ANTIOXIDATIVE ACTIVITY OF CITRIC AND ASCORBIC ACIDS AND THEIR PREVENTIVE EFFECT ON LIPID OXIDATION IN FROZEN PERSIAN STURGEON FILLETS

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***Abstract*— Persian sturgeon (*Acipenser persicus*) fillets were soaked in Citric acid, Ascorbic acid and combination of Citric and Ascorbic acid solutions and then were stored at frozen conditions (-18 ºC) up to 6 months. During storage, some general chemical analysis such as free fatty acids, primary and secondary oxidation products and sensory analysis were measured in order to study rancidity development. Results showed that antioxidant treatments had lower (P<0.05) lipid oxidation development in compare with control samples. Development of peroxides value in control samples was significantly higher (P<0.05) than antioxidants treatments after 6 months storage. Also other experiments showed that AA+CA treatment had the best effect (P<0.05) on delaying lipid oxidation in frozen fillets.**

***Keywords*— Antioxidant, Lipid oxidation, Persian sturgeon, Frozen storage, Fillets.**

I. INTRODUCTION

Most fish and other marine species give rise to products of great economic importance in many countries. Freezing and frozen storage have been largely employed to retain fish sensory and nutritional properties before they are consumed or used in other technological processes (Aubourg *et al.,* 2004). Nowadays fatty fish are attracting a lot of attention because the omega-3 poly unsaturated fatty acids, Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA), found in fish oil has important properties in nutrition and disease management. Because of this high unsaturated lipid content, fish products are very susceptible to loss of quality through lipid oxidation (Sánchez-Alonso and Borderias, 2008). Oxidation in food causes a number of changes such as improper changes in the product's sensory properties, decrease of nutritional value and economic losses (Amanatidou *et al*., 2000; Gramza *et al*., 2006). The main phenomenon which affects oil quality in fish during storage is auto oxidation. Some methods such as using low temperature storage, appropriate packaging and glazing with protecting chemicals or antioxidants are used for delaying improper changes in oils (Yildiz *et al*., 2006; Richards *et al.*, 1998; Lin and Lin, 2005; Reynolds *et al*., 2002).

The use of antioxidants is one of the most effective ways of increasing shelf-life and preserving quality of food (Serdaroğlu and Felekoglu, 2005). Antioxidants are substances that can delay or prevent oxidation caused by atmospheric oxygen in fats and oils and fatty components of food (Benjakul *et al.* 2005; Sarkardei and Howel, 2006). Antioxidants can slow down oxidation and rancidity development by reacting with free radicals and stabilizing hydroperoxides (Benjakul *et al.*, 2005).

Ascorbic acid (AA), Citric acid (CA) and their salts are widely known for their role as chelators and acidulants (Oktar *et al.*, 2001; Kim *et al*., 2006). The profitable effects of AA and CA on fish oil and emulsions (Osborn-Barnes and Akoh, 2003), minced fish (Stodolnik *et al.*, 1992) and fish fillets (Badii and Howell, 2002; Aubourg *et al.*, 2004; Pourashouri *et al*, 2006) have been observed.

Sturgeons are one of the oldest freshwater fishes still found living in the world, and Persian sturgeon *(Acipenser persicus*) is one of the most valuable species of them which lives in Caspian Sea. However not enough technological researches exists which accounts for its quality assessment. The present study, investigates the effect of AA, CA and combination of them on lipid stability of Persian sturgeon *(Acipenser persicus*) during frozen storage.

II. METHODS

A. Preparation of fish samples

Fresh Persian sturgeon *(Acipenser persicus)* was captured in October2006 and kept on ice (1h) till delivery to the laboratory. Then, were carefully gutted, dressed and filleted by hand. The weight of each fillet was 500 - 580 g. Fillets were then immersed either in water (blank control; BC treatment), 0.50% AA aqueous solution (AA treatment), 0.50% CA aqueous solution (CA treatment) and in a combination of 0.50% AA and 0.50% CA aqueous solution (AA+CA treatment). After 5 minutes, fillets were removed from all solutions, packaged in individual low density polyethylene bags and placed in a freezer at -40ºC. Antioxidants concentration and dipping time were chosen according to previous related research (Chapman *et al.*, 1993; Aubourg *et al.*, 2004; Pourashouri *et al.*, 2006). Treated fillets were kept in -40ºC for 24 h, and then were stored in a freezer at -18° C. Sampling was undertaken at 1st, 3ed and 6th months after frozen storage at -18ºC and on the raw

fish (initial material). For each treatment (BC, AA, CA and AA+CA), three different fish batches (n = 3) were considered and studied separately to achieve the statistical study. Chemicals (solvents and reactants) employed through the study were reagent grade (Merck, Darmstadt, Germany).

