INFLUENCE OF CHILLING AND SUPERCHILLING TEMPERATURES ON LIPID DEGRADATION AND QUALITY OF COBIA (RACHYCENTRON CANADUM) FILLETS DURING STORAGE

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Received: 29/7/2016; Revised: 22/8/2016; Accepted: 26/9/2016

ABSTRACT

Temperature is the most important factor affecting the shelf-life and quality of fishery products. In the present work, cobia fillets were air packaged and stored at chilling temperature of 2 - 4°C and superchilling temperature of -1 - -2°C to investigate the effects of storage temperatures on lipid degradation and physicochemical quality of cobia fillets. Lipid hydrolysis was evaluated by the measurements of free fatty acids (FFA) and phospholipid content. Lipid oxidation was determined by the peroxide value (PV) and thiobarbituric acid-reactive substances (TBARS) measurements. The physicochemical properties were assessed by the cooking yield (CY), total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) content. The results indicate that lipid degradation was significantly influenced by storage temperature (chilling and superchilling temperatures), as accelerated hydrolysis and oxidation in chilled cobia fillets. This led to higher FFA and TBARS contents and lower phospholipid values compared to those of superchilled cobia samples. Superchilling temperature seemed to slow down the physicochemical changes in the fish muscle, resulting in a higher CY value and lower TVB-N and TMA contents compared to those of chilled cobia fillets. Based on the TVB-N and TMA content, the shelf-life of superchilled cobia fillets was 16 days and 10-11 days for chilled cobia fillets.

Keywords: Cobia, chilling, superchilling, lipid oxidation, fish quality

I. INTRODUCTION

Cobia (Rachycentron canadum) is a relatively new species and is a promising candidate for marine culture, mainly due to its fast growth rate, white muscle, high nutritional value and high commercial price. Cobia fillets contain a high content of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic (EPA; 20:5n-3) and docosahexaenoic acids (DHA; 22:6n-3) (Taheri et al., 2012). The PUFAs have beneficial health effects to consumers (Murray and Burt, 2001), but they are highly susceptible to oxidation (Masniyom et al., 2005). The polyunsaturated fatty acids content of cobia fillets decreases significantly during frozen storage, mainly due to lipid hydrolysis and oxidation (Taheri et al., 2011, 2012). Lipid degradation consists of lipid hydrolysis caused by enzyme activity (e.g. lipase and phospholipase) and lipid oxidation. Lipid oxidation is one of the major deteriorative reactions taking place in fish muscle during processing and storage, limiting the shelf-life of fishery products. Lipid oxidation depends on different factors, such as the amount of lipid present, the degree of unsaturated fatty acids in the muscle, packaging method and conditions during processing and storage (Nguyen et al., 2012, 2015; Taheri et al., 2011, 2012). Temperature is the most important factor affecting the lipid oxidation, the growth of microorganisms and physicochemical changes during storage (Dunn and Rustad, 2008). In order to prolong the shelf-life of fresh fish products, different techniques have been applied such as chilling and superchilling
techniques, packaging methods in combination with active packaging materials and antioxidants. The extension of the shelf-life of fresh fish fillets will allow the transport of products to distant markets at lower cost (Olafsdottir et al., 2006) and increase profitability (Dunn and Rustad, 2008).

Superchilling is a process where the temperature of a food product is lowered to 1 - 2°C below the initial freezing point of the product. The initial freezing point is dependent on the food chemical composition and varies between -0.5°C and -2.8°C (Dunn and Rustad, 2007). Superchilling has been proven as a promising technique with the potential to maintain the prime quality of fresh fish (Wang et al., 2008). Superchilling prevents the fillets from rigor contraction, having an impact on the final fish quality (Bahuaud et al., 2008). For many fishery products, superchilling results in a better quality and increases the shelf-life compared to conventional chilling. This is believed to be due to the inhibition of microbial growth and retardation of biochemical changes in the fish muscle (Dunn and Rustad, 2007, 2008; Wang et al., 2008; Lauzon et al., 2009). There has not been much work done on lipid oxidation during superchilled storage; however it has been suggested that the increase in shelf-life and quality of fresh fish products is resulted from the reduction of lipid oxidation (Kaale et al., 2011). The shelf-life of vacuum packaged salmon increases two times compared to ice chilled storage (Dunn and Rustad, 2008). Huynh and co-workers (2007) found that superchilling (-2°C) of Arctic char fillets packed with dry ice results in 6 days extension of shelf-life compared to chilling (3°C). Superchilled storage compared with traditional chilled storage in polystyrene boxes increases the shelf-life of cod loins from 9 to 17 days (Wang et al., 2008). However, the formation of ice crystals in the outer layer of foods during superchilling may have negative effects on the quality of products, due to increased protein denaturation and structural damage (Kaale et al., 2011).

