen, & Skibsted, 2000) under HP. One of the key reasons for this uncertainty is that the relationship between lipid hydrolysis and oxidation under HP has remained unclear because oxidized fatty acids are mainly free fatty acids released from hydrolyzed lipids. Thus, it is important to understand the relationship between lipid hydrolysis and fatty acid oxidation to elucidate the causes of lipid oxidation induced by HP. A number of authors discussed the relationship between lipid hydrolysis and oxidation during dry-cured meat product processing. Some found that lipid hydrolysis could advance lipid oxidation (Coutron-Gambotti & Gandemer, 1999; Yang, 2005), whereas the others thought that lipid hydrolysis was little associated with lipid oxidation (Gandemer, 2002; Jin et al., 2010). On the basis of these studies, we found that phospholipid hydrolysis can induce lipid oxidation during smoke-cured bacon processing, whereas triglyceride not (Huang, Li, Huang, Li, & Sun, 2014). However, to our knowledge, there is no report on the relationship between lipid hydrolysis and oxidation under HP condition.

Therefore, the aim of this work was to evaluate the effects of HP (at 200–600 MPa) in combination with thermal treatment (at 20 and 50°C) on lipid hydrolysis and fatty acid oxidation in pork muscles, with a focus on their relationship.

2. Materials and methods

2.1. Preparation of pork samples

A total of 6 kg longissimus muscles from Rongchang pig weighing about 100 kg were provided by the Animal Sciences Academy of Chongqing City for this study. After 24 h of chilling at 4 °C, the visible fat and connective tissue were removed, following which the samples were vacuum-packed immediately and frozen at −20 °C until use.

2.2. HP-T treatment

Samples were thawed and minced. A total of 4200 g samples were prepared and randomly divided into seven equal groups, with six samples in each group and approximately 100 g for each sample. After being vacuum-packed in polythene bags, six groups of samples were randomly treated with combinations of different pressure levels (200, 400, and 600 MPa) and temperatures (20 and 50 °C) for 20 min in an HPP.L2 high pressure equipment (HTSM Bio-Tech Co., Ltd, Tianjin, China), using bis (2-ethylhexyl) sebacate as the pressure-transmitting medium. The capacity of the pressure vessel was 1 L, with maximum operating pressure and temperature of 800 MPa and 60 °C, respectively. The control group was kept for 20 min under ambient pressure (0.1 MPa). Following treatment, the lipid content, fatty acid profiles, POV, TBARS, and LOX activity in the samples were assayed immediately.

2.3. Intramuscular lipid content and fatty acid profile assay

The intramuscular lipid content and fatty acid composition were determined according to the procedures described in a previous study (Huang et al., 2012). Total intramuscular lipid content was measured by weighing after extraction. Fractions of intramuscular phospholipids and neutral lipids were prepared with silica cartridges (Sep-Pack, Waters, Milford, MA, USA). Neutral lipids were quantified by weighing and phospholipids were quantified by phosphorous determination. FFA were purified from neutral lipids using an anionic exchange resin (Amberlyst A26, Sigma Aldrich, St. Louis, MO, USA), and were quantified by gas chromatography of their methyl esters using methyl heptadecanoate (Sigma, St. Louis, MO, USA) as the internal standard. Triglycerides were defined as neutral lipids minus FFA. The total lipids, phospholipids, triglycerides, and FFA were methylated with boron fluoride–methanol (Sigma Aldrich, Buchs, Switzerland). The fatty acid methyl esters were analyzed with a QP-2010 gas chromatograph (Shimadzu, Kyoto, Japan). Identification of fatty acids was performed by comparison of the retention times with those of standards (Sigma). The contents of fatty acids were determined using area normalization method.

2.4. LOX activity assay

The lipoxygenase (LOX) was extracted using the method described by Jin, Zhang, Yu, Lei, and Wang (2011) with minor modifications. Samples were thawed at 4 °C for 24 h and minced, then 5 g of minced meat was mixed with four-fold volume of 50 mmol/L sodium phosphate buffer (pH 7.4), containing 1 mmol/L dithiothreitol (DTT) and 1 mmol/L ethylenediaminetetraacetic acid (EDTA). The mixture was homogenized using a Polytron homogenizer (Kinematica, Switzerland) for 4 × 10 s at 15,000 r/min under ice water bath. After filtration through four layers of gauze, the homogenate was centrifuged at 10,000 g for 1 h at 4 °C. The supernatant was filtered again through a filter paper and crude LOX solution was obtained.

140 mg of linoleic acid (Sigma) was dissolved in 5 mL of deionized water containing 180 μL of Tween 20. Then the pH of the solution was adjusted to 9.0 using 2 mol/L NaOH until linoleic acid was dissolved and the pH was stable. The solution was topped up to 50 mL with deionized water and stored under a nitrogen atmosphere at 4 °C until use.

0.2 mL of linoleic acid solution was mixed with 2.9 mL of 50 mmol/L citrate buffer (pH 5.5) at 20 °C. 0.1 mL LOX solution was added and mixed after the absorbance of the solution at 234 nm was stable. The increase of the absorbance in 1 min was then determined using a U–3900H spectrophotometer (Hitachi, Japan). The blank sample contained 0.2 mL linoleic acid solution and 3.0 mL citrate buffer. One unit (U) of LOX activity was defined as the amount of enzyme required for an absorption increment of 0.001 per minute and per g protein.

2.5. Lipid oxidation determination

Lipid oxidation was evaluated by measurement of thiobarbituric acid reactive substances (TBARS) number and peroxide value (POV). TBARS was determined using the method described by Siu and Draper (1978) and expressed as milligrams of malondialdehyde (MDA) equivalents per kilogram of muscle. POV was assessed according to AOAC method 965.33 and expressed as milliequivalents of peroxide/kg lipid.