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Stability of catfish methyl esters under normal versus accelerated storage conditions

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STABILITY OF CATFISH METHYL ESTERS UNDER NORMAL VERSUS ACCELERATED STORAGE CONDITIONS

By

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ACCELERATED STORAGE CONDITIONS

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Oxidative storage stability was conducted to determine the optimum antioxidant for the production of catfish methyl esters (CFME) for use as biodiesel. Peroxide value, anisidine value, 2-thiobarbituric reaction substances, acid value, iodine value, UV absorbance, and induction period were measured. Antioxidant, stability parameters changed over a storage time of one year when stored at 25^{o}C. The CFME were not stable to oxidation without the addition of TBHQ. When stored at 25 ^{o}C, oxidative parameters indicated that CFME was a stable product that met oxidative stability standards when TBHQ was added. When held at 80 ^{o}C for 28 days, the samples with TBHQ were stable to oxidation. However, the acid value rose above the maximum at 28 days. Thus, CFME can be stable to oxidative breakdown when THBQ was added at 800 ppm, but when exposed to high temperatures, one will have to limit water or protect CFME against hydrolytic rancidity.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Mississippi is the largest producer of channel catfish in the United States with more than 60 percent of the market (Crews, 2002; Craig, 2007). Channel catfish (*Ictalurus punctatus*) is the fourth most popular seafood product that is consumed in the United States (NASS, 2007). Approximately 55 % of the whole catfish is processing waste or offal containing frames, viscera, skin, and trimmings (Silva and Dean, 2001; Subramaniam et al., 2002). Drake (1971), Lovell (1980), Silva and Ammerman (1993), and Subramaniam *et al*. (2003) reported that skin and viscera of catfish contain 5.7 % and 34 % crude fat (wet basis), respectively, with 75% being unsaturated fatty acids. As a result, to increase the value of catfish offal, many researchers and commercial producers have become interested in producing catfish oil.

With the increase in fuel price and depletion of petroleum reservoirs, an alternate source of fuel is needed. Methyl ester fuels known as biodiesel are an alternative diesel fuel derived from renewable sources such as vegetable oils, animal fats, or cooking oils. Methyl ester fuels are environmentally safe, thus increased usage will lessen the emission of harmful pollutants from diesel engines. Methyl ester fuels are produced from

vegetable oils or animal fats transesterified with alcohol: ethanol or methanol (Maa and Hanna, 1990).

Vegetable oils and animal fats consist of saturated and unsaturated long-chain fatty acid alkyl esters (Dunn, 2005). Like all natural oils and fats, mono-alkyl esters are not stable over storage time due to autoxidation. Autoxidation of Mono-alkyl esters can cause motor damage because of debasement of fuel quality by increasing kinematic viscosity, acid value, and peroxide value (Canakci *et al*., 1999; Vicente *et al*., 1998). Oxidation stability is one of the most important criteria for methyl ester fuels quality according to the American Society for Testing and Materials, ASTM (Knothe, 2005). In an effort to prolong the storage stability of mono-alkyl esters, many antioxidants have been examined to determine their ability to increase the stability of mono-alkyl esters (Dunn, 2002; Liang *et al*., 2006).

Many publications have shown the influence of antioxidants on the oxidative stability of sunflower, soybean oils and other methyl esters during storage (Canakci *et al*., 1999; Du Plessis *et al*., 1985). Early reports (McGillivray, 2006; Danviriyakul *et al*., 2007) have shown that catfish oil and biodiesel are very unstable. These oils are made as a result of a rendering process (Sathivel et al., 2008) or as byproducts of hydrolysis during the production of fresh fertilizer (Ferguson, 1990).

Without antioxidants, the shelf-life of mono-alkyl esters from feedstock such as animal fats or waste frying oils is six months or less (Garba *et al*., 2006). Conducting storage stability tests take time. Therefore, accelerating oxidation by changing environmental conditions, such as increasing temperatures, takes less effort. Moreover, it

makes it more convenient and easier to predict the oxidation stability of methyl ester fuels (Bondioli *et al*., 2004).

The purpose of this research was to investigate the oxidative stability of catfish methyl esters (CFME) with and without antioxidants at room temperature (25^{o}C during one year) and accelerated aging conditions (80^{o}C for 28 days).

CHAPTER II

LITERATURE REVIEW

Production of Methyl Ester Fuels

Transesterification of vegetable oils and animal fats is the most common procedure to produce methyl ester fuels. Other processes such as direct use and blending, micro emulsions, and thermal cracking (pyrolysis) are also used. In the production of biodiesel, the vegetable oil or animal fat (triglycerides) mixes with the alcohol (commonly methanol or ethanol) in a 3:1 molar ratio of alcohol to triglycerides. Since the transesterification of triglycerides reaction is reversible, excess alcohol (6:1 molar ratio) is necessary to favor equilibrium yields toward the product side. Furthermore, a catalyst is employed to enhance the reaction rate and yield. There are three catalysts that can be used in the biodiesel reaction, which consist of alkalis (potassium hydroxide or sodium hydroxide), acids (sulfuric acid or hydrochloric acid), and certain enzymes (lipases). Commercial biodiesel production usually utilizes an alkali as a catalyst since it is cheaper, and its reaction time is much faster than the others. After transesterification of triglycerides, the products are esters, which consist of the biodiesel and the byproduct, glycerol. The final yield of mono-alkyl ester depends upon the quality of oils, which can reach 97-98% of the mono-alkyl ester fuel (Maa and Hanna, 1990).

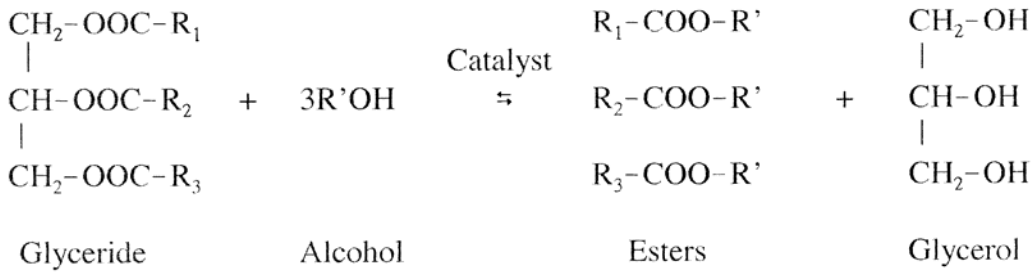

Figure 1. Transesterification of triglycerides (Maa and Hanna, 1990)

Oxidative Stability

Methyl ester fuels consist of long-chain fatty acids that are joined together by a glycerin backbond. For instance, soybean oil is composed of a triacylglyceride mixture that contains 53% linoleic (C18:2), 22% oleic (C18:1), 11% palmitic (C16:0), 8% linolenic (C18:3), and 4% stearic acids (C18:0) (Canakci *et al*., 1999; Domingos *et al*., 2007) while catfish fat contains 12% linolenic (C18:2 n6), 52% oleic (C18:1 n9), and 22% palmitic (C16:0) (Labuza, 1971; Worthington *et al*., 1972). Biodiesel from both of these materials contain predominantly C16 and C18 unsaturated ester components. Since both biodiesel materials consist of high concentrations of unsaturated fatty acids, they are susceptible to oxidation. Therefore, one of the main concerns for the methyl ester fuels' quality is its degree of saturation, which may directly correlate with its storage stability (Domingos *et al*., 2007).

There are two types of oil rancidity: hydrolytic rancidity and oxidative rancidity. Hydrolytic rancidity is the initial chemical reaction that leads to the development of rancid off-flavors. This occurs when water breaks down double bonds in triglycerides into free fatty acids and glycerol. Heat and free enzyme lipase also cause hydrolytic

rancidity (Murano, 2003). It is believed that methyl ketones and their esters may be derived primarily by this hydrolytic reaction. It has been claimed that the hydrolytic reaction, including lipolysis, provides the free oleic and linoleic acids, which then undergo more rapid autoxidation (Allen *el at.,* 1989). Moreover, enzymes at temperatures above 60 ^{o}C are usually inactive, but if oil or fat is contaminated with lipase-producing microorganisms during storage, lipolytic rancidity can occur (Kilcast and Subramaniam, 2000).

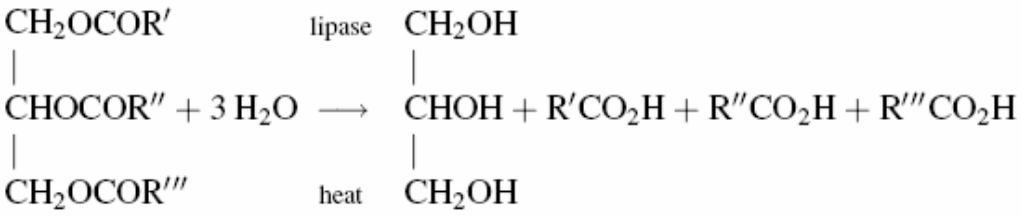

Figure 2. General hydrolytic reaction (Kilcast and Subramaniam, 2000)

Oxidative rancidity arises in three stages: an initiation process, a propagation process, and a termination process. The end result of these processes will gain and lessen over time. Therefore, lipid oxidation is difficult to quantify. Oxidative degradation starts with an initial chain reaction (RH + O_2 → R-OOH→ R*), where molecular oxygen joins with unsaturated fatty acids in order to produce very active hydroperoxides and free radicals. Then, the propagation stage occurs when hydroperoxides decompose into aldehydes, alcohols, and carbonic acids (R* + O_2 → ROO* and ROO* + RH → R-OOH + R*). Finally, the termination stage occur when free radicals interact to form nonradical end products (R* + R* → R-R and ROO* + R* → R-OO-R) (Bailey *et al.*, 1996; Murano

2003). Oxidative degradation of biodiesel fuel is caused by autoxidation, which is affected by such variables as time, oxygen, temperature, and metals during long term storage.

After methyl ester fuels are oxidized, hydroperoxides, aldehydes, ketones, and acids are produced, and they induce a change in fuel properties causing motor damage. Hydroperoxides may produce polymerization of esters and insoluble sediments clogging fuel filters and lines. Acid values also increase which might induce engine corrosion (Canakci *et al*., 1999; Vicente *et al*., 1998). Furthermore, ignition time is influenced by the cetane number and the peroxide value. The lower the cetane value and the peroxide value, the longer the ignition time is delayed (Knothe, 2005). Even in the initial state of degradation, peroxide values increase; but extensive degradation from decomposed hydroperoxides reduces the peroxide values (Dunn, 2005).

