Inhibitory impacts of natural antioxidants (ascorbic and citric acid) and vacuum packaging on lipid oxidation in frozen Persian sturgeon fillets

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Abstract

This study was aimed to investigate effects of aqueous citric acid (CA) and ascorbic acid (AA) on lipid oxidation in comparison with effect of vacuum packaging in order to find better treatment to delay improper changes in the Persian sturgeon (*Acipenser persicus)* fillets during frozen storage due to lipid oxidation. In this study traditional packaging, vacuum packaging, ascorbic acid solution (0.5 %) and citric acid solution (0.5 %) were considered as treatments. Rancidity development was measured by several biochemical indicators including Free Fatty Acids, Peroxide values and Thiobarbituric acid. Also pH, expressible moisture and sensory properties were measured during 6 months storage. Results showed that free fatty acid (FFA), 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 samples were significantly lower than those in control ($p<0.05$). However both antioxidants (AA and CA) extended shelf life of frozen fillets but rancidity development in CA treated samples was higher than other samples during storage. Results showed that all three treatments had significant effect on delaying lipid oxidation ($p<0.05$) but usage of AA and vacuum packaging had the best effect on delaying lipid oxidation and increasing shelf-life of fillets ($p<0.05$). Thus the employment of AA and vacuum packaging alone or in combination with other protective strategies is recommended.

Keywords: Persian sturgeon, Antioxidant, Citric acid, Ascorbic acid, Vacuum packaging, Rancidity

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Introduction

Fish are considered an important part of human nutrition, because of their high content in polyunsaturated fatty acids (PUFAs), especially of the ω -3 family. These unsaturated fatty acids are highly susceptible to oxidation (Sánchez-Alonso and Borderias, 2008). Deterioration of fat is not the only improper effect of oxidation and this phenomenon can cause some changes in color, texture and flavor of the product (Baker, 2001; Hamre & Sandnes, 2003). Lipid oxidation is a critical point during food processing, distribution, storage, and consumption because it decreases food quality, stability, safety, and nutritive value. Different methods have been used for extending fish products shelf life such as low temperature storage, proper packaging and glazing with solution of protecting chemicals and antioxidants (Richards *et al.*, 1998; Lin & Lin, 2005; Yildiz *et al*., 2006). Usage of antioxidants and vacuum packaging have the best influence on increasing shelf-life and delaying improper changes in sea food (Serdaroğlu & Felekoglu, 2005). Antioxidants block the formation of free radicals, stabilize hydroperoxides and thus slow down oxidation and rancidity development. Recently, the demand for novel natural antioxidants has increased; this is because of possible adverse side effects of synthetic antioxidants and beneficial effects of natural antioxidants (Benjakul *et al.*, 2005; Sarkardei & Howel, 2008). Ascorbic acid (AA) and citric acid (CA) and their salts are widely known for their role as chelators (Boyd *et al*., 1993; Oktar *et al.*, 2001; Kim *et al*., 2006) in biological systems and synergists of other antioxidants. The positive effects of AA and CA on fish oil and emulsions (Osborn-Barnes & Akoh, 2003), minced fish (Stodolnik *et al.*, 1992; Abdel-aal, 2001) and fish fillets (Badii & Howell, 2002; Aubourg *et al.*, 2004; Pourashouri *et al*, 2009) have been observed. Vacuum packaging is another way for delaying lipid oxidation (auto oxidation) because of limiting oxygen molecule. As reported by Anelich *et al.* (2001), Fagan & Gormley (2004) and Perez-Alonso *et al*. (2004), packaging under vacuum has positive effect on extended shelf life of fish fillets. In the present study, Persian sturgeon *(Acipenser persicus*) fillets were used. It is one of the most important fish species in the Caspian Sea and is exported to different countries, so should be frozen in order to delay improper changes due to lipid oxidation. Also it is better to use some antioxidants or other preservative methods to extend shelf life of frozen fillets. Thus in the present study, effect of ascorbic acid, citric acid (as natural antioxidants) and vacuum packaging on quality of fish fillets were investigated.

