Chemical Treatments for Reducing the Yellow Discoloration of Channel Catfish (*Ictalurus punctatus*) Fillets

by

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Abstract

Three sets of experiments were conducted to investigate effects of various chemical pretreatments on the color and carotenoid changes of yellow discolored channel catfish fillets. First, the relationship between fresh fillet color and carotenoid content was studied. The next set of experiments was to determine the color change and carotenoid contents in catfish fillets during storage. The final set of experiments was to evaluate the efficacy of chemical pretreatments for preventing or reversing yellow discoloration in channel catfish fillets during storage.

A strong linear relationship between the b-value of fresh whole fillets and total content of the three major carotenoids (lutein, zeaxanthin and alloxanthin) was found with a correlation coefficient of 0.76. The intensity of the yellow color of the fresh catfish fillets appears to be mainly related to the sum of the major carotenoid contents.

Yellow discolored catfish became darker and more yellow after 12 d refrigerated storage. With increased time, the sum of carotenoid contents (lutein, zeaxanthin and alloxanthin) of dark yellow discolored fillets decreased ($P<0.01$).

Ascorbic acid, BHA, citric acid and sodium metabisulfite were not successful at reducing the yellow discoloration. Sodium bicarbonate reduced the fillet yellowness, but the fillets turned darker after 12 days of storage. Sodium bisulfite gave the best results, with fillets being brighter and less yellow after storage. No evident improvement was found by combining these two chemicals together.

The sum of the carotenoid contents (lutein, zeaxanthin and alloxanthin) in untreated fillets significantly decreased compared to fresh fillets ($P<0.1$). However, the sum of the carotenoid contents in fillets treated by various chemical pretreatments did not differ significantly from the untreated fish.

The catfish industry may improve the color of their discolored fillets by using chemical pretreatments that include sodium bisulfite. The exact mechanism of bisulfite's action requires further study.

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Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

There are more than 39 species of catfish in North America. However, only six of them have been cultured for commercial production (Wellborn 1988). Channel catfish (*Ictalurus punctatus*), one of the most common catfish species, have been cultured for many years as the dominant aquaculture species in North America (Wolters and Johnson 1994).

The catfish industry, which is ranked high among aquaculture industries, is a very important industry in the United States. The catfish industry has shown growth over the past years with the increasing seafood consumption by Americans. In 2003, the volume of catfish processed increased to 660 million pounds from 225 million pounds in 1995 (Hanson and Sites 2011). However, in recent years, the catfish consumption has declined. Only 472 million pounds of catfish were produced in 2010, representing a decrease of about 30% since 2003 (USDA 2011). The product consumption is influenced by many factors, such as technological advances, market developments and catfish quality.

Yellow discoloration of channel catfish has been recognized as a quality problem in the catfish industry. Catfish processed in winter or early spring are more likely to have the yellow discoloration than fish processed during or after the growing season (Lovell 1984). This irregular yellow discoloration is mainly concentrated on the anterior, dorsal part of the fish near the backbone (Lovell 1984). Lee (1987) suggested that 0.6 µg carotenoid per gram of flesh would probably give yellow pigment spots in the flesh. Carotenoids cannot be synthesized by catfish, and thus carotenoids in the fillet must be

obtained from the diet. Thus, feeding strategies may be used to reduce the development of the yellow discoloration. It would be helpful to also have processing treatments that could be used to reduce or prevent the discoloration from occurring.

The purpose of this study was to evaluate the efficacy of various chemical treatments on the prevention or reduction of yellow spot formation on channel catfish. A secondary objective was to evaluate the relationship between fish fillet color and carotenoid content. Increasing the consumer acceptability of catfish fillets would be important to the catfish industry.

Chapter 2 Review of Literature

Channel catfish (*Ictalurus punctatus*)

Over 39 species of catfish exist in North America. However, only six of them have been cultured for commercial production (Wellborn 1988). Channel catfish (*Ictalurus punctatus*) is one of the most common catfish species and has been cultured for many years as the dominant aquaculture species in North America (Wolters and Johnson 1994).

Channel catfish (Fig 2.1) belongs to the Ictaluridae family of Siluriformes order. The catfish body is cylindrical and lacks scales. Several barbells exist around its mouth. Four of them are located under the jaw and one sits on each tip of the maxilla. A deeply forked tail is another special characteristic of channel catfish.

Fig 2.1-Channel catfish (*Ictalurus punctatus*).

Catfish usually feed on the muddy bottom of large reservoirs, lakes and ponds (Fig 2.2) and grow best at the temperature of 30 °C (Wellborn 1988). Their appetite increases with increasing water temperatures, and thus they grow faster in the summer than winter. According to the Vladykov's report (1951), the maximum age of channel catfish is nearly 40 years, but they are captured for trade before they are 2 years old.

Fig 2.2-Pond used for culturing channel catfish (*Ictalurus punctatus*).

Catfish Industry

The catfish industry (Fig 2.3) is ranked high among aquaculture industries and is a very important industry in the United States. It has grown over the past years as Americans increased their seafood consumption. The volume of catfish processed increased from 225 million pounds in 1995 to 660 million pounds in 2003 (Hanson and Sites 2010; USDA 1996). However, in recent years, the catfish consumption has declined with only 472 million pounds of catfish being processed in 2010 (USDA 2011). The

changes to product consumption are influenced by many factors, such as technological advances, international competition, alternative species availability and catfish quality.

Fig 2.3-Workers gathering channel catfish (*Ictalurus punctatus*) from pond.

The Discoloration Problem in Catfish Flesh

Consumers notice the color of seafood firstly, and thus color has an inevitable effect on the food products' acceptability and marketability. A deep pink color has a positive role on sailing salmon, while a small amount of pigmentation is undesirable in fish having a white flesh. For example, the intensity of red hue determines the grading and price of salmon, rockfish and snapper (Sacton 1986). Pen-reared salmon was considered the same as trout by sensory panels because of lacking typical salmon color (Sacton 1986). However, consumers may reject whitish fillets, such as channel catfish, with even a small amount of yellow pigment (Lovell 1984; Shahidi and others 1998).

In recent years, the yellow discoloration of catfish (Fig 2.4) has become a serious problem in the channel catfish industry. The intense yellow pigment develops mainly on the anterior, dorsal part of the fish near the backbone (Lovell 1984). This yellow discoloration occurs more frequently in winter or early spring when catfish have not been fed on regular diets rather than during or immediately following the growing season. Even though the pigment does not appear to affect the flavor or nutritional quality of the flesh, this problem needs to be solved in order to avoid economic loss due to consumers rejecting the product.

Reasons of Catfish Fillet Discoloration

Several theories are proposed about the cause of the yellow pigmentation in catfish. The main source of this pigmentation is carotenoids, primarily the yellow-orange xanthophylls. Astaxanthin and canthaxanthin are important to the pinkish color of other seafood, such as crab and salmon.

Carotenoids cannot be synthesized by fish, and thus the carotenoids found in seafood tissues must come from dietary sources, either natural food in the pond or commercial feed ingredients such as corn gluten meal or alfalfa meal, which are concentrated xanthophyll sources (Palmer 1915; Palmer and Eckles 1914; Steven 1947).

It has been shown that yellow discoloration started to appear in the skin of channel catfish after feeding certain amounts of lutein and/or zeaxanthin, but not in the fish on a low carotenoid diet (Lee 1987). After feeding 10 ppm dietary yellow pigment, the number of yellow fish increased and they appeared to be darker. However, 7 ppm dietary yellow pigment was acceptable. The unwanted pigments in the flesh of channel catfish came from carotenoids which were originally found in commercial fish feeds

Light yellow catfish

Medium yellow catfish

Dark yellow catfish

Fig 2.4-Catfish fillets with yellow discoloration.

containing corn, corn gluten meal, and alfalfa meal (Palmer 1915, Lovell 1984). Robinson and Li (1997) found that the yellow pigment content was 12.9 ppm in corn, 25.4 ppm in corn gluten, 15.4 ppm in canola meal and 28.6 ppm in distillers grain. Because the yellow pigments are lipid soluble, these ingredients could possibly influence the color of the fat in the fed fish. By consuming carotenoid-containing fish feed, fish absorbed these pigments which became distributed in the fish muscles, bound to actomyosin, and deposited in different tissues to cause red to yellow pigment in the flesh (Henmi and others 1989).

Additionally, another possible source of the yellow pigment could be a combination of natural organisms in the pond. During the cool season or restricted feeding period, catfish need to consume more naturally-present food from the pond. Lutein, β-carotene, violaxanthin, neoxanthin and zeaxanthin are most commonly distributed in green algae (Latscha 1990). In red algae, α-carotenes and β-carotenes are the main pigments (Shahidi and others 1998). In brown algae, β-carotenes, violaxanthin and fucoxanthin are the predominant pigments. The pigment level (more than 10 ppm) in these natural sources is sufficient to promote the accumulation of yellow pigment in catfish. However, this theory has a major limitation. When heavily fed on feed, fish consume little naturally occurring food, such as microorganisms, but can still develop yellow discoloration.

A third suggested explanation for the yellow pigment in catfish is that when fish fast, the body fat is consumed for energy but the pigments are not; as the fat disappears, the color becomes more concentrated. This suggestion is supported by the phenomenon that yellow discoloration of channel catfish is more common in the winter (Lovell 1984).

It is assumed that the carotenoid concentration increased because of the faster mobilization of fat than carotenoids from the flesh (Lee 1987). Therefore, fish would be expected to have more yellow pigmentation in the flesh after a period of fasting (Lee 1987; Lovell 1984). Genetics, environment, and other factors also could influence pigmentation.

Determinant of Cultured Fish Pigmentation

Pigmentation of cultured fish is mostly determined by the quantity of carotenoids in fish feeding, feed composition and type, size and age of fish.

After feeding on diets containing 60 and 90 mg carotenoid/kg, there is significant coloration on the fish weighing over 215 grams (Spinelli and Mahnken 1978). Only 6 to 7 mg carotenoid/kg can be deposited in the rainbow trout weighing between 100 to 500 g while 25 mg carotenoid/kg can be contained in larger trout. In addition, the contents of lipids in fish feeding have a positive role on carotenoid ingestion. The content of carotenoids in the flesh increased with increasing dietary lipids level (Storebakken and No 1992).

Astaxanthin is more efficient for trout flesh pigmentation than canthaxanthin. The trout fed astaxanthin appeared redder than the others fed canthaxanthin. In addition, the diet with astaxanthin and canthaxanthin together caused a higher level of total carotenoids deposited in the flesh than either single carotenoid alone (No and Storebakken 1991).

Fish deposit carotenoids differently depending upon their stage of life. Fry and fingerlings concentrate carotenoids mostly in their skin. Post juvenile fish in rapid growth periods concentrate carotenoids in their flesh. Salmonids deposited carotenoids in the

flesh and then transfer some of them to the skin and gonads (Sivtseva and Dubrovin 1981).

Properties of Carotenoids

Carotenoids, a group of pigmented isoprenoid compounds, are the most common pigment in bacteria, yeasts, mold, and all green plants, and they contribute to the yellow, orange, and red colors of the skin, shell, or exoskeleton of aquatic animals (Shahidi and others 1998). Depending on the degree of substitution, carotenoids can be divided into two categories, carotenes and xanthophylls. Carotenes do not have oxygen, are orange in color, and dissolve in petroleum ether. Xanthophylls contain at least one oxygenated substituent on the terminal rings.