Measures of Oxidative Stability

Acid value (AV)

Acid value is a measure of hydrolytic rancidity (41.1.21 AOAC, 1995). This value determines the quantity of free fatty acids (FFA) that are present during the initiation process. The FFA is determined in the percentage by weight of oleic acid, and AV is defined as the milligrams of potassium hydroxide needed to neutralize the free acids in one gram of fat of oil. Thus, percent of FFA (calculated as oleic acid) is two times the AV (AOAC, 1999; Nielsen 2003; Panda, 2003). This method computes percent free fatty acids as oleic acid and is indicated as percentage of the total lipids (AOAC, 1995;

Lakkakula *et al*., 2004). The AV increases due to exposure to light, heat, and moisture. Therefore, if AV increases during storage, it can indicate the degree of hydrolysis in the fuel. Increasing acidity level in fuels, causes increased engines corrosion (Osawa *et al*., 2007). According to the American Society for Testing and Materials (ASTM D6751) standard and the European biodiesel standard (EN 14214), the maximum allowable acid value is 0.5 milligrams (mg) potassium hydroxide (KOH) per gram sample (Table1) (Steinbach, 2007; Knothe, 2006).

Iodine value (IV)

Iodine value is used to determine the amount of double bonds in unsaturated fatty acids. Iodide is oxidized into iodine, and sodium thiosulphate is used to measure the level of iodine produced. IV is measured in centigrams of iodine absorbed per gram of sample (% iodine absorbed) (Steinbach, 2007; Knothe, 2002). A greater iodine value indicates a higher number of unsaturated fatty acids in the fuel. Thus, IV can be used as an indicator of fuel debasement instead of a predictor of methyl ester fuels' stability because it cannot locate the position and the number of double bonds per molecule. IV can determine the tendency of the fuel to polymerize and form engine precipitations (Bouaid *et a*l., 2001; Knothe, 2007). It is also used in European standards of biodiesel fuel, with 120 $gI_2/100g$ set as the maximum allowable value (Knothe, 2002). However, the correlation of structure and chemical properties with fatty acid formation is too broad to be indicated by IV since IV does not distinguish the structural differences in fatty compounds. It measures all double bonds, not the oxidization process of the fatty acid compounds that

are present. Furthermore, Knothe and Dunn (2003) also stated that there is no good correlation between IV and oxidative stability. In order to accurately determine the number and the position of double bonds in fatty acid chains in oil stability, the allylic position equivalent (APE) and the bis-allylic position (BAPE) are used. The APE and BAPE depend on the amount of reactive positions in oxidation processed since oxidative stability can be induced by a small amount of more unsaturated fats (Bouaid et al., 2001).

Peroxide value (PV)

Peroxide value is used as an indicator of the amount of hydroperoxides and peroxides that are produced in the initial phases of lipid oxidation. The PV is measured in milliequivalents of peroxide per kilogram of product by titration with an iodide ion (Allen and Hamiltom, 1994; Gotoh and Wada 2006).

The fluctuation of PV depends on oxidation stages. For example, hydroperoxides are produced in high concentration in the initial phase or induction period, and causes PV to increase. However, after the induction period, hydroperoxides decompose to secondary products such as aldehydes, alcohols, and carbonic acids causing PV to decline (Dunn, 2002). Furthermore, if oil is in the presence of oxygen, formation and decomposition of hydroperoxides are influenced by the free iodine from potassium iodide. Thus, if the peroxide value is high, it is a good sign of oxidation, and the chemicals (hydroperoxides) have been altered permanently. However, if the peroxide value is moderate or low, it does not mean that the fuel is good or in its final phase, and rancid. It might be due to

decomposition of peroxides after they have increased to high concentrations (Dunn, 2002; Warner and Eskin, 1995).

Peroxide value is not listed in biodiesel fuel standards. However, up to 70 meg/kg of PV can be used as an index of cetane number (CN) since as PV raises, CN increases (Domingos *et al*., 2007; Bouaid *et al*., 2001). If the PV is higher than 70 meq/kg, it cannot be used as an index of CN, since fuel starts to have a destructive effect (Gerpen *et al*., 1996). Canakci *et al* (1999) stated that peroxides are not heat tolerant. The level of PV decreases at higher temperatures while oxidation keeps going since hydroperoxides decompose dramatically.

UV absorbance

The UV absorption at 232-234 nm is used to monitor conjugated dienes (conjugated double-bond structures), that are caused by the formation of hydroperoxides from polyunsaturated fatty acids. UV absorbance at 234 nm indicates the initial phase of lipid oxidation of polyunsaturated fatty acids (Corongiu et al., 1983; Gunstone 2004; Pokorny *et al*., 2000; Sikorski and Kolakowska, 2002; Vieira and Regitano-d Arce, 1999). The UV absorbance at 234 nm is correlated with PV since PV also quantifies the amount of primary oxidation products (Jaswir *et a*l., 2000). However, PV is more specific than UV absorbance at 234 nm because UV absorbance at 234 nm measures not only hydroperoxides, but also measures other conjugated-structure formations such as 9-hydroxyoctadeca-10, 12- diencoic acid and 13-hydroxyctadeca-9, and 11-dienoic acid (Pokorny *et al*., 2000). In addition, Maskan and Bagci (2003) suggested that absorption at

270 nm can be used to determine aldehydes, ketones, and alcohols, which could be an indicator of propagation steps.

Anisidine value (AnV)

Anisidine value measures the total level of carbonyl compounds that are formed during secondary oxidation, since hydroperoxides decompose into volatile aldehydes, and the non-volatile portion of fatty acids (Contis, 1998; Saad *et al*., 2007). The *p*-anisidine reacts with saturated and unsaturated aldehydes. The result of the reaction produces a yellowish pigment which can be measured at 350 nm (Labrinea *et al*., 2001). Most oils and FAMEs value decrease during long term storage, whereas anisidine value increases, since it is a measure of secondary oxidation (Goth *et al*., 2006).

Oxidative Stability Index (OSI) and induction period (IP)

The oxidative stability index (OSI), that is measured using the Rancimat® test, is used to determine and predict the oxidative stability of biodiesel fuel according to EN 14112, ASTM D 6751, and the American Oil Chemists' Society (AOAC) Method Cd 12b-92 (AOAC, 1989; Steinbach, 2007). It is an automated replacement for the Active Oxygen Method (AOM) that requires less labor and is less time consuming (Coppin and Pike, 2001). The Rancimat® test measures the OSI of fuel by passing air through the biodiesel sample at constant temperature. The volatile products from lipid oxidation are dissolved in distilled water, and then measured by the increase in electrical conductivity of the water. The OSI measures the induction period with a plot of conductivity against

time, and calculates the inflection point in the conductivity curve. The induction period is the time up to the inflection point of the conductivity vs. time curve (Figure 3) (Anonymous, 1999). The results are expressed in hours on a curve where induction period (IP) is the interpret as the intercept of two tangent lines: the inclination and the curve level part (Coppin and Pike, 2001; Ferrari *et al*, 2005). Cleanliness of equipment used in the Rancimat® is very important to obtain reliable, precise, and accurate results (Ferrari *et al*, 2005). The minimum induction period in the European and the U.S. standards of biodiesel fuel is 6 and 3 h, respectively (Domingos *et al*., 2007; Kenneck, 2007; Liang *et al*., 2006). The lower the OSI value, the more likely the oil will be subject to oxidation.

Stability index (SI) can be used to evaluate the antioxidant's potential at preventing FAME oxidation. The SI helps determine which antioxidants/ antioxidant concentrations can be utilized to prevent oxidation and is used in conjunction with the Rancimat® (Kolb *et al*., 2002). It is the ratio between the induction period with and without the antioxidant. Danviriyakul *et al*. (2007b) used SI and IP to determine the effectiveness of antioxidants at inhibiting FAME oxidation and reported that increasing SI indicated that it took longer for the biodiesel to oxidize.

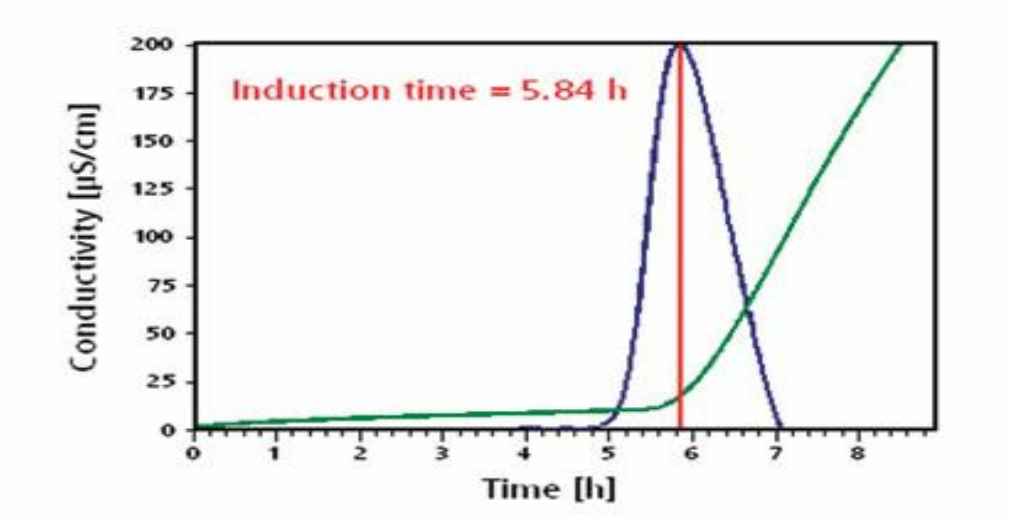

Figure 3. Calculation of inflection point of the conductivity vs. time curve for oil stability index, OSI (Steinbach, 2007)

2-thiobarbituric acid reaction substances (TBARs)

The TBARs method is used to measure lipid oxidation in muscle foods. It determines a reaction between 2-thiobarbituric acid and malondialdehyde, the product of secondary oxidation, which can be detected at 530 nm as mg malonaldehyde/kg sample (AOAC, 1993; Fernandez *et al*., 1997). The reaction proceeds by heating in an acidic solution to release malonaldehyde from triglycerides (Sikorski and Kolakowska, 2002). However, TBARs do not only interact with malonaldehyde, but also react with other aldehydes and ketones as well (Gomes *et al*., 2003). Furthermore, during production and storage stages, TBARs are low, as it should be, if aldehydes have been volatilized or have not yet been formed (Warner and Eskin, 1995). Therefore, after TBARs reach a maximum point, TBARs may drop (Silva and Ammerman, 1993).

Antioxidants

Dunn (2002), Knothe (2005) and other researches have studied the influence of antioxidants on the oxidation of biodiesel fuel. Sarin and the others (2007) recommended that antioxidants be utilized to help retard oxidative degradation since a higher antioxidant dosage results in a greater induction period. The induction period, according to both ASTM D6751 and EN14214 (Steinbach, 2007), can be described as the length of time that biodiesel will be stable towards oxidation. The higher the induction period, the better quality the methyl ester fuels will produce. Therefore, antioxidants are suggested to increase the commercial life of methyl ester fuels by increasing their shelf-life to at least six months.

Research that has been conducted to increase the stability of methyl ester fuels have compared synthetic antioxidants (tertiary-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate(PrG)), and natural antioxidants (α-Tocopherol, carotenes or vitamin E). These antioxidants tend to delay the oxidation reaction which prevents the formation and disturbs the propagation of free radicals. Consequently antioxidants act as oxygen scavengers or react with free radicals to form a stable molecule that prevents the oxidation reaction shown in Figure 4 (Danviriyakul *et al*., 2007b; Liang *et al*., 2006; Sarin *et al*., 2007). In addition, antioxidants also bind with metal into inactive forms to further enhance the prevention of oxidation (Pokorny *et al*., 2000).