Materials and methods

Fresh Persian sturgeon *(Acipenser persicus)* was captured and kept on ice (1h) till delivery to the laboratory. Then, the fish were gutted, dressed and filleted manually weighing 400-450 g. Then fillets were divided into 4 groups. First group was left

untreated and directly packaged traditionally in polyethylene bags (control samples; BC treatment). Samples of the second group were packaged under vacuum conditions in polyethylene bags (VP treatment). The third group was immersed in 0.50% aqueous solution of Ascorbic acid (AA treatment) and forth group was immersed in 0.50% aqueous solution of Citric acid (CA treatment). After 5 minutes, all treated samples were removed from the solutions and then were following previous similar studies (i.e., Chapman *et al.*, 1993; Aubourg *et al.*, 2004). Then, samples were immediately frozen at -40 ºC for 24 h and then kept in -18ºC. Samplings were carried out from the fresh fish (initial material) and then during frozen storage (at 1st, 3ed and 6th months). For each treatment (BC, AA, CA and VP), three different fish batches (totally 48 batches of fillets) were considered and examined individually. Chemicals (solvents and reactants) employed through the study were reagent grade (Merck, Germany).

For measurement of lipid hydrolysis, free fatty acid (FFA) content was determined in the lipid extract by the Egan et al. (1997) method. Results are expressed as grams of oleic fatty acid per kilogram lipids. For measurement of lipid oxidation, Peroxide value (PV) was determined in the lipid extract according to the method described by Egan et al. (1997). Results are expressed as meq oxygen kg^{-1} lipids. The thiobarbituric acid index (TBA-i) (mg malondi- aldehyde kg^{-1} flesh muscle) was determined in a 5% trichloracetic acid extract according to the method of Kirk & Sawyer (1991).

To measure the pH, five grams of fish mince was homogenized for 1 minute with 45 ml of distilled water. pH value was measured using a standardized portable pH meter (Metrohm) (Suvanich et al., 2000). Expressible moisture content was determined by weight difference between the mussel (1-2g) of fish before and after being pressed under 0.5 and 1 kg load for 5 and 20 minutes (Parvaneh, 1998).

Sensory analyses were conducted by a taste panel consisting of five to seven panelist, according to the guidelines presented in Table 1 (DOCE, 1989; Pourashouri *et al.,* 2009), 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 (Table .1). At each sampling, the different fish fillets were thawed and then analyzed in the same session. The fish fillets were served to the panel members in individual polyethylene bags in which they had been kept frozen and they were scored individually. Sensory analyses were carried out at 0, 1, 3 and 6 months after storage. Three replicates were used for each experiment.

Data from the different quality measurements were subjected to the ANOVA one-way method. Comparisons of means after the ANOVA test were performed using a least-squares difference (LSD) method.

