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Influence of Fresh and Processed Tannic Acid, and Fresh Tannic Acid, and Fresh Tannic Acid Plus Phosphate on Catfish Fillet Color, Microbial Shelf Life and Oxidation

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Influence of fresh and processed tannic acid, and fresh tannic acid, and fresh tannic acid plus phosphate on catfish fillet color, microbial shelf life and oxidation

By

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Influence of fresh and processed tannic acid, and fresh tannic acid plus phosphates on catfish fillet color, microbial shelf life and oxidation

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Fresh tannic acid (5%) (FTA), heated to 121C for 15 min (PTA), and the combination of FTA and phosphates (FPH) were vacuum tumbled with catfish fillets and compared to water tumbled fillets (CTL). Fillets treated with FTA or PTA had higher whiteness whereas all treated fillets had higher chroma values than CTL, resulting in a deeper yellow color fillet. Microbial shelf life (CTL, log CFU/g, APC) of FTA and PTA treated fillets was extended by two days, to11.8 d at 4C, but FPH fillets did not reach over 5 log CFU/g for 15 d of storage. Fat from FTA and PTA treated fillets had induction points (IP) of 4.0 and 3.4 h, respectively, whereas FPH and CTL samples had IP of 1.4 and 2.4 h. Thus, FPH seems to be the best antimicrobial treatment and FTA/PTA, the best antioxidant treatment.

DEDICATION

I would like to dedicate these years of my life and this work to my family and especially: Arthur Souto Maior Filizzola, Vera Lucia Lima Coelho Filizzola, Luiz Claudio peixoto Cury, Maria Auxiliadora Lika Coelho Filizzola who have bravely believed and supported me throughout these educational years.

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CHAPTER I

INTRODUCTION

Channel Catfish (*Ictalurus punctatus*) are particularly well adapted to warm waters and are farmed in the southeastern U.S. where they are grown commercially via extensive aquaculture. Fast growth rate, high feed turnover efficiency, mild flavor, combined with other social-economic factors has contributed to this being the most predominant aquaculture species in the USA (Hargreaves and Tucker, 2004). As a main important economical aquaculture product in the south, processed channel catfish in the USA reached 215,000 million kilograms (472,000 million pounds) in 2010, up 2.5 million (+1.2%) from 218,000 million kilograms produced in 2009 (USDA NASS 2010). Despite the success of the channel catfish industry, it is currently facing many challenges including market competition from Vietnam and channel catfish that are imported from China (USDA FAS 2008). Maintenance of the market is the predominate concern of the catfish industry in the USA. The number of U.S. operations decreased by 85 operations (-9%) from 2010 and is now at 909 operations (USDA NASS 2010). Production is seasonal and frozen storage is therefore necessary to maintain a uniform supply throughout the year.

Frozen catfish storage most often creates a change in product quality due to lipids degradation. The normal range of proximate composition in fish flesh is: 66-81% water, 16-21% protein, 0.2-2.5% lipid, 1.2-1.5% ash, and less than 0.5% carbohydrate. (Lovell and Ammerman 1974) This lipid percentage is dependent on feed, season and growing

environment (Lovell and Ammerman 1974). Changes during frozen storage include oxidative deterioration (lipid oxidation), particularly in fatty fish like catfish and toughening of fish muscle in lean species of fish (Ravesi and Andreson 1969). Fat autoxidation of polyenoic acid produces fishy flavors and other rancid undesirable flavors and aromas. In addition, lipid oxidation negatively affects the quality of foods, especially emulsion type products by altering appearance, odor, flavor, shelf-life and nutritional value. It may affect not only the nutritional and quality of fish, but also result in the involvement of protein on the sequence of autoxidative reactions (Braddock and Dugan, 1973). Untreated frozen channel catfish has a limited shelf-life (3-6 months) due to oxidation of lipids and development of rancid off-flavors (Erickson, 1993). Lipolysis occurs due to the increase of free fatty acids during storage (-6°C). Studies conducted to enhance the fat stability in catfish muscle tissue has focused on defining the contribution of different lipid classes to oxidation and concluded that higher temperatures have been associated with higher lipolysis rates (Lovern, 1962).

Antioxidants are defined as compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions (Velioglu et al., 1998). Antioxidants can also protect the human body from free radicals and Radical Oxygen Species (ROS) effects. They retard the progress of many chronic diseases as well as lipid peroxidation (Pryor, 1991; Kinsella et al., 1993; Gulçin et al., 2004, 2006). Furthermore, lipid oxidation is also associated with aging, membrane damage, heart disease and cancer (Ramarathnam et al., 1995). The problem of autoxidation can be addressed by introducing synthetic antioxidants. At the present time, the most commonly used antioxidants are BHA, BHT, propyl gallate (PG) and tert butylhydroquinone (TBHQ), which efficiently help quench radical molecules. Due to the

concern of possible toxicity of synthetic antioxidants (Sun et al., 1997), natural antioxidants have gained increasing attention among consumers.

Tannic acid's safe dosage ranges from 10 to 400 μg, depending on the type of food to which it is added (Chen and Chung, 2000). Also, several authors have demonstrated that tannic acid and other polyphenols have antimutagenic and anticarcinogenic activities (Andrade et al., 2005). In other studies, tannic acid inhibited skin, lung and stomach tumors that are induced by polycyclic aromatic hydrocarbon carcinogens and N-methyl-N-nitrosourea in mice (Vance and Teel, 1989). It was reported that the polyphenolic nature of tannic acid, it's relatively hydrophobic ''core'' and hydrophilic ''shell'' are the features responsible for its antioxidant action (Isenburg et al., 2006).

Consequently, finding a way with a "natural" compound to prolong the shelf-life of catfish is one of the main goals of this research. U.S farm raised catfish is the sixth most consumed fish and seafood product by Americans, who eat an average by 15.8 lb/year total fish and 0.85lb of catfish per year in 2009 (Hanson et al., 2011). Currently, catfish fillets have a maximum fresh distribution shelf-life of 4-10 days (Garcia 1999). Extending this shelf-life would expand the distribution range of fresh catfish, reduce shrink and spoilage, make it easier to offer fresh catfish on the market and increase the value of the product. It has been previously reported that tannic acid increases antioxidant capacity when added to soybean oil (Kim et.al. 2010).

The objective of this research was to determine the effect of tannic acid, fresh, heated or in combination with phosphates, on the quality of catfish fillets.

CHAPTER II

LITERATURE REVIEW

Catfish

Channel Catfish (*Ictalurus punctatus*) production and processing are concentrated in two main areas in the southeast United States with similar climate conditions: the Mississippi river alluvial valley, centralizing from the Mississippi Delta extending to the surrounding areas of Mississippi, Arkansas, and Louisiana; and a west-central Alabama area (Hargreaves and Tucker 2004). They are often referred as "trash fish" in the Northern and Midwest states, and are easy to supplement as the nickname refers. This fresh warm-water fish is well adapted to warm waters and are commercialized by aquaculture techniques. As a main important economical aquaculture product in the south, processed channel catfish in the USA reached 215,000 million kilograms (472,000 million pounds) in 2010, up 5.6 million (+1.2%) from 466 million pounds produced in 2009(USDA 2010). Maintenance of the catfish market is the main concern of the catfish industry in the USA. The number of catfish operations in the U.S. decreased by 85 (-9%) in 2010 and is now at 909 operations (USDA NASS 2010). A long-term competitive market requires producing a highly acceptable product. Production is seasonal and frozen storage is therefore necessary to maintain uniform supply throughout the year.

Moreover, consuming farm-raised catfish has been shown to have many positive health benefits. It is low in calories, total fat, saturated fat, and is a good source of lean protein (Al-Turk 2007). It has a moderate fat content, and is lower in cholesterol

concentration and higher in polyunsaturated fatty acid (PUFA) composition when compared to other muscle foods such as pork, beef and poultry (Silva, 1986). The PUFA is a positive attribute in our diet but it is also a main cause of problems of oxidation that leads to the spoilage of catfish.

Shelf Life

Catfish shelf life is primarily affected by microbiological spoilage and lipid oxidation. Although some other issues take place, one can summarize many factors that contribute to quality deterioration and the short shelf-life of fish and fish products as associated with fish species and size, their intrinsic properties (such as endogenous enzymes, microflora, sensitivity to light and oxygen and or oxidation), storage conditions (temperature), cross-contamination during harvesting, slaughter and processing, and packaging (Tozer, 2001).Shelf life is divided into high quality shelf-life (HQSL) and minimum acceptable quality shelf-life (MAQS). The HQSL can be regarded as the point at which a food product retains all its characteristic properties, whereas MAQS is the point at which a food product is considered to have become inedible (Graham et al., 1992). In addition, Potter and Hotchkiss (1998) defined the shelf-life of a food product as the time it takes a food product to decline to an unacceptable level. In general, shelf-life is a time point when the quality of a food product deteriorates during storage in the form of decreased nutritional values, color changes, off flavor developments and textural changes (Gulcin et al., 2009).

An end point indicator was established for fresh fish, where aerobic plate counts showing a count of 10^7 log CFU/cm^2 or grams at 20-25^oC was the upper limit of microbiological spoilage (ICMSF 1972). The shelf-life of raw, refrigerated channel

catfish (*Ictalurus punctatus*) fillets ranges from 4-14 days (Garcia 1999). Beuchat (1973) reported that refrigerated catfish fillets (4^{o}C) had a shelf-life of approximately 7 days. Gawborisut (2005) stated that shelf life of iced catfish fillets ranged between 8-10 days. The shelf-life of catfish fillets that were stored in crushed ice has been reported as 5-12 days (Heaton et. al 1972; Reddy et. al 1997).Currently, catfish fillets have a maximum fresh distribution shelf-life of 4-10 days (Garcia, 1999). Extending this shelf-life to 15 days would expand the distribution range of fresh catfish, reduce shrink and spoilage, make it easier to offer fresh catfish on the market and increase the value of the product.

Untreated frozen channel catfish has a limited shelf-life (3-6months) due to oxidation of lipids and development of rancid off-flavors (Erickson, 1993). Lipolysis has proven to occur due to the increase of free fatty acids during storage (-6°C). Higher temperatures have been associated with higher lipolysis rates (Lovern, 1962). Studies have been made to promote stability in catfish muscle tissue that is centered in defining the contribution of different lipid classes to oxidation. Erickson (1993) concluded that non-polar lipids were the major contributor to oxidation in catfish while acceleration in the rate of oxidation, on the other hand, was influenced by oxidation of phospholipids. Moreover, Erickson (1993) has proven that ascorbic acid protected the fatty acid membranes through regeneration of tocopherol in catfish treated samples.

Microbial Spoilage

Minimum acceptable shelf life is the time period beyond which fish products are no longer edible. With that said, it is very difficult to try to measure and indicate the shelf life or storage life of fishery products. There are no criteria for defining the end of shelf life of foods (Lu, 2004). Therefore, an end point indicator was established for fresh fish,

where psychotrophic plate counts (PPC) showing a count of 10^7 log CFU/cm^2 or grams at 20-25^oC was the upper limit of microbiological spoilage (ICMSF 1972). Fish exceeding this upper limit are considered to be spoiled or unacceptable. Even though bacteria are a major cause of deterioration and spoilage of fish and provide an index of freshness, there are many limitations in assessing the freshness of fish by PPC. The inclusion of total bacteria in the spoilage process but the exclusion of endogenous enzymatic and chemical activities from these processes makes microbiological methods unreliable. In addition to the end point sometimes ignored, product type, packaging method, contamination level and storage temperature are overlooked, Beuchat (1972) reported that refrigerated cat fish fillets at 4°C had approximately seven days of shelf life. Marroquin et al., (2004) studied different processing methods and found affecting the shelf life and found mechanically filleted and chilled fillets at 0^oC had a shelf life of nine days compared to fillets held at 18^oC. In addition, Reed et al., (1983) indicated spoilage as the bad odor and sliminess that was detected by the panelists and stated the shelf life by sensorial attributions of hand-dressed, ice-packed catfish was 11 days and 19 days for chilled packed (-2.2^oC ± 1.7^oC). Once again, these observations overlooked important endogenous characteristics that might have caused the bad odor by not only the psychotrophic micro flora, but also lipid oxidation.

