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Enhancement of the storage quality of frozen Persian sturgeon fillets by using of ascorbic acid

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Abstract: The effect of ascorbic acid (AA) soaking treatments on the rancidity development in Persian sturgeon (*Acipenser persicus*) fillets during frozen storage was studied. 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, primary and secondary oxidation products, expressible moisture and pH value of AA- treated samples were significantly lower than those in control samples($p<0.05$). Results indicate that AA was effective in reduce lipid oxidation of Persian sturgeon fillets and delayed lipid oxidation in frozen fillets. Thus the employment of AA alone or in combination with other protective strategies is recommended.

Keywords: *Persian sturgeon*, packaging, oxidation, antioxidant, ascorbic acid

Introduction

Fish lipids are known to be rich in polyunsaturated fatty acids (PUFAs), especially the n-3 PUFAs family including eicosapentaenoic acid (EPA or 20:5 n-3), docosapentaenoic acid (DPA or 22:5 n-3) and docosahexaenoic acid (DHA or 22:6 n-3). Marin lipids are known to be very prone to oxidation (Yildiz et al., 2006; Sánchez-Alonso and Borderias, 2008). Oxidation of lipids that occurs in raw material during storage, processing, heat treatment, and in the final products during subsequent storage, is one of the basic processes causing rancidity in food products (Selami and Sadok, 2008). Such oxidative deterioration may affect the organoleptic characteristics, including taste and aroma making the final product unacceptable for consumption. Rancid off-flavors are still the main objections in the production and commercialization of fish and foodstuffs containing fish oils (Pazos et al., 2005). Therefore, several investigations have been undertaken with the aim to enhance the shelf-life extension, the stability of lipid containing products and food quality (Selami and Sadok, 2008).

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 and Lin, 2005; Yildiz et al., 2006). The use of antioxidants is emerging as an effective methodology for controlling rancidity in oils and food (Frankel, 1998, Pazos et al., 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 and Howel, 2006).

Ascorbic acid (AA) is one of the natural antioxidant which used in order to protect meat products against oxidative change. Ascorbic acid removes oxygen and reduces the first step in the chain reaction of lipid oxidation. Moreover, ascorbic acid can shift the redox potential of food systems to the reducing range, act synergistically with chelators and regenerate primary anti-oxidants such as tocopherol (Mielnik et al., 2002). The positive effects of AA 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., 2009) have been observed.

This work aimed to study the antioxidant activity of ascorbic acid on extending shelf life of Persian sturgeon (*Acipenser persicus*) fillet because it is one of the most important fish species in Caspian Sea. In order to delay improper changes of unsaturated lipid during frozen storage some antioxidants or other preservative method should be used. Thus in this

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study effect of ascorbic acid (as natural antioxidants) on quality of fish fillets were investigated.

Material and Methods

Fresh Persian sturgeon (*Acipenser persicus*) was captured and kept on ice (1h) till delivery to the laboratory. Then, the fish were carefully gutted, dressed and filleted by hand and fillet as samples divided into 2 groups. Samples of the first group were left untreated (control samples; BC treatment) directly packaged traditionally in high density polyethylene bags and Second group were then immersed in 0.50% of Ascorbic acid aqueous solution (AA treatment). After 5 minutes, the samples removed out of the AA-solution, packaged in individual polyethylene bags. All samples were immediately frozen at -40 ºC. Antioxidant concentration and dipping time were chosen according to previous related research (Chapman et al., 1993; Aubourg et al., 2004). After stored at -40ºC for 24 h, all fish fillets were placed in a freezer at -18ºC. Sampling was undertaken at 1, 3 and 6 months after frozen storage at -18ºC and on the raw fish (initial material). For each treatment (BC and AA), 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, Germany).

Basic analyses

For measurement of 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).

Lipid damage measurements

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

Sensory analyses

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.

Statistical analysis

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 and Discussions

Evolution of general chemical parameters

The initial pH value of treated samples was lower than their corresponding control samples and this lower value was maintained during storage period (Figure 1); no significant differences were observed in the third and sixth months period among BC and AA treated samples. According to other researches, frozen storage did not have significant effect on pH changes during storage period (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 capacity due to denaturising of proteins (Suvanich et al., 2000). This phenomenon leads to reduction of flavour agents and nutrition value (Reddy and Srikar, 1991). In present study Expressible moisture content showed a gradual increase for all samples during the course of the study (Figure 2). Comparison of two treatments revealed that expressible moisture of AA treatment samples at 6th month of storage was lower than control samples ($p<0.05$). Similar results were reported by Chen et al. (1998) on milk fish, Pourashouri et al. (2009) on wells catfish and Garner et al. (2002) on channel catfish fillets.