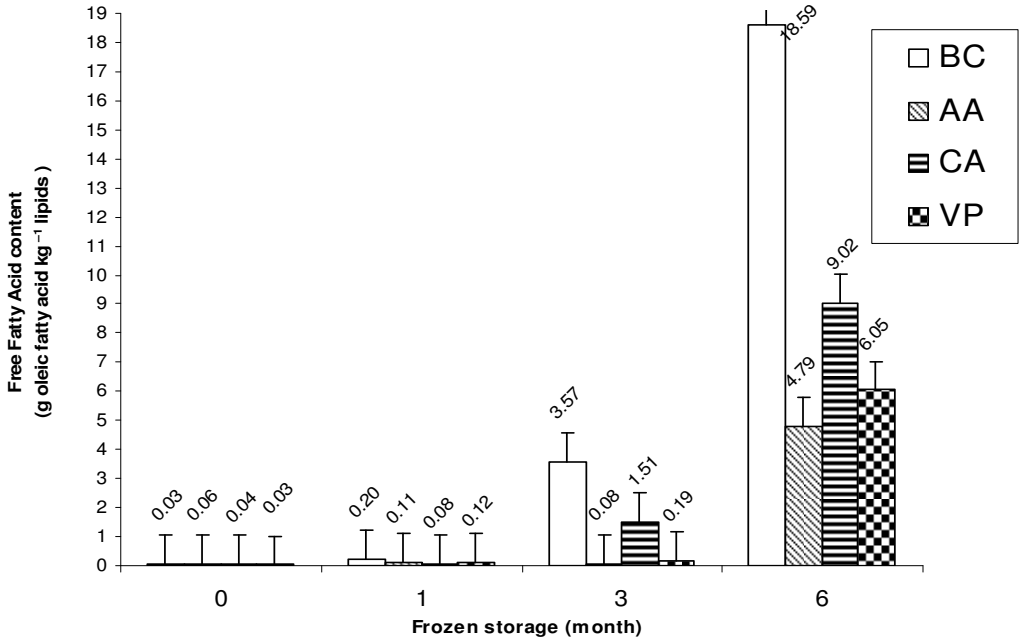
Results

Hydrolysis development (FFA content) increased ($p<0.05$) in all type of samples during frozen storage (Fig. 1). The antioxidant and vacuum packaging treatments led to lower value during the whole storage. Comparison of the different kinds of treatments led to higher ($p<0.05$) hydrolysis development at month 6 for BC samples while lower values were maintained throughout the whole experiment period for AA samples ($p<0.05$).

Table 1. 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)

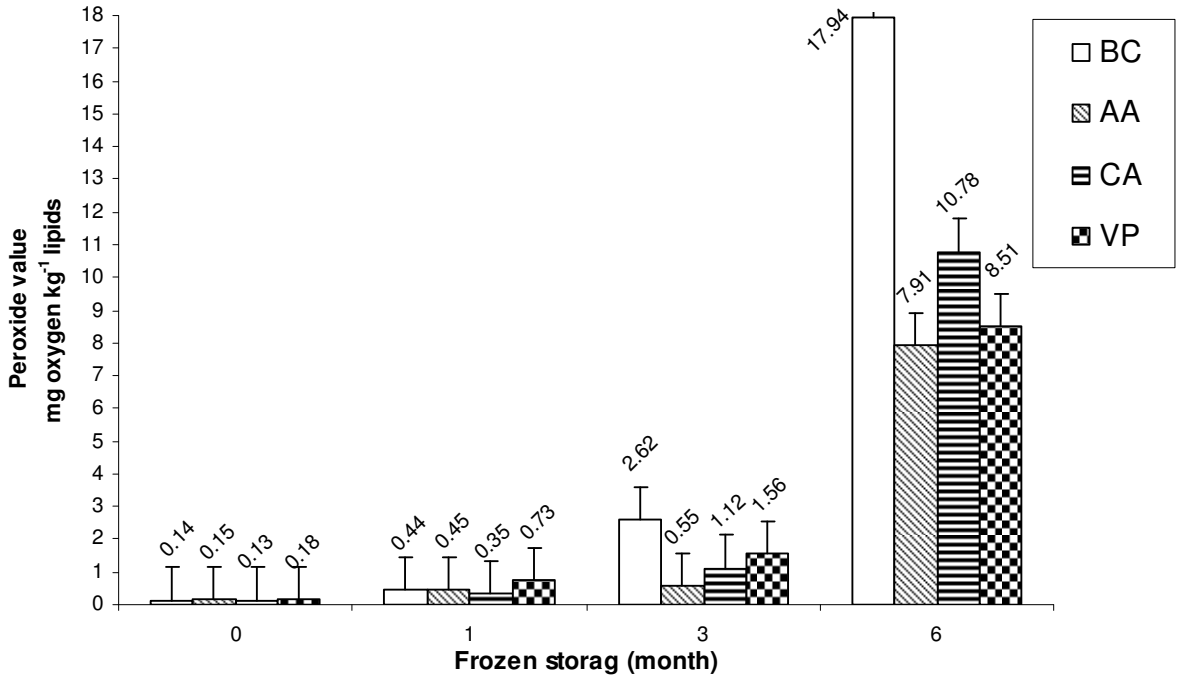


***** Bars denote standard deviation of the means.

Figure 1: FFA content of the Persian sturgeon fillet during frozen storage at -18 ºC

