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Pacific whiting frozen fillets as affected by postharvest processing and storage conditions

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ABSTRACT

Whole fish and H&G (headed and gutted) fish were stored under refrigeration (<4 °C) for 0, 2, and 5 days and subsequently filleted and frozen at −18 °C and −80 °C. Frozen fillets were analyzed during 24 weeks of storage. The activity of trimethylamine-*N*-oxide demethylase (TMAOase) decreased more quickly at −18 °C than −80 °C. TMAOase reduction was distinctively noted at −18 °C storage. Formaldehyde (FA) induced by TMAOase increased at all treatments at −18 °C as frozen storage extended to 24 weeks, but it was near zero at −80 °C up to 12 weeks of storage. Textural toughening, low water retention ability, and low salt soluble protein resulted from the denaturation function of FA. A sudden decrease in surface hydrophobicity at 24 weeks, when stored at −18 °C, resulted from FA-induced unfolding and subsequent aggregation. FA concentration appeared to affect protein aggregations and textual toughening of fillets during frozen storage.

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1. Introduction

TMAO, which is an important compound to maintain the physiological functions of fish, is widely found in saltwater fish. The TMAO content of fish may vary depending on diet, age and size of the fish, as well as the environmental salinity, temperature, and pressure of the fish (Haard & Simpson, 2000). Gadoid fish, like cod (Amano & Yamada, 1964) and red hake (Parkin & Hultin, 1982), have a high level of trimethylamine-*N*-oxide demethylase (TMAOase) that catalyzes the breakdown of trimethylamine-*N*-oxide (TMAO) to FA and dimethylamine (DMA). FA reacts with amino acid residues to promote covalent cross-links in the formation of inter- and intramolecular linkages between protein chains (Shenouda, 1980). However, the enzymatic mechanism of TMAOase and the role of TMAOase in frozen fillets have not been fully studied.

The textural quality of fishery products is commonly affected by the physiochemical changes of myofibrillar proteins that occur during processing and storage. In general, the textural deterioration of the frozen gadoid species, such as Pacific whiting and Alaska pollock, occurs faster than other species during frozen storage (Gill, Keith, & Lall, 1979). The textural quality reduction in frozen fish commonly results from the protein crosslinks caused by formaldehyde (FA) which is a strong denaturing agent to myofibrillar proteins (Castell, Smith, & Dyer, 1973).

Frozen storage is one of important and effective techniques for long-term preservation of fishery products. However, the textural toughening of fish muscle during frozen storage is unavoidable

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and mainly caused by the denaturation and aggregation of myofibrillar proteins (Huidobro & Mohamed, 1998). This is because FA induced by TMAOase activity during frozen storage leads to protein denaturation and subsequent aggregation by formation of methylene bridges between proteins. Moreover, TMAOase is still active below 0 °C and breaks down TMAO into DMA and FA, respectively. For that reason, textural toughening of frozen fish and fillets as affected by TMAOase enzymatic reaction has been an issue in the global seafood industry for the last couple of decades (Benjakul, Visessanguan, & Tanaka, 2004; Careche, Del Mazo, & Torrejón, 1998).

Controlling the time between harvest and processing is the first step to retard the quality deterioration of seafood products. Fast processing after harvest can minimize the negative postharvest changes occurring during processing and subsequent frozen storage. With the development of the Pacific whiting surimi industry in Oregon in 1991–1992, the Oregon State University (OSU) Seafood Lab worked with the Pacific whiting industry and demonstrated that Pacific whiting must be kept in champagne ice after harvest and process into surimi within 24 h postharvest to produce good quality of surimi (Park, Graves, Draves, Yongsawatdigul, 2014, Chapter 3). However, the effect of postharvest conditions (refrigeration before filleting and frozen storage after filleting) linked to the quality of frozen fillets has not been well examined. Our objectives were to investigate the physicochemical properties of Pacific whiting frozen fillets as affected by postharvest processing and storage conditions.

2. Materials and methods

2.1. Materials

Pacific whiting (*Merluccius productus*), which was kept in refrigerated seawater (RSW) for approximately 48–60 h postharvest, was offloaded at Jesse's Ilwaco Fish (Ilwaco, WA, USA). Fish samples were randomly collected and transported in ice to the OSU Seafood Laboratory (Astoria, OR, USA). Half of the fish samples were headed/gutted (H&G) and treated as H&G samples. Both whole fish (WF) and H&G fish (HF) were stored under refrigeration (<4 °C) and covered with ice for 3 holding times (0, 2, and 5 days), filleted, and subsequently frozen at −18 °C and −80 °C. Fresh fish (0 day holding) were analyzed immediately after filleting upon delivery. Two temperatures were selected based on the most common storage temperature (−18 °C) and the storage temperature that would give a minimum damage to our samples (−80 °C) (Baron, KjÆrsgård, Jessen, & Jacobsen, 2007; Shenouda, 1980). Frozen fillets after being thawed at 1, 4, 12, and 24 weeks of frozen storage, were analyzed. TMAO, FA, DMA, adenosine 5′-triphosphate (ATP), anilinon-apthalene-8-sulfonic acid (ANS), ascorbic acid, L-cysteine, ferrous chloride, carbon disulfide, acetylacetone, ammonium acetate, potassium carbonate, and copper sulfate pentahydrate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Bradford reagent was purchased from Bio-Rad Lab (Hercules, CA, USA).