Lipid Oxidation

Autoxidation is any oxidation that occurs in open air or in the presence of oxygen and or UV radiation and forms peroxides and hydroperoxides. Oxidation reactions are the result of many factors that might initiate and propagate up the process. Amount of oxygen present, degree of unsaturation in the lipids, presence of prooxidants: copper, iron

(heme ring of myoglobin), packaging material, light exposure, temperature of storage are the major components causing unwanted oxidation (Richards and Hultin, 2005). As part of the process of autoxidation, an initiation, propagation and termination process is developed and the food slowly becomes inedible. Unsaturated fatty acids can become a major problem if all of the above causing agents are not controlled since the double bonds can readily react with other compounds producing radicals. The mechanism is simple but to treat them can be very cost effective. More specifically, initiation of autoxidation (Figure 1) occurs when a hydrogen atom at the α-methylene group in double bonds of unsaturated fatty acids is removed to form an alkyl radical (R●).

$$RH \xrightarrow[\text{catalyst (metal ion)}]{\text{energy } (h\nu, \Delta)} R^{*} + H^{+}$$

Figure 1 Initiation oxidation reaction (Chemsketch Inc., 2010)

Initial generation of free radicals is slow. The reaction is initiated by singlet oxygen (1O2) excited energy state of O2. That is, singlet oxygen in the excited state where two electrons with different spin in one orbital initiates the reaction. Deterioration of singlet O2 is more electrophilic than triplet state oxygen (3O2), reacting approximately 1500 times faster at carbon double bonds when compared to the ground state or triplet O2. In addition, oxygen raised to the excited state (1O2) by light energy can cause photooxidation, which is promoted by pigments called sensitizers (e.g. chlorophyll, riboflavin).

$$\text{sens} \longrightarrow \text{sens}^{*}$$
$$\text{sens}^{*} + {}^{3}O_2 \longrightarrow \text{sens} + {}^{1}O_2$$

Figure 2 Initiation reaction of photooxidation (Chemsketch Inc., 2010)

Once free radicals build up, the oxidation reaction takes off. The next stage is developed; propagation and the chain reaction (autocatalytic) start (Figure 3). Oxidation from hydroperoxide decomposition, leads to aldehyde formation (e.g. alkanals, hexanal) and can cause rancid flavors and in addition, free radicals produced can damage other compounds including vitamins and proteins.

$$R^* + {}^3O_2 \longrightarrow ROO^*$$
$$ROO^* + RH \longrightarrow ROOH + R^*$$
$$ROOH \longrightarrow RO^* + OH$$
$$RO^* + R\text{-}H \longrightarrow ROH + R$$

Figure 3 Propagation reaction (Chemsketch Inc., 2010)

Oxidation rates are also affected by the number, position and geometry of double bonds. Conjugated double bonds are less reactive than non-conjugated double bonds. Fish oils are very susceptible (polyunsaturated) since, free fatty acids react faster than triglycerides. Oxidation is also accelerated during frying (180° - 200°C) since hydrolysis produces free fatty acids. There are prevention control mechanisms for each factor affecting lipid oxidation; some developed by humans and others found in nature. For singlet oxygen or the active species in photooxidation deterioration, the following mechanisms can prevent autoxidation:

– Vacuum packing or N_2 flush

– Oxygen scavengers

– Low film permeability

– Antioxidants

For metal ions:

– If the amount of free metal is restricted, the rate of lipid oxidation will be slower

– Use chelators (Antioxidants: e.g. tannic acid)

In muscle foods, including catfish, myoglobin and hemoglobin are promoters of lipid oxidation (Richards and Hultin 2002) as containing the metal ions mentioned above; thereby producing off odor and off-flavor that negatively affect muscle meat quality (Kanner 1994, Richards et al., 2005). In addition to lipid oxidation, the production of free radicals and hydrogen peroxide will further accelerate the autoxidation of myoglobin and hemoglobin (Grunwald and Richards 2006). Fatty fish contain a high concentration of unsaturated fatty acids that are susceptible to oxidation and can lead to the deterioration of texture and the production of undesirable rancid odors and flavor (Braddock and Dugan 1973, Hobbs and Hodgkiss, 1982). Fish lipids are more unsaturated than other muscle foods; they are more susceptible to oxidation which constitutes a problem in fatty fish in frozen storage like catfish. Fish with a high concentration of lipids and fish exposed to conditions, such as high heat, light or metal ions enhance oxidation and protein denaturation (Lu 2004).

Antioxidants

Antioxidants are substances that delay the onset of, or slow down the rate of oxidation. The most common types of lipid soluble antioxidants are mono or polyhydric phenols with ring substituent. These antioxidants function either by inhibiting the formation of free radicals in the initiation step or interrupting propagation of the free radical chains (Velioglu et al., 1998). Antioxidants can also protect the human body from free radicals and ROS effects. They retard the progress of many chronic diseases as well

as lipid peroxidation (Pryor, 1991; Kinsella et al., 1993; Lai et al., 2001; Gulcin et al., 2004, 2006). Most importantly, antioxidants have been widely used as food additives to provide protection against the oxidative degradation of foods (Gulcin et al., 2005, 2004).The proposed mechanism is believed to involve the antioxidant acting as a hydrogen donor and the phenol group forming radical intermediates that are relatively stable due to resonance delocalization (Kinsella et al., 1993). This reduces the number of positions suitable for attack by molecular oxygen. The mechanism is simple as it acts as a quencher:

$$R^* + AH \longrightarrow RH + A^*$$
$$RO^* + A\text{-}H \longrightarrow ROH + A^*$$
$$ROO^* + A\text{-}H \longrightarrow ROOH + A^*$$

Figure 4 Antioxidant quenching (Chemsketch Inc., 2010)

Lipid oxidation is associated with aging, membrane damage, heart disease and cancer (Ramarathnam et al., 1995).

Tannic acid (Antioxidants: Natural vs. Synthetic)

Fish oil is a rich source of eicosapentanoeic acid (EPA, C20:5 n-3) and decosahexaenoic acid (DHA, C22:6 n-3). However, the presence of multiple double bonds in polyunsaturated fatty acids (PUFA) makes them vulnerable to oxidation, which produces various aldehydes and ketones that result in unacceptable flavors, odors and colors in PUFA containing foods (Nawar et. al. 1996). Moreover, the products of lipid oxidation, such as malonaldehyde, can have adverse health effects to the consumer due to their cytotoxic and genotoxic effects (Esterbauer et. al. 1990; Fang et. al. 1996). The high rate of oxidation of PUFA can be controlled by the addition of synthetically produced

antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butyl hydroquinone (TBHQ) and synthetic or naturally sourced R-tocopherol. However, potential carcinogenic properties of the synthetic antioxidants have been reported and their use in food has been already limited in some countries (Sun and Fukuhara, 1997). Recently, consumer health consciousness has led to a demand for "natural" alternatives to synthetic food antioxidants such as BHT and BHA (Rupasingue et. al. 2010).

At the present time, the most commonly used antioxidants are BHA, BHT, propyl gallate (PG) and tert-butylhydroquinone (TBHQ). The safety of these antioxidants has recently been questioned due to toxicity (Sun and Fukuhara, 1997). Besides that BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Wichi, 1988; Sherwin, 1990). Also, BHT had minimal effect on mutagenicity at low concentrations, but significantly increased their mutagenicity at high concentrations (Shahidi and Wanasundara, 1992). Therefore, there is a growing interest on natural and safer antioxidants (Moure et al., 2001; Gulcin, 2006; Oktay et al., 2003). Natural antioxidants are known to exhibit a wide range of biological effects including antibacterial, antiviral, antiflammatory, antiallergic, antithrombotic and vasodilatory activities. In fact, a fundamental property important for life is the antioxidant capacity and this property may give rise to anticarcinogenicity, antimutagenicity and antiaging activity, among others (Cook and Samman, 1996). The antioxidant capacity of phenolic compounds is mainly attributed to their redox properties, which allow them to act as reducing agents, hydrogen donors and quenchers of singlet oxygen. In addition, they may also possess metal chelation properties (Rice-Evans, 1995; Liyana-Pathirana and Shahidi, 2006). Tannic acid is a plant polyphenol which is found, along with other tannins in

several beverages including red wine, beer, coffee, black tea, green tea, and many food products such as grapes, pears, bananas, sorghum, black-eyed peas, lentils and chocolate (Chung et al., 1998a; King and Young, 1999). This potent antioxidant has been topically applied showing wound healing capacity due to its high collagen cross-linking ability (Halkes et. al., 2001). Similar to many polyphenols, tannic acid has been proven to possess antioxidant properties (Lopes et al., 1999; Ferguson, 2001; Wu et al., 2004; Andrade et al., 2005), antimutagenic (Ferguson, 2001; Horikawa et al., 1994; Chen and Chung, 2000) and anticarcinogenic properties (Horikawa et al., 1994; Athar et al., 1989; Gali et al., 1992; Nepka et al., 1999). Tannic acid is composed of a central glucose molecule derivatized at its hydroxyl groups with one or more galloyl residues. It was reported that the polyphenolic nature of tannic acid, it's relatively hydrophobic ''core'' and hydrophilic ''shell'' are the features responsible for its antioxidant action where not only its hydophillic galloyl groups will quench radicals but also the hydrophobic quinones. (Isenburg et al., 2006). Its safe dosage ranges from 10 to 400 μg, depending on the type of food to which it is added (Chen and Chung, 2000). Moreover, the consumption of polyphenol-rich fruits, vegetables, and beverages, such as tea and red wine, has been linked with inhibitory and preventive effects in various human cancers and cardiovascular diseases, which may be related at least in part to the antioxidant capacity of polyphenols (Andrade et al., 2005). In other studies, tannic acid inhibited skin, lung and stomach tumors induced by polycyclic aromatic hydrocarbon carcinogens and N-methyl-N-nitrosourea in mice (Vance and Teel, 1989; Khan et al., 1988). Results reported by Gulcin (2009) indicated that metal chelating capacity was significant since it reduced the concentration of the catalysing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce

the redox potential thereby stabilizing the oxidized form of the metal ion. In addition, tannic acid demonstrates a marked capacity for iron binding, suggesting that their main action as a peroxidation protector may be related to its iron binding capacity. When tested for peroxide scavenging capacity, tannic acid had an effective hydrogen peroxide scavenging capacity. The hydrogen peroxide scavenging effect of tannic acid and four standard compounds decreased in the order of tannic acid >a-tocopherol> BHA > BHT> trolox. Tannic acid also demonstrated good results when tested for radical scavenging capacity, since radical scavenging activities are very important due to the deleterious role of free radicals in foods and in biological systems Gulcin and others (2009) used the DPPH radical method to show the scavenging ability of tannic acid and standards. The scavenging effect of tannic acid and standards on DPPH radical decreased in the order of BHT> tannic acid >BHA > a-tocopherol > trolox, once again showing great compatibility with synthetic reference antioxidants. After six months of storage, a study with frozen, reheated ground beef patties on oxidative stability using the following antioxidants: grape seed extract (GS), oleoresin rosemary (OR), water-soluble oregano extract (WO), propyl gallate (PG), butylated hydroxyanisole (BHA)), butylated hydroxytoluene (BHT)) was reached (Colindres and Brewer (2010)). These authors reported that PG and GS samples had lower rancid odor scores and TBARS than controls. Control samples and those containing BHT did not differ in sensory grassy or rancid odor, indicating that they were the most oxidized, showing that synthesize antioxidants are not always the best choice. TBARS correlated with grassy, rancid, cardboard and beef odors during the 6 month storage period.

Induction Point as a Measure of Many Methods Used to Measure Fat Oxidation

In general the Rancimat® instrument allows the automatic determination of the oxidative stability of oils and fats without the need for expensive and environmentally hazardous chemicals and time-consuming titrations. Six samples can be determined simultaneously side by side.

A Metrohm Rancimat® apparatus was used to determine the induction point of (IP) tannic acid mixed oil (Kim et al., 2010). The IP is defined as the time needed to produce volatile products such as aldehydes, acids, and alcohols, as a result of oil oxidation. Air is circulated through samples at 110 ±1.5oC. This air is then passed through deionized water, and the conductivity of the water is measured. The IP is measured as the intersection of the tangent lines first derivative (Brinkmann 2006).

Determination of the oxidative stability index (OSI) by the Rancimat® (AOCS, 1994) is the most widely applied standard method to determine susceptibility against oxidation of edible fats and oils under accelerated conditions (Antoun & Tsimidou, 1997; Hidalgo, Leon, & Zamora, 2006; Mancebo-Campos, Salvador, & Fregapane, 2007; Mariod, Matthäus, Eichner, & Hussein, 2006; Wan, 1995). Recently, an extensive analytical study on evolution of oxidation in sunflower and olive oils under Rancimat® conditions has been reported (Márquez-Ruiz, Martín-Polvillo, Velasco, & Dobarganes, 2008) and results were compared to those obtained at room temperature. The Rancimat test was also applied directly to samples of dried microencapsulated oils (DMOs) to evaluate the oxidative stability provided by different antioxidants. Results were consistent with those obtained in storage assays under ambient conditions. It was concluded that the Rancimat® test may find useful application in DMOs as a rapid method to assess the activity of added antioxidants (Velasco, Dobarganes, & Márquez-

Ruiz, 2000b). Moreover, phosphatidylethanolamine, phosphatidylcholine, and lysine were tested by Hidalgo et al., (2006) for antioxidative capacity in refined olive oil by the Rancimat® method to investigate the role of the chemical reactions produced in the Rancimat® vessel on the induction periods (IPs). Velasco and Dobarganes added that the Rancimat® may be a valuable tool for testing antioxidative activities of antioxidants produced during food processing if favorable conditions for antioxidant formation are employed. Other results have shown the compatibility of the Rancimat method with other methods. Kowalski and others (2004) reported that the results obtained from the determination of the oxidative stability of vegetable oils by Differential Scanning Calorimetry and Rancimat® measurements appeared to be consistent. In conclusion, assessment of oil stability for high temperature application (e.g. frying) based on these methods should lead to similar conclusions and recommendations. Results of stability evaluation based on accelerated lipid oxidation by Rancimat® methods on oils derived from poly-unsaturated fatty acids (PUFA), sometimes lead to a sudden change as it gets oxidized rapidly. Therefore, lipids highly sensitive to oxidation are often analyzed at lower temperatures, ranging between 60 to 80ºC. For example, fish oil was analyzed at 68 oC (Mendez et. al. 1996). The rate of oxidation may be limited by the mechanism of degradation because the rate of formation of volatile acids is likely reduced above a certain temperature. At high temperatures, the IP becomes too low for an accurate assessment of antioxidant efficacy due to underestimation. This phenomenon was observed during the Rancimat® experiments performed at 100 to 110ºC.