Carotenoids are able to combine with proteins (Lee 1966). These associations of carotenoids with proteins can provide a practical way for carotenoids becoming water-soluble and changing the color of the pigment. These complexes can be divided into three categories, which are carotenolipoproteins, chitinocarotenoids and real carotenoproteins. Carotenolipoproteins, which provide a blue, green, or purple color, are mainly found in the ovaries and egg of crustacea, blood, cuticle and epidermis. Chitinocarotenoids, found in the exoskeleton, consist of chitin and carotenoids by the Schiff base bonds or carbonyl-amino bonds. The real carotenoprotenis, always found on the external surfaces of crustacean, are formed from carotenoids, probably astaxanthin, and protein.

Carotenoids in Seafood

Of the more than 350 known carotenoids, β-carotene, lycopene, γ-carotene, β-cryptoxanthin, lutein, zeaxanthin, astaxanthin, canthaxanthin and tunaxanthin are the most common in seafood. The variety of carotenoids in seafood is quite similar as those

found in carotenogenic plants (Al-Khalifa and Simpson 1988). Astaxanthin is the most widely distributed carotenoid in invertebrate seafood such as shrimp, lobster and other crustaceans while zeaxanthin, lutein and astaxanthin are especially common in fish. Small amounts of xanthophylls were also detected in the wild salmon and Arctic char (Shahidi and others 1994).

Numerous carotenoids in seafood are responsible for the bright colors in lobster, shrimp, salmon and fish eggs (Shahidi and others 1998; Moretti and others 2006). For example, the majority of carotenoids (88% in the flesh of chum salmon, 99.8% in sockeye salmon, 95.7% in coho salmon, 82.1% in pink salmon, and 79.2% in masu salmon) are composed of astaxanthin in the flesh of salmonid, which causes them to have a red coloration (Shahidi and others 1998; Skrede and others 1989).

Function of Carotenoids

Carotenoids are reported to have diverse biological functions in seafood. They have various protective effects on seafood, such as absorbing and reflecting damaging radiation, destroying singlet oxygen and protecting from oxidation (Muller and others 1980; Tacon 1981). For example, carotenoids can not only strengthen the cytotoxic activity of killer cells, slow down tumor growth and promote wound healing but also ease bacterial and fungal disease (Czeczuga 1979; Goodwin 1986; Lee and Gilchrist 1975; Sivtseva and Dubrovin 1981).

Carotenoids also play an important role of being vitamin A precursors in seafood. Some plant carotenoids can be transformed into vitamin A which is an essential micronutrient in seafood. Seafood must obtain enough vitamin A from their diet or by

conversion of some provitamin A carotenoids. Vitamin A is critical for visual acuity, growth, normal development of the skin, reproduction and disease protection.

In addition, another important function of carotenoids is their influence on protection, courtship, reproduction and growth. Carotenoids provide a source of pigments to developing embryos in eggs to produce the characteristic pattern in the skin. Seafood has distinguishing coloration, which is from carotenoids and serve to gain the attention of a potential mate. In salmonids, carotenoids are transferred from the muscle to the integument and ovaries along with sexual maturation, in order to have a function in reproduction (Srivastava 1991). Astaxanthin has a positive influence on the growth rate of young salmon (Torrissen 1984).

Carotenoids have also been claimed to be involved in protecting cells from oxidation and improving cells' radiation resistance abilities. For example, in microorganisms and algae, carotenoids play a protective role because of not only acting as an antioxidant, but also quenching reactive species formed by photochemical reactions.

Although carotenoids are serving many biological functions in living seafood, their presence also impacts their color. As seafood is converted into edible product, this color may be desirable, as in salmon, or undesirable, as in the yellow discoloration of catfish.

Carotenoids Accumulation and Conversion in Fish

Various seafood species have the ability to store some pigments (Lovell 1984). Carotenoids were more concentrated in the front and back part of salmon near the back bone than in other parts, while catfish deposit carotenoids mainly on the ovaries and skin

(Lee 1987). It has been reported that just more than 0.6 µg carotenoid per gram of flesh would probably give yellow spots in the flesh (Lee 1987). This concentration of carotenoid in the fillet could be obtained from the diet of channel catfish in earthen ponds where fish were fed commercial fish food with various levels of xanthophylls during the summer growing season (Lee 1987).

Lee (1987) evaluated carotenoid distribution in catfish after feeding a carotenoid rich diet followed by a carotenoid-free control diet for 41 days. Lutein did not decrease in the flesh but decreased markedly in the liver, skin and abdominal fat. Zeaxanthin also clearly decreased in the flesh, liver, skin and abdominal fat. In catfish fed β-carotene, the carotenoid content decreased in the flesh, skin and the abdominal fat, but not in the liver (Lee 1987).

Through evaluating the fillet yellowness and the pigment content in the diets, Lee (1987) noted that only 11 mg dietary lutein and zeaxanthin/kg feed were enough to promote visible yellowing. However, Li and others (2007) thought that foods naturally existing in ponds, rather than fish diets, were the major sources of carotenoids in catfish fillets. When five carotenoids (lutein, zeaxanthin, canthaxanthin, astaxanthin and β-carotene) were added to catfish diets for a 12-week feeding study, large amounts of lutein and zeaxanthin concentrated in the catfish body while only trace amounts of canthaxanthin, astaxanthin and β-carotene appeared (Li and others 2007).

It has been shown that channel catfish can convert β-carotene into vitamin A; fish fed β-carotene had significantly higher amounts of vitamin A1 and A2 than the control group (Lee 1987). However, this result was not replicated in the groups fed lutein or

zeaxanthin. Fish fed on lutein and zeaxanthin had about the same amount of vitamin A1 and A2 as the fish fed the carotenoid-free control diet (Lee 1987).

Fish processing, such as baking, smoking and cooking, causes color changes in pigmented fish flesh (Skrede and others 1989). For example, the baked salmonid flesh appears more yellow and less red than raw flesh. Smoked flesh turned darker red and less yellow compared with raw flesh. Cooked flesh resulted in a shift in hue from red to pinkish from astaxanthin-pigmented trout and salmon flesh. The carotenoids in natural foods may be present in complexes with protein but the protein-carotenoid complex may be broken by cooking.

The prevention of yellow pigmentation in catfish raised in ponds is very challenging. Fortunately, the incidence of this problem can be reduced by making sure that the fish does not contain more than 7 ppm yellow pigments and that the fish are fed adequately. However, the development of additional strategies would be beneficial.

Pigment Analysis in Fish

Pigments within the fish flesh may be assessed by various analytical methods. Nowadays, pigments are tested by comparing the color of sample fish with that of standardized fish through sensory analysis, by instrumental analysis based on light reflected from flesh samples, or by quantitative carotenoid analysis of fish flesh (Skrede and others 1989). The results of the sensory testing have highly significant linear relationships with the value of instrumental color analysis (Erikson and Misimi 2008).

Before the carotenoids can be analyzed, the pigment must be completely extracted and concentrated. Carotenoids have a series of carbon-carbon double bonds. Therefore, carotenoids very readily break down in presence of light and oxygen, and thus the

carotenoids should be processed in the condition of no light and low temperature. An extraction procedure involving acetone has been described by Liu and others (2012).

High performance liquid chromatography (HPLC) is useful for the analysis of carotenoid pigments. Some of the methods used are outlined in Table 2.1. All methods used absorbance detectors. Carotenoid identification and quantitative analysis are able to be completed in a short time by HPLC (Moros and others 2002).

Table 2.1-HPLC carotenoid analysis methodologies.

	HPLC column	mobile phase	flow conditions
Lee (1987)	10 cm Radial-PAK C-18 (10 um)	acetonitrile: dichloromethane: methanol (70: 20: 10)	1.0 mL/min
Moros and others (2002)	25 cm C-30 Carotenoid column (4.6 mm)	mobile phase A : methanol/TBME [a]/water (81:15:4) mobile phase B : methanol/TBME (9:91)	95% A and 5% B to 50% A and 50% B 45 min 50% A and 50% B to 100% B 15 min (1.0 mL/min)
Liu and others (2012)	25 cm ProntoSIL C-30 (2.0 mm × 5 µm)	mobile phase A : methanol/TBME/water (81:15:4, v/v/v) mobile phase B : methanol/TBME (9:91, v/v)	95% A and 5% B to 50% A and 50% B 45 min 50% A and 50% B to 100% B 15 min (0.2 mL/min)

[a] TBME = tert-butyl methyl ether

Pigmentation can also be evaluated with a colorimeter. One example of a colorimeter is the Minolta Chroma Meter II Reflectance instrument. Chroma values C* (brightness) were calculated by a*(redness), b* (yellowness), and L* (lightness) values. However, a high-tech colorimeter needs to be used in this method. It is difficult to measure the color of a heterogeneous surface, like a catfish fillet with a colorimeter. Cline (2011) developed a method to analyze catfish color using a camera and calibrated computer software. An X-rite Checkerboard was photographed by a Cannon EOS500 D digital camera in the RAW image format. Then, under the same lighting conditions, a photograph of the entire catfish fillet was also captured. Using a standard color reference (ColorChecker Passport, X-rite, Grand Rapids, Michigan) and Adobe Photoshop CS5 (Adobe systems Incorporated, San Jose, CA), photographs were digitally converted into an overall average color. The average color was characterized by its L, a, b-values.

A quadratic relationship existed between visual scores and carotenoids in the diet while the carotenoid deposited in the tissue of channel catfish had a linear relationship with the carotenoid concentration in the diet (Lee 1987). This means that at low levels of pigmentation, color differences with small changes in carotenoid concentration could be easily and accurately detected, but abundant pigment was required to produce a detectable difference to the eye as the intensity of color increased. The visual judgment was no longer reliable when the concentration of carotenoid exceeded 1.3 µg carotenoid per gram of flesh (Lee 1987).

Objectives

The discoloration of catfish fillets is a topic requiring additional research. In this work, the relationship between carotenoid content and color of catfish fillets was studied.

More importantly, the effect of various chemical pretreatments on the improving the color of channel catfish was also investigated.

Chapter 3 Color Changes and Carotenoid Contents of Yellow Discolored Channel Catfish (*Ictalurus punctatus*) Fillets During Refrigerated Storage

ABSTRACT: The effects of refrigerated storage on the color and carotenoid content of three types of fresh yellow discolored channel catfish fillets were studied. The color and carotenoid content of the yellow fillets were analyzed using the L, a, b-color system and HPLC analysis, respectively. A strong linear relationship between the b-values (visual yellowness) of fresh catfish fillets and the sum of three major carotenoid contents was observed. Yellow discolored catfish fillets became darker and more yellow during 12 d of refrigerated storage. The redness of fillets did not change. The total carotenoid content of dark yellow fillets decreased during storage.

Introduction

The catfish industry has a large economic impact within the Southern United States. One threat to this industry is a yellow discoloration that develops on the surface of some fillets and worsens during storage. Even though some reports indicate that the pigmentation of channel catfish does not affect the flavor or nutritional quality of the flesh, consumers may still reject the channel catfish with even a small amount of yellow color (Lovell 1984; Shahidi and others 1998). Thus, yellow discoloration of channel catfish (*Ictalurus punctatus*) has been recognized as a problem in the catfish industry.