2.2. TMAOase activity assay

2.2.1. Preparation of TMAOase extract from frozen Pacific whiting fillet

The TMAOase extract was prepared using the method of Kimura, Seki, and Kimura (2000) with some modifications. First, 5 g of finely chopped muscle of partially thawed fillet were homogenized with 20 ml of chilled 20 mM Tris–acetate buffer (pH 7.0, 0.1 M NaCl) for 1 min with 30,000 rpm (Tissue Tearor Homogenizer, BioSpec Products Inc., Bartlesville, OK, USA). The homogenized sample was subsequently centrifuged at 3000*g* at 4 °C for 10 min using a Beckman J6-MI centrifuge (Beckman Coulter, Fullerton, CA, USA). The homogenate was washed twice with the same buffer by centrifuging at 3000*g* at 4 °C for 10 min and at 8000*g* at 4 °C for 20 min, respectively. After stirring the precipitates in 1 M NaCl (pH 4.5, 4 °C) for 30 min, the myofibrillar suspension was centrifuged at 15,000*g* for 30 min and centrifuged again at 105,000*g* for 1 h. The TMAOase extracts were obtained by neutralization with 1 M NaOH (Kimura et al., 2000).

2.2.2. TMAOase activity determination

TMAOase activity was measured using TMAOase assay mixture (pH 7.0), which contains 24 mM triacetate, 24 mM TMAO, 2.4 mM ascorbate, 2.4 mM cysteine, 0.24 mM $FeCl_2$, and 0.1 M NaCl. The TMAOase extract (0.5 ml) was mixed with 2.5 ml of TMAOase assay mixture and incubated at 25 °C water bath for precisely 80 min to activate the enzymatic reaction. The enzyme reaction was terminated by adding 1 ml of 5% trichloroacetic acid (TCA) before centrifuging at 8000*g* for 15 min and obtaining the supernatant for DMA determination. The enzyme unit was defined as the amount of μM DMA per min at incubation condition (Benjakul et al., 2004) and DMA was determined by copper-dithiocarbamate reaction (Dyer & Mounsey, 1945). One milliliter of copper-ammonia reagent and 4 ml of 5% CS_2–toluene solution were added into 2 ml of the supernatant in screw-capped glass tubes before incubating tightly capped tubes at 60 °C water bath for 2 min. After adding 30% acetic acid (400 μl), the mixtures were placed at room temperature, for approximately 10 min, until the layers were separated. The toluene part was then transferred to test tubes containing anhydrous Na_2SO_4 (approximately 0.5 g) to remove residual water from the toluene layer. Absorbance was read at 440 nm by UV–Vis spectrophotometer (UV 2401PC, Shimadzu Co, Kyoto, Japan) and protein content was determined by the Bradford method (Bradford, 1976).

2.3. FA determination

FA determination was performed according to the method of Benjakul et al. (2004). Finely chopped muscle (2 g) prepared from partially thawed fillet was homogenized with 8 ml of 5% TCA for 1 min (30,000 rpm) and subsequently centrifuged at 3000*g* for 15 min. The precipitate was homogenized with 4 ml of 5% TCA and centrifuged again with the same conditions as above. Each supernatant was combined together and neutralized using 0.1 N NaOH. Deionized water was added to make a final volume of 20 ml of FA extract. FA content was measured by the method of Nash (1953) modified by Benjakul et al. (2004). FA extract (1 ml) mixed with 1 ml of acetylacetone reagent was incubated at 60 °C for 5 min. The cooled reaction mixture was read at 412 nm and FA content was described as μM.

2.4. Surface hydrophobicity

Three gram of finely chopped muscle of partially thawed fillet were homogenized with 27 ml of 0.6 M KCl in 20 mM Tris–HCl (pH 7.0) and centrifuged at 20,000*g* at 4 °C for 30 min. Protein surface hydrophobicity (S_o) of the supernatant was determined by ANS probe containing 8×10^{-3} M ANS in 0.1 M phosphate buffer (pH 7.4) according to the method of Alizadeh-Pasdar and Li-Chan (2000). The protein concentration of the supernatant was diluted to 0.1, 0.2, 0.3, and 0.5 mg/ml, using the same buffer solution. Four milliliter of the sample mixed with 20 μl of ANS reagent were measured at wavelengths of 390 nm ($k_{excitation}$) and 470 nm ($k_{emission}$), respectively, using a luminescence spectrophotometer (Perkin Elmer LS-50B, Norwalk, Conn., USA). The protein surface hydrophobicity was expressed as the initial slope of the net relative fluores-

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