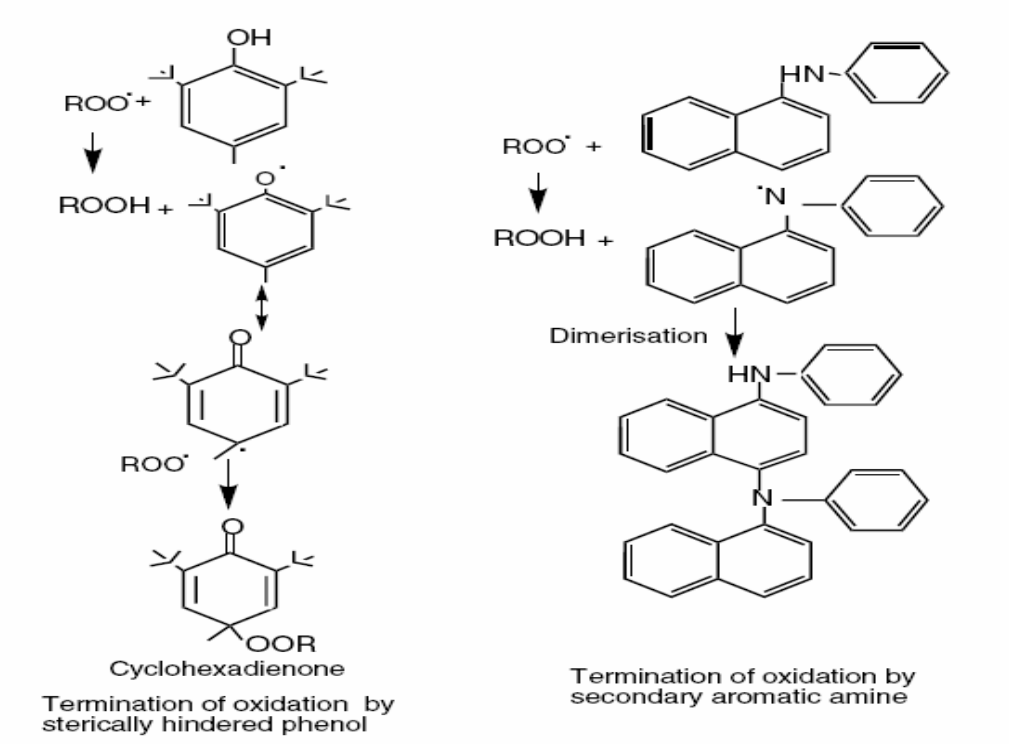

Figure 4. Mode of actions of antioxidants (Sarin *et al*., 2007)

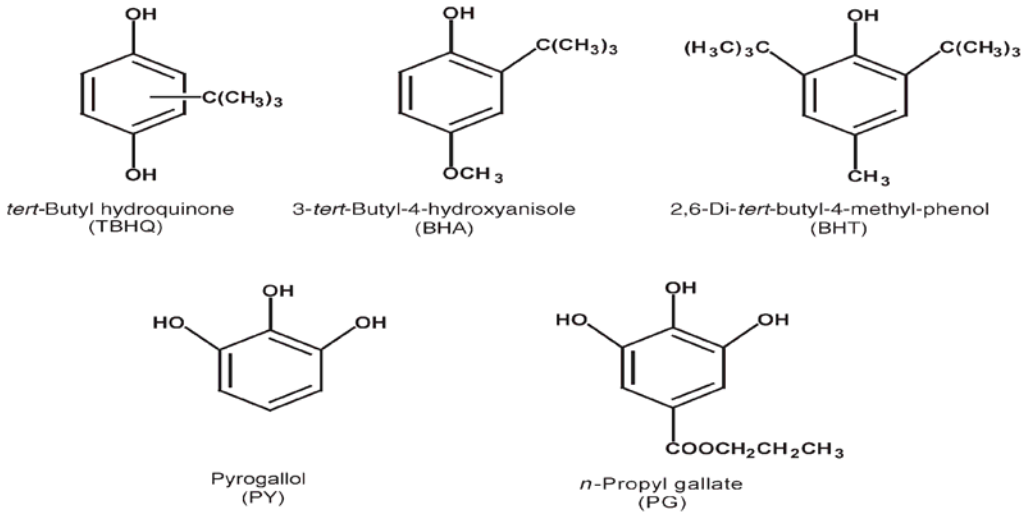

Figure 5. Structures of synthetic antioxidants (Hess *et al*., 2005)

Tertiary-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and propyl gallate (PrG) (Figure 5) are used as antioxidants in the commercial food industry. These substances are also used to prolong the shelf-life of methyl ester fuels by inhibiting autoxidation. There are many studies that show which antioxidants are the most effective at stabilizing methyl ester fuels. Liang et al. (2006) showed that TBHQ is more efficient at extending the induction period than BHT or α-Tocopherol, when used at the same concentration. TBHQ possesses two OH groups that are attached to the aromatic ring, but BHT has only one OH group that is attached to the aromatic ring. Thus, TBHQ can form a more complex structure between the antioxidant radices and the free radicals for lipid stabilization. Dunn (2005a) also compared the following five antioxidants for their ability to prevent oxidation: *Tert*-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PrG), and α-Tocopherol. These antioxidants were chosen based on their activity, in terms of increasing oxidation onset temperature, by non-isothermal pressurized-differential scanning calorimetry that was conducted in static (zero gasflow) and dynamic (positive gas flow) mode under 2000 kPa (290 psig) pressure and with a 5 ^{o}C/min heating scan rate. The results revealed that TBHQ and BHA, in dosages higher than 3000 ppm, were the most effective at increasing the oxidation stability of soybean oil fatty acid methyl esters (SME). However, BHA was more volatile than TBHQ. Results also showed that PrG was less soluble in vegetable oil and animal fat, and BHT operated best in dosages lower than 210 ppm after blending. These researchers also reported that

increasing antioxidant concentration showed significant increases in activity for loadings up to 1000 ppm followed by smaller increases in activity at higher concentrations.

Catfish as a Source of Oil

The byproducts from catfish processing, consisting of offal, frames, viscera, skin, and trimmings and make up 55 % of the total weight of live catfish (Silva and Dean, 2001). Viscera and skin of catfish contain 5.7 % and 34 % crude fat (wet basis), respectively (Lovell, 1980; Subramaniam *et al*., 2003; Drake, 1971). Silva and Ammerman (1993) reported that catfish lipids are made up of 25% saturated, 17% polyunsaturated and 58% monounsaturated fatty acids. These researchers also reported that C16:0 (18.3%) and C18:0 (4.2%) are the main saturated fatty acids whereas C18: l (51.4%), Cl6: 1 (4.8%), and C18:2 (14.8%) are the dominant monounsaturated compounds in catfish lipid. Subramaniam *et al*. (2002) also reported that the fatty acids that are present in crude catfish visceral oil are C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:2, C20:3, C20:4, and C 22:6. These researchers also stated that C18:1, C16:0, C18:2 and C18:0 are the major fatty acids in visceral oil. Moreover, these researchers reported that catfish visceral oil has high levels of unsaturated fatty acids. To extract oil from catfish wastes, the rendering process that is used is a conventional process. This process uses a large quantity of water to break down the fat cell walls, with steam under pressure, until they become liquefied and separate fat by skimming or centrifugation (Sathivel *et al*., 2008). McGillivray (2006) studied which extraction method (hexane, propane, and supercritical carbon dioxide ($SCCO_2$)) gives the

highest yield from soybean and catfish oil. He concluded that extraction yield from hexane was the highest, and oil extracted by $SCCO_2$ was oxidized more than others since $SCCO_2$ used a higher temperature and a longer extraction time.

Model and Prediction of Storage Stability or Shelf life

Storage and oxidative stability are the most important guidelines for the production methyl ester fuel with acceptable. Many researchers have tried to find a way to measure both parameters. Currently, there is no procedure for measuring storage stability; unlike thermal oxidative stability, which uses induction period as a method for measuring the fuel stability. However, the effect of thermal oxidative stability on engine and injection pump behavior is not clear since there are not enough data to support a theory. Thus, conducting research on the thermal oxidative stability of fuels during storage is necessary (Mittelbach and Gangl, 2001). Du Plessis *et al* (1985) and Canakci *et al* (1999) revealed a similar conclusion, adding that peroxide value, acid value, and viscosity all increase as temperature increases. To measure the thermal stability of biodiesel, the method described in ASTM-525 and ASTM 6418 is used (Mittelbach and Gangl, 2001; Dunn, 2008). This technique quantifies color degradation and gum formation, but does not determine oxidation stability. It is very important to use various techniques to measure the quality of biodiesel; IV, PV, UV etc. However, these methods can only quantify the level of degradation, but they do not predict the oxidation stability of biodiesel (Kenneck, 2007). The rate of resistance of fuel to oxidation depends on the degree of unsaturation, the presence of antioxidants, and prior storage conditions (Coppin and Pike, 2001).

The oxidative stability index (OSI) analysis gives a predictive value whereas PV or AV only determine how good or bad a fuel is at a certain time (Nelson, 2008). The OSI Rancimat® method quantifies the volatile compounds that occur due to the degradation of methyl ester fuels, under accelerated temperature and oxygen conditions. Therefore, the OSI method is considered a tool for predicting the oxidative stability of fatty acid methyl esters (FAMEs) (Coppin and Pike, 2001; Dunn, 2008; Kenneck, 2007; Nelson, 2008). The stability index is calculated from the ratio between the induction period with and without the antioxidant (Danviriyakul et al., 2007b). Knothe and Dunn (2003) investigated the influence of structure and amounts of fatty compounds on OSI by testing neat methyl 11-eicosenoate, methyl 13-docosenoate and methyl oleate. They found that the OSI value of methyl oleate (C18:1) was smaller than the OSI value of neat methyl 11-eicosenoate (C20:1) and methyl 13-docosenoate (C22:1). Their theory was that the effluent (the air flow constantly) produced by the procedure relies on the concentration of the samples that are being oxidized (on the number of double bonds per unit of sample). Thus, on a molecular basis, the OSI values of substances with an equal number of double bonds per molecule but different molecular weights (MW) were not exactly comparable except when a correction was calculated for the differing MW. These corrections may influence the interpretation of oxidative stability. Their equation was OSI = (-1.22 x M) + 15.68 (M= grams of sample).