Hydrolysis development

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 (Refsgaard et al., 2000). Also FFA has great influence on protein denaturation and texture deterioration by interaction with proteins (Losada et al., 2004). In this study, FFA (three glycerides and phospholipids groups) was measured in order

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)

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	BC	AA	BC	AA
1	A	E	A	E	A	A
3	B	B	B	A	B	A
6	C	B	C	B	B	B

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

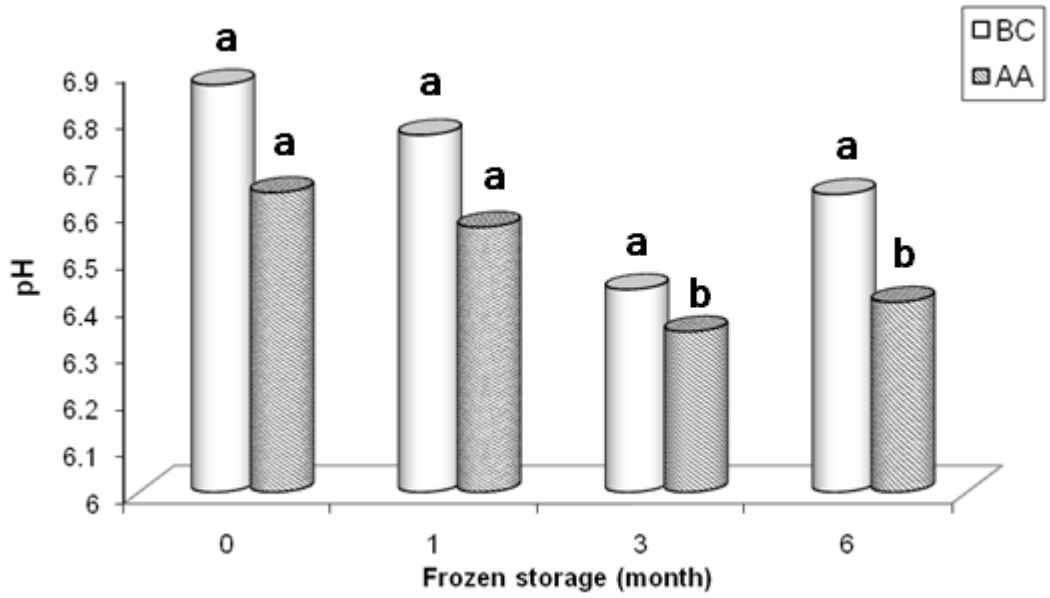

Figure 1. Changes of pH in Persian sturgeon fillets under different treatments during frozen storage. Mean values preceded by different letters denote significant differences ($P < 0.05$).

