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The effects of modified atmosphere packaging and enzyme inhibitors on protein oxidation of tilapia muscle during iced storage

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ABSTRACT

The objectives of this study were to investigate whether modified atmosphere packaging (MAP) and inhibitors of calpain, caspase-3, and cathepsins can reduce protein oxidation of tilapia muscle during iced storage. Parameters such as protein solubility, total carbonyl and sulfhydryl content as well as electrophoretic pattern of myofibrillar proteins from tilapia muscle were examined. We found that protein solubility, sulfhydryl group contents, and electrophoretic band intensities of myosin heavy chain and actin in MAP samples were significantly higher, whereas carbonyl content was significant lower than those in AP samples ($P < 0.05$). The extent of degradation in myosin heavy chain and actin in muscle tissue stored in AP was higher than that in MAP, suggesting that the latter was a better method to reduce protein oxidation during iced storage. After storage for 14 d with different enzyme inhibitors, carbonyl content, sulfhydryl content, and protein solubility in muscle samples were significantly affected by treatment ($P < 0.05$ for all three measures). The best preservation effect, similar to that of MAP, was achieved by the inclusion of the calpain inhibitor MDL-28170.

1. Introduction

Protein oxidation is a protein modification induced either by direct reactions with reactive oxygen species (ROS) or indirect reactions with secondary by-products of oxidative stress (Zhang, Xiao, & Ahn, 2013). Low oxidation may lead to changes in the structure and function of proteins by making them more prone to hydrolysis, polymerization, or cross-linking (Witko-Sarsat, Gausson, & Descamps-Latscha, 2003). Metabolic and other processes occurring in muscle tissue give rise to oxidative compounds, such as hydroxyl radicals, peroxyl radicals, superoxide anions, hydrogen peroxide, and nitric oxide (Rowe, Maddock, Lonergan, & Huffi-Lonergan, 2004a; Liu et al., 2015). After slaughter, antioxidant defense systems are attenuated and free radicals present in animal tissues increase the degree of lipid and protein oxidation (Morzel, Gatellier, Sayd, Renerre, & Laville, 2006). ROS preferentially attack side chains of amino acid residues, which can lead to carbonyl formation and decreased sulfhydryl content. These reactions, in turn, may cause a loss of protein catalytic activity, abnormal aggregation, cross-linking, fragmentation, and, as a result, lower solubility of the protein (Stadtman & Levine, 2003; Witko-Sarsat et al., 2003). Thus, the content of protein carbonyl and sulfhydryl groups as well as protein solubility are good markers of oxidation. Postmortem processing of meat is generally done to enhance or alter its sensory characteristics, functionality, and/or preservation conditions. Some of these processing manipulations may promote protein oxidation (Soladoye, Juárez, Aalhus, Shand, & Estévez, 2015), which is considered to be a very important factor of meat quality. Protein oxidation is associated not only with the deterioration of meat color and texture but also with the loss of nutrients, such as essential amino acids, and lower protein digestibility (Guyon, Meynier, & de Lamballerie, 2016; Martinaud, Mercier, Marinova, Tassy, & Gatellier, 1997).

The decrease in tenderness and juice loss of fish meat that occur during postmortem storage are often associated with unacceptable taste quality. Protein oxidation affects meat tenderness and water holding capacity (WHC) by two main mechanisms. The first mechanism is a synergistic effect of inhibition of proteolytic

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enzyme activity and oxidative modification of myosin and actin, which may lead to decrease of meat tenderness because the oxidative changes in the structure of myosin and actin reduce their sensitivity to proteolytic enzymes (Carlin, Huff-Lonergan, Rowe, & Lonergan, 2006; Rowe, Maddock, Lonergan, & Huff-Lonergan, 2004b). The second mechanism of protein oxidation action involves changes in the structure of myofibrillar proteins: cross-linking between proteins enhances muscle fiber structure and results in muscle tissue sclerosis and concomitant decrease in tenderness (Lametsch, Lonergan, & Huff-Lonergan, 2008). In addition, limited degradation of cytoskeletal proteins may result in increased shrinking of muscle cells, which ultimately translates into drip loss (Zhang, Lonergan, Gardner, & Huff-Lonergan, 2006). In addition, degradation of key cytoskeletal proteins by endogenous enzymes, such as calpain, caspase-3, and cathepsins, is known to play a key role in meat tenderness decrease and juice loss (Huang, Huang, Xue, Xu, & Zhou, 2011; Chéret, Delbarre-Ladrat, Lamballerie-Anton, & Verrez-Bagnis, 2007). However, it is unclear whether there is a correlation between the activity of endogenous enzymes and protein oxidation.

The application of modified atmosphere retail packaging, in which food does not contact with oxygen, is an effective method of food preservation that inhibits microbial growth (Cyprian et al., 2013). Whether such packaging can reduce protein oxidation of tilapia meat has not been elucidated. In addition, a certain degree of oxidation may favour proteolysis, since increased protein hydrophobicity might facilitate the degradation by proteases (Berardoa, Claeysa, Vossena, Leroyb, & De Smeta, 2015); and it is known that the proteasomal system is the major proteolytic pathway responsible for the removal of oxidized proteins (Breusing & Grune, 2008). Conversely, the effect of inhibitions of enzymes on protein oxidation has not been studied. Therefore, the aims of this study were to investigate whether modified atmosphere packaging (MAP) and the presence of inhibitors-MDL-28170, E-64, and AC-DEVD-CHO can reduce protein oxidation of tilapia muscle during ice storage.