Accelerated Shelf life (aging)

Methyl ester fuels that are derived from oil feedstocks are very prone to autoxidation (oxidation occurs by absorbing oxygen during long-term storage) since biodiesel contains highly unsaturated long chain fatty esters (Dunn, 2000; Canakci *et al*., 1999). Thus, it is recommended to store methyl ester fuels for less than six months if no antioxidants are added. Many researchers have reported that after methyl ester fuels are accelerated and oxidized by temperature, oxygen or copper; acid value (AV), kinematic viscosity (v), peroxide value (PV), UV-absorption, and anisidine value increase. This cause the FAMEs form gums and sediments, clogging filters which cause incomplete to poor combustion (Stombaugh *et al*., 2006; Dunn, 2002; Du Plessis *et al*., 1985; Thompson *et al*., 1998). There are no particular methods to measure oxidative stability of biodiesel in the ASTM standard. Then, in order to measure the rate of aging processes in a short amount of time, accelerating fuel at different temperatures or conditions has been studied by many researches. Du Plessis *et al*. (1985) reported that the presence of air, light, and copper in methyl and ethyl esters of sunflower-seed oil at 30^{o}C or higher for 90 d, increased AV, viscosity, PV, UV-absorption, and anisidine values and a decreased induction period. However, when TBHQ was added, oxygen was not present, and temperature was up to 50^{o} C, the oxidation rate increased more slower. Canakci *et al*. (1999) also accelerated the oxidation of soybean oil methyl esters (SMEs) by using different temperatures (60^{o}C, 80^{o}C, and 97.8^{o}C) and different oxygen flow rates ($0.25^{x}10^{-6}$ kg/s, $0.75^{x}10^{-6}$ kg/s, $1.38^{x}10^{-6}$ kg/s, and $1.85^{x}10^{-6}$ kg/s). Result revealed that higher temperatures and oxygen flow rates increased the AV, PV and viscosity of the SMEs.

Their studies also suggested that using TBHQ can prolong the oxidative stability of methyl ester fuels

Table 1. European and U.S. biodiesel standards (Steinbach, 2007; Knothe, 2006)

Biodiesel	EN 14214 /EN 14213		ASTM D 6751	
Property	Test method	Limits	Test method	Limits
Acid number [mg KOH/g]	EN 14104	<0.5	D 664	<0.5
Water content [mg/kg]	EN ISO 12937	<500	D 2709	<500
Iodine value [g I2/100 g]	EN 14111	120 -130		
Oxidative stability [h]	EN 14112	>6	EN 14112	>3
Group I metals (Na + K) [mg/kg]	EN 14108/EN14109	<5.0	EN 14538	<5.0
Group II metals (Ca + Mg) [mg/kg]	EN 14538	<5.0	EN 14538	<5.0
Flash point [^{0}C]	EN 3679	>120 min	D 93	>130min
Sulfated ash [%mass]	EN ISO 3987	>0.02	D 874	>0.02
Total Glycerol [%mass]	EN 14105	< 0.25	D 6584	<0.24
Free Glycerol [%mass]	EN 14105/06	< 0.02	D 6584	<0.02
Copper strip corrosion	EN 2160	Class 1	D 130	Class 3
Water and Sediment [% volume]			D 2709	<0.05
Sulfur content [mg/kg]	EN 20846/20884	10	D 5453	15/500
Cloud point [^{0}C]	EN 23015		D 2500	Report
Carbon residue [%mass]	EN 10370	-	D 4530	<0.05
Cetane number	EN 5165	51 min	D 613	47 min
Kinematic viscosity, 40^{0}C [mm^2/s]	EN ISO 3104	3.5-5.0	D 445	1.9-6.0
Distillation temp,90% recovered[^{0}C]			D 1160	>360

CHAPTER III

MATERIALS AND METHODS

Preliminary Study-Choosing Antioxidant

Two FAMEs were produced from catfish oil and soybean oil. Four types of antioxidant: tertiary-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA), propyl gallate (PrG) from Sigma-Aldrich (St. Louis, MO), and α-tocopherol from BASF (Florham Park, NJ) at different concentrations (100, 200, 400, 800 ppm) were used to investigate the stability of biodiesel as measured by the induction period (the Rancimat® method at 110 ^{0}C and airflow at 10 L/h according to pr EN 14112). Results showed that soy methyl esters were more stable than catfish methyl esters, 4.91 and 0.54 h, respectively. However, their stability of catfish oil was enhanced by the addition of antioxidants. Antioxidant's ability to prevent oxidation reactions was THBQ> PrG>BHA> α-Tocopherol. The induction period for catfish methyl esters increased to 40 h with the addition of 800 ppm of TBHQ.

Materials

Potassium iodide (KI), iodine monochloride 98% (ICl), sodium thiosulphate ($Na_2S_2O_3 \cdot 5H_2O$), methanol, glacial acetic acid, cyclohexane, chloroform, and isooctane (2,2, 4- trimethylpentane) were purchased from Fisher Scientific (Memphis, TN)

1-butanol, 2-thiobarbituric acid and *p*-anisidine were purchased from Sigma-Aldrich (St. Louis, MO).

Catfish methyl esters (CFME) were prepared by the transesterification reaction of triglycerides in catfish oils. This reaction used a molar proportion of 1:6 catfish oil to methanol, with 1% sodium hydroxide (NaOH) (Fisher Scientific, Memphis, TN) as a catalyst. The reaction lasted one hour at 30^{o}C under normal atmospheric pressure. The mixture was separated into two layers, "crude FAMEs" and glycerol, and washed with deionized water (around 10 washes) until the washed water was clear (Danviriyakul, 2007). The methanol residue was removed by passive evaporation (evaporated at 65^{o}C and 300 mbar in a rotary vacuum evaporator; Rotovapor® model R-250; Büchi, Flawil, Switzerland) overnight. The CFME samples were split into two samples. One set of samples was the control (no antioxidant added) and the other set had 800 ppm of TBHQ were added to it. These samples were aged (stored) at two temperatures (25^{o}C for 1 year and 80^{o}C for 28 days).

The samples were sampled after 0, 3, 6, and 12 months, were placed in brown-glass bottles, and incubated at 25^{o}C. Parts of the samples (at their sampling time) were kept at an ultra-low temperature (Scien Temp, ADRIAN, MI) at -65$\pm$0.5^{o}C until they were analyzed. The CFME samples were aged at accelerated temperatures and placed in a brown glass bottles. These samples were incubated at 80^{o}C under static conditions. The samples were withdrawn at days 1, 3, 5, 7, 14, 21, and 28 and analyzed.

Methods

The samples were examined for free fatty acids (FFA) (AOAC official Method 940.28, 1999), iodine value (IV) (AOAC official Method 993.20, 1999), peroxide values (PV) (AOAC official Method 965.33, 1999), UV absorbance at 234 nm (Sergent *et al*., 1993), anisidine value (AOAC official Method Cd 18-90, 1995), induction period (pr EN 14112), stability index (Danviriyakul *et al*., 2007; Kolb *et al*., 2002), and 2-thiobarbituric acid value (TBARS) (AOAC official Method Cd 19-90, 1993).

Acid value (AV)

The acid value (AV) determines the quantity of free fatty acids (FFA) in a sample. This is the result of hydrolytic rancidity of the oil (Lakkakula *et al*., 2004) and occurs prior to the manufacture of FAMEs with FFA being the amount of potassium hydroxide (or equivalent alkali) required to neutralize FFA in one gram of sample (Panda, 2003). The FFA is determined in percent by weight of oleic acid, while AV can be defined as the milligrams of potassium hydroxide needed to neutralize the free fatty acids in one gram of fat of oil. The AV is two times of the % FFA (as oleic) in a sample. The free fatty acids (FFA) were determined by weighing 7.05 g of sample into a beaker, adding 2 mL of phenolphthalein indicator, and 50 mL of pre-neutralized 95% ethanol. The solution was mixed thoroughly and titrated with 0.025N NaOH until a pink color appeared at least for one minute. As the molecular weight of oleic acid and KOH are 283 and 56 g/mol, respectively, the FFA (calculated as oleic acid) was reported as

% FFA = Molecular weight of oleic * Normality *mL titrate*100/ grams of sample

The AV was calculated as:

AV = Molecular weight of KOH * Normality * Volume/ grams of sample

AV = % FFAx2

Iodine value (IV)

Iodine value (IV) measures the amount of double bonds of unsaturated fatty acids (Steinbach, 2007; Knothe, 2002). It was tested by weighing three milligrams of sample into a 500 mL Erlenmeyer flask, adding 15 mL of cyclohexane-glacial acetic acid reagent (1:1 v:v), and swirling. Twenty five milliliters of Wijs solution (0.1N ICl in glacial acetic acid) was pipetted into the sample solution and swirled. The sample solution was placed in the dark for one or two hours (determined by type of oil samples). Twenty milliliters of fifteen percent of KI and 150 mL of freshly boiled and cooled water were added within three minutes and mixed. The sample solution was titrated with 0.1N sodium thiosulfate until the yellow color almost disappeared, then 1 mL of starch solution was added, and the titration was continued until the blue color disappeared entirely. The IV was calculated as:

IV (cgI_2/g) = [(Blank-Sample)*Normality*12.69]/ Weight of sample

Peroxide value (PV)

Peroxide value (PV) measures the amount of hydroperoxides and peroxides that are produced in the initial phases of lipid oxidation (Allen and Hamiltom, 1994; Gotoh and Wada 2006). Five grams of sample were weighed in a 250 mL Erlenmeyer flask, and 30 mL of glacial acetic acid and chloroform (3:2 v/v) were added and shaken. One half

milliliter of saturated KI solution was added and the solution was stirred for one minute prior the addition of 30 mL of distilled water. The solution was titrated with 0.1N (or 0.01 N when applicable) sodium thiosulfate until the yellow color almost disappeared. One half mL of one percent starch (potato starch) indicator was added and the solution was titrated until the blue color disappeared. The PV was computed as

PV (meq/kg) = mL sodium thiosulfate* Normality *1000/g sample

UV absorbance

UV absorbance at 234 nm indicates the initial phase of lipid oxidation. UV absorbance was measured by weighing 0.01 g of sample into a test tube and adding 5 mL of methanol. The sample solution was shaken and the absorbance was measured at 234 nm by using a spectrophotometer (GENESYS, Spectronic Instruments, and Rochester, NY). The result was calculated as

UV units = absorbance of sample * gram of sample

Anisidine value (AnV)

Anisidine value (AnV) measures the total level of carbonyl compounds (volatile aldehydes and non-volatile portion of fatty acids) that are present during secondary oxidation (Contis, 1998; Saad *et al*., 2007). AnV was measured using two solutions that included a blank (Ab) and as a p-anisidine solvent (As), in which the absorbance was measured at 350 nm using a spectrophotometer (GENESYS, Spectronic Instruments, Rochester, NY). The Ab solution was prepared by weighing 0.4 g of sample into a 25 mL volumetric flask, and dissolving and diluting it with isooctane. The As solution was

prepared by removing precisely five mL from the Ab solution and placing it in a test tube, and then adding one mL of anisidine solution that was 0.25 g of p-anisidine in 100 mL glacial acetic acid, and gently swirling. After the Ab solution was incubated for 10 min, the solution was measured at 350 nm with a spectrophotometer. The AnV was computed as

$$\text{AnV (mmol/kg)} = [25*(1.2*\text{As}-\text{Ab})]/ \text{ mass sample}$$

Oxidative stability index (OSI) and Induction period (IP)