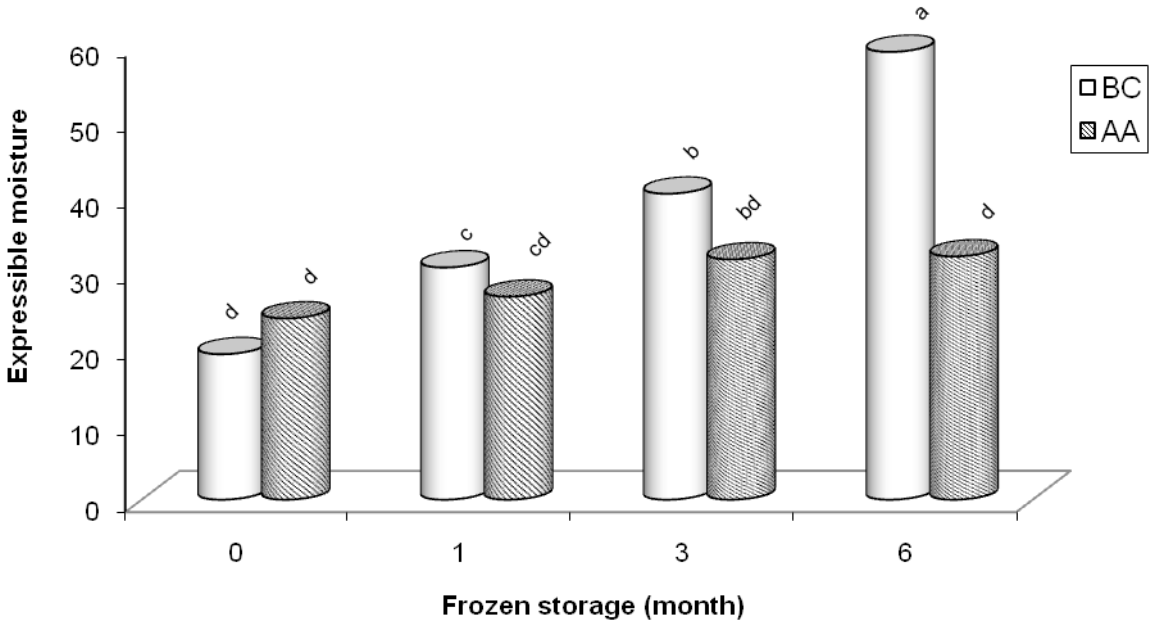

Figure 2. Changes of Expressible moisture in Persian sturgeon fillets under different treatments during frozen storage
Mean values preceded by different letters denote significant differences ($P < 0.05$).

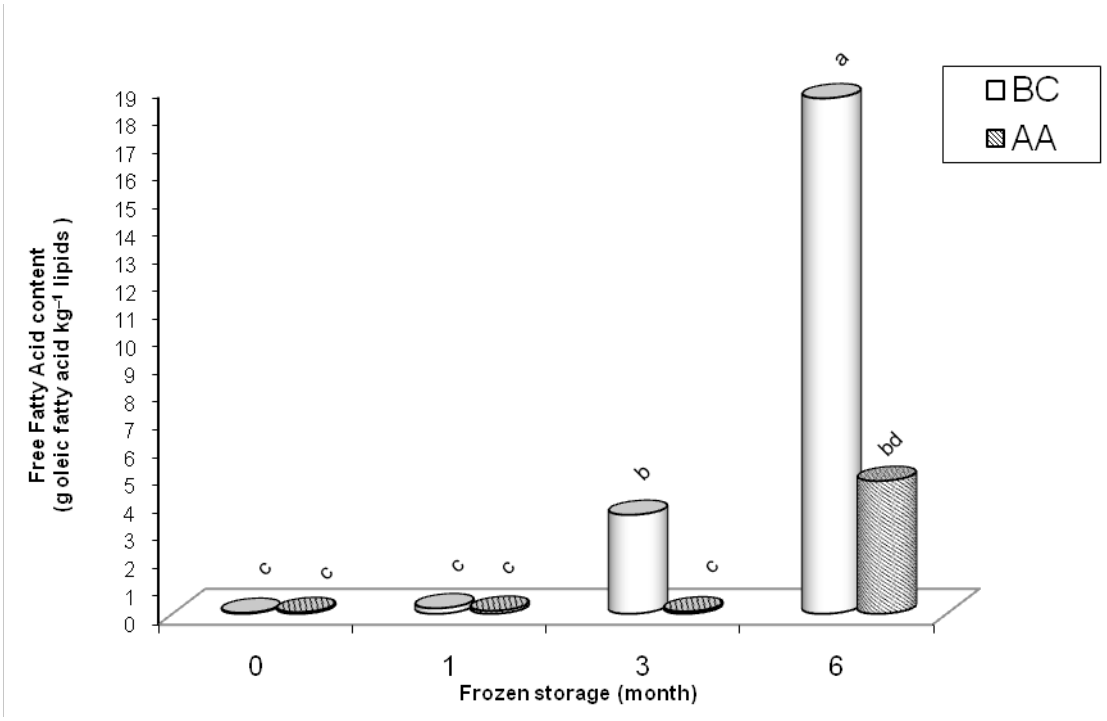

Figure 3. Changes of Free fatty acid in Persian sturgeon fillets under different treatments during frozen storage
Mean values preceded by different letters denote significant differences ($P < 0.05$).