During frozen storage, a slow increase on the basis of primary oxidation products (peroxide values, PV) values was observed for each treatment, at sixth month a marked increase was observed for Blank control (Fig. 2). In Blank control significant difference ($p<0.05$) were obtained at 3 and 6 months and antioxidants and vacuum packaging treatments only after 3 month had significant change($p<0.05$). From the results, it is concluded that all three treatments had significant effect on delaying lipid oxidation but AA and vacuum packaging were the most effective treatments among them.

Secondary lipid oxidation products, as reported by the TBA-i, presented low values at the beginning of the study (Fig. 3) and gradually increased during frozen storage (as in the case of PV). A significant increase in Thiobarbituric acid TBA-i value was observed for control and CA-treated samples ($p<0.05$) compared with the other treatments during storage. pH values ranged between 6.15 and 6.92 among samples and decreased at during storage at freezer but no statistical difference were observed among treatments and Blank control($p<0.05$). The initial pH value of treated samples was lower than that in their corresponding control samples and this lower value was maintained during the 3-6 months period (Fig. 4).

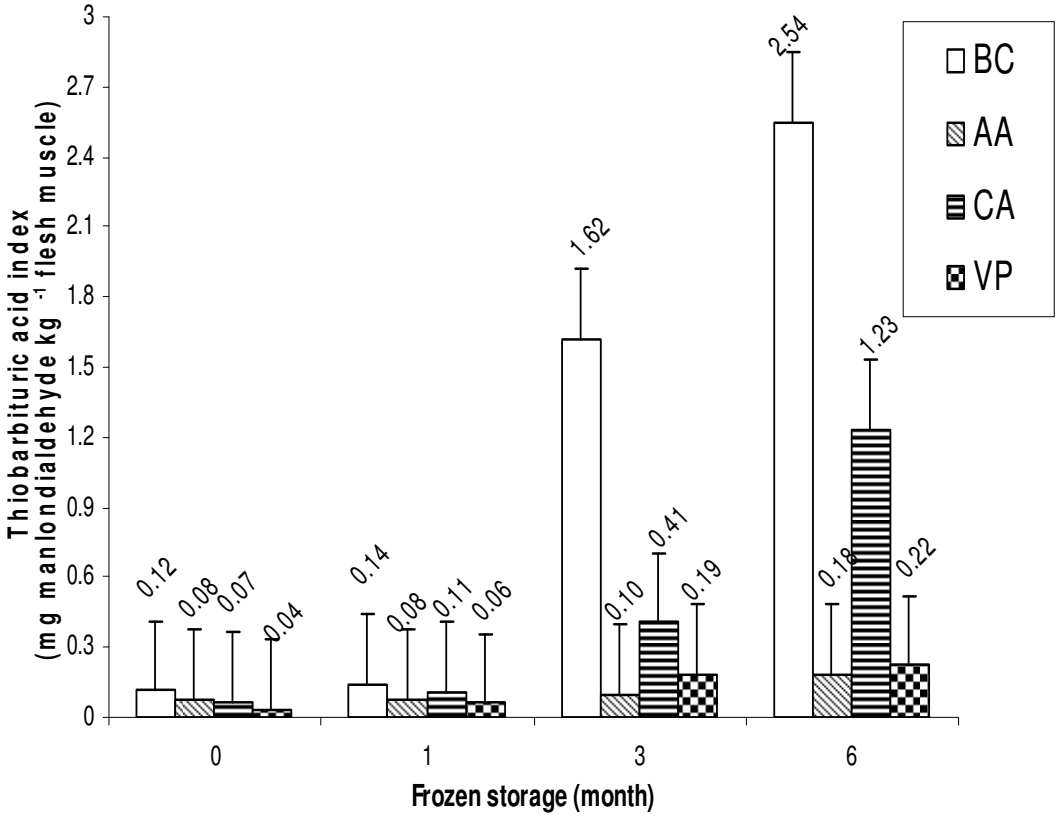


* Bars denote standard deviation of the means.

