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Effects of Antioxidants on Copper Induced Lipid Oxidation During Salting of Grass Carp(*Ctenopharygodon idella*) Fillets Under Refrigerated Storage

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Abstract
This study was aimed to investigate of the effects of ascorbic acid, citric acid and ethylene diamine tetra acetic acid EDTA with copper in the brine on lipid oxidation of the grass carp (*Ctenopharyngodon idella*) fillets during 15 days of refrigeration storage. In this study traditional ascorbic acid, citric acid and EDTA solutions (10, 30 ppm) respectively with copper (5ppm) were considered as treatments. Rancidity development was measured by several biochemical indicators including peroxide values and thiobarbituric acid. Also pH expressible moisture NaCl and copper content were measured during 15 days storage. Results showed that primary and secondary oxidation products of control samples were significantly higher than those in other treatments ($p<0.05$). Also, expressible moisture and pH value of treated sample were showed significant difference during storage. Results showed that all three treatments had significant effect on delaying lipid oxidation ($p<0.05$) but using of EDTA had the best effect on delaying lipid oxidation and increasing shelf-life of fillets ($p<0.05$) when similar concentration (10, 30 ppm) of EDTA, ascorbic acid and citric acid were included with 5 ppm copper in the bring EDTA was the antioxidant that effectly inhibited copper-induced lipid oxidation.
Key words: Citric acid , EDTA, Ascorbic acid , grass carp, lipid oxidation , copper (11) catalysis.

Introduction

Fish is an excellent protein source with high nutritive value due to a favorable essential acid composition (Jannat *et al*.,2010). Fish are considered as an important part of human nutrition because of their high content in poly unsaturated fatty acid (PUFAS), especially of the ω-3 family these un saturated fatty acid are highly susceptible to oxidation (Sānchez-Alonso and Borderias, 2008). Deterioration of fat is not the only improper effect on oxidation and this phenomenon can cause some changes in color, flavors and a decrease in the ω-fatty acids that are known to be beneficial to human health. Dietary lipid oxidation products may even accelerate atherosclerotic processes, coronary heart disease (Kubow, 1992 ; Baker, 2001 ; Turan *et al*.,2007). Lipid oxidation is one of the major problems in the fish industry, due to the resultant flavor deterioration and loss of nutritional value (Petlersen *et al*.,2004). In order to minimize such undesirable effects, different technological strategies have been applied such as low temperature storage , proper packaging , glazing including chemicals and incorporation of antioxidants (Medina *et al*.,2009 ; Teheri *et al*.,2012). In food industry , metal chelators are commonly used to minimize the catalytic effect of transition metals in the peroxidation process (Pokorny,1987; Loliger,1991). The most important chelators are water-soluble compounds such as citric acid, ethylene diamine tetra acetic acid (EDTA), ascorbic acid and some amino acids (Holmer, 1995). These function either by coordinating the metals and changing their potential by suppressing the redox reaction producing peroxyl and alkoxyl readical or by blocking complex formation with hydroperoxides and preventing their decomposition (Willcox *et al*.,2004 ; Selfried *et al*.,2007). The effectiveness of various antioxidant appears to be influenced by either the phase of reaction systems or the type of radicals (Lin and Liang, 2002). Antioxidant effects on transition metal induced lipid oxidation have been extensively studied using oils or fatty acids, proteins, polysaccharides model systems (Harel, 1994 ; Jacobsen, 1999). The objectives of the present study were to evaluate the effect of different antioxidants on lipid oxidation of common carp fillets, started at refrigerated condition were investigated.

Materials and Methods

Preparation and treatment of fish sample from common carp fish (*Clenophorynodor idella*) was obtained from Basrah market and transported in iced containers to the laboratory, carp fish (about 1-2 kg each) were gutted, headed , skinned and filleted manually. Before preparing the treatment , the protein lipid , ash and moisture were assessed in the fresh sample. The fillets were divided into uniformly sized pieces (thickness-2cm. width-4cm and length-6cm) of 80g each prior to the treatment with antioxidants. Then fillets divided into groups.

Code1 (25% NaCl + 5 ppm $CuCl_2$ + 10 ppm ascorbic acid). Code2 (25% NaCl + 5 ppm $CuCl_2$ + 30 ppm ascorbic acid). Code3 (25% NaCl + 5 ppm $CuCl_2$ + 10 ppm citric acid). Code4 (25% NaCl + 5 ppm $CuCl_2$ + 30 ppm citric acid). Code5 (25% NaCl + 5 ppm $CuCl_2$ + 10 ppm ADTA).