Typically, a more yellow fillet contains a larger amount of carotenoids (Lee 1987). Three major carotenoids (lutein, zeaxanthin and alloxanthin) and several minor carotenoids were identified in discolored catfish fillets (Liu and others 2012).

Carotenoids cannot be produced by catfish because only plants and microorganisms have abilities to synthesize them (Shahidi and others 1998). However, catfish can assimilate carotenoids, which appear to be obtained through their diet, giving fillets a yellowish color (Lee 1987; Li and others 2007). The carotenoids become deposited on the ovaries and skin (Lee 1987).

Reducing the amount of tissue carotenoids may improve the fillet color. However, the relationship between the yellow color intensity and the carotenoid content has not been clarified. Also, changes in the fillet color and carotenoid content during storage have not been evaluated. Understanding the relationship between catfish color and carotenoid content will provide the basic information necessary for the industry to solve the discoloration problem.

Therefore, the objectives of this study were to determine the relationship between catfish yellowing and carotenoid content, quantify the color change of catfish fillets during storage, and evaluate carotenoid contents in catfish tissue during storage.

Materials and Methods

Materials

Acetone (analytical grade), ethanol (analytical grade), ethyl ether (analytical grade), hexane (analytical grade), BHA: 3-tert-butyl-4-hydroxyanisole (analytical grade), Sudan I (analytical grade), methanol (HPLC grade), TBME: tert-butyl methyl ether (HPLC grade) and acetonitrile (HPLC grade) were purchased from VWR (Radnor, PA, USA).

Lutein (purity ≥ 95%) and zeaxanthin (purity ≥ 95%) standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Alloxanthin standard solution (purity: 99.6%) was obtained from ChromaDex (Irvine, CA, USA).

Deionized water was used for the pigment extraction. High-purity water produced with an Alpha-Q system (Millipore, Marlborough, MA, USA) was used for HPLC analysis.

Yellow discolored channel catfish fillets were obtained from two processors in west Alabama.

Sample handling

The catfish industry classifies discolored fillets as being dark yellow, medium yellow or light yellow. Six whole fillets of each type were obtained. Before storage, three fillets of each type were individually color evaluated, ground, extracted and analyzed by HPLC. The other three fish fillets of the same type were placed in a zippered plastic bag and stored in a refrigerator at 2-4 °C for 12 days. Color was evaluated every three days. On day 12, the three whole catfish fillets were ground, extracted and analyzed for their carotenoid contents by HPLC.

An additional 25 catfish fillets displaying a range of yellow intensities were also obtained. Each whole fresh catfish fillet was color evaluated first, and then ground, extracted and analyzed for their carotenoid contents by HPLC. Therefore, the total number of fresh fillets for correlating color and carotenoid content was 34.

Color evaluation

The photo-based color evaluation method used in this work was a modification of that described by Cline (2011). Whole fillets were placed on a white plastic board in a

controlled-lighting white box. Both right and left sides were lit using 3150 K video spotlights. In order to avoid color contamination from outside objects, three white poster boards were separately placed under, in front of, and behind the white box. A standard color reference (ColorChecker Passport, X-rite, Grand Rapids, Michigan) was photographed using a Cannon EOS500 D digital camera in the RAW image format. Under the same lighting conditions, the image of each whole catfish fillet was individually captured. Using the standard color reference and Adobe Photoshop CS5 (Adobe Systems Incorporated, San Jose, CA), photographs were digitally converted into an overall average color, which was characterized by its L, a, b-values. This methodology eliminates problems associated with the heterogeneous distribution of color patterns on the fillet surface when using traditional colorimeters.

Carotenoid extraction

The carotenoid extraction procedure was based on the general method described by Rodriguez-Amaya (2001) and subsequently modified by Liu and others (2012). Before the carotenoids can be analyzed, the pigment must first be extracted and concentrated.

Samples were cut into small pieces and homogenized in a Waring blender using intermittent bursts to minimize heat evolution. Five-gram aliquots of the ground fillet and 25 mL acetone containing 6 mg BHA/mL were placed in an Erlenmeyer flask, which was shaken on an orbital shaker at 150 rpm for 1.5 hour. The mixture was decanted into a glass funnel containing qualitative (medium fast) filter paper. The filtrate was stored at -20 ºC until two additional extraction steps were completed. The remaining residue was extracted two more times using 25 mL fresh acetone with 6 mg BHA/mL. The three extracts (≈75 mL) were combined and transferred into a separatory funnel. Then 20 mL

hexane/ethyl ether (1:1, v/v) and 110 mL water were added until two layers formed. The upper layer was removed and 20 mL fresh hexane/ethyl ether (1:1, v/v) were added. Again, the top layer was removed and another 20 mL hexane/ethyl ether (1:1, v/v) were added. The three upper layers were combined and concentrated under flowing nitrogen until all of the solvent evaporated. The residues were stored at -20ºC and analyzed within 15 days.

Carotenoids may break down in the presence of light. Thus, during this extraction period, the funnel was covered with aluminum foil to minimize carotenoid degradation. All the extraction steps were carried out at room temperature.

Carotenoid analysis

Carotenoids in the flesh of channel catfish were analyzed by a LC-20 series high performance liquid chromatography (HPLC) system with a photodiode array (PDA) detector, which was produced by Shimadzu (Kyoto, Japan). Carotenoids were detected by applying 20 µL sample to a 250 mm × 2.0 mm × 5 µm ProntoSIL C30 reverse-phase column (MAC-MOD Analytical Inc., Chadds Ford, PA, USA). A 10 mm × 2.0 mm guard cartridge was installed ahead of the column.

The multiple-step linear gradient used in this experiment was based on that described by Moros and others (2002), who analyzed carotenoids in corn and subsequently used by Liu and others (2012) for carotenoids in catfish. Two separate mobile phases were used for gradient elution. Mobile phase A was composed of methanol/TBME/water (81:15:4, v/v/v) and mobile phase B was composed of methanol/TBME (9:91, v/v). The initial mobile phase was 95% A and 5% B changing linearly to 50% A and 50% B over 45 min followed by increasing to 100% B for 15 min

The flow rate was 0.2 mL/min. UV-Vis spectrum was recorded from 190 to 800 nm by the PDA detector, and the chromatogram at 450 nm was used for quantitation.

After thawing the extraction sample at room temperature, it was dissolved in 2 mL acetone and filtered through a 0.2 µm PTFE syringe filter before injection. Sudan I (100 µL, 0.10 mg/mL) was added as the internal standard for determining the quantity of each carotenoid. The average contents and standard deviations of lutein, zeaxanthin, and alloxanthin were determined.

Our lab has used the above method previously and found the recoveries from ground fillets spiked lutein and zeaxanthin to be 94.7 and 99.9% respectively, with the coefficient of variation being less than 6.5% (Liu and others 2012).

Statistics

Correlations were evaluated using R-values. Differences between the average total carotenoid contents after 12 d storage were evaluated using a two-tailed t-test (Microsoft Excel, San Diego, CA, USA).

Results and Discussion

Yellow discoloration in relation to carotenoid contents

Positive b-values indicate the extent of yellowness in the L, a, b-color system, and thus b-values were used to measure visual yellowness of the fresh catfish fillets. The b-value ranged from 11 to 36 in the tested fillets. The carotenoids (lutein, zeaxanthin and alloxanthin) were analyzed in these catfish fillets. A strong proportional relationship between the b-value and the sum of these three major carotenoid contents (lutein, zeaxanthin and alloxanthin) was found with an R-value of 0.76 (Fig 3.1). The correlations between b-value and individual carotenoid contents were not as strong as the sum of the

carotenoids (Table 3.1). The intensity of the yellow color of the fresh catfish fillets appears to be mainly related to the sum of the major carotenoid contents, with zeaxanthin appearing to have a large effect on the R-value. Thus, strategies for reducing tissue carotenoid contents would be expected to improve the fillet color.

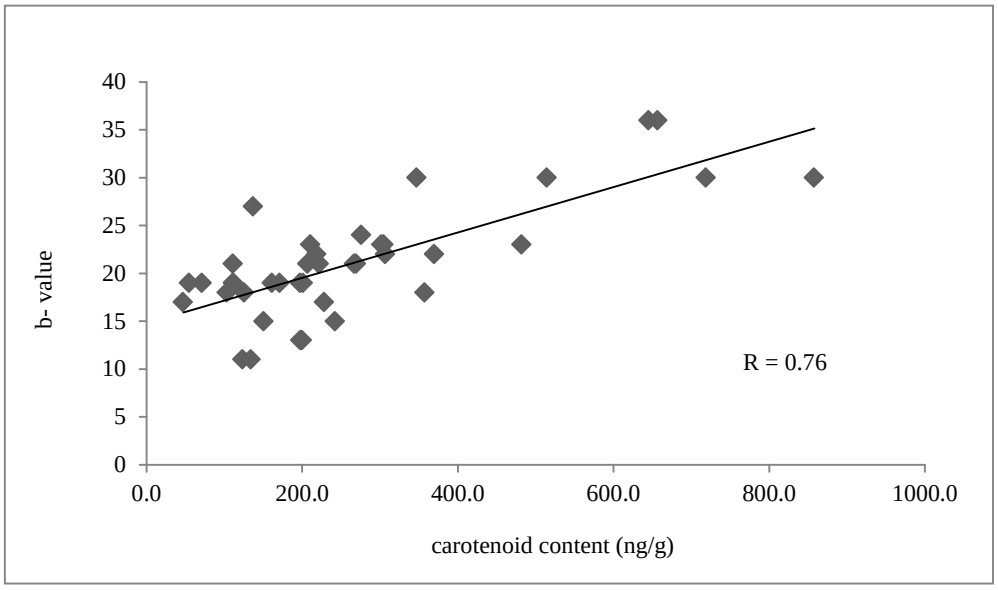

Fig 3.1-Relationship between the b-value and the sum of three major carotenoids (lutein, zeaxanthin and alloxanthin) in fresh channel catfish fillets.

Table 3.1-Correlation coefficients (R-values) of linear relationships between the b-value and various carotenoid contents of fresh channel catfish fillets.

	Lutein	**Zeaxanthin**	**Alloxanthin**	**Sum**
R value	0.57	0.74	0.42	0.76

Color changes during storage

The a- and b-values were larger for the fillets classified as “dark yellow” than the other two fillet types meaning the former was redder and more yellow. Similarly, the L-value was lower for the fillets considered dark yellow as compared to those classified as medium and light yellow.

Figure 3.2 shows the color changes that occurred to the yellow discolored fillets during storage for 12 d. During the first 6 days, the b-value of medium yellow and light yellow types of channel catfish increased moderately, which means these two types of channel catfish were becoming slightly more yellow during storage. The b-value of the dark yellow type did not change over the first 6 days. During the next six days, the degree of visual yellowness (b-value) of all types of fillets, especially for the light yellow type, increased rapidly. The L-value of all three types of discolored channel catfish fillets gradually decreased during 12 d. The a-values were approximately constant for all of the fillets indicating redness remained virtually unchanged during storage.