Color

When a beam of light strikes an object's surface, it is separated into three parts at different directions: reflective (scatter), absorptive and transmitted (Francis and Clydesdale 1975). Light scattering: the perception of color from a piece of food, is a physiological phenomenon for the interpretation of reflective light and is determined by the spectra of incident light, and the spectra of reflective (scatter) light, absorptive light and transmitted light from the meat surface. Meat is an opaque object with little transmitted light (AMSA1991). The substances on the meat surface selectively absorb light at the wavelength based on their available energy difference (Francis and Clydesdale 1975). For the most part, fresh meat color is determined by myoglobin and hemoglobin concentrations, oxidation state, and ligands that are bound to their heme groups. In addition, protein denaturation also increases light scatter (Offer and Trinick 1983). Thus, the color of meat in an observer's mind is the result of the interpretation of the spectra of the reflective light signal and his experience.

The visible wavelength absorption properties or the color of the myoglobin and hemoglobin is determined by the binding of a ligand to the iron atom, and the interchangeable states of ferrous and ferric iron in the porphyrin ring. That is, deoxygenated myoglobin and hemoglobin, where, the sixth coordination position of the porphyrin iron is free from binding; as an example as seen immediately after fresh cutting or vacuum-packaged meat (Mancini and Hunt 2005). Moreover, binding to other molecules such as chelating molecules like tannic acid is very important. Color protection may be achieved by the addition of tannic acid to wines, which promotes polymerization with anthocyanins to form stable reaction products (Marquette and Trione, 1998). It was also observed by Berg and Akiyoshi (1956) that tannic acid increased browning in white

wines. The use of mordant dyes has decreased in recent years, owing to environmental concerns regarding the use of heavy metals, including chromium (Arroyo-Ortiz, 2008). To reduce the impact of these products on the environment, it has been recommended that natural mordants be used. For example, tannins (Quercus spp., Enterolobium cyclocarpum and Caesalpinia coriaria) can be used as natural mordants, as they can provide fabrics with characteristics similar to those provided by synthetic mordants (Arroyo-Ortiz, 2008).

Proteins and lipids are the major energy reserves in fish (Love, 1997). Fish have less reserve glycogen than their terrestrial counterparts (Huss, 1994), but an acute stress causes even greater disturbances to fish than those observed in mammals because in burst exercise, the fish use all white muscle (Bone, 1978). Fish flesh color is also affected by pre-slaughter stress. Increasing the rate of pH decline postmortem resulted in higher Hunter 'L', hue and chroma values in rainbow trout fillets (Robb, 1998) and a high 'L' value in striped bass (Eifert et al., 1992). This may be the result of increased protein denaturation. Pre-slaughter stress decreased sensory scores in salmon (Sigholt et al., 1997). Nunez (1997) reported that pre-slaughter stressed channel catfish had lower ATP in postmortem muscle, a shortened onset of rigor, a reduced duration of rigor and an increased rate of ATP degradation. Silva and others (2001) reported that pre-slaughter chilling of channel catfish resulted in a red, soft and exudative muscle (RSE), and that the fish achieved rigor mortis faster when exposed to lower pre-slaughter chilling temperatures. Bosworth and Small (2004) compared the transport methods in channel catfish and concluded that an increase in transport stress increased drip-loss and surface L value in catfish fillets due to muscle protein denaturation. During refrigerated storage, fish muscle is characterized by a loss of shininess (Suvanich and Marshall, 1998), water

loss, shrinkage of myofibrils, denaturation of protein (Offer and Knight, 1988) and possible pH changes. In addition, autoxidation of myoglobin and hemoglobin occurs on the muscle surface. All these factors may affect the light scattering properties which would affect the color of fresh catfish fillets. Even though most fresh catfish muscle is characterized by a pale and slightly red color, variations in fillet color sometimes occur that may affect the uniformity of fillets and consumer acceptability.

Vacuum tumbling

Tumbling is a physical process that involves meat rotating, falling, and contacting with metal walls and paddles in a drum. This process provides a transfer of kinetic energy to extract protein that forms a binding agent for muscle fibers (Addis and Schanus, 1979).When pieces of meat are tumbled, some of the salt soluble proteins that include actin, myosin, actomyosin, and other sarcoplasmic proteins, migrate to the outer surface of the meat and a tacky white exudate that includes fat, water, and proteins is formed (Marsh, 1977). The functions of tumbling are to improve yield, increase tenderness and cohesiveness, and gain faster and more uniform ingredient distribution in the meat (Cassidy et. al., 1978; Krause et. al., 1978). There are two major types of tumbling, vacuum and non-vacuum processing. The application of vacuum to tumbling promotes good outcomes. According to Chiralt and Fito (1997) the salting process (brine vacuum impregnation: BVI) has been reported to reduce salting time in Manchego type cheeses and ham for curing (Barat et al., 1998), while promoting more even salt distribution in the product. Ockerman and Organisciak (1978) reported that the tumbling process increased the migration of sodium chloride, dextrose, and sodium nitrite in tumbled meat. Moreover, combination of tumbling with certain additives has been reported to reduce

lipid oxidation, including use of egg white on tumbling catfish (Yetim & Ockerman, 1994) and the use of CaCl2 and NaCl with tumbling has been effective in reducing oxidation in spent fowl meat (Woods, Rhee, & Sams, 1997). According to Katsaras and Budras (1993) many meat product manufacturers are using vacuum tumblers, mainly in order to accelerate the solution penetration and homogeneity in cooked hams. Moreover, vacuum tumbling increased cure penetration rate and color stability (Marriott et. al., 1984; Solomon et. al., 1980). The application of vacuum tumbling has been found to produce more extractable protein in beef than non-vacuum conditioned beef (Ghavimi et al., 1986; Wiebe and Schmidt, 1982). Specifically, vacuum conditions increase the amount of crude myosin extracted to the meat surface. However, if meat is over-tumbled it results in over extraction of myosin from the surface layers of the meat Treharne (1971). Vacuum processing promotes the expansion and discharge of occluded gases from the product. The subsequent return to atmospheric pressure compresses residual gas in the pores, thus prompting infiltration of exogeneous solution via pores of the product (Deumier, et. al., 2003).

Organic Solvent Extraction

Organic solvent fat extraction is widely used in the industries. Previous researchers have developed and tested the efficacy of different solvent systems; some pure others mixed. When extracting fats from foods, both the method and the solvents must be taken under consideration for a complete and clean extraction. As lipids are relatively non-polar molecules, they can be pulled out of a sample using non-polar solvents. With a non-polar solvent, only non-polar molecules in the sample dissolve while polar ones do not. Problems arise however, in cases where lipids are bound in

animal or plant cell membranes. Animal and plant cell membranes are made up of molecules that have both polar and non polar regions such as triglycerides (molecule with polar glyceride heads and non-polar fatty acid tails) and phospholipids (similar to triglycerides, but a phosphate group replaces the fatty acid tail). These molecules end up grouping together with their polar heads sticking outwards and non-polar tails inwards making it difficult for non-polar solvents to interact with the non-polar tails and extract them. As these molecules are part non-polar and part polar, we need a solvent that presents some of these same characteristics. Benning (2010) use a mixture of two solvents such as hexane and isopropanol. The isopropanol demonstrated to be polar enough to interact with the polar regions and help "pull apart" the cell membrane while also being non-polar enough to help in extracting and being soluble in the hexane. The ratio was determined to be a 3:2(iso:hex) since the isopropanol component of the solution makes it more polar. Although, too much of it and the solution will be too polar to extract fat properly. With this ratio the solution maintains enough of its non-polar characteristic to work well for extractions while also being just polar enough to work well in situations such as those mentioned earlier.

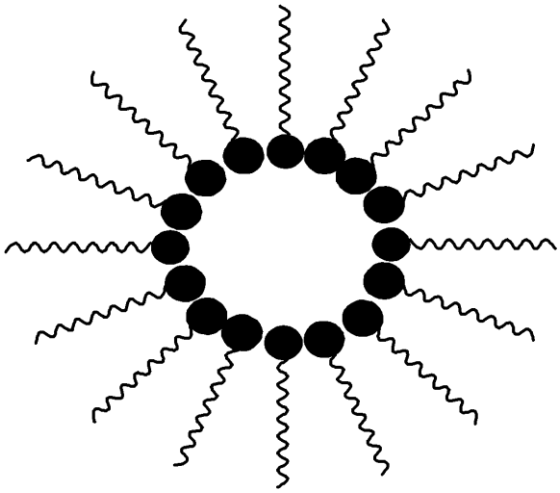

Figure 5 Molecule containing polar and non-polar regions (Chemsketch Inc., 2010)

Bergeron and Benning (2010) also demonstrated by experiment using the synergistic characteristics of the solvent mixture compared to that of the Soxhlet experiment, that the results were the same and even better in percent efficiency on fat separation. In addition, Erickson (1993) tested various organic solvents in a comparison on the lipid extraction from channel catfish muscle. In all criteria on which Hexane and isopropanol mixture were compared, it showed the higher quantity of lipid extraction.

CHAPTER III MATERIALS AND METHODS

Materials and Chemicals

Catfish fillets were obtained from Superior Catfish Products in Macon, Mississippi after chilling. The fillets were transported on ice to Mississippi State University's Ammerman-Hearnsberger Food Processing Laboratory. The catfish fillets were enhanced with tannic acid solutions using a vacuum tumbler. Catfish fillets for PPC and color measurements were stored under refrigeration at 4°C and evaluated at 5 day intervals. Catfish fillets for oxidation measurements were stored for one month at -18°C. The Least Significant Difference (LSD) test was utilized to separate main effect treatment means ($P<0.05$) when significant differences occurred among treatments (SAS Version 9.2, Cary, NC, USA).

Tannic, gallic acid standards and anhydrous magnesium sulfate were purchased from Sigma-Aldrich Inc. (St. Louis, MO). An Agglomerated blend of sodium phosphates was BRIFISOL®550 from BK Giulini Corp. (Simi Valley, CA), potassium lactate (PL) Ultra Pure Bestate-P4218 from Hawkins (Minneapolis, MN), and sodium chloride Culinox® 999® food grade salt from Morton International Inc. (Chicago, IL). Acetonitrile, methanol, acetic acid, water, isopropanol and hexane (ACS reagent grade) were purchased from Fisher Scientific Inc. (Pittsburgh, PA).

Sample Preparation

A total of four treatment solutions were prepared. The control (CTL) treatment was prepared with only distilled water; fresh (FTA) and processed (PTA) tannic acid were prepared in different manners prior to the addition of 25g of tannic acid into 500mL of distilled water. Five percent processed tannic acid solutions (PTA) were prepared in the same manner as FTA and autoclaved for 15 minutes (Kim et. al 2010). The fourth treatment was FTA plus a phosphate formulation (FPH). The formulation (Kim et. al, 2011) was prepared by the addition of grams agglomerated sodium phosphate blend and 5% sodium chloride Culinox® 999® food grade salt (Morton International Inc., Chicago, IL, USA) to the FTA. These were all manually mixed in a sterilized 500mL stock glass bottle until the salt was dissolved into solution to form FPH solution.

Catfish fillets were placed in a vacuum tumbler (Lyco Model 40 Columbus, WI, USA), with 10 ml of each solution for 10 min at a speed of 20 rpm and a pressure of 20 mmHg (2.67Kpa). After tumbling, fillets were drained for 10 min, placed in plastic Ziplock® bags and labeled with type of treatment and date of sampling. The samples were placed in an ice chest and stored under refrigeration at Mississippi State University's pilot plant at a temperature of 4°C for PPC, color measurements. Samples used for oxidation on the Rancimat® were placed in a deep freezer at -18°C for 1 month and unthawed for further processing.

Psychotrophic Plate Counts (PPC)

Twenty five grams of catfish was obtained under sterile conditions for every treatment in triplicate (3 fillets per treatment, n=12), by cutting the same center fraction of the fillet aseptically. Each 25g bag was placed in Stomacher 400 Laboratory Blender (A. J. Seward and Co. Ltd., London, England) with 225mL of 0.1% sterilized peptone

solution, for 60 sec. Dilutions were made by transferring 1 ml of the homogenate into dilution tubes with 9 ml of 0.1% sterilized peptone solution. Plating was conducted on aerobic (PPC) count PetrifilmTM (3M Co., St. Paul, MN, USA) which was incubated at 20°C for 72 h (Dormedy et al., 2000; Marroquin et al., 2004). Pschytrophic plate counts were obtained every five days up to the fifteenth day.

Catfish fillet color was measured with a Labscan Model 6000 0/45o Spectrocolorimeter (Hunter Associates Laboratory, Inc. Fairfax, VA, USA) with option CMR 559 (a 0/45 instrument with circumferential viewing illumination). Computations were performed in accordance with ASTM E 308 for the 2 degree observer equipped with a computer with HunterLab Universal software version 1.4 (Hunter Associates Laboratory). The equipment was calibrated with two blank plates (white and black) before measuring color; a portion of the rest of the fillet taken from the PPC was used at the same location (head side of the fillet) on the surface of each fillet (3 fillets, 3 parts of each/treatment); Catfish fillets were placed on Hunter lab glass on top of a 2.54 cm port. Hunter L, a and b values were measured. The triplicate readings of each fillet head side part were obtained and the average values were recorded. Hue angle, hue = arctan(b/a); chroma or saturation index, SI= (a2 + b2)1/2 (Francis 1998); and value or whiteness, WI =100-[(100-L)2 + a2 + b2]1/2 (Reppond and Babbitt 1997) were calculated.