Code6 (25% NaCl + 5 ppm $CuCl_2$ + 30 ppm ADTA). Code7 (25% NaCl (w/v). Cod8(0%NaCl). All treated samples were from the solution. Then samples were storage at 5C° for 15 days (Lauritzen and Olsen, 2004).

General chemical Analyses:
The moisture content, crude protein , fat and ash were carried out according to the AO AC (1990).

Measurement of pH
For pH measurement 5g of fish muscle was wall mixed with 45 ml of distilled water and them the pH values recorded through a pH meter (Suvanich *et al*.,2000).

Thiobarbituric Acid Reactive Substances TBARS Analysis
TBARS assay was measured according to the procedure described by Tarladgis (1969) 20g of fish fillet was homogenized with 100 of 7.5 trichloracetic acid solution (TCA) for 10 minutes and filtered. The obtained liquid 5ml was added 10 5ml of solution containing 0.0288g thiobarbituric acid and 90% acetic acid the mixture was heated in boiling water bath for 30 minutes and then cooled to temperature -18C°. TBARS was measured 538 nm.

Salt determination
The NaCl content was carried out according to AOAC (1990).

Peroxide Value (PV) determination
Peroxide Value (PV) was determined in the sample according to the method described by AOAC (2000).

Copper determination
A sample 2g placed in a high farm porcelain crucible. The furnace temperature was slowly increased room temperature to 450 C° in 7h. The sample were a shed for about 6h until or grey ash residue was obtained. The residue was dissolved in 5ml of HNO_3 (25% v/v) on the mixture. Where necessary, was heated slowly to dissolve the residue. The solution was transferred to a 25ml volumetric flask and made up to volume (Vaidga and Rontala, 1996). Stock standard solution of copper was used to prepare calibration solution to obtain calibration curve. The metal analyses of samples were carried out by using flame atomic absorption spectrophotometer.

Statistical analysis
The experimental design was factorial 6 × 3 × 5. ANOVA was employed to fish the interaction between value of different analyses and storage time. The results were subjected ANOVA followed by beast significant difference lest (LSD) with significant level of ($p<0.05$) used to compare sample means by using SPSS.

Results
Results of chemical compound (protein lipid, ash and moisture) show in muscle tissue of *Ctenopharynodon idella* in table 1. Amount moisture, protein fat and ash were 77.58%,16.35%, 2.15% and 0.97% g/100g muscle, respectively.

Table1:Chemical compound of the meat grass carp

Chemical composition	%
Moisture	77.58
protein	16.35
fat	2.15
ash	0.97

Changes in pH
The changes of pH value of the fillets during the 15 day are presented in (Figure 1). It can be seen that the pH of the EDTA samples remained at as significantly higher level than all samples. The muscle pH decreased rapidly from 6.72 in fresh muscle to values in the range of 6.61 to 6.68 during the first day of storage. All samples slightly decreased with the refrigerated storage time ($p<0.05$). the control sample at the lower level 5.22 and the EDTA samples at the upper level 5.81.

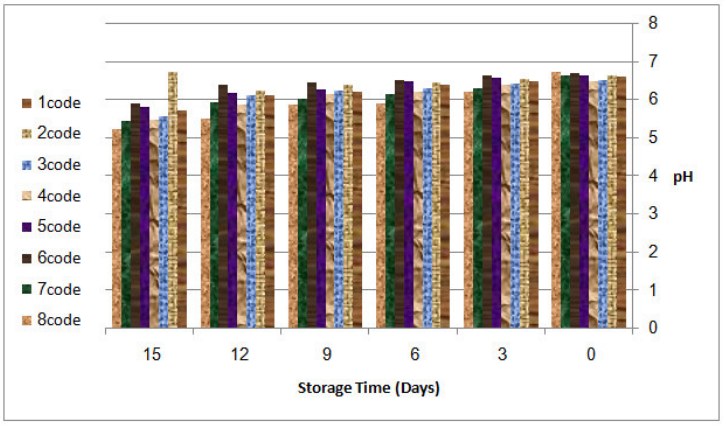

Figure 1: pH content of the grass carp fillet during storage

Total NaCl content

The NaCl concentration of the muscle samples was also determined initially during refrigerated storage as shown in (Figure 2). We notice NaCl content increased rapidly from 0.13% in fresh fillets to 7.30% after salting. The samples without additives had the highest NaCl concentration which was 8.87% at the end of storage period, and followed by the citric acid sample of concentration 10,30 ppm and the NaCl content of them was 8.45% , 8.60% respectively.