Oxidative stability index (OSI) is a measurement of the induction period (IP) by calculating a plot of conductivity against time, and determining the inflection point in the conductivity curve (Coppin and Pike, 2001). The IP measures volatile products such as aldehyde, acid, and alcohol that occur during the secondary phase of lipid oxidation. The OSI determines the resistance of fuel to oxidation. A Metrohm Rancimat apparatus (743 Rancimat®, Brinkmann Instruments, Inc., Westbury, NY) was used to determine IP. Air flow was circulated at 10$\pm$ 0.2 L/ hr through three gram samples at 110$\pm$ 0.2 ^{0}C. Air was then passed through deionized water and the conductivity of the water was measured. The IP was measured with a computer as the intersection of the tangent lines (first derivative) by the software provided (Brinkmann, 2006). The stability index (SI) is the ratio between the induction period with and without the antioxidant (Danviriyakul *et al*., 2007; Kolb *et al*., 2002). The SI was calculated as

Stability index = Induction period [CFME + antioxidant] /induction period [pure CFME]

The 2-thiobarbituric acid reaction substances (TBARs)

The 2-thiobarbituric acid reaction substances (TBARs) analysis measure secondary oxidation products. TBARs was analyzed by weighing 0.2 g of sample into a 25mL volumetric flask, diluted with 1-butanol, and mixed thoroughly. The TBA reagent was prepared by using 2 mg of 2-thiobarbituric acid dissolved in 100 mL of 1-butanol. After removing 5 mL from both solutions, the mixture was and heated in boiling water at 95^{o}C for 120 min. The mixture of the two solutions was then cooled in an ice bed, and the absorbance was measured at 535nm using a spectrophotometer (GENESYS, Spectronic Instruments, Rochester, NY). The result were calculated as

TBARS value (mg malonaldehyde/g) = 50 *(absorbance of test solution- blank)/gram

Statistical Analysis

Two separate experiments were conducted, one at room temperature (25^{0}C) over one year and the other at accelerated temperature (80^{0}C) for 28 days. The samples were arranged in a split-plot in time with three replications in a randomized complete block design (RCBD). The RCBD was used to determine differences ($p \leq 0.05$) among incubation times for each treatment, with treatment as the main plot and time as the subplot.

The generalized liner model (GLM) from Statistical Analysis Software (SAS, 2007) was used to compute data. Means of all treatments and times were separated by employing the least significant difference test ($p \leq 0.05$).

CHAPTER IV

RESULTS AND DISCUSSION

To determine the best antioxidant for prevention of the oxidation of catfish methyl esters (CFME), four antioxidants were tested at four concentrations. TBHQ was the most effective antioxidant tested (Figure 6). A minimum of 200 ppm of TBHQ were needed to meet the six hour induction time (IP) minimum required by the standards. Propyl gallate (PrG) and BHA also showed significant improvement in IP beyond 200 ppm, with α-tocopherol showing no improvement. These results are similar to Liang *et al*., (2006). When calculating stability index (SI), TBHQ was at least twice as effective as any of the other antioxidants tested (Table 2). From this data and the literature, it was concluded that 800 ppm TBHQ should be sufficient to protect CFME from oxidation, even under accelerated aging conditions. Mittelbach and Schober (2003) recommend an antioxidant concentration that can provide an induction period of at least 10h. This should be sufficient to prevent the deterioration of biodiesel from the oxidation stability for a longer storage time. Dunn (2005a) also suggested that addition of TBHQ at a concentration up to 3000 ppm for will prevent the autoxidation of biodiesel during storage.

When CFME without antioxidants (Control) were stored at 25^{o}C, all oxidation measurement parameters increased to unacceptable levels after six months of storage (Table 3). The IP was above the minimum 3h required to meet ASTM D 6751

(Kenneck, 2007). However, the acid value, AV was above the maximum 0.5 mg KOH/g (ASTM D 664) since the beginning.

When 800 ppm TBHQ was added to the CFME (TBHQ), all parameters remained constant except for IV and TBARs (Table3). The IV of these samples increased after three months of storage while TBARs increased after 12 months of storage. The latter indicates some oxidation of the CFME after 12 months storage at 25^{o}C. The IP with TBHQ was always above the 6 h European standard (Domingos *et al*., 2007; Kenneck, 2007), showing that the product with antioxidant stored at 25^{o}C can last for at least 12 months at 25^{o}C. TBHQ boosted the IP up, which is a similar to results reported by Liang and Schwarzer (1998). The AV was also below the maximum 0.5 mg KOH/g (ASTM D 664) when 800 ppm TBHQ was added to the FAMEs.

When exposed to 80 ^{o}C for 28 days, all oxidation parameters increased, especially by the 28th day (Table 4). The IP was well below the 3 h or 6 h minimum European standard (Domingos *et al*., 2007; Kenneck, 2007). When TBHQ was added, the IP did not decrease below 6 h. However, the FFA and thus the acid value, AV, increased above the maximum 0.5 mg KOH/G (ASTM D 664) since the CFME were produced from a result of a rendering process that used a large amount of water and steam at high pressure (Sathivel *et al*., 2008). Besides, high temperature and air flow will increase the rate of hydrolytic rancidity of oils, in which case the antioxidant will have little effect after long exposure times.

The AV decreased after the antioxidant was added. It can be assumed that the antioxidant inhibited the lipase in CFME that catalyze the hydrolytic rancidity (Pokorny

et al., 2000). Water content and moisture level in CFME from long term storage are very important since these factors will enhance CFME degradation due to the hydrolytic rancidity reaction (Hamilton, 2005; Leung *et al*., 2006). This may have caused the AV of the control CFME to increase rapidly after 6 months. Furthermore, hydrolytic rancidity is catalyzed by heat (Murano, 2003). Thus, the AV, stored at 80^{o}C for 28 days is higher than the AV, stored at 25^{o}C for one year. Moreover, lipases can be thermally inactivated above 60°C (Pokorny *et al*., 2000).

During decomposition of hydroperoxide (initial step), there are some products that are created from hydroperoxide decomposition such as 9-hydroxyoctadeca-10, 12-dienoic acid and 13-hydrooxyoctadeca-9, 11-dienoic acid. These products have a double bond structure, so when they are present in the system, double-bond structures increase in the system. Therefore, IV from the control also increased during storage one year. However, Pokorny et al (2000) stated that formation of hydroperoxides decompose significantly at temperatures of 80 to 90^{o}C. As a result, at accelerated condition storage (80^{o}C), double-bond structures that are caused by the decomposition of hydroxides did not affect IV as much as long term storage at room temperature (25^{o}C).

The PV and the UV measurements had similar results since both analyses quantify the amount of primary oxidation products (Jaswir *et a*l., 2002; Lakkakula *et al*., 2004; Allen and Hamiltom, 1994; Gotoh and Wada 2006). For CFME (no antioxidant), PV and UV decreased after 12 months of storage whereas the AnV measures the secondary oxidation products and increased after 12 months of storage.

The AnV and the IP showed similar results since both analyses measure the amount of secondary oxidation products (Contis, 1998; Saad *et al*., 2007; Steinbach, 2007). The SI measures the ability of antioxidants to inhibit oxidation. SI was decreased over time, which demonstrates that the antioxidant either deteriorated or lost its effectiveness during storage.

Even though the AnV and the TBARs measured the amount of secondary oxidation products, the TBARs of the control CFME decreased rapidly after 12 months unlike the AnV. The TBARs measured the malonaldehyde concentration that is released from triglycerides by the heating in an acidic solution (Sikorski and Kolakowska, 2002). It can be assumed that most of triglycerides break down into smaller compounds after 12 months and that some of these volatile compounds evaporate from the system during the heating process.

Table 2. Stability index[1] (SI) of catfish methyl esters (CFME) in the presence of antioxidants

Antioxidants	Concentration (ppm)			
	100	200	400	800
TBHQ	14.33	26.35	44.08	74.73
PrG	10.01	16.64	25.07	35.52
BHA	8.38	13.07	17.45	22.75
α-Tocopherol	4.87	6.8	7.22	7.79

SI[1] = Induction period [CFME + antioxidant] /induction period [pure CFME]

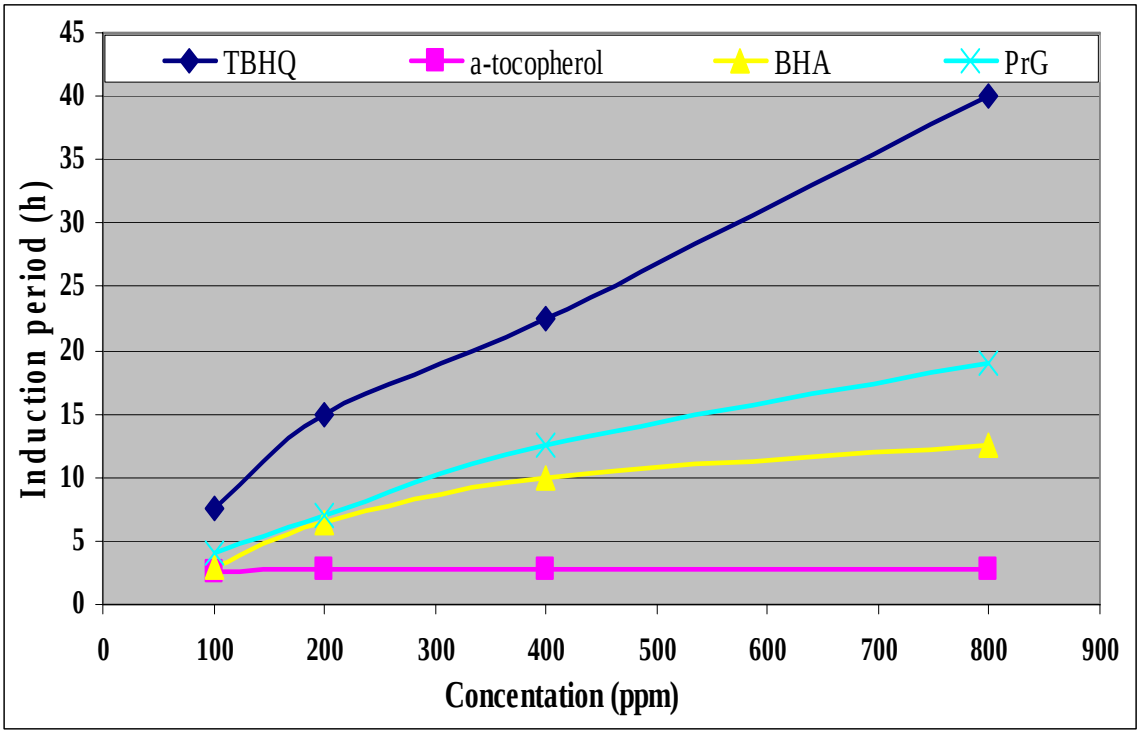

Figure 6. Effect of antioxidant type and concentration on the induction period (IP) of catfish methyl esters (CFME)

Table 3. Free fatty acids (FFA), acid value (AV), iodine value (IV), peroxide value (PV), UV absorbance (UV), anisidine value (AnV), induction period (IP), stability index (SI[1]), 2-thiobarbituric acid reactive substance (TBARs) of catfish methyl esters (CFME) with tertiary-butylhydroquinone (TBHQ) and without TBHQ, stored at 25°C for one year.