Figure 3.3 shows the average change in the L, a, b-values from time 0. On average, the ΔL-value decreased initially, then plateaued while the Δa value remained constant during storage. The Δb-value increased consistently during storage. Overall, the fillets generally became darker and more yellow during refrigerated storage

Carotenoid content changes during storage

Previous studies have related catfish fillet color to carotenoid content (Lee 1987; Li and others 2007). A more recent study also has suggested that lutein, zeaxanthin, alloxanthin, and diatoxanthin are most probably the carotenoids responsible for the yellow discoloration of fresh catfish fillets (Liu and others 2012). The best correlation

with yellow color was the total carotenoid content of the fillets as shown in Figure 3.1. The sum of the carotenoid contents (lutein, zeaxanthin and alloxanthin) in the dark yellow fillets significantly decreased ($P<0.01$) during 12 d of storage (Table 3.2). The sum of the carotenoid contents in light and medium yellow fillets did not significantly change during storage.

Table 3.2-Total carotenoid content (ng pigment/g fillet) of three types (dark yellow, medium yellow and light yellow) of whole yellow discolored catfish fillets during storage at 2-4 °C.

Type	Fresh	Day 12	significance level
Dark	398 ± 166 (n=15)	124 ± 23 (n=3)	<0.01
Medium	178 ± 59 (n=10)	178 ± 32 (n=3)	0.98
Light	128 ± 53 (n=9)	153 ± 73 (n=3)	0.63

Results are presented as mean ± std

Although catfish fillets became more yellow and darker during storage, the carotenoid contents appeared to decrease, suggesting that freely available carotenoids may not be directly responsible for the color changes. Carotenoid oxidation during storage would typically reduce coloration, rather than increase it (Schwartz and others 2008). Therefore, other unknown chemical changes may be occurring to cause the enhanced yellowing that develops during storage. Further research on the mechanism of discoloration and the potential relationship between color changes and carotenoid contents during storage is necessary.

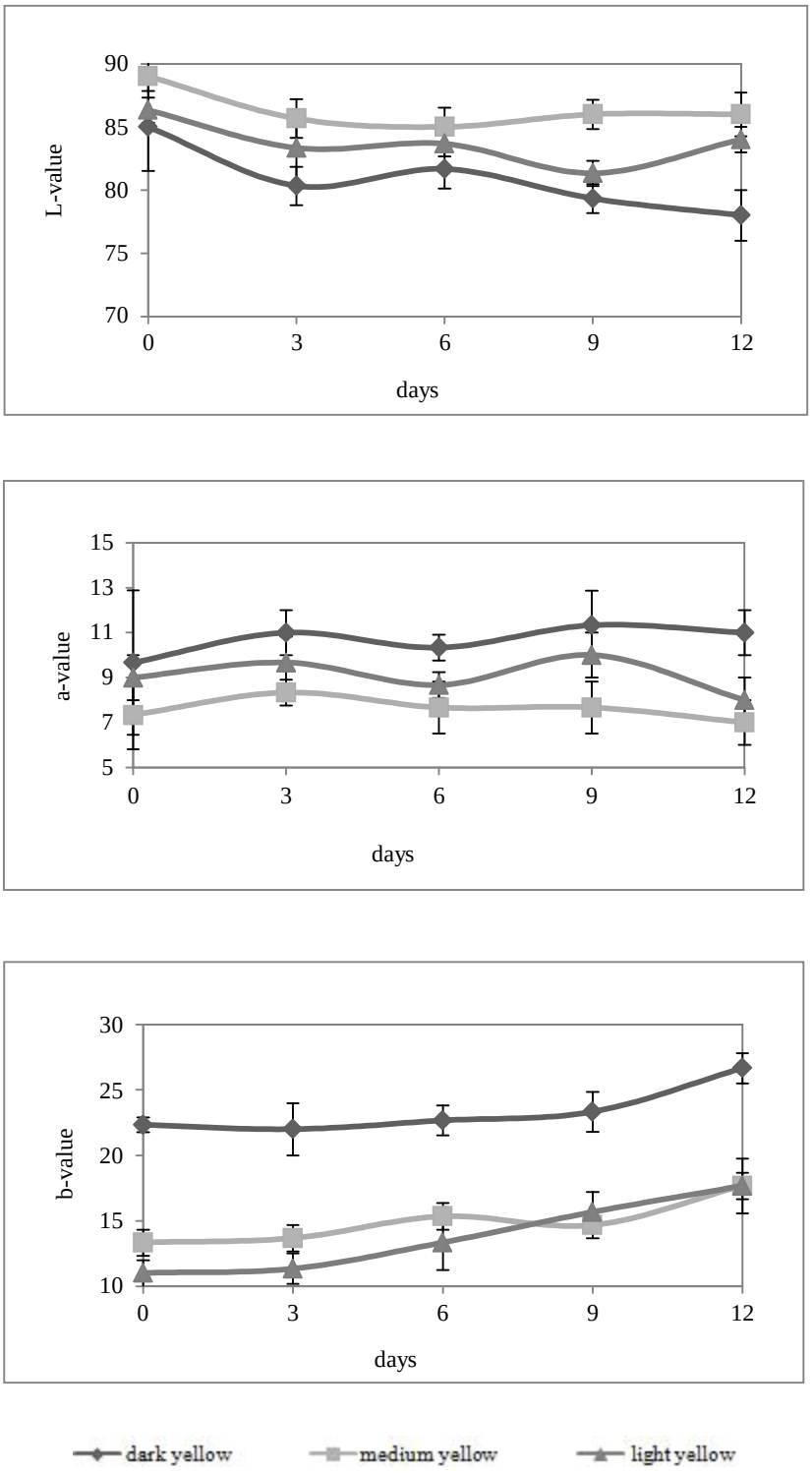

Fig 3.2-Average L, a, b-values with standard deviations of three types of yellow discolored fillets during storage at 2-4 °C (n=3).

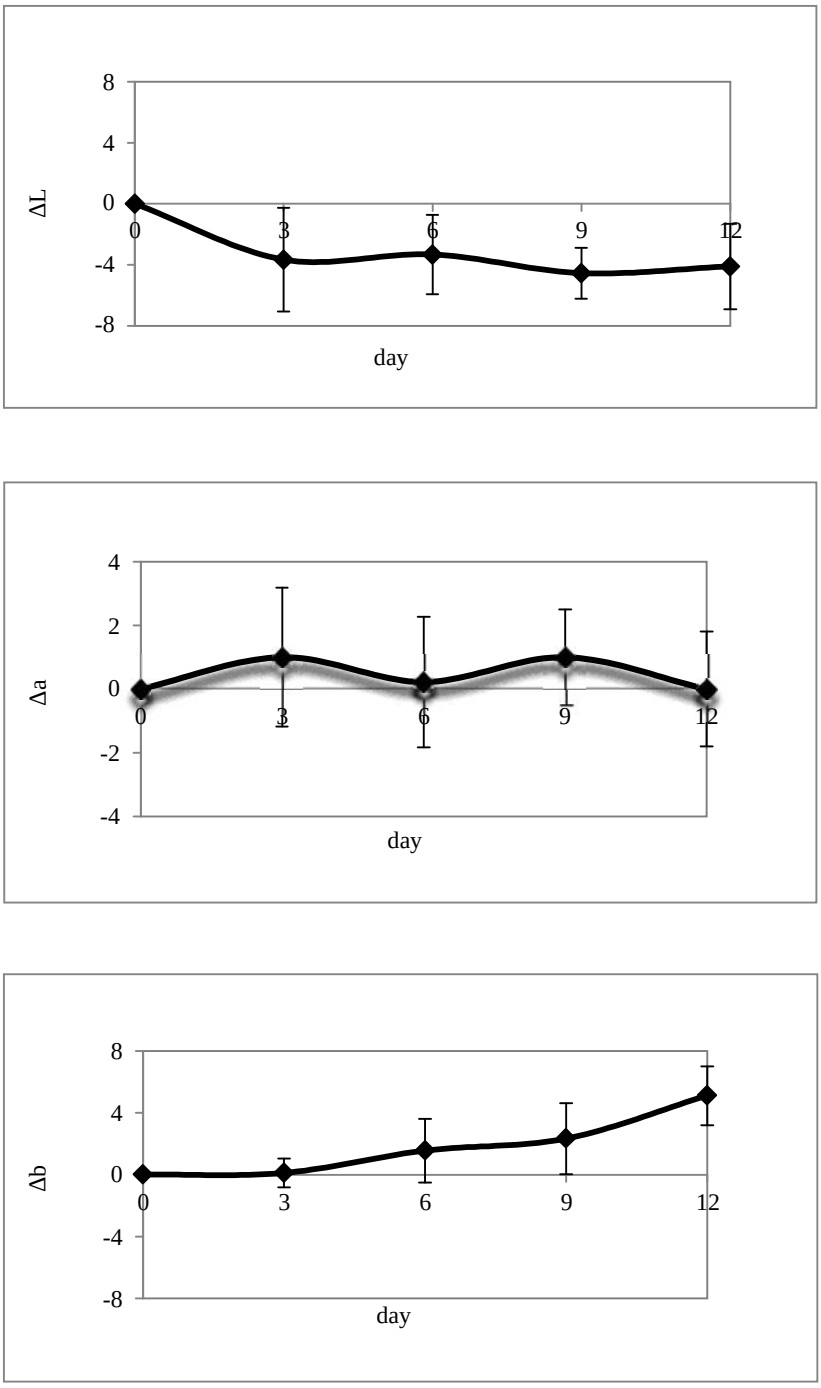

Fig 3.3-Average ΔL, Δa, Δb-values (difference from time 0 to time 12) with standard deviation of three types of yellow discolored fillets during storage at 2-4 °C (n=9).

Summary

For fresh catfish fillets, the intensity of the yellow color appears to be mainly related to the sum of the major carotenoid contents, with zeaxanthin appearing to have greater importance. During storage, the yellow discolored catfish fillets became darker and more yellow. The total carotenoid content of the dark yellow fillets decreased significantly ($P<0.01$) during 12 d of storage while the carotenoid contents of light and medium yellow fillets did not significantly change during storage.

Although the coloration of fresh fillets appears related to their carotenoid contents, the discoloration during storage worsens while carotenoid contents decrease. The chemical change causing the fillets to become darker and more yellow during storage remains unknown and requires more research.

Acknowledgements

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Chapter 4 Chemical Treatments for Reducing the Yellow Discoloration of Channel Catfish (*Ictalurus punctatus*) Fillets

ABSTRACT: The effect of chemical pretreatments on the color and carotenoid content of yellow discolored channel catfish fillets was studied. The color and carotenoid content of the fillets were analyzed by the L, a, b-color system and HPLC, respectively. Ascorbic acid, BHA, citric acid and sodium metabisulfite were not successful at reducing the discoloration. Sodium bicarbonate had a beneficial effect on reducing the degree of yellowness, but the fillets still turned darker after 12 d storage. Sodium bisulfite gave the best results, with fillets becoming brighter and less yellow after storage. The sum of carotenoid contents of untreated fillets decreased significantly ($P<0.1$) during storage as compared to the fresh fillets. However, the sum of the carotenoid contents of fillets treated by various chemicals were not significantly ($P>0.1$) different from the fresh or untreated fillets.