B. Sensory analyses

Sensory analyses were conducted by a sensory panel consisting of five to seven panelist, according to the guidelines presented in Table 1 (DOCE, 1989; Pourashouri *et al.,* 2006), four categories were ranked: highest quality (E), good quality (A), fair quality (B) and poor quality (C). Sensory assessment of the fish fillet included the following parameters: flesh appearance, rancid odor and flesh consistency (The evaluation of consistency was performed by hand).

At each sampling, different fish fillets were thawed and then analyzed in the same session. Each fillet was tested and scored by panelists individually. Sensory analyses were carried out at 0, 1st, 3ed and 6th months after storage. Three replicates were used for each experiment.

C. General chemical analyses

For measuring pH, 5 g of Persian sturgeon mince was homogenized for1 min with 45 ml of distilled water. Then pH was measured with a standardized portable digital pH meter (Beckman U40, Krefeld, Germany) (Suvanich *et al*., 2000).

Expressible moisture was determined according to the method of Suvanich *et al.* (2000) and the drip was calculated as g kg^{-1} flesh muscle.

D. Lipid damage measurements

Peroxide value (PV) and free fatty acid (FFA) content were determined in the lipid extract by the Egan *et al*. (1997) method.

a) Muscle samples (30-150 g) were blended with 250 ml of chloroform for 2-3 min and filtered through a large fluted paper. Then the samples were re-filtered through a paper containing a small amount of anhydrous sodium sulphate. Portions of this second filtrate (A) were used for other measurements ("b" to "d").

b) Weight of fat in the solution: Transferred 10 ml of filtrate A into a weighted metal dish; removed the solvent and dried at 100°C. Then samples were cooled in deciccator and weighted. This weight was used for calculation FFA and Peroxide value in the methods below.

c) FFA**:** 25 ml of 95% alcohol with a few drops of 0.1 N NaOH after adding phenolphthalein was neutralized. Then this solution was added to 25 ml of "A" and titrated with 0.1 N NaOH until the pink color neutralize for 10 s. The FFA were calculated as oleic acid as a percentage of the oil (Egan *et al.,* 1997).

d) Peroxide value: 25 ml of filtrate "A" was transferred into a 125-ml stopper conical flask; 37 ml of glacial acetic acid and 1 ml of freshly prepared saturated potassium iodide solution were added. Allowed the solution to stand with occasional swirling for exactly 1 min, then 30 ml of water was added and titrated with 0.01 N sodium thiosulphate. Starch was used as indicator (Egan *et al*., 1997).

Peroxide value was calculated as:

1) ml of 0.002 N sodium thiosulphate/g

2) $\frac{\text{Titration} \times \text{N} \times 1000\ \text{mequiv/kg}}{\text{Wt. of sample}}$

where N is the normality of sodium thiosulphate, mequiv is the milliequalivalents. Results were expressed as meq oxygen kg^{-1} lipids.

e) Thiobarbituric acid

The thiobarbituric acid index (TBA-i) (mg malondialdehyde kg^{-1} flesh muscle) was determined in a 5% trichloracetic acid extract according to the method of Kirk and Sawyer (1991). Muscle samples (10.0 g) were blended with distilled water (30 ml) for 2 min. The sample was then transferred to a 500 ml distillation flask with 47.5 ml of distilled water and the pH was adjusted to 1.5 with 2.5 ml of 4 N HCL, and a drop of silicone antifoaming agent were added. The flask was connected to a distillation apparatus consisting of a Y-type connector, dropping funnel, splash head and condenser. The mixture was boiled until 50 ml of distillate was collected. In a screw capped test tube, 5 ml of the distillate was reacted with 5 ml of TBA reagent (0.02 M TBA in 90% acetic acid) and placed in a boiling water bath for 35 min. a control made up of 5 ml distilled water and 5 ml of TBA reagent was also boiled for 35 min. the tubes were cooled to room temperature and the absorbance was read at 538 nm with a DU640 UV/Vis Spectrophotometer (Beckman Coulter, Inc., Harbor Boulevard, Fullerton, CA, USA). The TBA were calculated by multiplying the absorbance reading by factor of 7.8 and expressed as mg malondialdehyde (MDA) kg^{-1} meat sample.

E. Statistical analysis

Statistical analysis was conducted with the SAS software package (SAS, 1998). Data from the different quality measurements were subjected to the one way ANOVA analysis. Comparisons of means after the ANOVA test were performed using least squares difference (LSD) method.