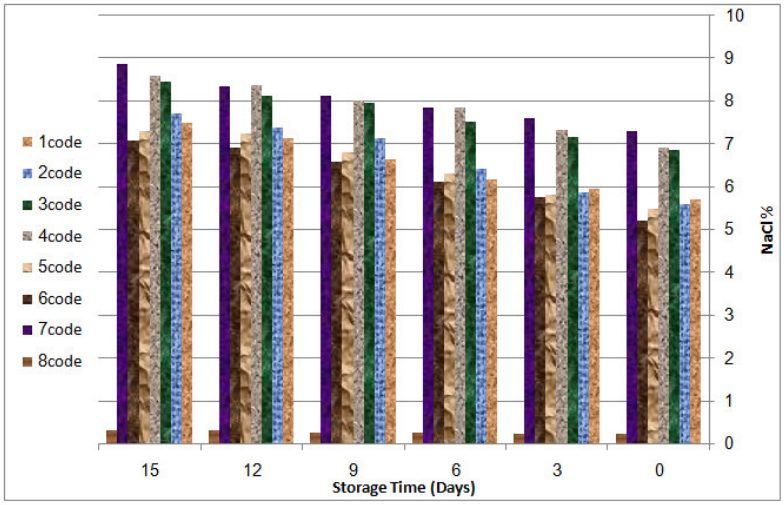

Figure 2: NaCl content of the grass carp fillet during storage

Changes in moisture

The results of statistical analysis showed the total amount of moisture that there was significant difference in samples as shown in (Figure 3). The moisture content reduced from 77.58% in fresh muscle to 71.50% after salting. During the storage period the water content of the citric acid samples decreased to a significantly ($p<0.05$) lower level than the EDTA and ascorbic acid samples. The highest water content was found in the EDTA (10 and 30 ppm) 60.94% and 61.23%.

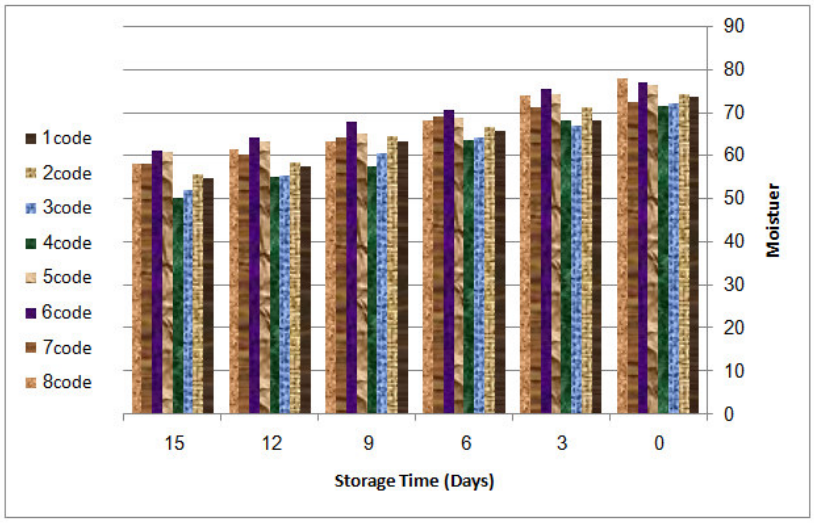

Figure 3: Moisture content of the grass carp fillet during storage

Lipid oxidation

Lipid oxidation development was measured according to the PV formation (Primary oxidation compounds) and TBARS (secondary oxidation compounds). The main use of a peroxide value is to determine the quality of oil samples changes in PV values of samples and during refrigerated storage at 5C° for 15 days are shown in (Figure 4). All samples showed significant increase in PV value and the highest value was in control it increase from 0.12 meg O_2 kg on the early storage time to reach 2.10 meq O_2/kg after 15 days. When EDTA treatment samples showed a lower ($p<0.05$) PV value compared with other treatments, the EDTA concentration at 10,30ppm of PV values were 1.24 and 1.19 meq O_2/kg respectively. All the samples show secondary lipid oxidation products, as reported by the TBARS. Presented low values at the beginning of the study (Figure 5) and gradually increased during refrigerated storage. A significant increase in the TBARS values of control was found to be 0.850 mg MAD/Kg and increased to 7.88 MAD/Kg at the end of storage. When the EDTA concentration increased from

10 to 30 ppm, the TBARS values decreased to approximately 3.51 MAD/g after 15 days. Also ascorbic acid at the same concentration the TBARS decreased to approximately 4.12 MAD/g after 15 days. Higher concentration of EDTA and ascorbic acid present during the initial storage inhabited present and that is clearly appear in the low TBASR.