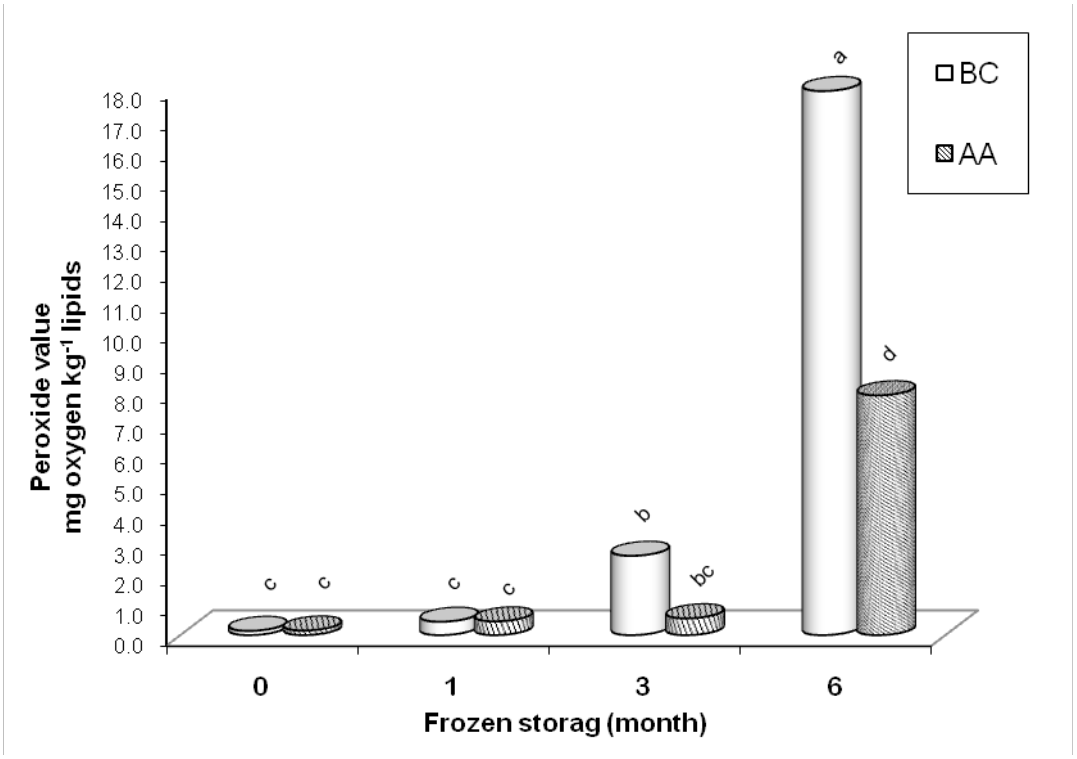

Figure 4. Changes of Peroxide value in Persian sturgeon fillets under different treatments during frozen storage
Mean values preceded by different letters denote significant differences ($P < 0.05$).