Figure 2: PV content of the Persian sturgeon fillet during frozen storage at -18 ºC

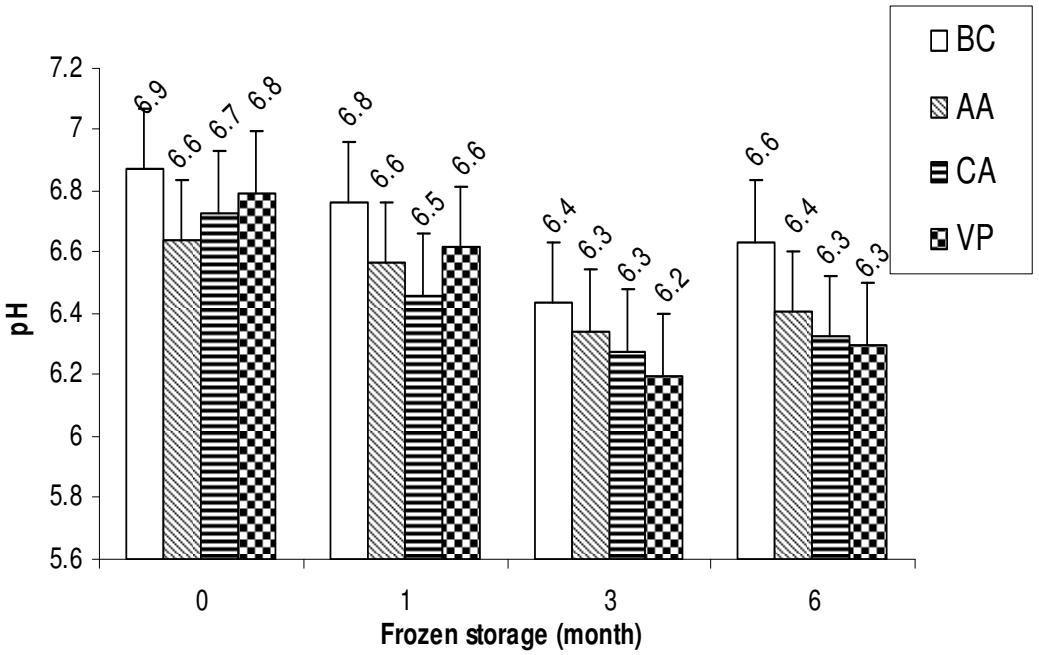
When vacuum packaging (VP) treatment samples showed a lower ($p<0.05$) pH value compared with other treatments, no significant differences were observed in the 3-6 months period among different treatments. Expressible moisture content

showed a gradual increase for all samples during the course of the study (Fig. 5). Comparison of the different treatments revealed that the antioxidant and vacuum packaging treatments had lower Expressible moisture content values compared to the Blank control but no significant differences were detected among the samples of antioxidant and vacuum packaging treatments throughout the whole experiment. Initially, odor, taste, color and appearance of fillet were natural and fresh. However, their quality deteriorated with time. Scores given to the four sensory indices decreased as storage time increased (Table 2). Flesh appearance assessment showed a lower ($P<0.05$) score at month 6 for the BC samples than other treatments. Odor analysis led to a better quality score ($P<0.05$) at month 3 for AA -treated samples than that for BC, CA and VP treatments. Flesh odor and flesh appearance in control samples at month 6 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.