III. RESULTS AND DISCUSSION

A. Evolution of general chemical parameters

The initial pH value of antioxidant treated samples was lower than their corresponding control samples and this lower value was maintained during storage time (Fig.1). AA+CA treated samples showed a lower ($p<0.05$) pH value in compare with other antioxidant treated samples during frozen storage, no significant differences were observed in the third and sixth months period among BC, CA and AA treated samples. According to previous researches, frozen storage dose not lead to pH differences between fresh muscle at different frozen storage times (Aubourg *et al*., 2004).

Water holding capacity in meat tissue is strongly related to myofibril proteins. Increase of expressible moisture is a sign of reduction of water holding capability by

Table1. Scale employed for evaluating the sensory quality of frozen Persian sturgeon fillets

Attribute	E (Highest quality)	A (Good quality)	B (Fair quality)	C (poor quality)
Flesh appearance	Strongly hydrated and pink; myotomes totally adhered	Still hydrated and pink; myotomes adhered	Slightly dry and pale; myotomes adhered in groups	Yellowish and dry; myotomes totally separated
Rancid odor	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
Flesh consistency	Presence or partial disappearance of rigor mortis symptoms	Firm and elastic; pressure signs disappear immediately and completely	Presence of mechanical signs; elasticity notably reduced	Important shape changes as a result of mechanical factors

*Adapted from DOCE (1989)

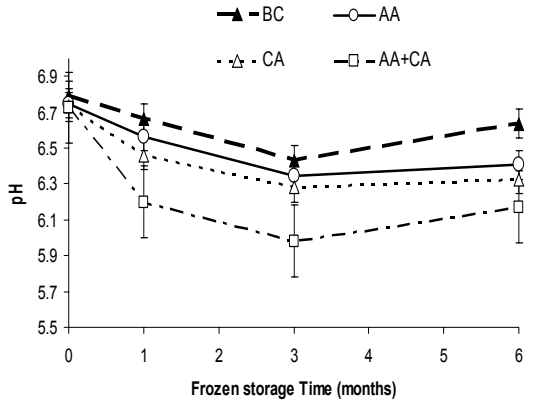

Fig. 1. Changes of pH in Persian sturgeon fillets under different treatments during frozen storage

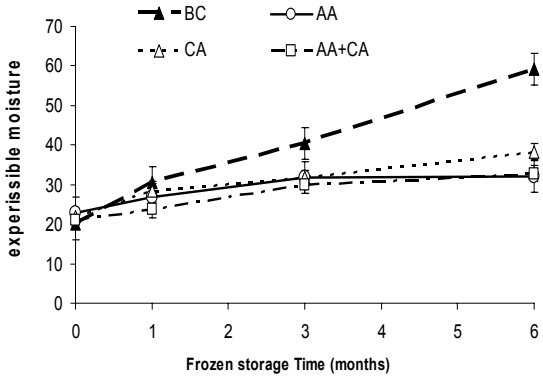

Fig.2. Changes of Expressible moisture in Persian sturgeon fillets under different treatments during frozen storage

tissue due to denaturizing of proteins (Suvanich *et al.*, 2000). This phenomenon leads to reduction of flavour agents and nutrition value (Reddy and Srikar, 1991).

In this study expressible moisture content showed a progressive increase in all samples during frozen storage (Fig. 2).

Comparison among the different treatments showed that expressible moisture of BC samples at 6th month of storage was higher than other treatments ($p<0.05$), while no significant differences were detected among the samples of the three kinds of antioxidant treatment throughout the whole experiment.

This result can be explained according to the previous related researches on milk fish (Chen *et al*., 1998), wells catfish (Pourashouri *et al*., 2006) and channel catfish fillets (Garner *et al*., 2002). In these studies, as the results of this experiment, expressible moisture of antioxidant treated samples was lower than control samples.

B. Lipid hydrolysis development

Lipolysis leads to production of free fatty acids during storage. FFA (three glycerides and phospholipids groups) was measured in order to investigate deterioration of fats during storage. Accumulation of FFA in frozen samples is attributed to the hydrolysis of lipids (Aubourg *et al.,* 2002).

As a result of frozen storage, a gradual increase in FFA formation was observed for all samples ($p<0.05$) as indicated in previous research on other frozen fish species (Aubourg *et al.,* 2002; 2005).