Introduction

Although over 39 species of catfish exist in North America, only six of these have been cultured for commercial production (Wellborn 1988). Channel catfish (*Ictalurus punctatus*), one of the most common catfish species, have been cultured for many years as the dominant aquaculture species in North America (Wolters and Johnson 1994). These popular fish typically feed on the bottom of large reservoirs, lakes and ponds (Wellborn 1988). Their diet appears to influence the coloration of their fillets (Lee 1987, Li and others 2007).

The catfish industry, which is ranked high among aquaculture industries, is a very important industry in the United States. As seafood consumption by Americans increased, the catfish industry has also grown. The volume of catfish processed increased from 220 million pounds in 1995 to 660 million pounds in 2003 (Hanson and Sites 2011). However, the catfish consumption has declined recently with only 472 million pounds produced in 2010 (USDA 2011). The product consumption is influenced by many factors, including technological advances, economical developments and catfish quality.

In recent years, yellow discoloration of catfish fillets has become a major quality issue to the channel catfish industry. Lee (1987) and Li and others (2007) showed dietary carotenoids influenced the yellow coloration of fillets. The intense yellow pigmentation generally concentrates on the anterior, dorsal part of the fish near backbone (Lovell 1984). In addition, channel catfish fillets often become increasingly dark yellow during storage.

Consumers may reject white fillets, such as those from channel catfish, if they contain regions of yellow discoloration (Lovell 1984; Shahidi and others 1988). Therefore, although the pigmentation does not appear to affect the flavor or nutritional quality of the fillet, the industry needs to solve this problem in order to avoid economic loss. The objective of this project was to evaluate the efficacy of various chemical pretreatments for preventing or reversing yellow discoloration in channel catfish fillets during storage.

Materials and Methods

Materials

Acetone (analytical grade), ethanol (analytical grade), ethyl ether (analytical grade), hexane (analytical grade), BHA: 3-tert-butyl-4-hydroxyanisole (analytical grade), Sudan I (analytical grade), methanol (HPLC grade), TBME: tert-butyl methyl ether (HPLC grade) and acetonitrile (HPLC grade), ascorbic acid (analytical grade), citric acid (analytical grade), sodium bisulfite (analytical grade), sodium bicarbonate (analytical grade), sodium metabisulfite (analytical grade) were purchased from VWR (Radnor, PA, USA).

Lutein (purity ≥ 95%) and zeaxanthin (purity ≥ 95%) standards were obtained from Sigma-Aldrich (St. Louis, MO, USA). Alloxanthin standard solution (purity: 99.6%) was obtained from ChromaDex (Irvine, CA, USA).

Deionized water was used for the pigment extraction. High-purity water produced with an Alpha-Q system (Millipore, Marlborough, MA, USA) was used for HPLC analysis.

Discolored channel catfish fillets classified by the industry as “dark yellow” were obtained from Harvest Select in west Alabama.

Sampling handling

Triplicate fresh fillets were immersed in different treatment solutions for 10 min. These solutions included 0.5% ascorbic acid (water soluble antioxidant), 0.5% BHA (lipid soluble antioxidant), 0.5% citric acid (acidic dip), 0.5% sodium bicarbonate (alkaline dip), 1.0% sodium metabisulfite, and 1.0% sodium bisulfite as well as blends of sodium bicarbonate/sodium bisulfite. After immersion, fillets were drained, placed into

zippered plastic bags, and stored at 2-4ºC for 12 days. Three untreated fillets (i.e., control) were also stored for 12 d at 2-4ºC. Catfish fillets were color evaluated every three days. On day 12, these fillets were ground, extracted and analyzed for their carotenoid contents by HPLC. Fresh dark yellow fillets (n=13) were also evaluated for carotenoid contents (not stored; day 0).

Color evaluation

The photo-based color evaluation procedure used for catfish fillets was based on the general method described by Cline (2011). Whole fillets were positioned on a white plastic board in a controlled-lighting white box. The right and left sides were lit using 3150 K video spotlights. Three white poster boards were placed under, in front of and behind the white box to prevent color contamination from outside objects.

A standard color reference (ColorChecker Passport, X-rite, Grand Rapids, Michigan) was photographed using a Cannon EOS500 D digital camera in the RAW image format. Under the same lighting conditions, the image of each whole catfish fillet was individually captured. Using the standard color reference and Adobe Photoshop CS5 (Adobe Systems Incorporated, San Jose, CA), photographs were digitally converted into an overall average color. The average color was characterized using the L-a-b color system. This methodology eliminates problems associated with heterogeneously distributed color patterns on the fillet surface when using traditional colorimeters.

Carotenoid extraction

The carotenoid extraction procedure was based on the general procedure described by Rodriguez-Amaya (2001) and subsequently modified by Liu and others

(2012). The pigments must first be extracted and concentrated prior to carotenoid analysis.

Fillets were cut into small pieces and homogenized in a Waring blender using intermittent bursts to minimize heat evolution. Five-gram aliquots of the ground fillet and 25 mL acetone containing 6 mg BHA/mL were placed in an Erlenmeyer flask, which was shaken on an orbital shaker at 150 rpm for 1.5 hour. The mixture was decanted into a glass funnel containing a qualitative (medium fast) filter paper. The resulting filtrate was stored at -20 ºC until two additional extraction steps were completed. The remaining residue was extracted two more times using 25 mL fresh acetone with 6 mg BHA/mL. The three extracts (≈75 mL) were combined and transferred into a separatory funnel. Then, 20 mL hexane/ethyl ether (1:1, v/v) and 110 mL water were added creating two layers. The upper layer was removed and 20 mL fresh hexane/ethyl ether (1:1, v/v) were added. Again, the top layer was removed and another 20 mL hexane/ethyl ether (1:1, v/v) were added. The three upper layers were combined and concentrated under flowing nitrogen until all of the solvent evaporated. The residues were stored at -20ºC and analyzed within 15 days.

Because carotenoids may break down in the presence of light, the funnel was covered with aluminum foil. All the extraction steps were carried out at room temperature.

Carotenoid analysis

Carotenoids extracted from the flesh of channel catfish were analyzed using a LC-20 series HPLC (high performance liquid chromatography) system with a photodiode array (PDA) detector, which was produced by Shimadzu (Kyoto, Japan). Carotenoids

were analyzed by injecting 20 µL sample on to a 250 mm × 2.0 mm × 5 µm ProntoSIL C30 reverse-phase column (MAC-MOD Analytical Inc., Chadds Ford, PA, USA). A 10 mm × 2.0 mm guard cartridge was installed ahead of the column.

The multiple-step linear gradient used in this experiment was based on that described by Moros and others (2002) and Liu and others (2012). Two separate mobile phases were used for gradient elution. Mobile phase A was composed of methanol/TBME/water (81:15:4, v/v/v) and mobile phase B was composed of methanol/TBME (9:91, v/v). The initial mobile phase was 95% A and 5% B changing linearly to 50% A and 50% B over 45 min followed by increasing to 100% B for 15 min. The flow rate was 0.2 mL/min. UV-Vis spectrum was recorded from 190 to 800 nm by the PDA detector and the chromatogram at 450 nm was used for quantitation.

After thawing the extraction sample at room temperature, it was dissolved in 2 mL acetone and filtered through a 0.2 µm PTFE syringe filter before injection. Sudan I (100 µL, 0.10 mg/mL) was added as the internal standard for determining the quantity of each carotenoids. The average contents and standard deviations of lutein, zeaxanthin and alloxanthin were determined.

Our lab has used the above method previously and found the recoveries of ground fillets spiked with lutein and zeaxanthin were 94.7 and 99.9%, respectively; the coefficient of variation was less than 6.5% for this method (Liu and others 2012).

Data analysis

The average change in the L, a, b-values between day 0 and 12, and the average carotenoid contents were determined. Data comparisons were analyzed for significance ($P < 0.1$) using one-way ANOVA with Tukey HSD (SPSS, IBM, New York, NY).

Results and Discussion

Color changes

Table 4.1 shows the effects of various treatments on the color characteristics of catfish fillets. Untreated fillets became darker (ΔL = -7.0) and more yellow (Δb = +4.3) after storage for 12 d. Ascorbic acid, citric acid, BHA and sodium metabisulfite were not successful at reducing nor preventing the intensification of the yellow discoloration (P>0.1). In addition, citric acid and BHA did not lessen fillet darkening during storage (P>0.1).

Table 4.1-Average change in L, a, b-values of whole yellow discolored fillets between day 0 and day 12 as affected by chemical pretreatment.

	Δ L (d12 – d0)	**Δ a (d12 – d0)**	**Δ b (d12 – d0)**
Untreated	-7.0±2.0 ^{a}	1.3±2.3^{a}	4.3±1.5^{ac}
Ascorbic acid 0.5%	-3.0±1.0^{bd}	0.7±0.6^{a}	4.0±1.7^{ac}
BHA 0.5%	-4.7±1.5^{ab}	1.0±1.0^{a}	5.0±2.0^{ac}
Citric acid 0.5%	-4.0±0.0^{ab}	1.7±2.3^{a}	7.7±0.6^{a}
Sodium bicarbonate 0.5%	-5.0±1.0^{ab}	-10.3±1.2^{b}	-3.3±4.6^{bde}
Sodium metabisulfite 1.0%	-3.0±1.0^{bd}	1.0±1.5^{a}	3.0±1.0^{ace}
Sodium bisulfite 1.0%	1.3±1.5^{ce}	-6.7±3.1^{b}	-3.7±4.0^{bd}
Sodium bicarbonate 0.5% & sodium bisulfite	-0.7±6.6^{de}	-10.0±2.6^{b}	-0.7±1.5^{cd}

Results are presented as mean ± std (n=3); values with same superscript letters are not significantly different (p>0.1)
Negative ΔL= darker; positive Δa= redder; positive Δb= more yellow

Sodium bicarbonate had a beneficial effect on reducing the degree of yellowness (Δb = -3.3), but the fillets darkened similarity to the untreated fillets (ΔL = -5.0) after 12 d storage. Sodium bisulfite gave the best results, with fillets becoming brighter (ΔL = +1.3) and less yellow (Δb = -3.7) after storage. However, no evident improvement was found by combining these two chemicals together. The fillets pretreated with the

combination of sodium bicarbonate and sodium bisulfite were significantly lighter than the untreated fillets ($P<0.1$) but were non-significantly less yellow. The last three treatments appear to improve color of the fillets during the storage as compared to the untreated fillets.

Carotenoid content

Previous studies have related catfish fillet color to carotenoid content (Lee 1987; Li and others 2007). A more recent study has suggested that lutein, zeaxanthin, alloxanthin and diatoxanthin are most likely responsible for the yellow discoloration of fresh catfish fillets (Liu and others 2012). Chapter 3 showed a strong proportional relationship between the b-value and the sum of three major carotenoid contents (lutein, zeaxanthin, alloxanthin) of fresh fillets with an R-value of 0.76.

Although the color of freshly harvested catfish fillets appears to be influenced by their carotenoid content, the relationship is less clear after storage. The contents of lutein and alloxanthin of untreated fillets and various chemically-treated fillets after 12 d of storage were all not significantly different from the fresh fillets ($P>0.1$). The contents of zeaxanthin, the richest carotenoid, in untreated fillets, fillets treated by 0.5% sodium bicarbonate and fillets treated with 0.5% bicarbonate/1.0% sodium bisulfite combination after 12 d of storage were significantly lower ($P<0.1$) than those in fresh fillets. The sum of carotenoid contents of untreated fillets significantly decreased ($P<0.1$) during storage as compared to the fresh fillets (Table 4.2). However, the sum of the carotenoid contents of fillets treated by various chemicals were not significantly ($P>0.1$) different from the fresh or untreated fillets.