2. Materials and methods

2.1. Tilapia samples

Ten tilapias (weight 650 ± 36 g) were killed by a head blow, filleted, and skinned. Then, fillets of ten different tilapia were bagged individually in polyethylene bags with a modified atmosphere (70% N_2, 30% CO_2) created by a Map-D400 gas mixer (Senrui, Suzhou, China). Another ten fillets were also bagged individually in polyethylene bags containing normal atmosphere. All bags were kept in ice for 3, 7, 11, or 14 d.

For experiments involving enzyme inhibitors another ten tilapias (weight 670 ± 42 g) were killed by a head blow. Subsequently, approximately 30 g of muscle tissue was frozen rapidly in liquid nitrogen as “0 d” samples. The remaining muscles were subdivided into four fractions and soaked in one of the following four buffers at 1:1 ratio (meat/buffer, w/v):

(1) 0.1 mol/L NaCl and 2 mmol/L NaN_3 (control);
(2) control + 1×10^{-4} mol/L MDL-28170 (ENZO Life Sciences; Switzerland);
(3) control + 1×10^{-4} mol/L E-64 (MCE, USA);
(4) control + 1×10^{-4} mol/L AC-DEVD-CHO (ENZO Life Sciences, Switzerland).

The samples were bagged in individual polyethylene bags and kept for 1, 3, 7, 11, or 14 d on ice. At the end of each storage period, the samples were retrieved individually and frozen at −80 °C until further analysis.

2.2. Protein solubility

Protein solubility of muscle was evaluated according to the method of Eymard, Baron, and Jacobsen (2009) with slight modification. Five hundred milligram of fish meat was homogenized in 10 mL of 0.05 mol/L Tris−HCl (containing 0.6 mol/L KCl, pH 7.4) for 1 min. The mixture was centrifuged at 16,600×*g* for 15 min at 4 °C. The supernatant was diluted tenfold with 0.6 mol/L KCl and the protein concentration was determined using a BCA kit (Jiancheng Co., Ltd, Nanjing, China). Protein solubility was expressed in mg of soluble protein per 1 g of meat.

2.3. Determination of sulfhydryl group content

5,5′ -dithio-bis (2-nitrobenzoic acid) was used to determined the total sulfhydryl group content according to Eymard et al. (2009) with some modifications. Five hundred milligrams of the sample was homogenized in 4.5 mL of normal saline (0.86 g/100 mL) for 1min. Subsequently, the mixture was centrifuged at 600×*g* for 15 min at 4 °C. Sulfhydryl group content was detected by using a Total mercapto (-SH) measurement kit (Jiancheng Co., Ltd, Nanjing, China) according to the manufacturer's instructions. The optical density was measured using a Synergy H1 Microplate reader (BioTek, USA) at 405 nm. Total protein content was detected according to the instructions of the Bradford kit (Jiancheng Co., Ltd, Nanjing, China) and measured by a Synergy H1 Microplate reader (BioTek, USA) at 562 nm. The results were expressed in mmoles of SH per 1 mg of total protein.

2.4. Determination of total protein carbonyl content

Protein carbonyl content was measured as described by Oliver, Ahn, Moerman, Goldstein, and Stadtman (1987) with some modifications. One milliliter of protein-containing solution (5 mg/mL) was added to1mL of 0.01 mol/L 2,4-dinitrophenylhydrazine solution in 2 mol/L HCl and incubated in a water bath for 30 min in the dark at 37 °C. Then, 3 mL of 20 g/100 mL trichloroacetic acid was added to the mixture, which was centrifuged at 8500×*g* for 5 min. The supernatant was removed and the pellet was dissolved in a 1:1 mixture of ethyl acetate and ethanol six times. Finally, the pellet was dissolve in 5 mL of 6 mol/L guanidine hydrochloride solution, incubated in a water bath for 15 min at 37 °C, and centrifuged at 8500×*g* for 10 min. Optical density of the supernatant was measured by a Synergy H1 Microplate reader (BioTek, USA) at 370 nm. Results were expressed in nano-moles of carbonyl per 1 mg of total protein.

2.5. Sodium dodecyl sulfate polyacrylamide gel electrophoresis

Muscle protein extraction and preparation of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) samples and gels were carried out as described by Subbaiah et al. (2015) with some modifications. Five hundred milligrams of minced fish meat was homogenized for 2 min in 10 vol of extraction buffer A (0.05 mol/L PBS, pH 7.4). The mixture was centrifuged (8500×*g* 10 min, 4 °C), the pellet was retained, resuspended in another 10 vol of extraction buffer A, the above steps were repeated. At the end, the pellet was retained and resuspended in 10 vol of extraction buffer B (0.1 mol/L PBS containing 1.1 mol/L NaCl, pH 7.4). Finally, the suspension was centrifuged (8500×*g*, 10 min, 4 °C) and the supernatant was retained. The content of myofibrillar protein was detected according to the instructions of the Bradford kit (Jiancheng Co., Ltd, Nanjing, China) and measured by a UV3000 spectrophotometer (Mapada, Shanghai, China) at 595 nm. Myofibrillar protein was mixed in a loading buffer (5 ×) containing 1.5 mol/L