Results showed that, amount of FFA in antioxidant treated samples, never reached to 10 g oleic fatty acid kg^{-1} lipids, but control samples had 18.59 g oleic fatty acid kg^{-1} lipids at the end of storage period (Fig. 3). Comparison between treated samples, showed that BC treated samples had the highest hydrolysis development at sixth month of storage while lower values were maintained throughout the whole experiment period for AA+CA samples ($p<0.05$). Increasing in FFA formation is due to the hydrolysis of phospholipids and triglyserides by the action of lipases and phospholipases (Serdaroğlu and Felekoglu, 2005). Results of this study showed that antioxidants could decelerate developing process of FFA production during storage. Same results were reported by Aubourg *et al*. (2004) and by Pourashouri *et al.* (2006).

Formation of FFA, itself does not lead to nutritional losses. However, examining the progression of lipid hydrolysis is important because FFA are known to undergo further oxidation to produce low molecular

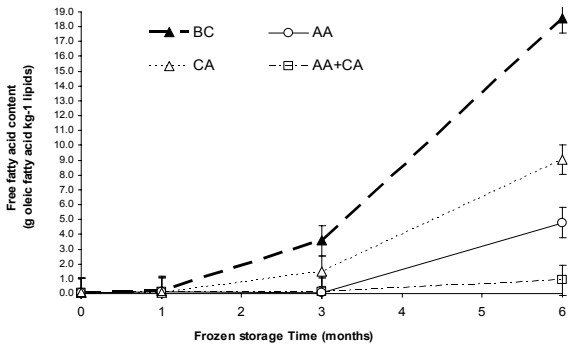

Fig. 3. Changes of Free fatty acid in Persian sturgeon fillets under different treatments during frozen storage

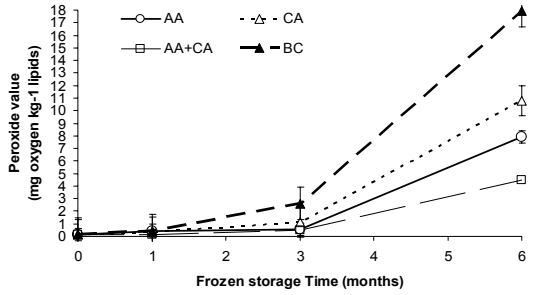

Fig. 4. Changes of Peroxide value in Persian sturgeon fillets under different treatments during frozen storage

weight compounds which are responsible for the rancid off-flavor and taste of fish and fish products (Refsgaard *et al.*, 2000). This phenomenon has great influence on protein denaturation and texture deterioration by interacting with proteins (Lodasa *et al*., 2004).

C. Lipid oxidation development

Fats in fish are inclined to oxidation during storage. This is one of the most important reasons of meat spoilage, production of poisonous compounds and reduction of meat quality and nutrition value (Sahoo *et al.*, 2004). Hydroperoxides are the output of oxidation. Reaction between these products with other molecules leads to deterioration off colour and off odour formation (Lee *et al.*, 1998).

In this study, PV was low at month 1 for all samples but increased at month 3 (Fig. 4). Compared to month 3, a marked increase ($p<0.05$) was observed at month 6 for different kinds of treatments. Comparison of the different treatments showed significantly higher peroxide formation at month 6 for the control samples than other treatments ($p<0.05$). However, AA- and CA-treated samples showed a progressive increase ($p<0.05$) during frozen storage; no significant differences were detected between AA- and CA-treated samples.

Combination of citric acid and ascorbic acid (CA+AA) was found to be the most effective treatment for controlling oxidation in compare with other treatments ($p<0.05$).

Similar results were reported by Aubourg *et al*. (2004) and Pourashouri *et al*. (2006). In those studies peroxide value of antioxidant treatments were lower than control samples and AA+CA treatment showed the best inhibitory effect on lipid oxidation too.

Other studies on the effects of flaxseeds on rancidity development in frozen whole mackerel (Stodolnik *et al.*, 2005) and ascorbic acid in frozen herring (Hamre *et al.*, 2003) and so also the effects of vitamins C, E and ascorbic acid in frozen horse mackerel (Sarkadei and Howell, 2008) reported significant increase of peroxide value in all samples during storage period. But increase of peroxide value in antioxidant treated samples was significantly lower than that reported for control samples. PV measurements are not reliable for assessing oxidation progress of highly unsaturated oils such as fish oils. This is probably because the peroxides that form