Induction Point (oxidation)

Frozen catfish fillets were thawed in a cold water bath for seven hours and homogenized (Polytron®, Brinkmann Instruments Co.) for approximately five minutes or until the fillet was completely broken down. Homogenized catfish fillets were transferred to a 1000mL round bottom flask (RBF). A hexane and isopropanol mixture, 3:2 ratio was

added at a 1:1 (w:v) and the mix was rotated at 40 rpm for 10 h for fat extraction (Rancimat® Metrohm Co., Basel, Switzerland 2000). The RBF was wrapped with aluminum foil during extraction to prevent radical formation and consequently fat oxidation from UV light. After rotating for 10 h the organic solvent was vacuum filtered using Whatman® #42 Ashless (12.5cm) filter paper (Whatman Ltd, England). The aliquot was washed three times with 50mL of a hexane and isopropanol mixture (3:2,v:v) and dried with anhydrous magnesium sulfate (Erickson, 1993). Anhydrous magnesium sulfate was added until it was free flowing in the bottom of the beaker. Using a Whatman® #42 (12.5cm) ashless filter, the anhydrous magnesium sulfate was gravity filtered into a RBF, the remaining organic phase was set to a rotovap and the fat (oil) was directly placed into a Rancimat® Methrohm glass test tube and taken immediately for induction point analysis.

Oxidative stability from the extracted fat (oil) was evaluated using an OSI instrument (automated Metrohm Rancimat® model 743) and the AOCS Official Method Cd 12b-92 AOCS 1997. The operating condition of the instrument was 110 oC ±1.5oC, and samples were held for an equilibration time of 30-60 min while an air flow rate of 10 L/h bubbled through the oil (3g). In this process, the volatile oxidation products were stripped from the oil and dissolved in the water thus increasing the water conductivity. The oxidative stability is the time necessary to reach the conductivity curve inflection point; also known as the induction point (IP) and the IP wasdetermined by intersecting the baseline with the tangent to the conductivity curve.

Tannic/Gallic acid Determination

Fifteen grams of catfish fillet from each of the treatments were cut from the same central mid-section of the fillet and homogenized (Polytron®, Brinkmann Instruments Co.) in 50ml centrifuge plastic vials. (Fisher Scientific Inc., Pittsburgh, PA). The homogenized fillets were spun using a rotary mixer from Dynal® Inc. (New Hyde Park, NY) for 12 hours at 21rpm, to extract tannic/gallic acid. The centrifuge plastic vials were then centrifuged in a CU-5000 Curtin Matheson Scientific, Inc. centrifuge for 10 min at 7000rpm and two mL of the top aqueous layer was collected into HPLC amber vials using C18 silica, Florisil® Alumina syringe filter Sep-Pak® (Waters Corp., Milford, MA). A gallic acid standard curve (Figure 8) was developed for quantification using pure gallic acid dilutions: 100ppm, 200ppm, 500ppm, 1000ppm using HPLC (Fig. 12). Vials were placed in the auto sampler and injected (25μL) into a Gemini C18, 250 x 4.6 mm column (Phenomenex Inc., Torrance, CA) in an Agilent HPLC 1100 series equipped with a diode array detector (Agilent Technologies Inc., Santa Clara, CA). The two mobile phases were solvent A: methanol/acetic acid/water (10:2:88, v/v/v), and solvent B: acetonitrile (Harborne 1998; Lee 2000). A linear gradient for phenolics separation was used as follows: at 0 min, 95% solvent A, 5% solvent B; at 1 min, 90% solvent A, 10% solvent B; at 30 min, 30% solvent A, 70% solvent B; at 31 min, 90% solvent A, 10% solvent B; at 32 min, 95% solvent A, 5% solvent B with 5 min post run. The flow rate was 1 mL/min. Individual phenolics were detected at 260 nm.

Experimental design and statistical analysis

The microbial load (PPC) and Hunter color data were evaluated as a two-way factorial arrangement (treatments=4 and storage time: 1, 5, 10, 15 days) with three

replications in a randomized complete block (RCB) design. Fillets for each replication were obtained at different times from the same point in the processing line.

Catfish from each replication (initial time) was frozen for one month and used for induction point and determination of tannic acid concentration. The data was evaluated as a one-way anova (treatments) analyzed using a one-way anova in a randomized complete block design, with three replications as described previously.

Fisher's protected Least Significant Difference (LSD) test was utilized to separate main effect treatment means ($P<0.05$) when significant differences occurred among treatments (SAS Version 9.2, Cary, NC, USA).

CHAPTER IV RESULTS AND DISCUSSION

Hunter color values

There were no significant ($p \geq 0.05$) interactions between treatment and storage time for Hunter color values (L, a, b). Hunter 'L', 'a' and 'b' values were different ($p \leq 0.05$) between treatments (Table 1) but only 'L' and 'b' values were different ($p \leq 0.05$) between days of storage (Table 2).

Hunter 'L' values for catfish fillets were higher ($p \leq 0.05$) than the control for the tannic acid treated fillets (FTA, PTA) but lower ($p \leq 0.05$) than the control when the phosphate blend (FPH) was incorporated into the formula. The reason for the increased 'L' value with tannic acid was probably due to the attachment of tannic acid to proteins, causing denaturation of protein (Eifert et al., 1992). As a result, light scattering is increased and the interpretation of the observer becomes more intense.

Hunter 'a' values for catfish fillets were higher ($p \leq 0.05$) than the control for PTA treated fillets but the difference between treatments are not practical as almost all treatments were slightly close to zero (Berns 1996; CIE 2001). Kin et. al (2010) reported that Hunter 'a' values in catfish fillets were not affected when treated with a phosphate blend. This was likely due to the fact that catfish fillets have minimal pigmentation so slight changes in moisture content and yield will have minimal effects on red color (Lu 2008). Small changes in fillet color could have been contributed by the tannic acid's acidification of the catfish fillets and low variability among replications. This result was

different from Hayes et al., (2006), who reported that ‘a’ values of pork loin were affected by the enhancement of salt, sodium tripolyphosphate, and milk protein.

Hunter ‘b’ values were higher ($p \leq 0.05$) than the control but showed no difference within treatments due to the fact that all treatments contained tannic acid (yellowish/brown mixture) which causes denaturation and decreased pH, thus affecting the reflecting color observed by the human eye (Offer and Trinick 1983) and consequently yellowness. Values for pH showed no difference ($p > 0.05$) between tannic acid solutions, FTA and PTA (2.35, 2.32) but was more (6.17, $p \leq 0.05$) acidic than FPH treated fillets. Kin et. al (2010) reported that Hunter ‘b’ values were lower for phosphate treated fillets. Phosphate effects on Hunter ‘b’ values agreed with the findings of Hayes and others (2006) who reported that Hunter ‘b’ values of pork loin were decreased through enhancement with salt, sodium tripolyphosphate, and milk protein. In addition Lu (2008) and Kin et. al (2010) indicated that the majority of Hunter ‘a’ and ‘b’ values for catfish fillets treated with and without phosphate blend were close to zero. In this study mean Hunter ‘b’ values for all treated fillets ranged between 11.22 and 13.02. This may be caused by slight changes in light reflection that are associated with a more open protein structure after the addition of tannic acid (Damodaran and others 2008).

Hunter ‘L’ values of catfish fillets decreased ($p \leq 0.05$) after 10 d at 4oC (Table 2). The difference was less than 3 units making these practically similar when approximating minimal elliptical tolerance (Berns 1996; CIE 2001). Marroquin and others (2004) observed that Hunter ‘L’ values of channel catfish fillets that were stored on ice increased until the third day, but decreased by the sixth day during refrigerated storage. These results similar to our results as the days proceeded Hunter ‘L’ values decreased ($p \leq 0.05$). Hunter ‘L’ value is related to the physical property of the myofibrils

(Offer and Trinick 1983). Myofibrils continue to shrink during storage, thus, increasing the contrast of myofibrils and the sarcoplasm. Consequently, the Hunter 'L' values increased due to increased light scattering. Erikson and Misimi (2008) also observed that lightness of Atlantic salmon fillets increased during ice storage and coincided with the development of rigor. This was not the case in this study, due to the addition of phosphate which tends to increase water holding capacity. Not causing the myofibrils to shrink and changing the light scattering of the catfish fillet resulted in an opposite effect; decreased Hunter 'L' values. Hunter 'a' values were close to zero and showed a constant ($p \geq 0.05$) value for all treatments including the CTL.

To further comprehend these differences an investigation of how colors are perceived in meat sources and the chemistry involving tannic acid protein affinity is explained here. Nunez (1997) reported that Hunter 'L' values in bone side hand filleted channel catfish ranged from 43.4 to 53.1. These numbers were lower than our results due to the fact that our fillets were sampled from mechanically processed fillets; seasonal fish differences, and or rigor mortis. Fillet freshness also could have played a role in color differences of these studies. Willits (1992) reported that Hunter 'L', 'a', 'b' values of fresh channel catfish muscle from catfish steaks were 50.4, 1.8, and 3.5, respectively. Bosworth et al. (2004) reported that different catfish strains averaged L values were 73.92 in USDA-103, 75.31 in Norris and 72.06 in Channel x Blue; the average a values were 8.35 in USDA-103, 8.81 in Norris and 9.25 in Channel x Blue; and the average b values were 22.01 in USDA-103, 25.74 in Norris and 22.75 in Channel x Blue. These numbers were greater than our results. Another explanation is that the scale of processing machines resulted in different quality of catfish fillets since fillets in our experiment were processed in a commercial processing line; whereas their fillets were

processed in a pilot plant processing (Bosworth et al., 2004). Increase of the dropping rate of pH in postmortem results in higher Hunter 'L', hue and chroma values in rainbow trout fillets (Robb 1998) and a high 'L' value in striped bass (Eifert et al., 1992). This may be the result of increased protein denaturation. Bal'a and Marshall (1998) reported that dipping catfish fillet strips in 2% organic acid and inorganic acid (acetic acid, lactic acid, hydrochloric acid, citric acid, malic acid or tartaric acid) resulted in a noticeable bleaching effect (higher 'L' value). They also reported that acid treatments rendered the catfish fillets redder and yellower (higher 'a'and 'b'values) than the untreated control. This explains the same effect as acidifying the fillets with tannic acid in this experiment. In addition, protein denaturation was observed for all acid treatments who also increases light scatter (Offer and Trinick 1983).

Tannic acid is perceived as a yellow-brownish color (FTA) in solution and when thermally processed (PTA) turns into a dark brown solution. Moreover, when mixed with the phosphate blend it transforms into beige, opaque color (personal observations). Denaturation of protein is observed when tannic acid is added to the catfish fillet, as a result as mentioned before, light scattering is increased and the interpretation of the observer becomes more intense (Offer and Trinick 1983).

A more scientific chemistry approach for the binding of tannic acid can be reached by further analyzing the characteristics that define color. The visible wavelength absorption properties or the color of the myoglobin and hemoglobin is determined by the binding of a ligand to the iron atom, and the interchangeable states of ferrous and ferric iron in the porphyrin ring. As an example, deoxygenated myoglobin and hemoglobin, on the sixth coordination position of the porphyrin iron is free from binding; as seen immediately after fresh cutting or vacuum-packaging meat (Mancini and Hunt 2005).

Tannic acid has the ability to bind to proteins and organometallic molecules, acting as a strong chelating agent. Tannic acid has a much greater relative binding efficiency to iron than propyl gallate, methyl gallate or gallic acid (Chung et. al., 2005) which is found in the metalloproteins or hemoproteins. Therefore, tannic acid not only attaches but also chelates the iron in the porphyrin ring through the hydrophobic and hydrophilic interaction of the protein side chains (Fig. 14).

Since the angle of refraction affects the perception of color to consumers, hue, chroma (saturation index) and value were calculated since light scattering was increased by tannic acid through a proposed tannic acid attachment mechanism that was explained previously (Tang et al., 2003). Hue and chroma of catfish treated fillets were greater ($p \leq 0.05$) than CTL fillets, with no significant differences between other treatments (Table 3). Treatment of catfish fillets with TA and/or FPH leads to a deeper more intensified yellow color than the CTL fillets. Moreover, the addition of the phosphate blend FPH to the fillets resulted in higher ($p \leq 0.05$) whiteness/value. A major difference from the CTL to the other treatments was observed within the chroma or saturation index (SI). The treatment variables showed similar ($p > 0.05$) intensity results. An increased pH drop rate in postmortem muscle resulted in higher hue and chroma values in rainbow trout fillets (Robb 1998) and a high 'L' value in striped bass (Eifert et al., 1992) which directly affects the chroma. This may be the result of increased protein denaturation as the treated fillets were all vacuum tumbled with tannic acid. In conclusion, there were major differences on hue angles between the tannic acid treated fillets and the control, in which tannic acid treated fillets were closer to the yellow hue with more intensity (saturation/chroma) than the control. On the other hand FTP fillets were no different than

the CTL value/whiteness, but were more white ($p \leq 0.05$) when compared to FTA and PTA.

No changes ($p \geq 0.05$) in hue were observed in storage time of catfish fillets when compared to the CTL and or within treatments. Chroma on catfish fillets was higher ($p \leq 0.05$) after 10 d when compared to day 1 whereas value/whiteness also increased ($p \leq 0.05$) after 10 d of storage.