Treatment	Time at 25°C (months)	FFA (mg NaOH/g)	AV (mg KOH/g)	IV (cgI_2/g)	PV (meq/kg)	UV (234nm)	AnV (mmol/kg)	IP (h)	SI	TBARs (mg malonaldehyde/g)
Control	0	0.3a	0.6a	87.1a	9.8a	0.8a	18.7a	0.3a	NA	28.1a
	6	1.6b	3.2b	128.4c	35.6b	6.1b	61.9b	2.0b	NA	201.6b
	12	2.1b	4.2b	115.3b	34.1b	5.3b	76.5b	2.8b	NA	168.03b
TBHQ	0	0.3a	0.6a	87.1a	9.8a	0.8a	18.7a	14.8c	49.6	28.2a
	3	0.1a	0.2a	132.9cd	10.4a	0.8a	10.7a	11.5c	NA	44.7a
	6	0.2a	0.4a	134.3cd	9.4a	0.8a	14.6a	10.2c	7.1	46.3a
	12	0.2a	0.4a	145.4d	7.9a	0.8a	14.2a	11.8c	4.3	66.0b

a-d Means with the same letter within each column are not different ($P > 0.05$).
NA- Not available.
SI[1] = Induction period [CFME + antioxidant] /induction period [pure CFME].
LSD (0.05)
CV (%)

Table 4. Free fatty acids (FFA), acid value (AV), iodine value (IV), peroxide value (PV), UV absorbance (UV), anisidine value (AnV), induction period (IP), stability index (SI[1]), 2-thiobarbituric acid reactive substance (TBARs) of catfish methyl esters (CFME) with tertiary-butylhydroquinone (TBHQ) and without TBHQ, stored at 80°C for 28 days.

Treatment	Time at 80°C (day)	FFA (mg NaOH /g)	AV (mg KOH/g)	IV (cgI_2/g)	PV (meq/kg)	UV (234nm)	AnV (mmol/kg)	IP (h)	SI	TBARs (mg malonaldehyde/g)
Control	0	0.3a	0.6a	87.1b	9.8a	0.8a	18.7a	0.3a	NA	28.1b
	1	0.3a	0.6a	87.2b	15.0a	0.6a	25.8a	0.2a	NA	58.3e
	3	0.4b	0.8b	87.1b	14.0a	0.8a	26.4a	0.5a	NA	25.8b
	5	0.4b	0.8b	87.8b	9.8a	0.8a	28.9a	0.6a	NA	22.7ab
	7	0.4b	0.8b	87.9b	10.1a	0.8a	29.5a	0.7a	NA	25.2b
	14	0.6b	1.2b	87.7b	6.5a	0.9a	33.8a	0.8a	NA	19.4a
	21	0.6b	1.2b	87.7b	4.1a	0.8a	34.8a	0.8a	NA	32.7c
	28	1.4c	2.8c	85.0a	28.7b	1.5b	72.5b	0.7a	NA	19.0a
TBHQ	0	0.3a	0.6a	87.1b	9.8a	0.8a	18.7a	14.8c	49.6	28.2b
	1	0.3a	0.6a	87.1b	9.5a	0.6a	20.0a	12.3bc	61.7	41.2d
	3	0.4b	0.8b	87.6b	10.4a	0.7a	22.4a	11.4b	23.3	22.6ab
	5	0.4b	0.8b	87.2b	10.0a	0.7	22.1a	11.9bc	20.4	24.9b
	7	0.4b	0.8b	86.9b	10.5a	0.7a	21.8a	13.7bc	20.3	22.3ab
	14	0.5b	1.0b	87.9b	6.0a	0.8a	27.6a	14.0bc	18.3	23.9ab
	21	0.5b	1.0b	87.6b	5.4a	0.7a	27.9a	13.4c	17.6	27.6b
	28	0.7b	1.4b	87.5b	7.8a	0.8a	29.1a	11.3b	16.8	20.0a

a-d Means with the same letter within each column are not different ($P > 0.05$).
NA- Not available.
SI[1] = Induction period [CFME + antioxidant] /induction period [pure CFME].
LSD (0.05)
CV (%)

CHAPTER V

SUMMARY AND CONCLUSIONS

When catfish methyl esters (CFME) are made from byproducts of a catfish operation, the oil that it is produced is usually is of poor quality with respect to its oxidative stability (McGillivray, 2006; Danviriyakul *et al*., 2007a). To protect CFME, synthetic antioxidants at different levels were tested. It was concluded that TBHQ at 800 ppm provided the best protection against oxidation.

The CFME made without antioxidant proved to be below the minimum 3 to 6 of induction period needed to pass national or European standard. However, when the antioxidant was added, the CFME was stable to oxidation for up to 12 months at 25^{o}C. When stored at 80^{o}C, the CFME was proved to be stable to oxidation as proved by the high IP, even after 28 days. However, liberation of free fatty acids leads to an acid value that is higher than the maximum standard limit after 28 days of storage at 80^{o}C.

Result showed that when an antioxidant is added to CFME, they are stable for up to one year at 25^{o}C. However, if they are exposed to high temperatures or stored at high temperatures for over 28 days, there can be some hydrolytic rancidity, even though the IP will still be within the standards.

It can be assumed that TBHQ may assist the CFME by binding with free radicals and/or metal and thus improving the stability of the CFME during storage or accelerated condition.

Further research could extend time of aging with accelerated temperature (80^{o}C), or store in various temperatures such as 60 through 100^{o}C. Further research should also be conducted in which samples are collected every month over one year at room temperature (25^{o}C).

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APPENDIX

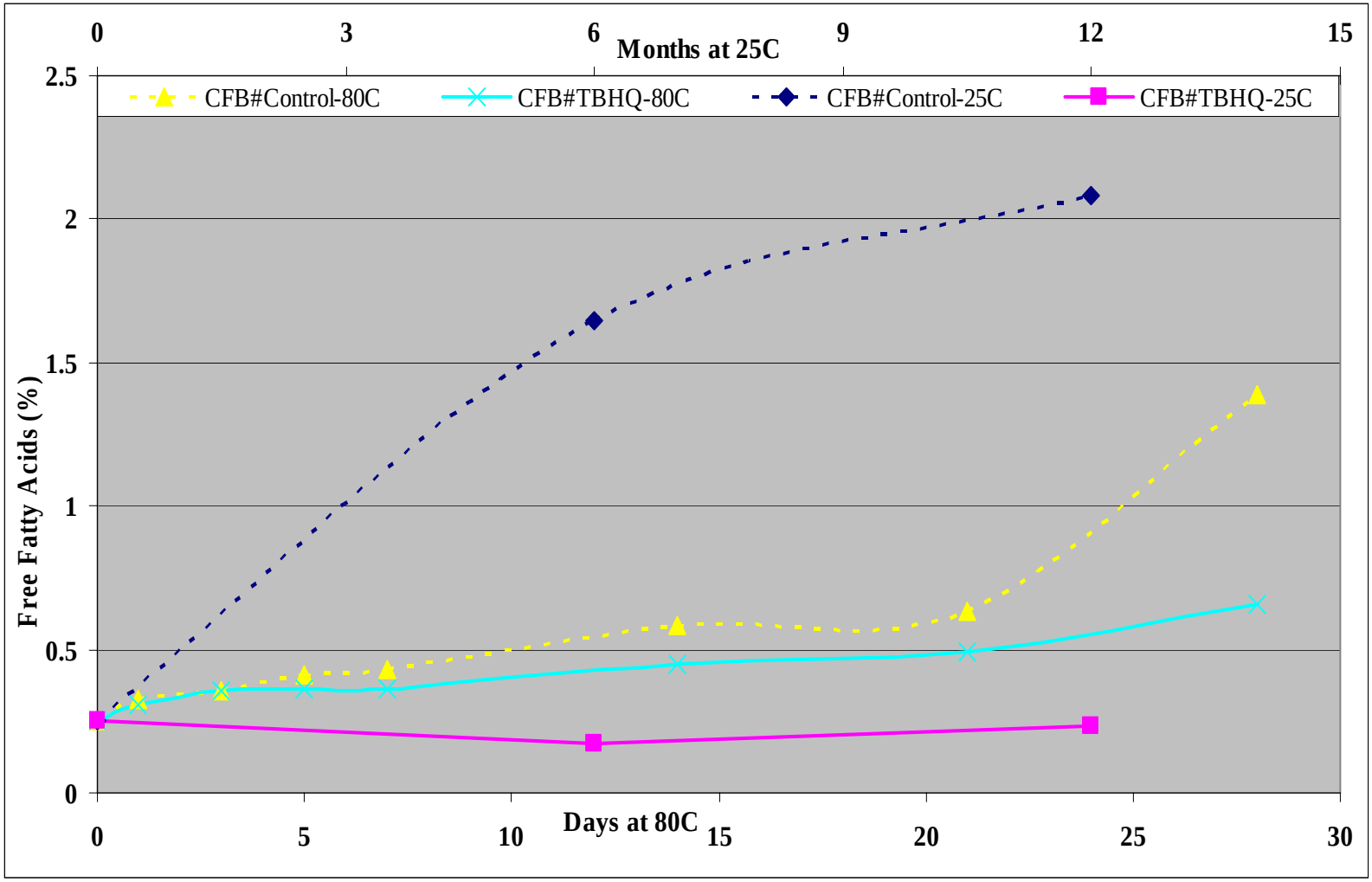

Figure 7. %Free fatty acids (FFA) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