The aim of this study is to investigate the effects of chilling and superchilling temperatures on lipid oxidation, quality and shelf-life of cobia fillets. Lipid oxidation and physicochemical degradation of the fish muscle were observed during storage time by measurements of CY, TVB-N, TMA, FFA, phospholipid, PV and TBARS.

II. MATERIALS AND METHODS

1. Materials

1.1. Sample preparation

Farmed cobias were caught from the cages in Nha Trang bay, Vietnam in March, 2015. The average weight of the fish was about 4.0-5.0 kg. The fish were bled in refrigerated water at the temperature of 4 ± 1 °C for 15 minutes. The bled fish were immediately chilled and transported to the JK Fish Processing Company in Nha Trang, Vietnam, where the fish were gutted and filleted. The fillets were individually air packaged in polyethylene bags and randomly divided into two groups for storage at chilling temperature of 2 - 4°C and at superchilling temperature of -1 - -2°C for 16 days. Before storage, packaged fillets were cooled to storage temperatures in a freezer at the temperature of -35°C.

1.2. Sampling

For all groups, analyses were carried out on day 0 and every two days of storage. At each sampling point, three fillets in each group were randomly taken and determined separately for cooking yield, total volatile basic nitrogen, trimethylamine, free fatty acid, phospholipid, peroxide value and thiobarbituric acid-reactive substances. All measurements were done in three replications.

2. Methods

2.1. Cooking yield (CY) determination

Three slices (30 g ± 5 g) in the middle part of each fillet (n=3) were individually weighed
in aluminium boxes and steam cooked in a steamed pot for 10 min. All cooked samples were drained from the excess liquid in a plastic grid for 10 minutes and weighed again. CY was expressed as the percentage of retaining weight compared to the weight of the sample before cooking. The average cooking yield value of three samples for each fillet was used to calculate the mean and standard deviation.

2.2. Total volatile basic nitrogen (TVB-N) and Trimethylamine (TMA) determination

The method of Malle and Poumeyrol (1989) was used for measuring total volatile basic nitrogen (TVB-N) and trimethylamine (TMA). TVB-N was measured by direct steam distillation into boric acid using a Kjeldahl-type distillatory (Vapodest 40 distillatory, Gerhardt, Bonn, Germany) and titration, after extracting the fish muscle with 7.5% aqueous trichloroacetic acid solution. The acid was back-titrated with diluted sulphuric acid solution. To determine TMA, the same method was used as for TVB-N but adding 20 mL of 35% formaldehyde to the distillation flask to block the primary and secondary amines, TMA being the only volatile and measurable amine. The TVB-N and TMA content were expressed in mg N/100 g sample.

2.3. Free fatty acid (FFA) determination

Free fatty acid (FFA) content was determined on lipid extract according to the method of Bernardez et al. (2005), based on complex formation with cupric acetate-pyridine, followed by absorbance reading at 710 nm (Spectrophotometry, Carry 50, Varian, USA). The results were expressed as grams FFA/100 g of lipid using standard curve prepared from oleic acid.

2.4. Phospholipid determination

Phospholipid content of the fish muscle was determined according to the method of Steward (1980), based on complex formation of phospholipid with ammonium ferrothiocyanate, followed by absorbance reading at 488 nm (Spectrophotometry, Carry 50, Varian, USA). The results were expressed as a percentage of total lipid content and calculated using a standard curve prepared from phosphatidylcholine.

2.5. Lipid hydroperoxide (PV) determination

Lipid hydroperoxide was determined by the ferric thiocyanate method (Santha and Decker, 1994). 500 µL of lipid extract was added to 500 µL ice-cold chloroform:methanol solution (1/1, v/v). A total amount of 5 µL of ammonium thiocyanate (4 M) and ferrous chloride (80 mM) mixture (1:1, v/v) was finally added. The samples were then brought to room temperature for 10 minutes and the absorbance was read at 500 nm (Spectrophotometry, Carry 50, Varian, USA). A standard curve was prepared using cumene hydroperoxide. The results were expressed as µmol lipid hydroperoxides per g of sample (µM/g).

2.6. Thiobarbituric acid-reactive substances (TBARS) measurement

Thiobarbituric acid-reactive substances (TBARS) were measured by method of Lemon (1975) with modifications as described in Nguyen et al. (2012). The results were expressed as µmol malondialdehyde per kg of sample (µmol MDA/kg).