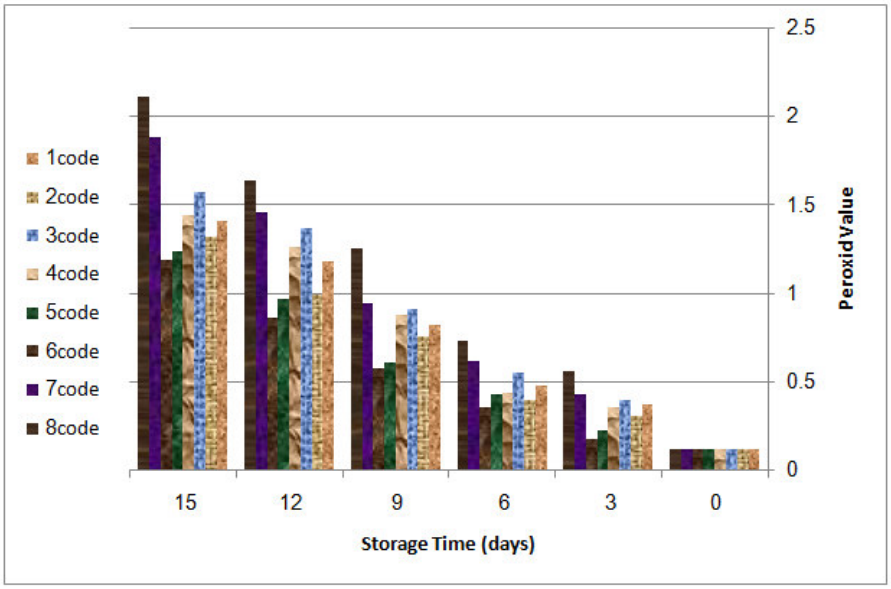

Figure 4: PV content of the grass carp fillet during storage

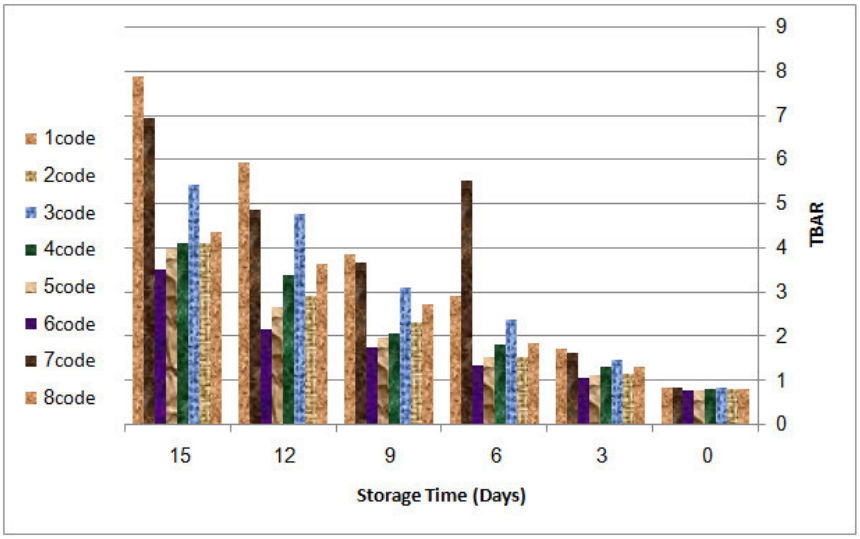

Figure 5: TBAR content of the grass carp fillet during storage

Copper content

The levels of tissue copper in grass carp is presented in(Figure 6).The uptake of copper ions was significantly($P<0.05$) effected by different antioxidants. The copper content of the Control without any additives lower than in all samples after 3 hours 3.19 ppm. The copper content of the EDTA samples 10 and 30 ppm at 5.20 and 4.84 ppm respectively, than salted samples except control. The higher content of copper were show in citric acid samples ,where increase from to 4.67 ppm at 30 ppm concentrate after one hour from salting to 6.25 ppm at the end salting period. All samples show significantly ($P<0.05$) increase in copper content after the end salting time.