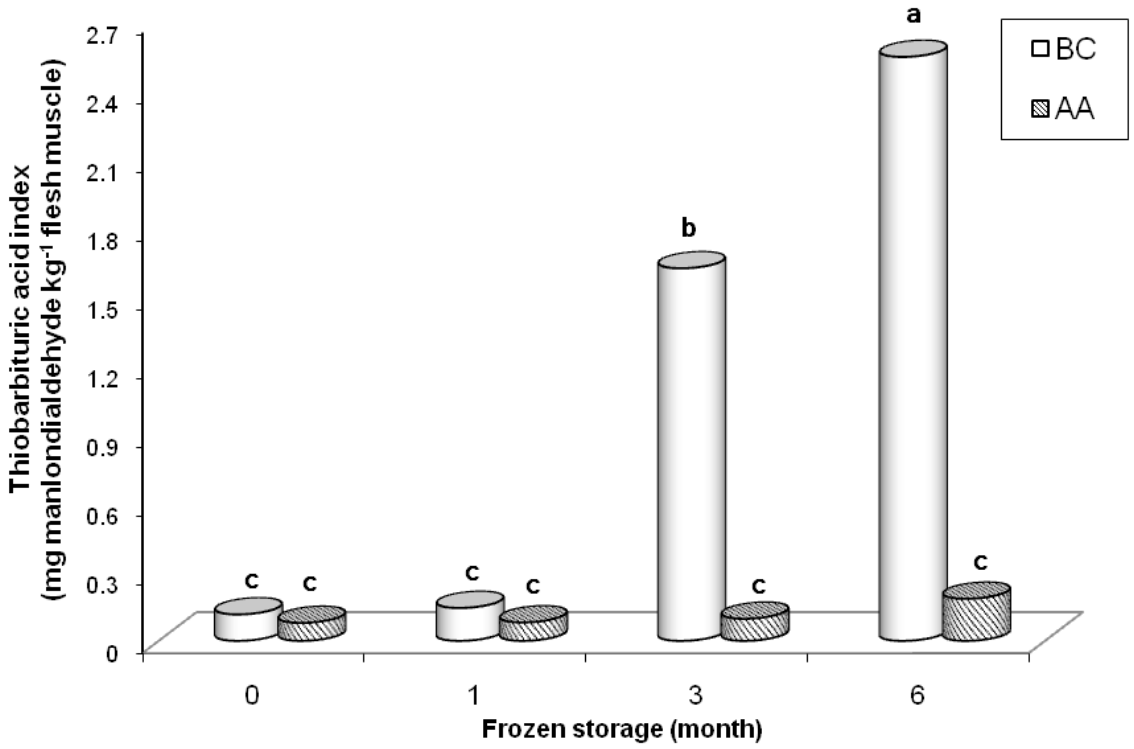

Figure 5. Changes of Thiobarbituric acid index in Persian sturgeon fillets under different treatments during frozen storage.
Mean values preceded by different letters denote significant differences ($P < 0.05$).

to investigate deterioration of fats. Results showed a gradual increase in FFA formation in all samples due to hydrolysis of phospholipids and triglycerides because of lipases and phospholipases (Serdaroğlu and Felekoglu, 2005) but AA treatment, decelerated developing process of FFA production during storage. Similar results were reported by Aubourg et al. (2004) and Pourashouri et al. (2009).

Lipid oxidation

In this study, PV was low at month 1 for all samples but increased at month 3 (Figure 4). Compared to month 3, a marked increase ($p<0.05$) was observed at month 6 for control samples. Comparison among two treatments showed significantly higher peroxide formation at month 6 for the control samples than AA treatment ($p<0.05$). From the results, it is concluded that AA treatments had significant effect on delaying lipid oxidation. 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 and Borderias, 2008). The thiobarbituric acid (TBA) 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 (Figure 3) and gradually increased during frozen storage (as in the case of PV). A significant increase in TBA-i value was observed for control samples ($p<0.05$) compared with the AA treatment during storage. Totally, results showed that Usage of ascorbic acid had positive influence on delaying lipid oxidation and increasing shelf-life of fillets ($p<0.05$). Benjakul et al. (2005), Pourashouri et al. (2009), Sarkadei and Howell (2008) and Sánchez-Alonso and Borderias (2008) reported lower TBA values in samples which were treated by antioxidants in compare to control samples.

Sensory analysis

At the beginning of the storage time odor, taste, color and appearance of fillet was natural and fresh. But their quality deteriorated with time. Scores given to the four sensory indices (odor, color of fillet, appearance and firmness of fillet) decreased

with storage time (Table 2). Flesh appearance assessment showed a lower ($P<0.05$) score at month 6 for the control samples than AA treatment. Also flesh consistency assessment showed a better score at month 3 for AA-treatment samples. In the end, Odor analysis led to a better quality score ($P<0.05$) at month 3 for AA -treated samples than that for control samples. 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. Sensory analyses of attributes considered indicate that ascorbic acid can slow down quality loss during frozen storage.

Results of sensory evaluation tests indicated that usage of ascorbic acid can slow down improper changes during frozen storage. Some similar results were reported by Leaflet (2004) and Fagan and Gormley (2004) who found that antioxidant treatment 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).

Conclusion

The effect of ascorbic acid in order to delay lipid oxidation was studied in Persian sturgeon fillets. Usage of ascorbic acid, 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 ascorbic acid (o.5%) had significant differences in biochemical parameters which were studied in compare with control during frozen storage. This can be due to using of AA which is oxygen scavenger and can delay lipid oxidation by reducing necessary agents like oxygen and metals. The use of ascorbic acid led to preservation of fillet quality, partial inhibition of quality loss and increase of shelf-life. In the end, the employment of ascorbic acid 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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