3. Data analysis

The data sets obtained were calculated means and generated the graphs using Microsoft Excel 2013 (Microsoft Corporation, USA). Means were compared by using ANOVA and Duncan’s Multiple-Comparison Test using NCSS 2000 software (NCSS, Kaysville, Utah, USA). Significance of differences was defined at the 5% level (p < 0.05).
III. RESULTS AND DISCUSSION

1. Effects of chilling and superchilling temperatures on physicochemical properties

1.1. Changes in cooking yield (CY)

Cooking yield (CY) is an important quality characteristic that reflects the structural changes in the fish muscle during processing and storage. The CY of cobia fillets was significantly (p < 0.05) affected by the storage temperature. Generally, the CY of all samples decreased throughout the storage period, but in different extents (Figure 1). The CY values of the superchilled cobia samples remained remarkably higher compared to those of chilled cobia fillets during storage. The decrease in CY is thought to be due to the biochemical changes occurring in the fish muscle such as protein denaturation/oxidation, lipid oxidation and enzyme activity that had negative effects on water holding capacity of the fish muscle during heating (Dunn and Rustad, 2007, 2008; Bahuaud et al., 2008). The decrease in CY was in negative correlation with the increase in TVB-N and TMA content (Figure 2A and 2B, respectively). Higher CY values obtained in the superchilled samples may be due to the lower storage temperature (i.e. -1 - -2°C vs. 2 - 4°C), resulting in the lower protein denaturation and lipid oxidation rates. Since temperature is one of the most important factors affecting the development of protein denaturation and lipid oxidation in the food muscle during storage (Dunn and Rustad, 2008). Huynh and co-workers (2007) reported that the CY of Arctic char fillets after superchilling was higher than that of the samples after chilling. However, the storage temperature did not have significant effects on the CY. This may be due to the different fish species used, resulting in different chemical composition of the fish muscle.

1.2. Changes in total volatile basic nitrogen (TVB-N) and trimethylamine (TMA)

The total volatile basic nitrogen (TVB-N) and trimethylamine (TMA) are the most widely used as biochemical indicators for assessment of shelf-life of fresh fish products. TVB-N includes trimethylamine, dimethylamine, ammonia and other volatile basic nitrogenous compounds. TMA is a pungent volatile amine often associated with the typical fishy odors and flavors of fish spoilage (Connell, 1995).

Generally, the storage temperature significantly affected (p < 0.05) the TVB-N and TMA in the fish muscle. The TVB-N of all samples increased with increasing storage time (Figure 2A). The TMA content of all groups remained stable during the first 6 days of storage and then remarkably (p < 0.05) increased during the subsequent storage time (Figure 2B). Significantly lower TVB-N and TMA values (p < 0.05) were observed in the cobia fillets stored at superchilling temperature.

Figure 1. Changes in cooking yield of cobia fillets during storage as a function of temperature
in comparison with those of the samples stored at chilling temperature. This revealed that superchilling temperature is an effective condition to delay the muscle protein degradation. High TVB-N production was generally correlated with high TMA production. The increase in TVB-N could be promoted by the trimethylamine oxide (TMAO) breakdown catalyzed by endogenous enzyme (trimethylamine oxide demethylase - TMAOase) present in muscle tissue and bacterial activity (Connell, 1995). Both H₂S-producing bacteria, mainly Shewanella putrefaciens, and P. phosphoreum are able to reduce TMAO to TMA but the latter is usually a considerable part of TVB-N (Lauzon et al., 2009). The pattern of changes in TVB-N and TMA content was in agreement with that noted in previous publications (Wang et al., 2008; Lauzon et al., 2009; Olafsdottir et al., 2006).

Levels of 30 mg N/100 g for TVB-N and 10 mg N/100 g have been considered the upper limit, above which fishery products are considered spoiled and not suitable for human consumption (Connell, 1995; EC Directive 95/149/EC). According to this, the cobia fillets stored at chilling temperature were unfit for human consumption after storage for 10-11 days, but cobia fillets stored at superchilling temperature were still fit after storage for 16 days. This was in harmony with the sensory evaluation results - Torry score (data not shown). Olafsdottir and co-authors (2009) stated that the shelf-life of superchilled cod based on Torry score and TVB-N is 15 days at -1.5°C compared to 11 days for iced chilled cod. The shelf-life of cod loin stored at superchilling temperature (-0.9°C) was 16 or 17 days, but increased to 21 days when MAP and superchilled storage were combined (Wang et al., 2008).