As described before, the differences in color, especially chroma between the CTL and the treated fillets is due to the attachment of the tannic acid molecule. First, by the fifth coordination of iron perpendicular to the four-nitrogenous coordination (Mancini and Hunt 2005). The sixth coordination of the iron atom that is opposite to the fifth coordination is free to reversibly bind small ligands when the iron is in ferrous state (Fe+2). When the iron atom is in the ferric state (Fe+3), the binding of molecular oxygen is limited. The sixth coordination of iron opposite to the fifth coordination is free to reversibly bind oxygen or other small molecules, thus forming the derivatives of myoglobin and hemoglobin. This binding of small ligands to the sixth position is greatly influenced by the valence state of the porphyrin iron (Mancini and Hunt 2005). The wavelength absorption properties, thus the visible color of myoglobin and hemoglobin are determined by the ligands that are bound to the iron atom, and the redox states of the iron atom in the porphyrin ring (Mancini and Hunt 2005). In this case tannic acid chelates iron (Fe+2) by iron-binding galloyl groups (Fig. 6), leading to differences in saturation index (chroma), which is associated with yellowness, and value.

Secondly, the protein side chain is bound (Fig. 14) by the collagen proposed mechanism (Tang et al., 2003). Therefore, since the raw tannic acid molecule has a yellowish color (FTA) and a brownish color when processed (PTA), chroma was affected

and yellowness was greater with the attachment of tannic acid to catfish fillets. Similar results were obtained from previous experiments with gelatin-tannin films (Pena et. al., 2010) and chitosan-starch blends (Mathew and Abraham 2008).

Psychotropic Plate Counts (PPC)

There was a significant interaction ($p \leq 0.05$) between treatments and storage time on psychotropic plate counts of catfish fillets (Table 2). Addition of FTA and PTA to catfish fillets reduced initial plate counts by approximately 0.2 log CFU/g and reached its greatest difference on day 10 where FTA difference to CTL doubled (0.4 log CFU/g) and PTA 1.5 times higher. The microbiological spoilage point of 7 log CFU/g (Silva and White 1994; Silva et al., 1993) for CTL catfish fillets were reached at 9.6 d. Addition of tannic acid extended microbiological life (ML) at 4◦C by two days, to 11.8 d. The addition of the phosphate mix prolonged the lag phase of the catfish fillets for 15 days of storage never reaching over 5 log CFU/g. Therefore, the FPH treatment was the best antimicrobial treatment. The intercept of the microbial growth curves indicate little or no difference in initial counts between all treatments but the slope (Table 6) for the FPH treated fillets was much lower (P 0.05) than the others. Kin et al. (2010) used the same phosphate blend with no tannic acid on catfish fillets kept under the same conditions and reported that catfish fillets spoiled between day 7 and 10 when stored at 4◦C. It appears that tannic acid and the phosphate blend have a strong synergistic effect on microbial growth due to the inhibition of growth when compared to the other treatments.

Minimum acceptable shelf life is the time period beyond which fish products are no longer edible. It is very difficult to try to measure and indicate the shelf life or storage life of fishery products. A major one is that there are no criteria for defining the end of

shelf life. Therefore, an end point indicator was established for fresh fish, where aerobic plate counts (PPC) showing a count of 107 log CFU/cm2 or grams was the upper limit of microbiological spoilage (ICMSF 1972). Fish exceeding this upper limit are considered to be spoiled or unacceptable. However, increased PPC may also be the result of lactic acid bacteria growth which indicates that the fish may not be spoiled when PPC reaches 7 log CFU/g (Silva and White 1994; Silva et al., 1993). These researchers reported that spoilage occurs at 8log CFU/g when LAB is the predominant micro flora in a seafood product under reduced oxygen conditions for example.

The antimicrobial capacity of tannins has been well documented throughout literature. Chung et al. (1996) found that tannic acid and propyl gallate, inhibited growth of food-borne bacteria including *Alcaligenes faecalis, Enterobacter aerogenes, Escherichia coli, K. pneumonia, Listeria monocytogenes., Proteus vulgaris, Pseudomonas fluorescens. Salmonella enteritidis, Salmonella paratyphi, Shigella flexneri, Staphylococcus aureus, Streptococcus faecalis, Streptococcus pyogenes and Yersinia enterocolitica. Aquatic fish pathogens such as Aeromonas hydrophila, Aeromonas sobria, Edwardsiella ictaluri, Edwardsiella tarda, Escherichia coli* and *Pseudomonas fluorescens* were inhibited by tannins (Chung et. al., 1995). The antimicrobial properties of tannic acid are associated with the ester linkage between gallic acid and polyols (Chung et. al., 1995). The ester bonds are hydrolyzed upon ripening of many edible fruits. Therefore, the presence of tannins in fruits serves as a natural defense mechanism against microbial infections. Similarly, since tannic acid is highly water-soluble and relatively rigid and spherical (Taguri et al., 2004), thermal processing could breakdown the ester bonds of polygalloyls and enhance hydrolysis of tannic acid. The hydrolysis ends up forming one more gallic acid and other galloyl groups on gallotanins

which could enhance tannic acid's antimicrobial capacity by producing more hydroxyl groups that are able to acidify the bacteria's environment. This mechanism could be explained by hydrogen binding of polygalloyl glucopyranose and hydrophobic interactions by gallic acid with surface proteins on bacteria cells (Kim and Silva, 2009).

The antimicrobial capacity was greatly enhanced when antimicrobials such as potassium lactate and acetate were added together with the tannic acid within the formula. Conjugate bases such as sodium lactate, potassium lactate, sodium acetate, and potassium acetate have been utilized in pork and poultry products to extend shelf-life by reduction of bacterial load and to decrease food safety risks through inhibiting the growth of pathogens, especially Listeria spp. (Mendonca et al., 1989).The weak acids associated with these bases have also been utilized to extend the shelf-life of pork and poultry. Salts of organic acids such as lactic, acetic and citric are used as antimicrobial agents. Previous studies indicated that these organic acid salts have the ability to control microbial growth, improve sensory attributes, and extend the shelf-life of various food products such as beef, pork, poultry and fish products (Zhuang et al., 1996; Kilinc et al., 2009). The salts of these organic acids are included in product formulations to prevent outgrowth of spoilage bacteria and pathogens in a variety of ready-to-eat (RTE) foods. One proposed mechanism suggests that the perturbation of membrane function may be the primary cause of growth inhibition by weak acid salts while other mechanisms suggest that growth inhibition might result from the accumulation of inhibitory levels of the weak acid anion within the cytoplasm of the cell (Roe et al., 2002; Hirshfield et al., 2003). It has also been demonstrated that a large pH difference between the intracellular and extracellular environment of bacterial cells leads to greater inhibition by weak acid anions such as lactates (Pieterse et al., 2005).

The antimicrobial effect of lactates may be due to the specific ability of sodium lactate to lower the water activity (Chirife and Fontan 1980) and the ability of weak lipophilic acids to pass across the cell membrane in their undissoicated form, within the cell and acidifying the cell interior (De Wit and Rombouts 1990; Shelef 1994). A sufficient extracellular anion concentration is necessary to maximize antimicrobial effectiveness since the presence of extracellular anions leads to the accumulation of intracellular anions (Romick and Fleming 1998).

Sodium acetate is sometimes used in food and would be expected to have the same antimicrobial properties as acetic acid at the same pH values, approximately 2.88 for 0.1M acetic acid solution (Hoffman et al., 1939). However, sodium diacetate has commonly been used and is more effective than acetic acid against bacteria. The inhibitory effect of sodium diacetate has been attributed to the diacetate moiety rather than pH alone (Doores 2005). Sodium diacetate at concentrations of 0.1% to 0.3% and sodium lactate at concentrations of 2% to 3% were equally effective as antilisterial compounds in meat with a minimal effect on pH and sensory characteristics (Mbandi and Shelef 2001).

However, more attention has been focused on the combined application of lactate and diacetate due to their synergistic inhibitory effect against the growth of pathogenic organisms in meat products (Zhu et al., 2005). Moreover, the attention was shifted not only to the synergistic inhibitory effects of the conjugate bases but that of the bases and tannic acid together. Although this coupling showed the best results in the inhibition of psychotropic microbial flora, results were better than when used without the coupling of tannic acid (Kin, 2011). Consequently, the dissociation of the diacetate and lactate showed a dominant competitive nature and also chelating ability with tannic acid when

mixed together; chelating metal ions that are essential in bacterial metabolism and growth (Molins 1991).

Antioxidant Capacity and Tannic Acid Quantification

Induction point was used to evaluate the oxidation of catfish fat from the treated fillets (Table 5). FTA was the most effective treatment against oxidation; with the highest ($p \leq 0.05$) induction time (It) followed by PTA, control, and FPH. FTA reduced auto-oxidation 1.08h (41.6%) longer than the CTL treated catfish fillets and 0.86hrs (21.6%) more efficient than PTA. In addition, PTA reduced auto-oxidation 0.82h (25.8%) longer than the control. The FPH blend showed no advantageous inhibition to oxidation as its induction point was even lower ($p \leq 0.05$) than the CTL group. In addition the European Committee for standardization has established a minimum limit 3-6 h for the IP, as determined by an oxidative stability instrument (Rancimat®) (Lacoste and Lagardere, 2003). The IP for the CTL and FPH were well below the 3 h or 6 h minimum European standard (Domingos et al., 2007; Kenneck, 2007) but when tannic acid was added, it was well in range with the standard after a storage time of one month (Table 5). All of the above observations can be explained by analyzing the affinity of the tannic acid/gallic acid (hydrolyzed radical scavenging tannic acid units) molecules to proteins and its chelating ability to inorganic elements.

Tannic acid has long been used as an essential agent in the leather industry because of its ability to bind and precipitate proteins (Zhu et al., 2005). Its capacity in cross linking makes it a more stable system, both thermally and mechanically. In this investigation, it was not different and due to the interactions of the tannic acid molecules, by first hydrogen bonding to the amino groups and also bonding to the hydrophobic

groups (Tang 2003). Since tannic acid can chelate the iron in the porphyrin ring of the hemoglobin, the antioxidant capacity of the catfish fillets containing tannic acid was increased. Tang and coworkers (2003) deduced that there are four major factors that govern the interactions among tannins and proteins;

I. Galloyl groups of polyphenols act as functional groups.

II. Strength of interactions is dependent upon molecular weight, number of galloyl groups.

III. Hydrophobic interactions are significant.

IV. Strength of interaction is dependent upon the flexibility of galloyl groups.

As observed in Figure 8 tannic acid is classified as a hydrolysable tannin, and it is comprised of a pentagalloylglucose core esterified at all hydroxyl functional groups by an additional gallic acid molecule (Mueller-Harvey 2001). The increase in induction point is well illustrated as one can observe the numerous hydroxyl groups in the gallic acid portion of the polymer and the hydrolysable ester bonds that can be broken to attach to the protein molecules in catfish fillets. The hydroxyl groups not only act as radical scavengers that reduce auto-oxidation but also as main molecules in the hydrogen bonding attachment of the amino groups. The shaded region surrounds the pentagalloylglucose (PGG) core (Figure 9). To be more specific a proposed mechanism was developed by Heijmen et al. (1997) to explain the attachment of tannic acid to protein (Figure 10). Lower aggregation of natural actomyosin was noticeable in the presence of sarcoplasmic protein, which was enhanced and preferably cross-linked with tannic acid (Balange et al., 2009).Tannic acid demonstrates stabilization attachment and consequently anti-oxidation properties, even under thermal stress. Thermal stress brings instability to the hydrogen bonds unfolding the proteins on the meat, exposing

hydrophobic portions (Niwa, 1992). As a result tannic acid will aggregate via hydrophobic interactions by quinones, and an electrophilic group able to attach to the amino group, a nucleophillic counterpart (Strauss and Gibson 2004). Fat from fillets treated with FTA and PTA showed a higher ($p \leq 0.05$) induction point since tannic acid attachment was not only made by hydrogen bonding, but also hydrophobic interactions occurring between phenolic compounds and hydrophobic amino acids such as valine, isoleucine, leucine, methionine, phenylalanine, tyrosine, tryptophan, cysteine and glycine (Prigent 2005); all found in catfish. As a result, protein-tannic acid interactions were suggested as a surface phenomenon where tannic acid molecules effectively coat the surface of the catfish proteins (Spencer et. al., 1988). These results were also in agreement with Silva et al. (2009). The treated tannic acid catfish fillets were higher in anti-oxidant capacity, higher IP when compared to the CTL ($p \leq 0.05$). Moreover, Gulcin and others showed that tannic acid's capacity to chelate to metal ions; more specifically iron, was compatible to that of strong synthetic antioxidants such as BHA and BHT. Unlike the findings of Kim and Silva (2010), the FTA treatment had a longer induction period of 1.08h (21.3%) than PTA. This is because the concentration of tannic acid on PTA was less than that of FTA. Moreover, the new group mixed with phosphate blend showed a lower ($p \leq 0.05$) induction point than the control (Table 5).

To understand the low anti-oxidant (low IP) capacity of the FPH treatments, the chemicals structures of the blend are very important. Complexes of phosphate tannic acid (P-T) were formed and chemical charge repulsion and attraction played a major role in the inhibition of the antioxidant capacity of this treatment group (Rao et al., 2000). The ionic binding of the hydroxyl molecules (OH-) of the galloyl groups to the free bound molecules including sodium (Na+) and potassium molecules (K+), introduce the adhesion

of tannic acid into larger molecules bonded by ionic forces and a proposed mechanism for the observed phenomena of the low induction point of the FPH. Moreover one of the lesser known uses for sodium hexametaphosphate (Figure 11) is as a deflocculant that causes heavy particles to drop to the bottom. In this case, proteins secure the tannic acid by hydrogen bonding. Another issue is the use of potassium acetate (Figure 11) which is often used to precipitate dodecyl sulfate (DS), DS-bound proteins, allowing the removal of proteins; thus, once again taking away the aggregation ability of tannic acid with the meat source by having an affinity with the protein.