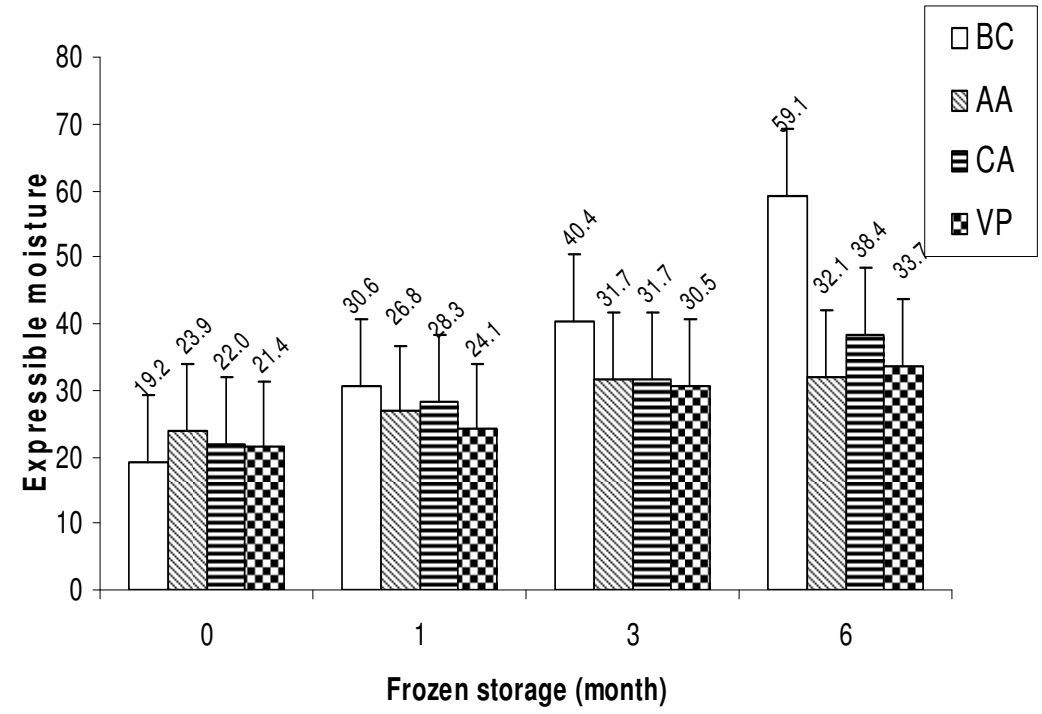
* Bars denote standard deviation of the means.

Figure 3: TBA content of the Persian sturgeon fillet during frozen storage at -18 ºC.



* Bars denote standard deviation of the means.

Figure 4: pH content of Persian sturgeon fillet during frozen storage at -18 ºC



* Bars denote standard deviation of the means.

Figure 5: Expressible moisture (%) of Persian sturgeon fillet during frozen storage at -18 ºC

In the end, flesh consistency assessment showed a better score at month 3 for AA- and vacuum packaging treatment samples, while at the end of the storage time no differences were obtained among the four kinds of the samples. Sensory analyses of attributes considered indicate that antioxidants particularly AA and packed under vacuum can slow down quality loss during frozen storage.

Table 2: Changes in 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	VP	BC	AA	CA	VP	BC	AA	CA	VP
0	E	E	E	E	E	E	E	E	E	E	E	E
1	A	E	E	E	A	E	E	E	A	A	A	A
3	B	A	B	A	B	A	B	B	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.

Discussion

Fats in fish are inclined to oxidation during storage. This is one of the most important reasons of spoilage of fish products due to formation of poisonous compounds and reduction of nutrition value (Sahoo *et al.*, 2004). Hydroperoxides are considered as primary products of lipid oxidation. Reaction between these products with other molecules leads to “off colour” and “off odour” in fish products (Lee *et al.*, 1998). Results showed that usage of all three treatments, 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 were decreased in compare with control samples ($p<0.05$) and AA and vacuum packaging treatments showed the best inhibitory effect on lipid oxidation. Anelich *et al*. (2001), Losada *et al*. (2004), Aubourg *et al*. (2004) and Fagan *et al*. (2004) reported that usage of antioxidants and packaging have positive effect on delaying fat spoilage. Also effects of ascorbic acid on frozen herring (Hamre and Sandnes, 2003), flaxseeds on rancidity development of frozen mackerel (Stodolnik *et al.*, 2005) and vitamins C & E on frozen