Figure 2. Changes in TVB-N (A) and TMA (B) of cobia fillets during storage as affected by temperature

2. Effects of chilling and superchilling temperatures on lipid degradation

2.1. Changes in free fatty acid (FFA) and phospholipid content

The changes in free fatty acid (FFA) and phospholipid (PL) content of fish samples during storage as influenced by different temperatures are described in Figure 3A and 3B, respectively. As expected, the FFA content of fish samples in all treatments increased throughout the storage period, whereas the PL content of all samples dramatically (p<0.05) decreased. The results indicate that storage temperature and storage time strongly affected the rate of lipid hydrolysis in the fish muscle. The FFA in both superchilled and chilled samples increased slightly during the first 8 days of storage and then rapidly increased during the subsequent storage time (Figure 3A). This may be due
to the temporary inactivation of enzyme and microorganisms at early stages of storage caused by low temperatures. It has been well demonstrated during storage of fish products, lipid in the fish muscle is hydrolyzed mainly caused by microbial enzyme activity such as lipases and phospholipase (Nguyen et al., 2012) and non-microbial enzyme activity (i.e., natural lipase present in the fish muscle) as well as spontaneous lipid hydrolysis (Jin et al., 2010; Nguyen et al., 2012). At subsequent storage time, enzymes and microorganisms accustom to living conditions, resulting in increased lipid hydrolysis rate. The increase in FFA during storage was in accordance with the decrease in phospholipid content. It has been suggested in literature that majority of FFA evolving in fish during storage is derived from phospholipids (Lopez-Amaya and Marangoni, 2000). The decrease in phospholipid is proved to be due to the reaction of phospholipase enzyme in the fish muscle during storage (Jin et al., 2010). Significantly (p < 0.05) lower FFA values and higher phospholipid content were obtained in the superchilled samples compared to those of the chilled samples. This can be explained by the lower temperature leads to a lower enzymatic and microbial activity, because temperature is one of the most important factors affecting their activity (Lauzon et al., 2009; Wallenstein et al., 2012).

2.2. Changes in peroxide value (PV) and TBARS content

Figure 3. Changes in FFA (A) and phospholipid content (B) of cobia fillets during storage as function of temperature

Figure 4. Changes in peroxide value (A) and TBARS (B) of cobia fillets during storage as influenced by temperature
The PV (primary product) and TBARS (secondary product) are commonly used as lipid oxidation indicators for food products during processing and storage. In general, the development of PV and TBARS was significantly influenced by storage temperature and storage time. The PV content of chilled samples increased remarkably during the first 8 days of storage and then tended to decrease. Whereas, the PV content of superchilled samples increased throughout the storage time (Figure 4A). The increase in PV is well-known to drive from the preferential oxidation of phospholipids during early stages of autoxidation (Hernandez-Herrero et al., 1999). Moreover, hydroperoxides are primary lipid oxidation products, and their content depends much on the ratio between formation and decomposition of hydroperoxides (Jin et al., 2010). This explains the PV content of chilled samples tended to decrease after storage for 8 days. The pattern of changes in PV was in harmony with the changes in TBARS as presented in Figure 4B. The TBARS content of the chilled cobia fillets slightly increased during storage for 8 days and after that significantly increased during the last 8 days of storage. The significantly increased TBARS in chilled samples towards the end of storage may be attributed to the faster decomposition of existing hydroperoxides into TBARS since the corresponding PV content was decreasing. The TBARS content of the superchilled samples increased gradually throughout the storage period and remained significantly lower than that of chilled samples. The increase in TBARS content during storage may be due to the decomposition of hydroperoxides in the fish muscle (Chaijan et al., 2006). The formation of FFA could also contribute an effect to decomposition of hydroperoxides to form free radicals (Yoshida et al., 1992). Since FFA are known to undergo oxidation to produce low molecular weight compounds that are responsible for the rancid off-flavor and taste of fish and fish products (Aubourg et al., 2004). The increase in TBARS content was in correlation to the increased FFA content of the fish muscle during storage. As expectation, the superchilling temperature appeared to be effective in retardation of lipid oxidation. Similar results have been published by Huynh and co-authors (2007) for superchilled storage of Arctic char fillets. They found that the lowest TBARS content is observed in superchilled fillets packed with dry ice.

IV. CONCLUSION

The results of the present study indicate that temperature significantly affected the lipid degradation and physicochemical quality of cobia fillets during storage. Superchilling temperature slowed down lipid degradation and physicochemical changes in the fish muscle, resulted in lower FFA, PV, TBARS, TVB-N and TMA content; higher cooking yield and phospholipid values compared to those of chilled samples. Based on lipid degradation and physicochemical data, superchilled storage demonstrated as an effective means to extend the shelf-life of cobia fillets. The shelf-life of superchilled samples was 16 days compared 10-11 days of chilled samples. In order to obtain high quality products, optimization of superchilling technique is needed to prevent irreversible destruction of the myofibres caused by formation of ice crystals.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support of the Ministry of Education and Training Research Fund (Project No. B2014-13-11).
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8. European Communities Directive 95/149/EC: Commission Decision of 8 March 1995 fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of fishery products and specifying the analysis methods to be used.