Table 4.2-Carotenoid content* (ng pigment/g tissue) of whole yellow discolored catfish fillets as affected by chemical pretreatment.

Treatment**	Lutein	Zeaxanthin	Alloxanthin	Total
Fresh, day 0 (n=13)	110±42[a]	290±151[a]	55±38[a]	454±210[a]
Untreated, day 12 (n=3)	55±16[a]	50±7[b]	20±3[a]	124±23[b]
Treatment 1, day 12 (n=3)	47±12[a]	89±31[ab]	23 ±15[a]	159±57[ab]
Treatment 2, day 12 (n=3)	71±14[a]	144±50[ab]	32±19[a]	246±48[ab]
Treatment 3, day 12 (n=3)	71±25[a]	133±60[ab]	31±20[a]	235±93[ab]
Treatment 4, day 12 (n=3)	55±16[a]	50±7[b]	91±125[a]	195 ±124[ab]
Treatment 5, day 12 (n=3)	81±52[a]	190±129[ab]	21±7[a]	291±172[ab]
Treatment 6, day 12 (n=3)	93±21[a]	95±25[ab]	50±3[a]	238±47[ab]
Treatment 7, day 12 (n=3)	48±22[a]	48±32[b]	68±64[a]	164±114[ab]

*Results are presented as mean ± std; values with same superscript letters are not significantly different (p>0.1);
**Treatment 1: 0.5% ascorbic acid; Treatment 2: 0.5% BHA; Treatment 3: 0.5% citric acid; Treatment 4: 0.5% sodium bicarbonate; Treatment 5: 1.0% sodium metabisulfite; Treatment 6: 1.0% sodium bisulfite; Treatment 7: 0.5 % sodium bicarbonate/1.0% sodium bisulfite

Summary

Sodium bisulfite gave the best results, with fillets becoming brighter and less yellow after storage. Therefore, the appearance of discolored fillets could be improved by using chemical pretreatments containing sodium bisulfite. The color change over time appeared not to be directly dependent on the carotenoid content. Thus, more research is required to understand the mechanism of bilsulfite's action on the fillet color and the role of carotenoids on catfish color changes during storage.

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Chapter 5 Summary

A strong linear relationship between the b-value of fresh whole catfish fillets and the total of the three major carotenoid contents (lutein, zeaxanthin and alloxanthin) was found with an R-value of 0.76. The intensity of the yellow color of the fresh catfish fillets seems to be mainly related to the sum of the major carotenoid contents rather than individual carotenoids.

Yellow discolored catfish became darker and more yellow after 12 day refrigerated storage. The sum of carotenoid contents (lutein, zeaxanthin and alloxanthin) of dark yellow discolored fillets decreased ($P<0.01$) during storage.

Ascorbic acid, BHA, citric acid and sodium metabisulfite were not successful at reducing nor preventing the yellow discoloration ($P>0.1$). Sodium bicarbonate had a beneficial effect on reducing the degree of yellowness, but the fillets turned darker after 12 days of storage. Sodium bisulfite gave the best results; the treated fillets become brighter and less yellow after storage. However, no evident improvement was found by combining sodium bicarbonate and sodium bisulfite together.

The sum of the carotenoid contents (lutein, zeaxanthin and alloxanthin) of untreated dark fillets significantly decreased during 12 d storage compared to fresh fillets ($P<0.1$). However, the sum of carotenoid contents of fillets treated by chemicals did not change significantly. The relationship between the color change and carotenoid contents of fillets during storage requires additional research.

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Appendix A-Ground Fillet Sub-study

Changes in color and carotenoid content during storage cannot be continuously evaluated in a single fillet due to the fillet being destroyed by the carotenoid extraction procedure. Therefore, fillets were ground and placed into 10 uncovered petri dishes, which were stored uncovered in a refrigerator at 2-4 °C for 12 d. Duplicate samples were photographed and analyzed for carotenoid contents, as described in chapters 3 and 4.

Very little changes to the color were observed (Fig A.1, A.2) during storage. The surface yellowing got blended into the non-yellow regions, diluting its intensity. Potential changes were more difficult to identify. Similarly, carotenoid contents changed minimally during storage (Table E.3).

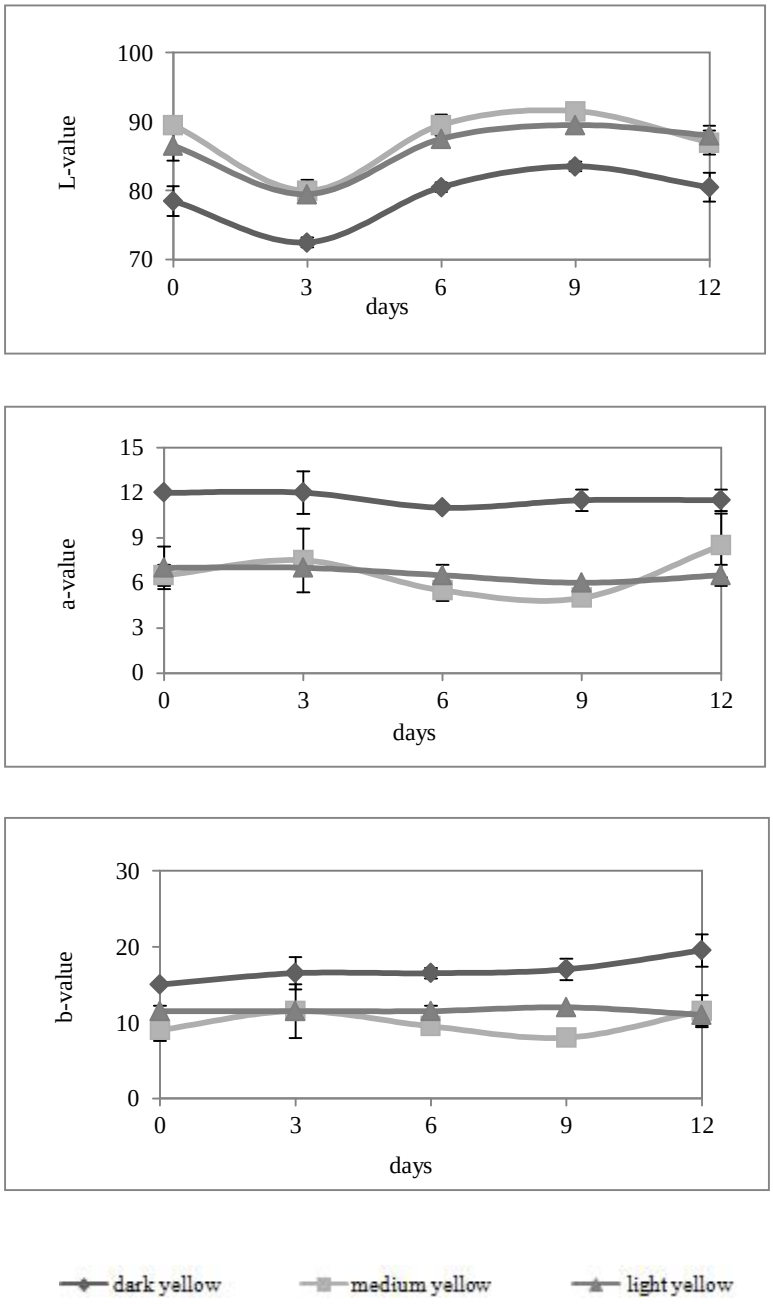


Fig A.1-Average L, a, b-values with standard deviations of three types of ground yellow discolored fillets during storage at 2-4 °C (n=2).

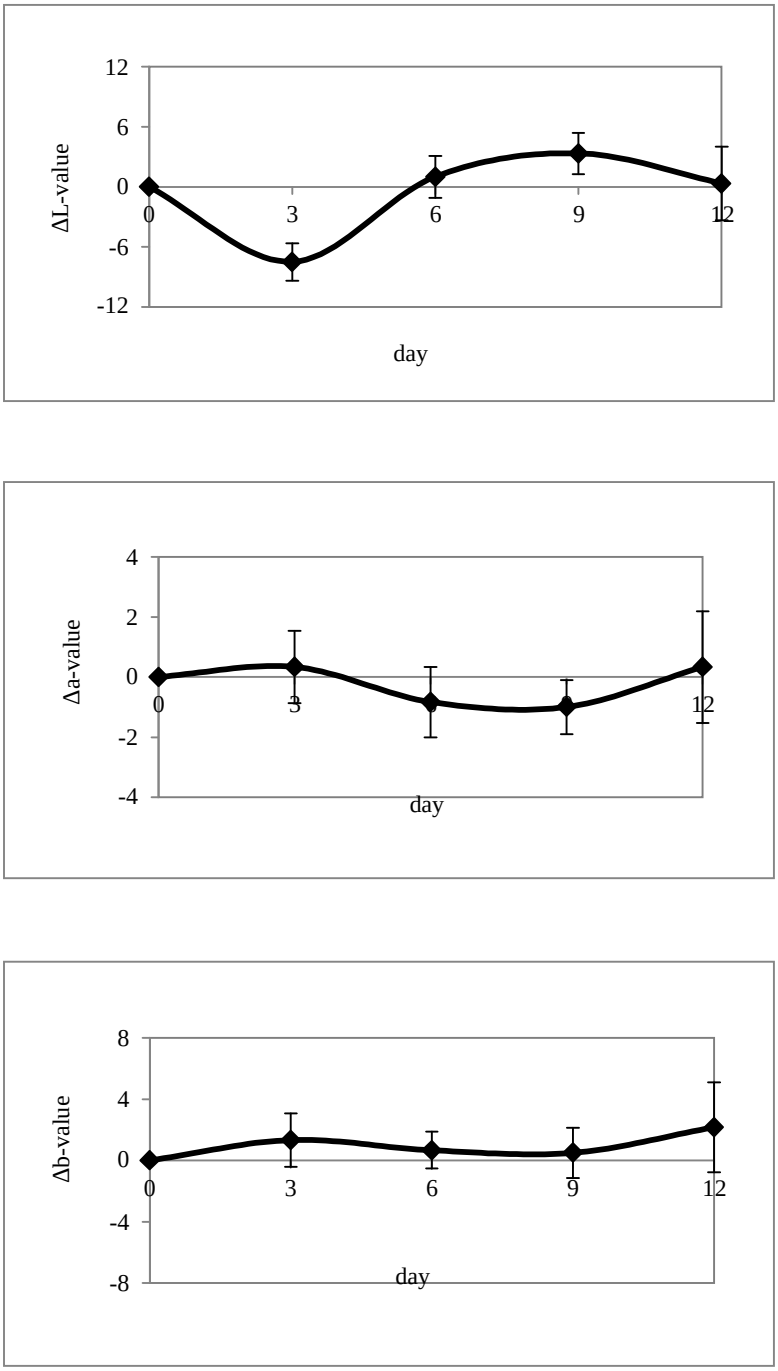


Fig A.2- Average ΔL, Δa, Δb-values (difference from time 0 to time 12) with standard deviations of three types of ground yellow discolored fillets during storage at 2-4 °C (n=6).