The difference in the findings between induction points in the tested treatments was reached by the quantification of tannic acid derivatives expressed as gallic acid units using the HPLC. The FTA treated fillets had a higher ($p \leq 0.05$) concentration of tannic acid solution than all other treatments. Moreover, PTA treated fillets were the second highest in tannic acid which is directly related in comparison to the induction points (Table 5). Even though the FPH shows concentration of tannic acid, the tannic acid did not contribute to the antioxidant capacity. This can be maybe explained by tannic acid chelation of inorganic elements prone to nucleophillic attack. Thus, chelating to the phosphate forming ligands (P-T) causes less adhesion to the proteins. This leads to protein precipitation of the acetate/lactate and sodium/potassium bonding leaving less galloyl free radical scavenging units to quench radicals (Rao et al., 2000). In addition, percent absorbance quantification also helped explain the differences in results to Kim and Silva (2010) research, where PTA had a greater induction point rather than FTA (Table 5).

The FTA treatment had a greater ($p \leq 0.05$) ability to attach by the catfish fillets while vacuumed tumbled (Table 5) as indicated by the quantification with HPLC. This

greater absorption directly affects the oxidation of the extracted catfish fat oil and consequently the induction point differences between the FTA and PTA treatments. In this investigation, tannic acid was not added directly to the oil as previous experiments (Kim et.al. 2010); therefore, since it was added to catfish fillets results differ where FTA absorptivity and affinity to attach and penetrate the meat was greater than that of PTA. It is plausible that PTA would have more attachment sites since the thermal process will break tannic acid into gallic acid units, which produces more OH groups and consequently attachment sites for bonding (Kim and Silva 2010). Catfish proteins seem to neutralize those smaller molecules and cause it to not be as effective as the tannic acid polymer structure. In conclusion FTA treated catfish fillets shows 72.8% absorption compared to that of PTA, 43.8% and FPH, 18.1% (Table 5).

Table 1 Surface Hunter color values of vacuum-tumbled catfish fillets as affected by treatment, regardless of storage time.

Treatment	L-value (Lightness)	a-value (Redness)	b-value (yellowness)
CTL	54.2^{b}	-0.67^{b}	6.52^{c}
FTA	60.1^{a}	-0.41^{ab}	12.5^{ab}
PTA	58.2^{a}	0.07^{a}	13.0^{a}
FPH	48.2^{c}	-0.69^{b}	11.2^{b}
STDEV	2.5	0.56	1.8
LSD (0.05)	2.10	0.38	1.65
C.V	4.50	47.5	15.2

CTL = no Tannic acid added, tumbled only with distilled water; FTA = Fresh 5% Tannic acid solution; PTA = Thermally processed 5% Tannic acid solution; FPH= phosphate= 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.
ab = Means with the same letter within each column are not significantly different by treatment ($P<0.05$)

Table 2 Surface Hunter color values of vacuum-tumbled catfish fillets as affected by storage time at 4°C, regardless of treatment.

Storage time (days)	L-value (Lightness)	a-value (Redness)	b-value (yellowness)
1	56.8[a]	-0.39[NS]	9.65[b]
5	56.5[a]	-0.12	10.63[ab]
10	53.9[b]	-0.62	11.79[a]
15	53.7[b]	-0.64	11.29[a]
STDEV	2.46	0.64	1.65
LSD (0.05)	2.05	0.53	1.38
C.V	4.46	46.4	15.2

ab = Means with the same letter within each column are not significantly different by treatment ($P \leq 0.05$)
[NS]No significant ($p \geq 0.05$) differences.

Table 3 Surface color (hue, chroma, whiteness) of vacuum-tumbled catfish fillets enhanced with 5% tannic acid solutions with or without phosphate.

Treatment	Hue	Chroma	Whiteness
CTL	78.6[b]	6.7[c]	53.7[b]
FTA	87.3[a]	12.6[ab]	58.2[a]
PTA	86.8[a]	13.0[a]	56.18[a]
FPH	85.6[a]	11.3[b]	47.0[c]
STDEV	2.89	1.63	2.49
LSD (0.05)	2.40	1.40	2.10
C.V	3.40	15.0	4.60

CTL = no Tannic acid added, tumbled only with distilled water; FTA = Fresh 5% Tannic acid solution; PTA = thermally processed 5% Tannic acid solution; FTP = phosphate= 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.
ab = Means with the same letter within each column are not significantly different by treatment ($P<0.05$)

Table 4 Surface color (hue, chroma and whiteness) of vacuum-tumbled catfish fillets over 15 days of storage at 4°C, regardless of treatment.

Storage time (days)	Hue	Chroma	Whiteness
1	83.3[b]	9.72[b]	55.5[a]
5	86.7[a]	10.6[ab]	55.1[a]
10	85.6[ab]	11.8[a]	52.2[b]
15	83.9[ab]	11.4[a]	52.2[b]
STDEV	2.93	1.63	2.49
LSD(0.05)	2.40	1.40	2.10
C.V	3.41	15.0	4.72

ab = Means with the same letter within each column are not significantly different by treatment ($P<0.05$)

Table 5 Induction point, tannic acid concentration and absorbance of vacuum-tumbled catfish fillet after one moth storage, enhanced with 5% tannic acid with or without phosphate.

Treatment	Tannic acid (ppm as Gallic acid)	Induction point (hrs)	Absorbance (%)
CTL	0[d]	2.36[c]	0
FTA	520[a]	4.04[a]	72.8
PTA	313[b]	3.44[b]	43.8
FPH	129[c]	1.37[d]	18.1
STDEV	46.0	0.30	
LSD (0.05)	93.8	0.59	
C.V	19.3	10.5	

abcd = Means with the same letter within each column are not significantly different (P<0.05).
CTL = no Tannic acid added, tumbled only with distilled water; FTA = Fresh 5% Tannic acid solution; PTA = Thermally processed 5% Tannic acid solution; FTP= phosphate= 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.
**Total amount of tannic acid was calculated to be 715.3ppm on the catfish evaluated from HPLC results.

Table 6 Intercept (initial count) and slope of microbial growth curves of treated catfish fillets stored for 15 days at 4°C.

Treatment	Slope	Intercept
CTL	0.290	4.20
FTA	0.287	3.79
PTA	0.294	3.71
FPH	0.071	3.96

CTL = no Tannic acid added, tumbled only with distilled water; FTA = Fresh 5% Tannic acid solution; PTA = Thermally processed 5% Tannic acid solution; FTP= phosphate= 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.

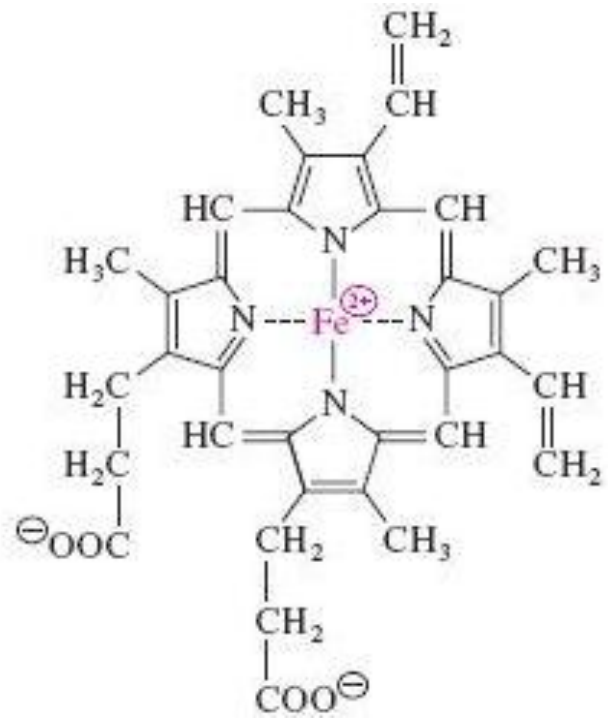

Figure 6 Chemical structure of the Fe(II)-protoporphyrin IX heme group in myoglobin and hemoglobin.

(http://ead.univ-angers.fr/~jaspard/Page2/TexteTD/7ModuleS6BG3/1Exo14/4HemeFer.gif)

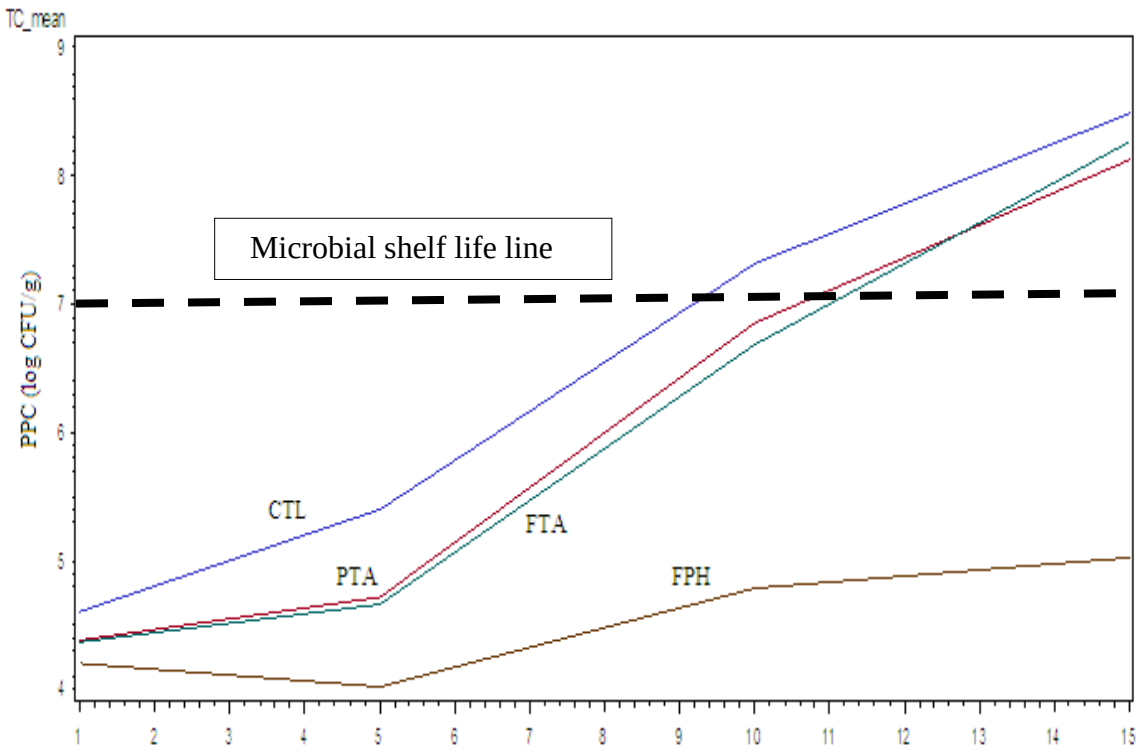

Figure 7 Psychotropic plate counts (PPC) of vacuum-tumbled catfish fillets, enhanced with 5% Tannic acid solutions over 15 days storage at 4°C.

CTL = no Tannic acid added, tumbled only with distilled water; FTA = Fresh 5% Tannic acid solution; PTA = Thermally processed 5% Tannic acid solution; FTP= phosphate= 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.

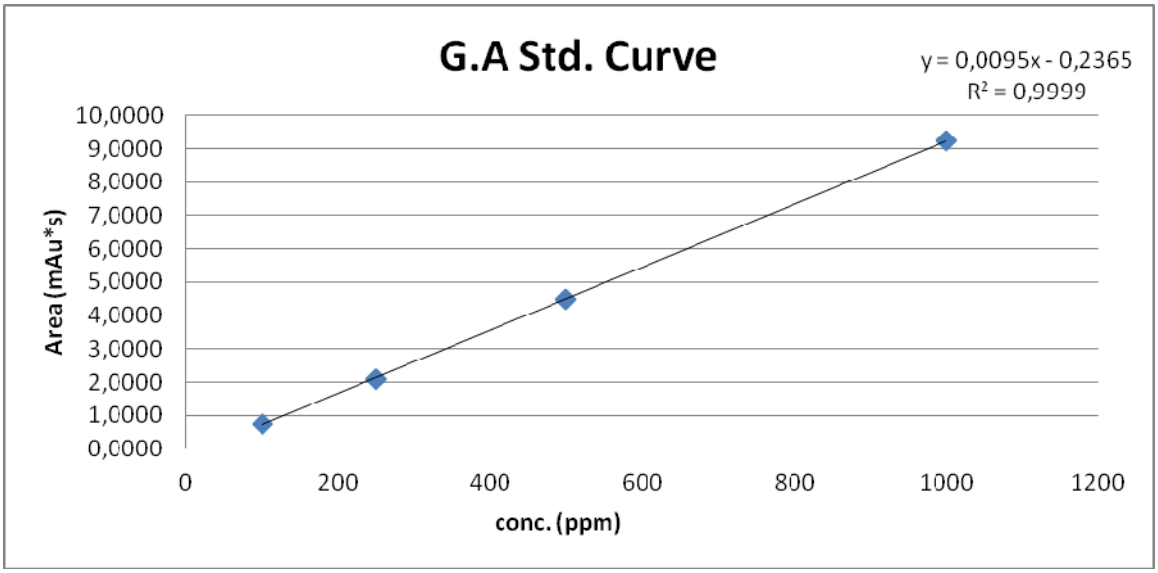

Figure 8 Best gallic acid standard curve used for the quantification of tannic acid of after chilling catfish fillets, kept under refrigeration (4^{o}C).