horse mackerel (Sarkardei and Howel, 2008) were investigated and results showed that increase of peroxide value in samples which were treated by antioxidants, was significantly lower than control samples.One of the most widely used tests to quantify lipid oxidation products in fish meat is thiobarbituric acid (TBA) test. In fact PV measurements are not reliable in assessing the oxidation of highly unsaturated oils such as fish oils. This is probably because the peroxides that form 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 & Borderias, 2008). In this study, results showed that TBA-i value of control and CA-treated samples were significantly higher than AA and vacuum packaging treatments ($p<0.05$). Usage of AA and vacuum packaging had the best influence on delaying lipid oxidation and increasing shelf-life of fillets ($p<0.05$). Benjakul *et al.* (2005), Sarkadei & Howell, (2008) and Sánchez-Alonso & Borderias, (2008), Pourashouri *et al.* (2009) reported lower TBA values in samples which were treated by antioxidants in compare to control samples.

Lipolysis of fats, leads to production of free fatty acids during storage. FFA are known to undergo further oxidation to produce low molecular weight compounds that are responsible for off-flavor and undesirable taste of fish and fish products (Vidya Sagar Reddy and Srikar, 1996; Refsgaard *et al.*, 2000). Also FFA has great influence on protein denaturation and texture deterioration by interaction with proteins (Mackie, 1993; Sikorski and Kolakowska, 1994; Lodasa *et al*., 2004). Results showed a gradual increase in FFA formation in all samples due to hydrolysis of phospholipids and triglycerides because of lipases and phospholipases (Serdaroğlu & Felekoglu, 2005). In this study use of antioxidants and vacuum packaging decelerate the developing process of FFA production during storage; same results were reported by Aubourg *et al*. (2002, 2005), Pourashouri *et al.* (2009) and Fagan *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 capacity due to denaturising of proteins (Suvanich, *et al.*, 2000). This phenomenon leads to reduction of flavour agents and nutrition value (Reddy & Srikar, 1991). In this study expressible moisture content showed a progressive increase in all samples during frozen storage. No significant differences were detected among all three treatments throughout the whole experiment. Similar results were reported by Chen *et al*., 1998 (on milk fish) and Pourashouri *et al*., 2009 (on wells catfish). Also Ozogol *et al* (2004) showed that samples which were packed under vacuum conditions had lower expressible moisture compared with control samples. Results of pH measurements showed that pH of antioxidant treated samples was lower than control samples in fresh and frozen fish fillets during six months storage. According to other researches, frozen storage did not have significant effect on pH changes during storage period (Aubourg *et al*., 2004).Results of sensory evaluation tests indicated that usage of antioxidants,

particularly AA, can slow down improper changes during frozen storage. Some similar results were reported by Fagan *et al.,* (2004) and Leaflet (2004) who found that antioxidant treatment and usage of vacuum packaging increased shelf-life and preserved sensory attributes during storage. Also previous experiments about effect of AA and CA treatments 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 kinds of samples (Pourashouri *et al.,* 2009).As a conclusion, results showed that the samples which were soaked in solutions of AA and CA, had significant differences in biochemical parameters which were studied in compare to control samples at 0, 1^{st}, 3^{ed} and 6^{th} months. This can be due effect of AA and CA as oxygen scavengers which can delay lipid oxidation by reducing necessary agents like oxygen and metals. Usage of AA, CA and vacuum packaging led to partial inhibition of quality loss and increased shelf-life of fillets and among all treatments ($p<0.05$), AA yielded the best results in preventing lipid oxidation development in frozen fillets. Thus the employment of AA and vacuum packaging alone or in combination with other protective strategies is recommended for the Persian sturgeon.

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