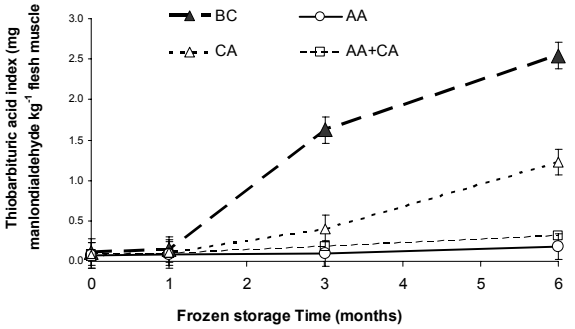

Fig. 5. Changes of Thiobarbituric acid index in Persian sturgeon fillets under different treatments during frozen storage

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). The thiobarbituric acid (TBA) test is widely used to quantify lipid oxidation products in fish meat. Secondary lipid oxidation products, as reported by the TBA-i, presented low values at the beginning of the study (Fig. 5) and gradually increased during frozen storage (as in the case of PV). A significant increase in TBA-i value was observed for control and CA-treated samples ($p<0.05$) in compare with other treatments (Fig. 5). AA and the combination of AA and CA (AA+CA) were found to be the most effective treatments for delaying oxidation ($p<0.05$).

Result of other studies by Benjakul *et al.* (2005), Pourashouri *et al.* (2006), Sarkadei and Howell (2008) and Sánchez-Alonso and Borderias (2008). Aubroug *et al*. (2004) showed that TBA values of antioxidants treated samples were lower than control samples.

D. Sensory analysis

In fresh fish, odor, color and appearance of fillets were natural, but their quality deteriorated during storage (Tab 2).

Flesh appearance assessment showed a lower ($P<0.05$) score at 6th month in control samples in compare with antioxidant treatments.

At third month, AA+CA-treated samples had better odor according to sensory scores in compare with other treatments.

Flesh odor and flesh appearance in control samples at 6th month of storage was considered a limiting factor. Among different kinds of molecules produced as a result of lipid oxidation, secondary ones are considered the chief compounds responsible for oxidized flavors (White, 1994). A close relationship between the rancid odor development and the TBA-i assessment has been obtained in the present study.

In the end, flesh consistency assessment showed a better score at 3rd month for AA- and AA+CA-treated samples, while at the end of the storage time no significant differences were obtained among treatments.

These results conform to those of previous research by Leaflet (2004) who found that antioxidant treatment

increased shelf-life and preserved sensory attributes during storage. Other studies about effect of Ascorbic acid and Citric acid on Wells catfish fillet showed significant differences in flesh odor of treated and untreated samples at the end of storage time, although no differences were obtained for other attributes (consistency, color and flesh appearance) for both treated groups of samples (Pourashouri *et al.,* 2006).

Table 2. Evolution of sensory parameters during frozen storage of Persian sturgeon fillets that were pretreated under different conditions

Frozen storage time (months)	Flesh appearance				Rancid Odor				Flesh consistency			
	BC	AA	CA	AA+CA	BC	AA	CA	AA+CA	BC	AA	CA	AA+CA
1	A	E	E	E	A	E	E	E	A	A	A	A
3	B	B	B	B	B	A	B	E	B	A	B	A
6	C	B	B	B	C	B	B	B	B	B	B	B

Freshness categories: E (excellent), A (good), B (fair) and C (poor).
*All fish were category E for all attributes initially.

Sensory analysis indicated that antioxidants particularly a combination of Ascorbic and Citric acid (AA+CA) can slow down quality loss during frozen storage.

IV. CONCLUSION

In this study AA, CA and combination of them were used. Usage of both antioxidants, led to reduction of rancidity of fats in frozen Persian sturgeon fillets. As a sign of this phenomenon, primary and secondary lipid oxidation compounds formation was decreased in compare with control samples ($p<0.05$). Results showed that the samples which were soaked in solutions of acids had significant differences in biochemical parameters which were studied in compare with control during frozen storage. This can be due to using of AA and CA which are oxygen scavengers and can delay lipid oxidation by reducing necessary agents like oxygen and metals. Also as it was reported in previous research (Aubourg *et al.*, 2004), AA and CA have synergistic effect on each other. According to the results of this study, it was confirmed and samples which were soaked in a solution of 0.5% CA and 0.5% AA had the lowest improper changes of fats due to reduction of lipid oxidation($p<0.05$).

The use of AA, CA and AA+CA led to preservation of fillet quality, partial inhibition of quality loss and increase of shelf-life. AA treatments yielded better results as compared to that for CA treatments and a combination of AA and CA proved more effective in preventing lipid oxidation development in freeze stored fillets. In the end, the employment of AA and CA and AA-CA mixture as a previous treatment to the frozen storage, alone or in combination with other protective strategies such as modified atmosphere and vacuum packaging, etc., is recommended.

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