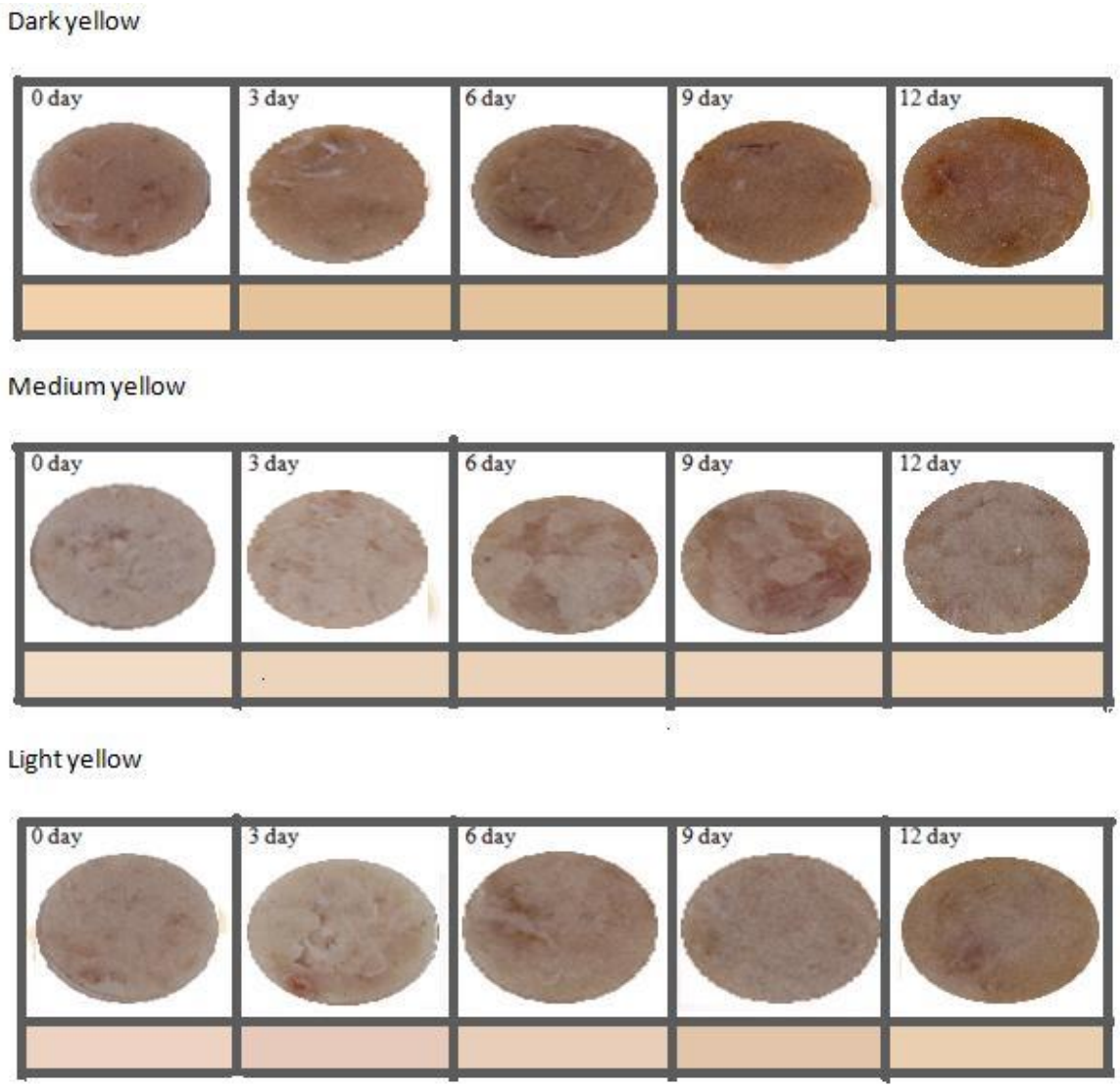


Fig A.3-Photo and corresponding average color of three types of ground yellow discolored fillets during storage at 2-4 °C.

Appendix B-Color Method

The photo-based color evaluation steps used in this work were a modification of the general steps described by Cline (2011). Photographs were digitally converted into an overall average color and the average color was characterized by its L, a, b-values. An example appears in Figure B.1.

L=81; a=16; b=20

Fig B.1- Original photograph and the digitized average color of a yellow discolored fillet.

Appendix C-Color Data

Table C.1- L, a, b-values of three types (dark yellow, medium yellow and light yellow) of whole fresh channel catfish fillets. Data were used in chapter 3.

	L	a	b
Light yellow 1	89	10	19
Light yellow 2	88	13	19
Light yellow 3	86	14	17
Light yellow 4	86	9	11
Light yellow 5	86	9	11
Light yellow 6	83	12	18
Light yellow 7	76	24	17
Light yellow 8	80	18	19
Light yellow 9	78	25	27
Medium yellow 1	87	14	19
Medium yellow 2	86	14	21
Medium yellow 3	84	18	21
Medium yellow 4	86	13	23
Medium yellow 5	86	15	19
Medium yellow 6	84	15	18
Medium yellow 7	83	13	19
Medium yellow 8	82	12	21
Medium yellow 9	84	11	15
Medium yellow 10	84	14	15
Dark yellow 1	89	15	23
Dark yellow 2	85	15	21
Dark yellow 3	85	16	22
Dark yellow 4	89	11	23
Dark yellow 5	85	10	22
Dark yellow 6	85	10	22
Dark yellow 7	85	19	36
Dark yellow 8	85	19	36
Dark yellow 9	84	16	30
Dark yellow 10	85	18	30
Dark yellow 11	85	18	30
Dark yellow 12	79	23	18
Dark yellow 13	80	17	24
Dark yellow 14	80	18	23
Dark yellow 15	79	18	19

Table C.2- L, a, b-values of three types (dark yellow, medium yellow and light yellow) of whole fillets during storage at 2-4 °C.

	Day	0	3	6	9	12
Untreated dark-1	L	87	80	80	80	80
	a	6	12	11	10	10
	b	22	22	22	25	28
Untreated dark-2	L	87	79	82	80	78
	a	11	11	10	11	11
	b	22	20	22	23	26
Untreated dark-3	L	81	82	83	78	76
	a	12	10	10	13	12
	b	23	24	24	22	26
Untreated medium-1	L	90	83	84	87	87
	a	6	8	7	7	6
	b	14	14	19	15	18
Untreated medium-2	L	89	85	86	86	86
	a	7	8	7	7	7
	b	14	14	16	15	18
Untreated medium-3	L	88	89	85	85	85
	a	9	9	9	9	8
	b	12	13	11	14	17
Untreated light-1	L	87	85	85	82	83
	a	9	9	8	9	9
	b	10	10	14	17	17
Untreated light-2	L	87	82	84	82	83
	a	10	11	9	10	7
	b	11	12	11	14	20
Untreated light-3	L	85	83	82	80	86
	a	8	9	9	11	8
	b	12	12	15	16	16

Table C.3- L, a, b-values of whole dark yellow fillets during storage at 2-4 °C as affected by chemical pretreatment.

	Day	0	3	6	9	12
Ascorbic acid 0.5% -1	L	89	89	88	87	87
	a	13	14	14	12	13
	b	17	18	20	18	20
Ascorbic acid 0.5% -2	L	88	88	87	84	85
	a	14	16	15	14	15
	b	19	24	25	23	25
Ascorbic acid 0.5% -3	L	87	87	86	84	83
	a	12	14	13	11	13
	b	16	18	19	18	19
BHA 0.5% -1	L	92	92	91	89	89
	a	9	8	9	8	10
	b	23	22	24	23	26
BHA 0.5% -2	L	85	85	85	80	80
	a	12	11	9	10	14
	b	17	20	22	23	24
BHA 0.5% -3	L	83	84	82	80	77
	a	17	15	13	14	17
	b	23	25	29	27	28
Citric acid 0.5% -1	L	91	90	91	89	87
	a	9	5	8	10	12
	b	15	15	20	20	23
Citric acid 0.5% -2	L	89	89	87	86	85
	a	13	12	12	13	12
	b	18	20	25	26	26
Citric acid 0.5% -3	L	88	88	89	85	84
	a	10	11	10	11	13
	b	18	20	24	24	25

Sodium bicarbonate 0.5% -1	L	84	84	79	80	79
	a	14	9	8	5	3
	b	20	16	14	13	14
Sodium bicarbonate 0.5% -2	L	79	80	77	75	75
	a	20	17	16	14	11
	b	20	19	19	20	22
Sodium bicarbonate 0.5% -3	L	80	81	76	75	74
	a	16	14	11	7	5
	b	31	27	25	25	25
Sodium metabisulfite 1.0% -1	L	89	88	88	87	85
	a	14	15	15	13	14
	b	26	28	31	29	30
Sodium metabisulfite 1.0% -2	L	89	89	88	87	86
	a	12	13	12	10	11
	b	22	21	23	24	24
Sodium metabisulfite 1.0% -3	L	88	88	88	85	86
	a	14	14	12	11	12
	b	18	20	20	20	21
Sodium bisulfite 1.0% -1	L	85	85	84	82	85
	a	19	17	18	14	13
	b	27	26	27	26	27
Sodium bisulfite 1.0% -2	L	81	84	82	80	84
	a	23	19	18	16	13
	b	37	35	32	29	29
Sodium bisulfite 1.0% -3	L	89	90	88	88	90
	a	13	11	13	10	9
	b	27	28	26	24	24
Sodium bicarbonate 0.5% & sodium bisulfite 1.0% -1	L	77	78	75	75	76
	a	24	23	21	17	11
	b	21	22	19	21	22

Sodium bicarbonate 0.5% & sodium bisulfite 1.0% -2	L	75	76	72	73	74
	a	24	22	20	18	16
	b	23	22	21	22	22
Sodium bicarbonate 0.5% & sodium bisulfite 1.0% -3	L	82	84	80	81	82
	a	16	11	11	10	7
	b	20	17	12	17	18

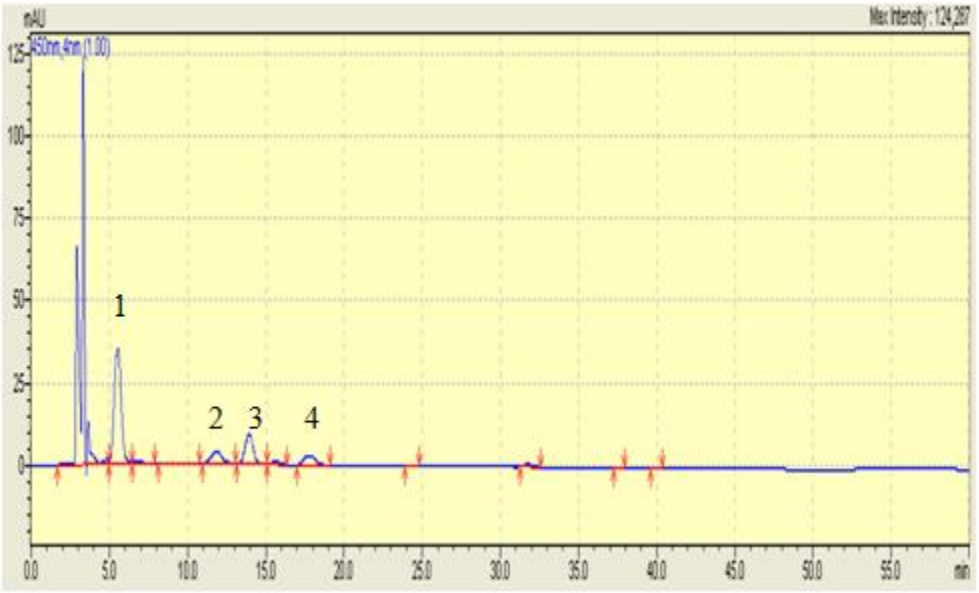
Table C.4- L, a, b-values of three types (dark yellow, medium yellow and light yellow) of ground fillets during storage at 2-4 °C.