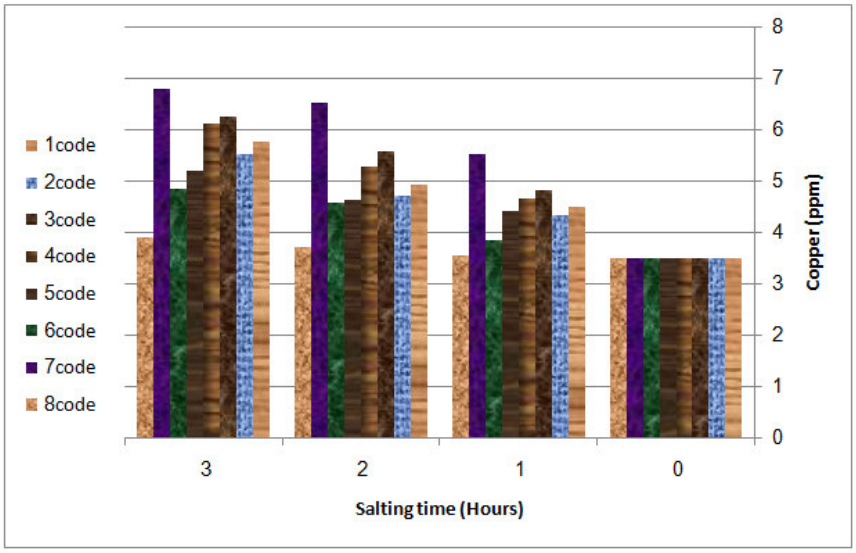

Figure 6: Copper content of the grass carp fillet during salting time

Discussion

Investigating chemicals characteristic of freshwater fishes very important, because useful information for expert related to food resources having low fat, high protein and being easily accessible. Results of this study shown level of the moisture, protein, fat and ash contents in *Ctenopharynodin idella* was a quite similar to Afkami *etal*.(2011). Results of pH measurements showed that pH of antioxidant treated samples higher than control sample in refrigeration fish fillets during 15 days storage (Lauitzsen and Olsen, 2004). The lowered water content of the samples were thought to be explained by an increased uptake of NaCl, when the NaCl concentration of the muscles the muscle tissue start to shrink and the salting- out effects that place. Peroxide value samples indicates the concentrations of peroxides and hydroperoxides that are produced during the early stages of lipid oxidation. The peroxide values are monitored for a samples and when it sharply increases it indicates the end of the self-life for that sample Similar result were reported by others. According to Layrizsen and Olsen (2004) showed the lowest rate of peroxide formation and the highest values were found in control sample during any time of storage. One of the most widely used tests to quantify lipid oxidation in fish meat is TBARS test. In fact PV measurements are not reliable in assessing the oxidation of highly unsaturated oil such as fish oil. This is probably because the peroxides that from initially are unstable and react quickly to form secondary oxidation products. For this reason, the PV should be used in conjunction with other methods(Sánchez-Alonso and Borderias, 2008). As assign of this phenomenon, primary and secondary lipid oxidation compounds formation were decreased in compared with control samples ($P<0.05$) and the antioxidant treatments showed the best inhibitory effect on lipid oxidation Rostamzad *etal.* (2010), Kashiri *etal.* (2011) reported that usage of antioxidants and chilling have positive effect on delaying fat spoilage. In water solution EDTA, Ascorbic acid and citric acid are known by their multifunctional effects as an antioxidant, a prooxidant, a metal chelator, a reducing agent or as an oxygen scavenger, they compounds interact with Cu^{+2} to accelerate the decomposition of linoleate hydroperoxides (Huttin, 1994). EDTA and other antioxidant inhibit enzyme-catalysed lipid oxidation by removing iron and reducing hydrogen peroxide (Hölmer, 1995). The presence of antioxidant inhibited copper induced oxidation during the storage period. This may be explained by direct participation of the reduced copper in a Fenton-type reaction where hydro peroxides are reduced to hydroxyl radicals or lipid alkoxy radicals. Potential sources for copper contaminations with fish muscle during salting are the top water, salt and fish processing equipments (Lauritzsen, 2004). Similar result were reported by other researcher Lauirtzsen, (2004), Kashiri *et al*.,(2011). EDTA is a chelator that forms thermodynamically stable complexes with transition metal ions inhibiting electron transfer and thus oxidation (Pribil, 1972 : Dokrony, 1987). The effectiveness of EDTA in inhibiting copper induced lipid oxidation has been shown in several studies. Examples are inhibition of the development of off-flavour in margarines during storage (Melniek, 1961). Inhibition of copper induced rancidity in cod muscle blends at 12% NaCl concentrations (Costell *et al*.,1965) and inhibition of metal-induced lipid oxidation of fish oil mayonnaise products (Jacobsen and Timm, 2001) ineffectiveness as an antioxidant. The reason why citric acid sample appeared to facilitate the uptake of copper is not known. One can speculate that the binding of copper to citric neutralizes the positive charge of the metal and thereby makes it easier to penetrate into the muscle (Goldstein and Czapski, 1986 ; Kanner, 1994). At the end of the salting time the increased copper concentration observed in the samples could be explained by the loss of moisture during studying. Potential sources for copper contaminations with the fish muscle during heavy

curing are the tap water, salt and fish processing equipment.

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