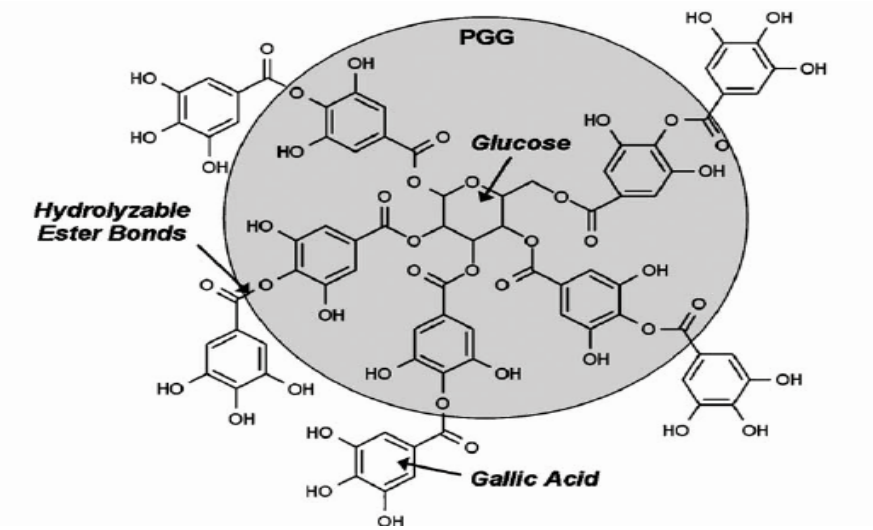

Figure 9 The structure of tannic acid.

The shaded region surrounds the pentagalloylglucose (PGG) core. (Mueller-Harvey, 2001)

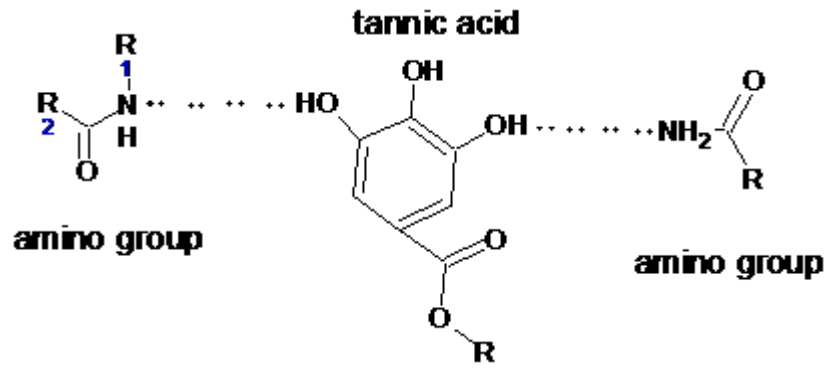

Figure 10 Proposed crosslinking mechanism of tannic acid with amino groups.

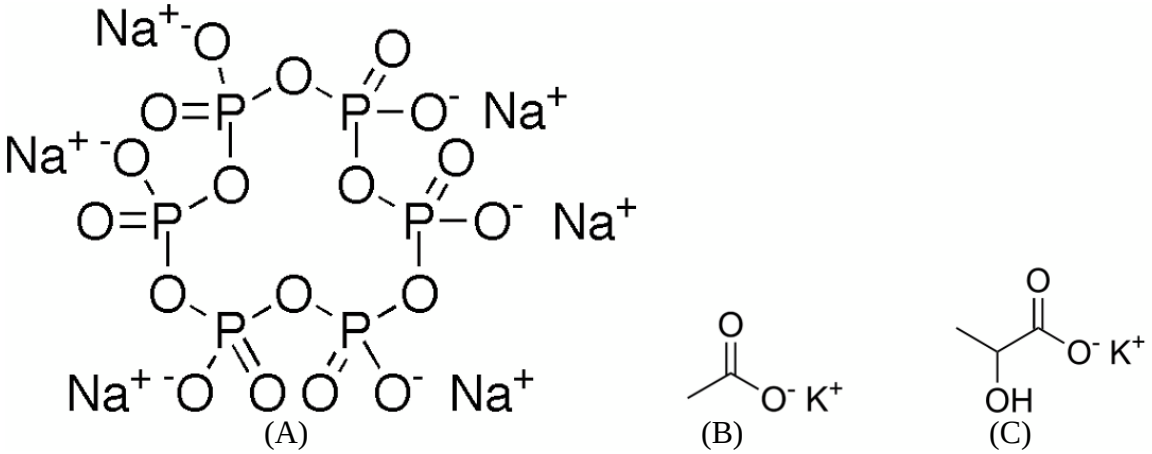

Figure 11 Structure of (A) Sodium hexametaphosphate, (B) potassium acetate, (C) potassium lactate (Merck Index, 12th Edition)

CHAPTER V

SUMMARY AND CONCLUSION

In conclusion all treatments showed a significant ($p \leq 0.05$) difference in psychotropic plate counts, with FPH being the most effective, demonstrating an average 3.7 times slower microbial growth rate than the other treatments. However, FPH showed no advantageous inhibition to oxidation as its induction point was lower than the CTL group. On the other hand FTA did not only show positive microbiological growth inhibition but also a reduced auto-oxidation 1.08hr (41.6%) longer than the CTL treated catfish fillets and 0.86hr (21.6%) more efficient than the thermally processed tannic acid (PTA). Also explained by its ability to attach to the fillet, FTA shows a 78.3% absorption rate compared to that of PTA, 43.8% and FPH, 18.1%. Thus, FTA was the best treatment choice in this investigation to increase catfish shelf life by antimicrobial capacity and induction point (IP). Furthermore, based on the discussion above, FTA showed it can be used for minimizing or preventing lipid oxidation in food products, maintaining nutritional quality and prolonging the shelf life of foods in catfish fillets. Catfish color was a minor concern since catfish fillets treated with tannic acid derivatives contained a more saturated (chroma) yellowish (hue) color than catfish consumers might be used to buy and/or prefer. In addition, one might recommend marinating the catfish to explain such colors and or label as a natural antioxidant product emphasizing the phenolic characteristics of tannic acid in our diet. Also future research could be conducted by

combining with artificial antioxidants such as BHA and BHT to investigate for further synergistic effects.

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APPENDIX A

FIGURES

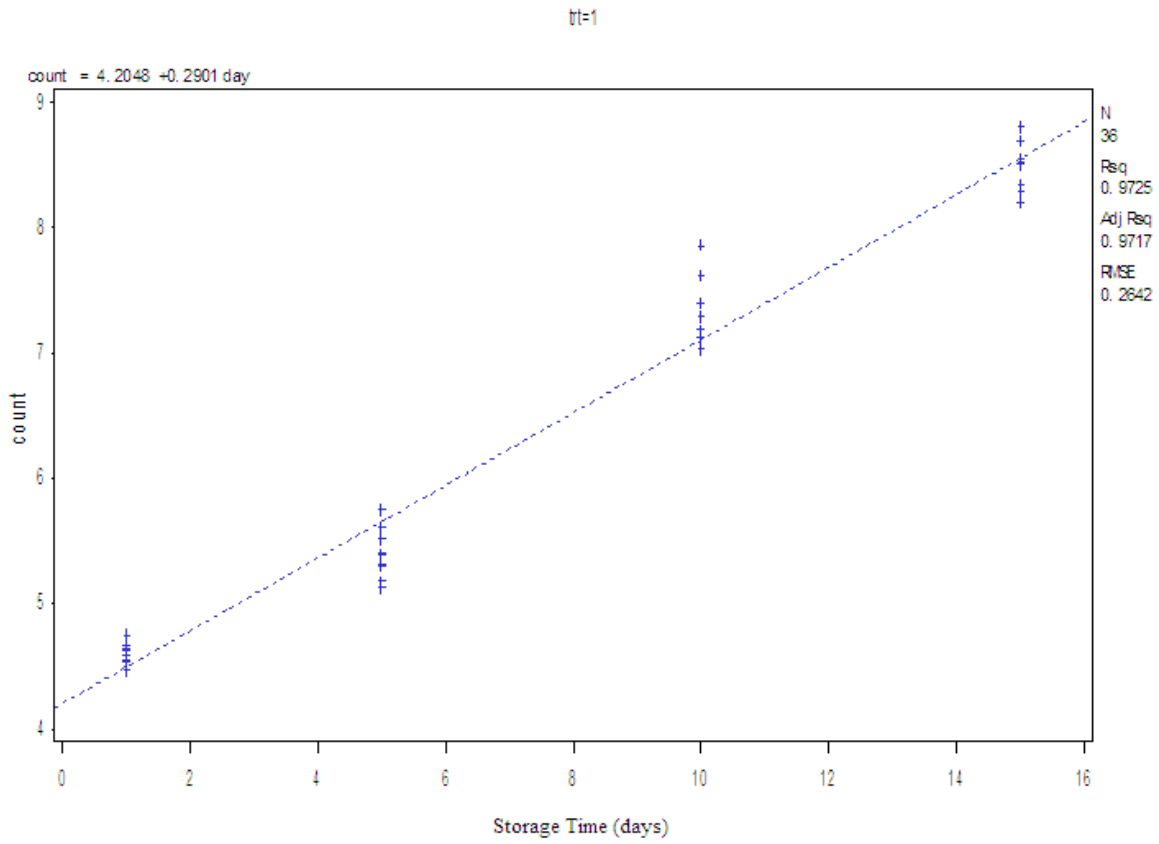

Figure 12 Exponential regression of psychotropic plate count (PPC) of vacuum-tumbled catfish fillets with water (CTL) as affected by storage time (days) at 4°C.

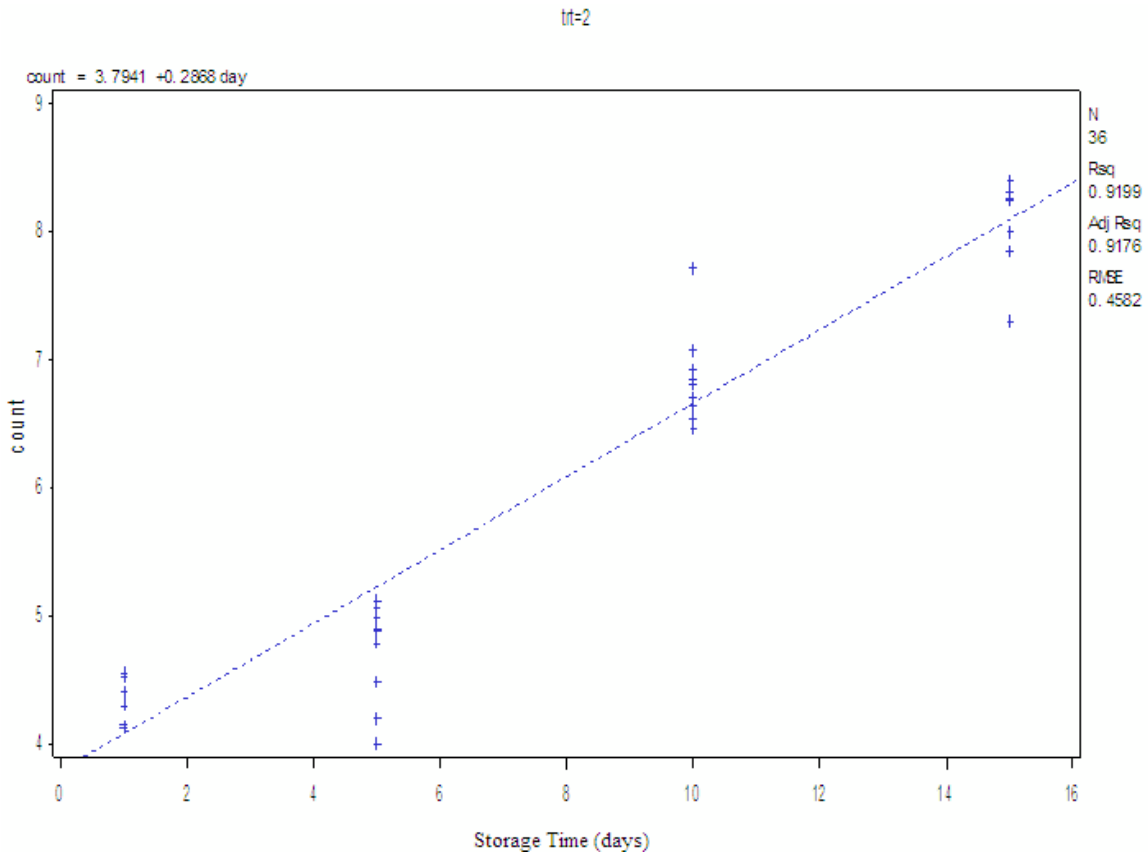

Figure 13 Exponential regression of psychotropic plate count (PPC) of vacuum-tumbled catfish fillets enhanced with 5% fresh tannic acid solutions, (FTA) as affected by storage time (days) at 4°C.