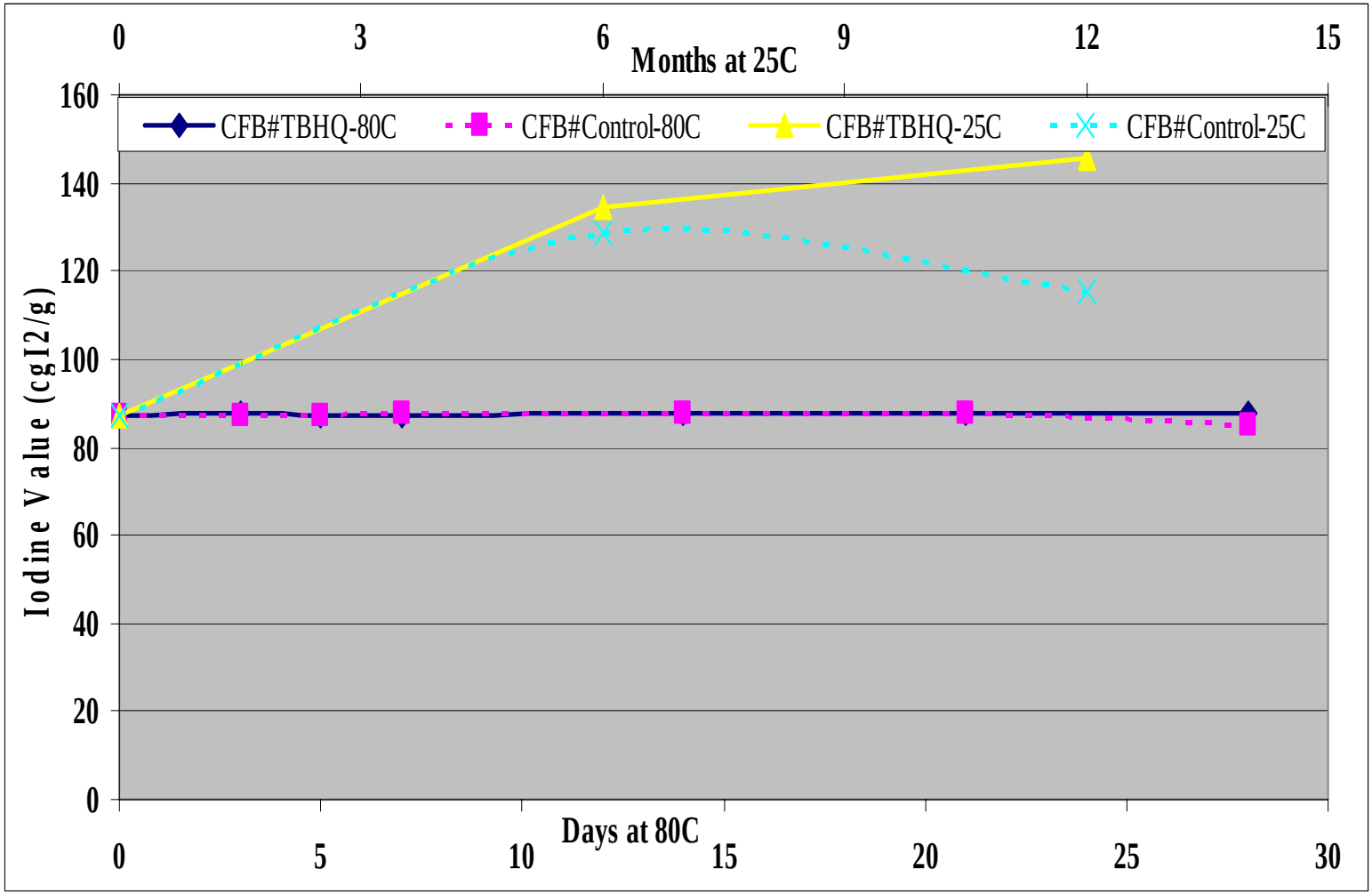

Figure 8. Iodine value (IV) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

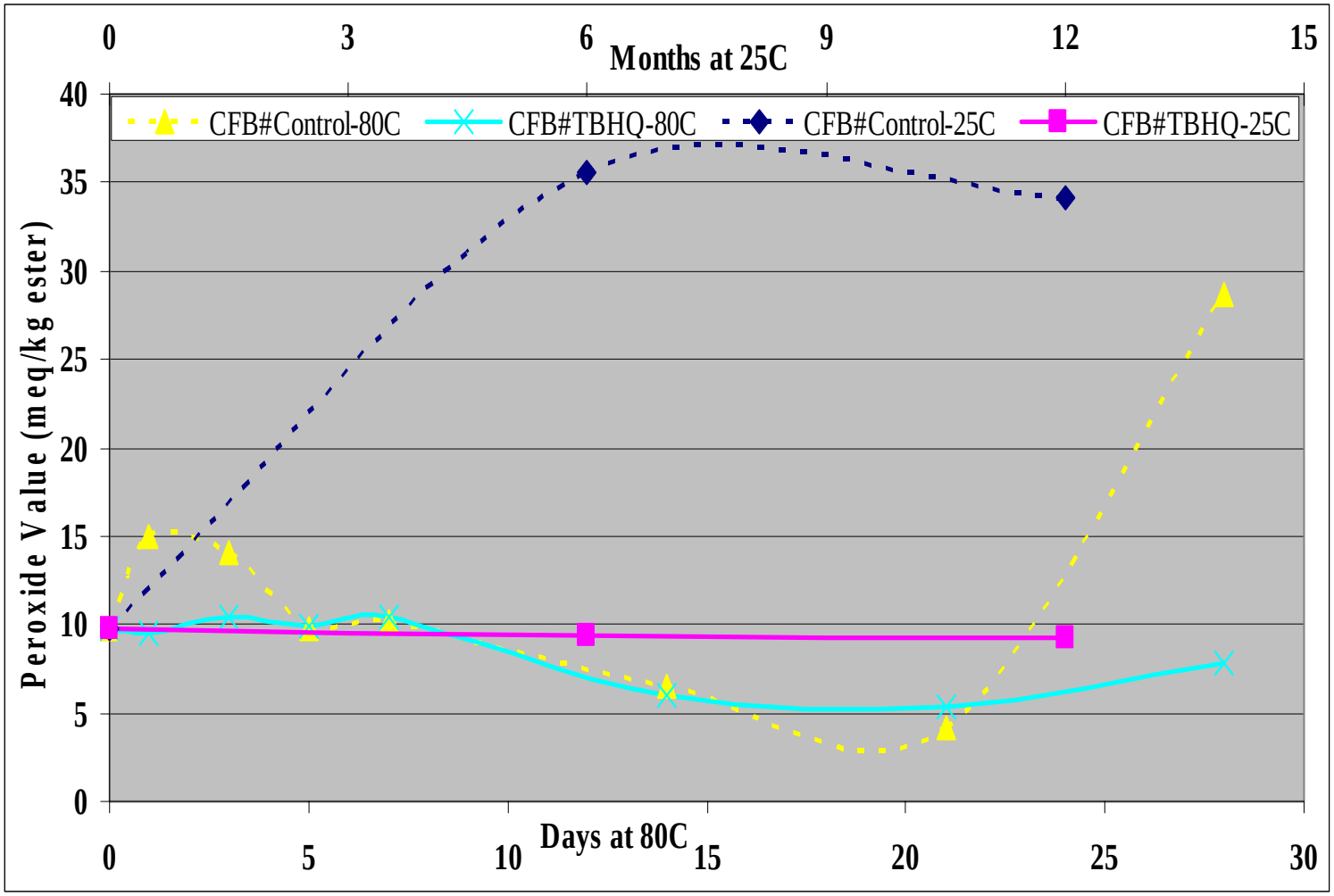

Figure 9. Peroxide value (PV) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

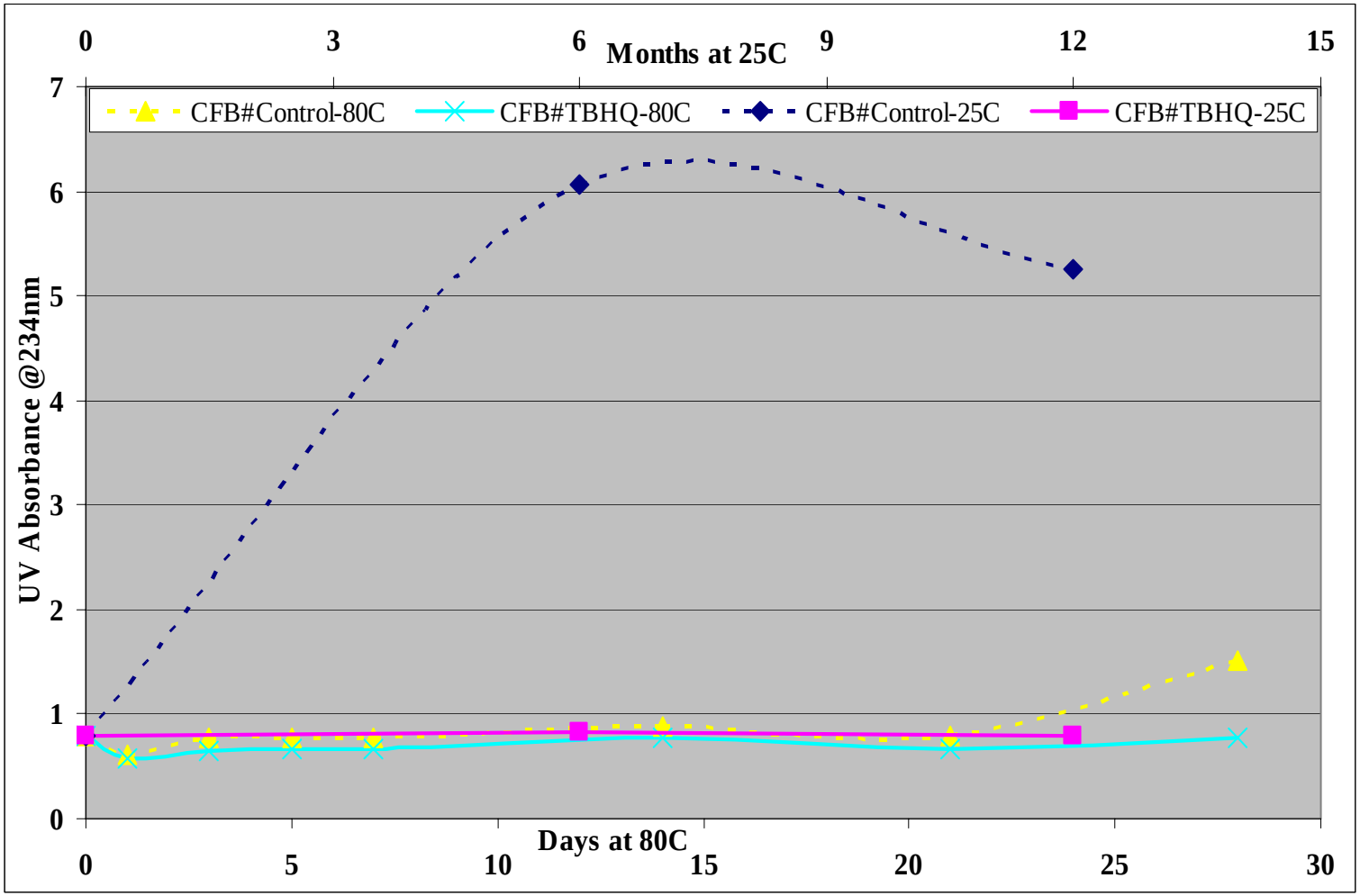

Figure 10. UV absorbance at 234 nm of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