	Days	**0**	**3**	**6**	**9**	**12**	**15**
Untreated dark-1	L	80	73	81	84	79	75
	a	12	11	11	11	12	15
	b	15	15	16	16	21	26
Untreated dark-2	L	77	72	80	83	82	74
	a	12	13	11	12	11	15
	b	15	18	17	18	18	24
Untreated medium-1	L	91	82	89	92	85	84
	a	6	6	6	5	10	7
	b	8	9	10	8	13	12
Untreated medium-2	L	88	78	90	91	89	85
	a	7	9	5	5	7	6
	b	10	14	9	8	10	12
Untreated light-1	L	88	80	87	89	89	81
	a	6	7	7	6	6	8
	b	11	11	11	12	10	18
Untreated light-2	L	85	79	88	90	87	82
	a	8	7	6	6	7	8
	b	12	22	12	12	12	16

Appendix D- Sample HPLC Data

HPLC chromatograms were used to determine the carotenoid contents of yellow discolored channel catfish fillets. The method was described in chapter 3 and 4.

0 day



12 day

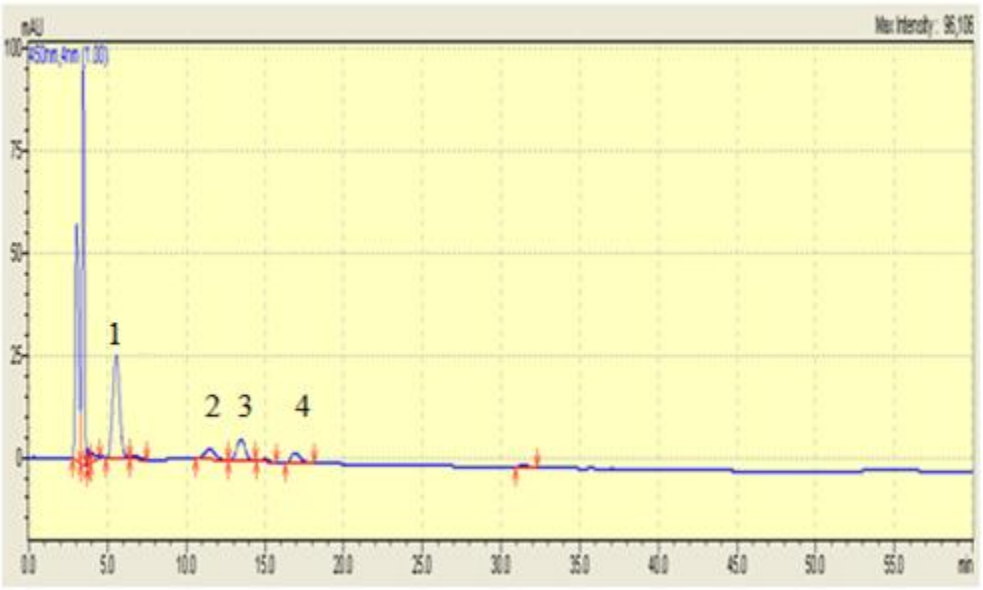


Fig D.1-HPLC chromatogram (absorbance at 450 nm vs. retention time) of carotenoids extracted from channel catfish fillets. Peak1: Sudan I; Peak 2: Lutein; Peak 3; Zeaxanthin; Peak 4: Alloxanthin.

Appendix E-Carotenoid Content

Table E.1-Carotenoid contents (ng pigment/g fillet) of three types (dark yellow, medium yellow and light yellow) of whole fresh catfish fillets. Data were used in chapter 3.

	Lutein	Zeaxanthin	Alloxanthin	Sum
Light yellow 1	33.4	70.5	2.6	111.7
Light yellow 2	38.1	29.2	0.8	71.5
Light yellow 3	22.5	21.8	5.8	47.2
Light yellow 4	38.0	71.3	4.0	123.7
Light yellow 5	42.1	75.9	4.2	134.3
Light yellow 6	67.7	44.7	4.3	125.8
Light yellow 7	78.0	101.4	9.2	228.6
Light yellow 8	71.6	68.3	6.8	171.3
Light yellow 9	47.8	57.2	6.3	137.0
Medium yellow 1	50.5	100.2	5.7	161.2
Medium yellow 2	55.1	149.7	6.1	222.0
Medium yellow 3	35.6	66.8	3.3	111.1
Medium yellow 4	57.4	132.1	14.2	210.6
Medium yellow 5	39.0	62.3	3.4	111.8
Medium yellow 6	36.9	56.5	3.0	103.5
Medium yellow 7	66.9	75.1	7.6	197.7
Medium yellow 8	135.4	98.9	9.2	267.3
Medium yellow 9	118.5	80.6	8.8	242.5
Medium yellow 10	55.5	61.1	62.9	150.6
Dark yellow 1	115.9	337.0	20.5	482.2
Dark yellow 2	86.5	139.5	29.2	269.4
Dark yellow 3	67.7	116.0	11.3	218.6
Dark yellow 4	73.3	213.6	16.0	304.7
Dark yellow 5	86.0	164.6	14.3	307.1
Dark yellow 6	103.9	197.9	17.7	370.0
Dark yellow 7	135.7	405.7	38.9	645.4
Dark yellow 8	141.5	409.0	39.8	657.1
Dark yellow 9	173.1	542.7	128.7	719.1
Dark yellow 10	64.6	232.7	19.2	347.2
Dark yellow 11	104.1	340.0	29.2	514.7
Dark yellow 12	81.3	115.6	18.5	357.7
Dark yellow 13	56.2	47.3	12.0	276.2
Dark yellow 14	198.0	85.1	5.2	302.1
Dark yellow 15	93.3	68.2	7.4	201.1

Table E.2-Carotenoid contents (ng pigment/g fillet) of whole yellow discolored catfish fillets as affected by chemical pretreatments. Data were used in chapter 4.

	Lutein	Zeaxanthin	Alloxanthin	Sum
Fresh-1 (day 0)	115.865	336.968	29.364	482.2
Fresh-2 (day 0)	86.491	139.468	43.412	269.4
Fresh-3 (day 0)	67.666	115.986	34.979	218.6
Fresh-4 (day 0)	73.309	213.576	17.836	304.7
Fresh-5 (day 0)	76.275	124.446	6.389	207.1
Fresh-6 (day 0)	85.983	164.594	56.566	307.1
Fresh-7 (day 0)	103.880	197.855	68.290	370.0
Fresh-8 (day 0)	135.729	405.700	103.962	645.4
Fresh-9 (day 0)	141.484	409.031	106.545	657.1
Fresh-10 (day 0)	196.761	539.884	121.331	858.0
Fresh-11 (day 0)	173.148	542.726	3.257	719.1
Fresh-12 (day 0)	64.566	232.660	49.936	347.2
Fresh -13 (day 0)	104.110	340.014	70.602	514.7
Untreated-1 (day 12)	70.822	57.795	18.851	147.5
Untreated-2 (day 12)	38.753	44.219	18.557	101.5
Untreated-3 (day 12)	54.063	46.395	23.475	123.9
Treatment 1-1 (day 12)	39.893	64.813	7.656	112.4
Treatment 1-2 (day 12)	61.354	123.865	36.533	221.8
Treatment 1-3 (day 12)	39.965	77.521	23.885	141.4
Treatment 2-1 (day 12)	85.275	200.718	14.739	300.7
Treatment 2-2 (day 12)	57.950	125.537	28.227	211.7
Treatment 2-3 (day 12)	68.603	105.040	51.975	225.6
Treatment 3-1 (day 12)	44.152	88.413	9.394	142.0
Treatment 3-2 (day 12)	92.754	200.470	34.661	327.9
Treatment 3-3 (day 12)	75.980	109.081	48.502	233.6
Treatment 4-1 (day 12)	70.822	57.795	18.851	147.5
Treatment 4-2 (day 12)	38.753	44.219	18.557	101.5
Treatment 4-3 (day 12)	54.063	46.395	234.745	335.2
Treatment 5-1 (day 12)	60.759	265.296	17.597	343.7
Treatment 5-2 (day 12)	139.669	263.395	28.896	432.0
Treatment 5-3 (day 12)	41.831	41.662	15.627	99.1
Treatment 6-1 (day 12)	116.095	123.099	51.124	290.3
Treatment 6-2 (day 12)	74.780	80.777	47.598	203.2

Treatment 6-3 (day 12)	86.538	80.387	52.479	219.4
Treatment 7-1 (day 12)	59.283	66.917	132.675	258.9
Treatment 7-2 (day 12)	62.389	66.479	66.526	195.4
Treatment 7-3 (day 12)	22.475	11.202	5.149	38.8

Treatment 1: 0.5% ascorbic acid; Treatment 2: 0.5% BHA; Treatment 3: 0.5% citric acid; Treatment 4: 0.5% sodium bicarbonate; Treatment 5: 1.0% sodium metabisulfite; Treatment 6: 1.0% sodium bisulfite; Treatment 7: 0.5 % sodium bicarbonate/1.0% sodium bisulfite

Table E.3- Carotenoid contents (ng pigment/g fillet) of three types (dark yellow, medium yellow and light yellow) of ground fillets during storage at 2-4 °C.

	day	**0**	**3**	**6**	**9**	**12**
Dark yellow-1	Lutein	85.9	84.0	85.5	88.2	87.0
	Zeaxanthin	163.9	158.3	129.7	113.8	145.5
	Alloxanthin	56.6	55.4	55.9	57.9	57.3
Dark yellow-2	Lutein	103.8	83.8	87.8	87.4	88.5
	Zeaxanthin	197.3	160.6	142.7	104.1	147.4
	Alloxanthin	68.3	55.9	35.7	62.4	59.1
Medium Yellow-1	Lutein	57.6	52.5	54.2	64.8	54.6
	Zeaxanthin	112.3	100.2	93.9	113.8	94.6
	Alloxanthin	29.2	25.9	24.9	60.5	25.2
Medium Yellow-2	Lutein	56.3	58.3	54.4	62.0	62.7
	Zeaxanthin	114.5	121.5	94.6	104.1	115.7
	Alloxanthin	26.4	23.3	28.4	31.2	1.6
Light yellow-1	Lutein	38.0	44.9	29.8	29.0	36.4
	Zeaxanthin	70.2	83.2	48.9	51.0	61.9
	Alloxanthin	14.4	4.5	10.9	13.1	13.3
Light yellow-2	Lutein	42.1	44.6	48.0	29.8	44.3
	Zeaxanthin	74.9	82.6	81.6	51.6	71.8
	Alloxanthin	16.3	16.6	17.3	12.5	15.5