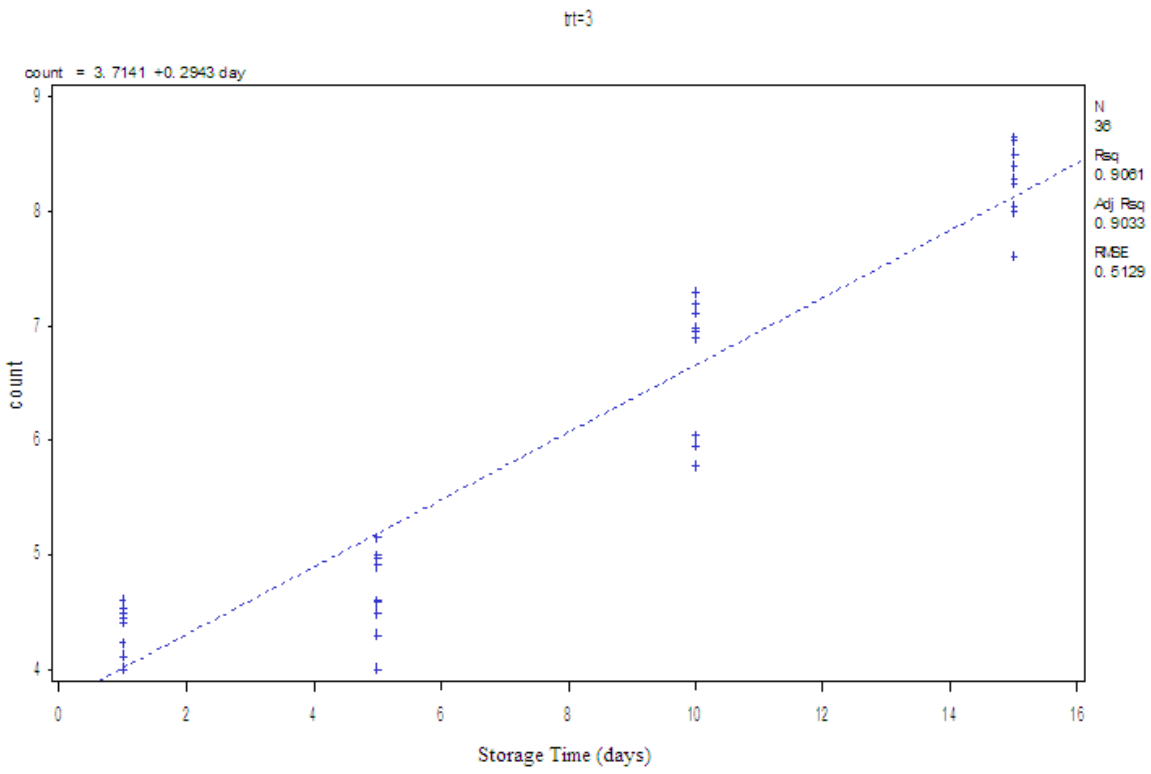

Figure 14 Exponential regression of psychotropic plate count (PPC) of vacuum-tumbled catfish fillets enhanced with 5% processed tannic acid solutions, (PTA) as affected by storage time (days) at 4°C.

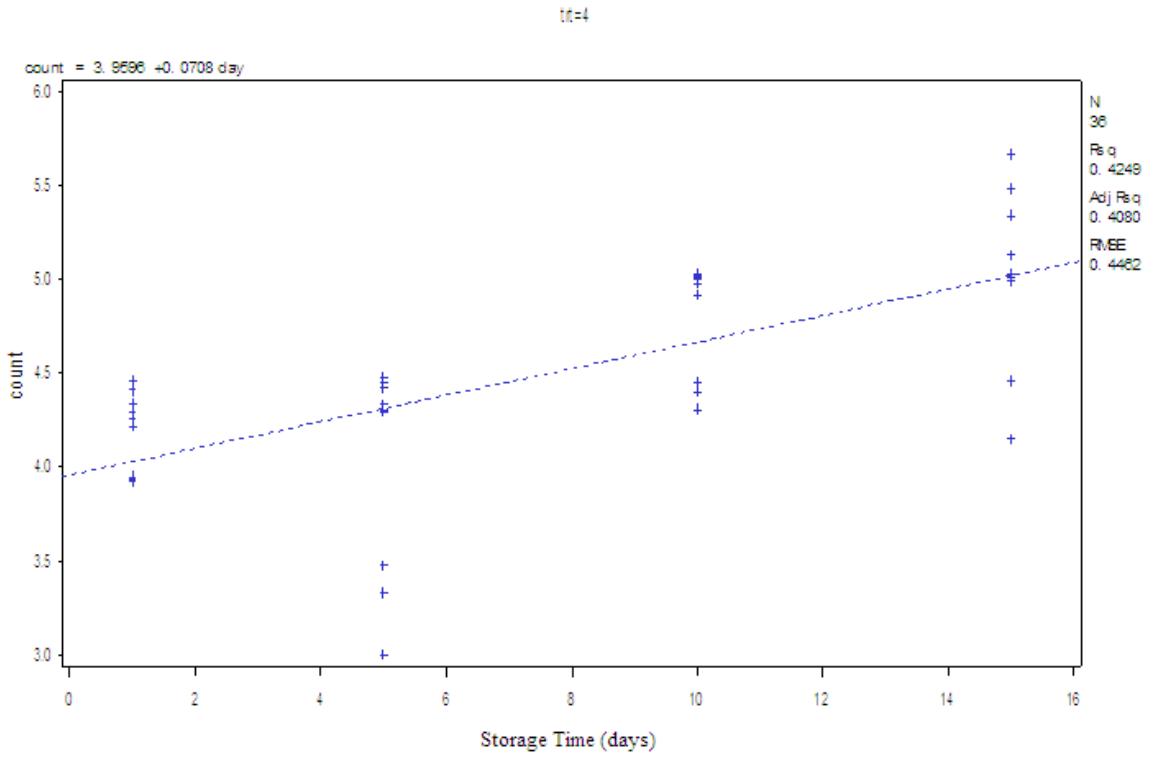

Figure 15 Exponential regression of psychotropic plate count (PPC) of vacuum-tumbled catfish fillets enhanced with 5% fresh tannic acid and phosphate blend solutions, (FPH) as affected by storage time (days) at 4°C.

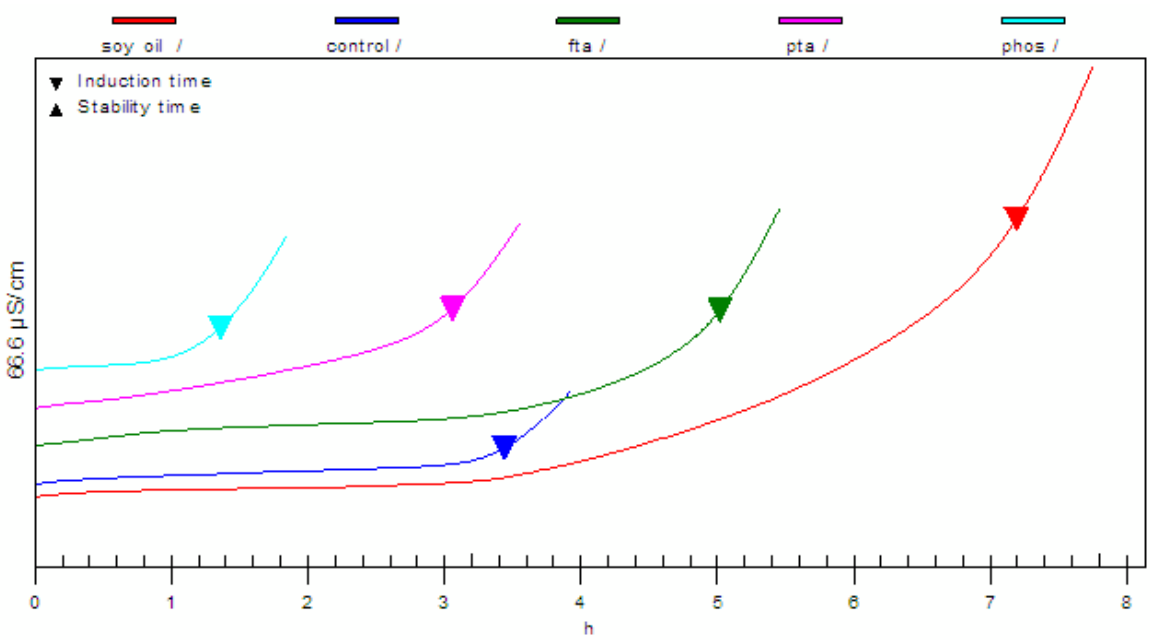

Figure 16 Rancimat graph illustrating the effect of treatments solutions with or without phosphate on the induction point (h) of catfish oil after one month storage time.

Control = no Tannic acid added, vacuum tumbled only with distilled water. FTA = Fresh 5% Tannic acid solution, PTA = Thermally processed 5% Tannic acid solution, FTP = 5%FTA + phosphate + AGSP combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride.

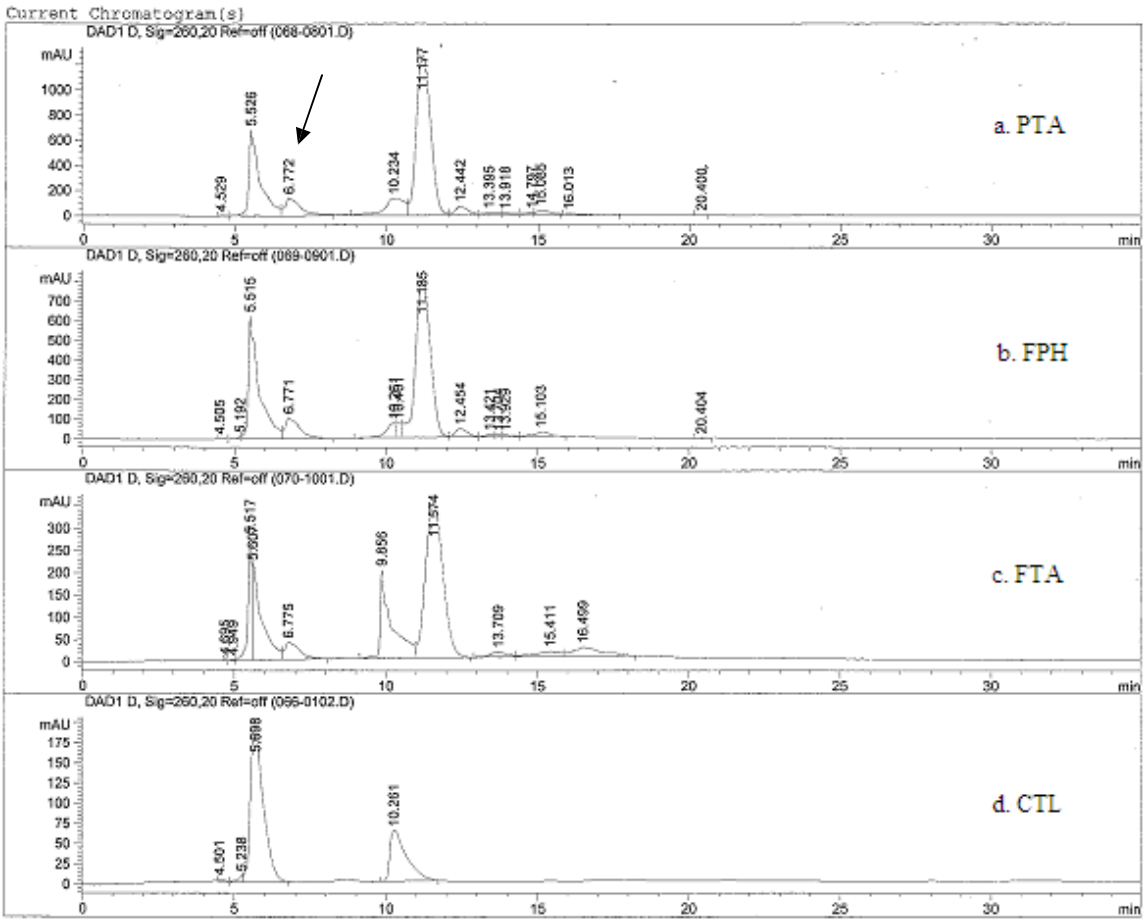

Figure 17 HPLC Chromatograms measuring the concentration of tannic acid on treated cat fish fillets (ppm of gallic acid units).

(a)PTA = Thermally processed 5% Tannic acid solution, (b)FTP = 5%FTA + AGSP + combination of 0.25% potassium acetate and 0.58% potassium lactate and sodium chloride, (c)FTA = Fresh 5% Tannic acid solution, (d) CTL = no Tannic acid added, tumbled only with distilled water; respectfully starting from the top of the chromatogram.

$$\text{eq. 1: total conc. (ppm)} = \left(\frac{\text{solution added to each fillet (mL)}}{\text{solution added (mL) + catfish weight (g)}} \right) \text{ x total solution concentration}$$

$$\text{eq. 2: total conc. (ppm) in HPLC} = \left(\frac{\text{weight of the treated fillet (g)}}{\text{weight of the treated fillet + HPLC extrating solvent (mL)}} \right) \text{x eq. 1}$$

$$\text{eq. 3: absorbance \%} = \left(\frac{\text{HPLC mean conc. (ppm)}}{\text{total conc. (ppm) in HPLC}} \right) \text{ x 100}$$

Figure 18 Equations quantifying the concentration (ppm) of tannic acid in catfish fillets used in HPLC quantification.

HPLC extracting solvent = water + methanol