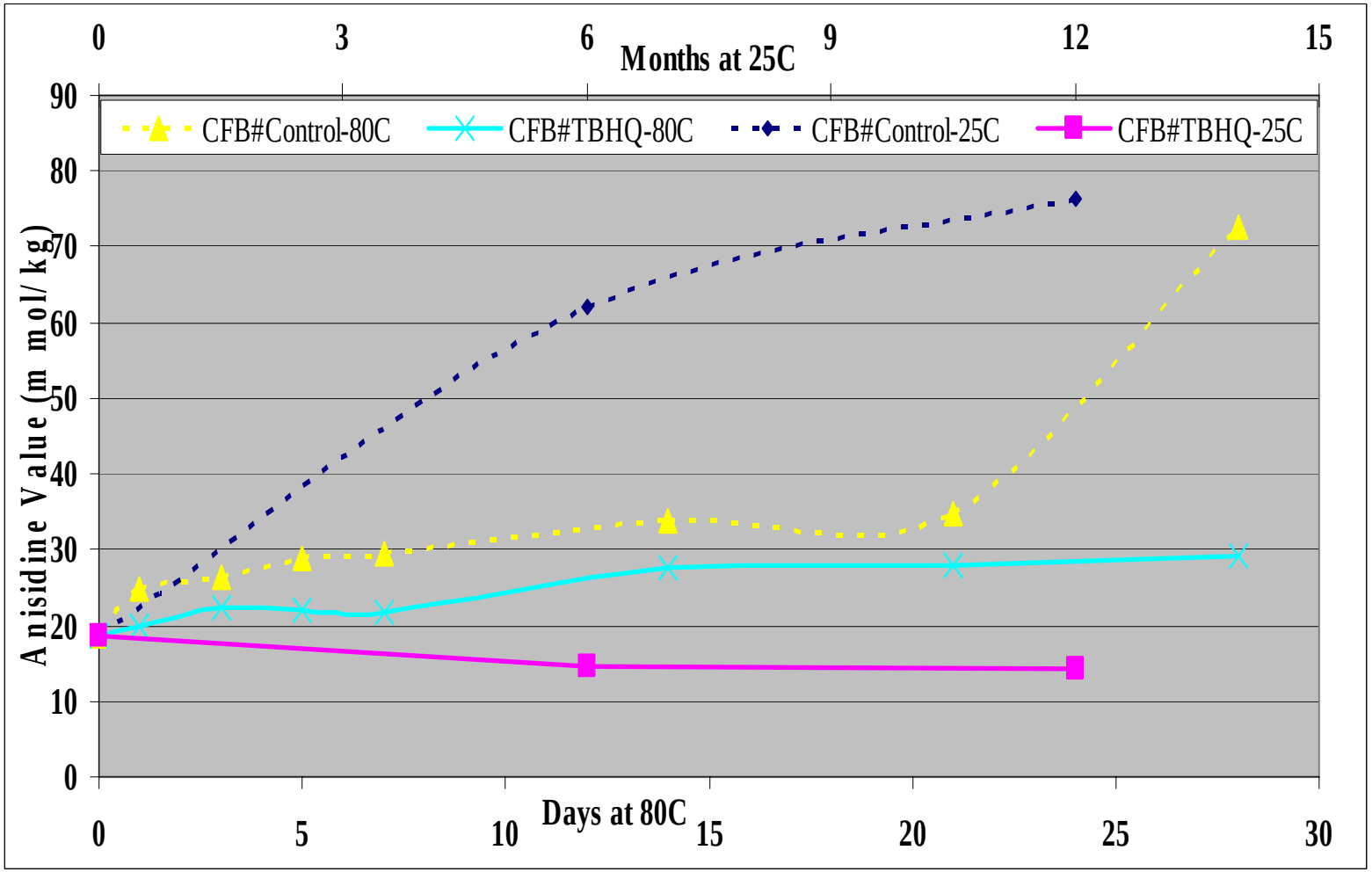

Figure 11. Anisidine value (AnV) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

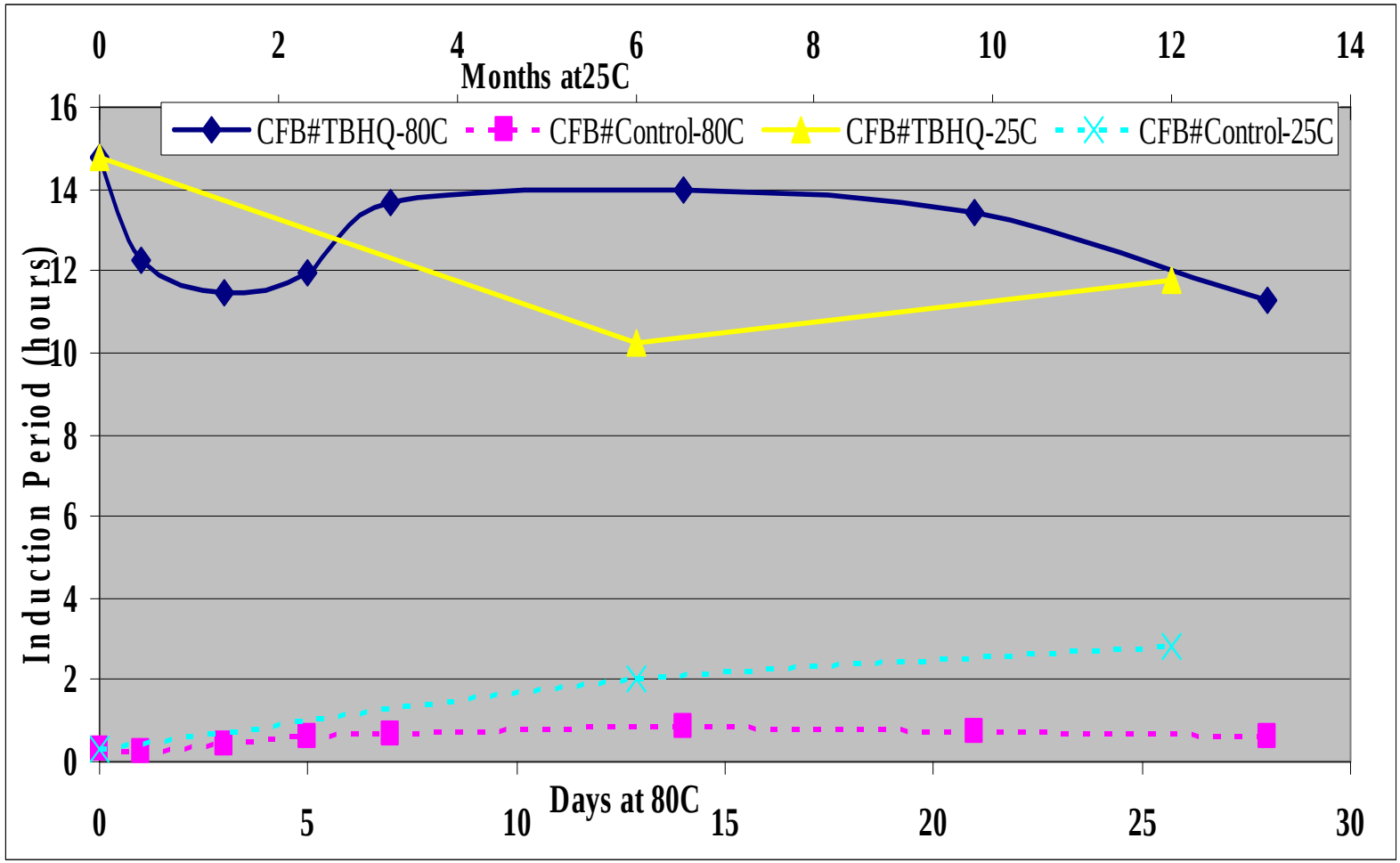

Figure 12. Induction period (IP) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C

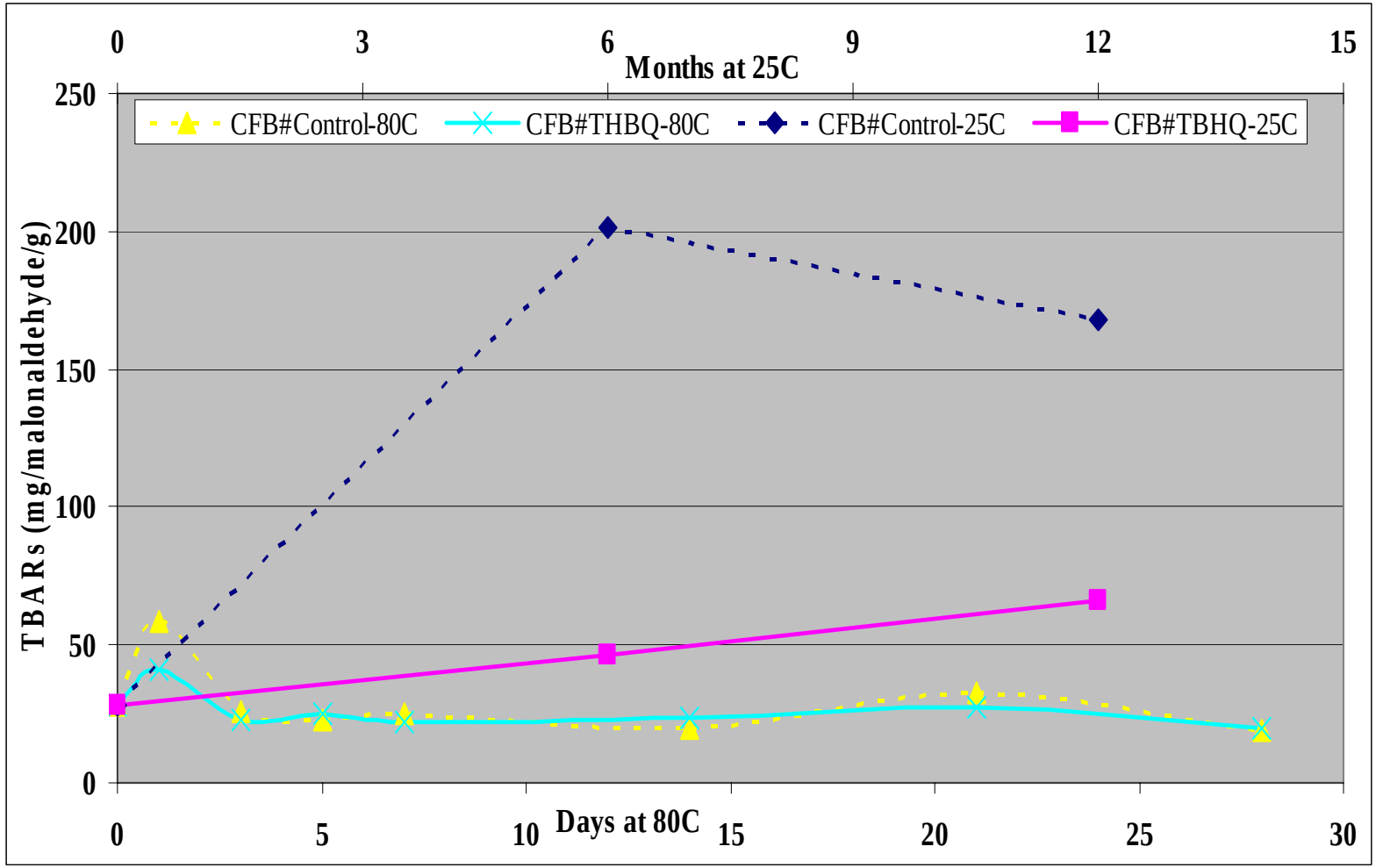

Figure 13. 2-Thiobarbituric acid reactive substances (TBARs) of catfish methyl esters, CFME, stored at 80^{o}C and 25^{o}C