

Inhibitory effect of citric acid on rancidity of frozen catfish (*Silurus glanis*) fillets

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Abstract: The effect of aqueous solution of citric acid (CA) on the lipid stability of the European catfish (*Silurus glanis*) fillets during frozen storage (up to 6 months) was investigated. Rancidity development was measured by several biochemical indices (free fatty acids, peroxides and TBA) and complemented by the measurement of expressible moisture and sensory evaluation (flesh odor, consistency and flesh appearance). The CA treatment led to some lower free fatty acids (6.43% oleic acid in total lipid at month 6), peroxides (7.98meq oxygen/kg lipids, at month 6) and secondary compounds formation (2.16mg malondialdehyde/kg fish sample). Lower peroxide, thiobarbituric acid-reactive substance (TBA), free fatty acids (during 0, 3 and 6 months frozen storage), and expressible moisture (month 6) values were obtained from CA-treated fish fillets than the untreated (blank control) ones ($P<0.05$).

Keywords: Catfish, Lipid oxidation, Citric acid, Shelf life

Introduction

Lipid oxidation is one of the most important factors responsible for quality deterioration of fish during frozen storage due to its detrimental effects on color, flavor, texture, and nutritional value (Hettiarachy *et al.*, 1996; Serdaroglu &

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Felekoglu, 2005). The lipid fraction is therefore, the subjects of a great deal of attention since PUFAs are highly prone to oxidation (Perez-Alonso *et al.*, 2003). Reactions between the by-products derived from lipid oxidation and proteins cause undesirable changes of food properties including protein denaturation, loss of protein solubility, alteration of texture and functional properties of protein and destruction of nutrient components (Serdaroglu & Felekoglu, 2005). Addition of synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxy toluene (BHT), and tertiary butylated hydroquinone (TBHQ) can control lipid oxidation in foods (Hettiarachchy *et al.*, 1996). Recent efforts have been focused on the replacement of synthetic antioxidants by natural ones. Reports have shown that synthetic antioxidants may act as mutagenic and carcinogenic agents, while natural ones may provide nutritional and therapeutic effects (Aubourg, 2000). Natural antioxidants have been successfully employed with filleted fish (Aubourg, 2000; Saeed & Howell, 2002). In this work, acetic acid is selected as a natural antioxidant for being easily and cheaply available.

Large European catfish (wels or sheatfish), *Silurus glanis*, is distributed in the Caspian Sea and some other inland water basins in Iran (Abdoli, 1999). This species is selected for having a high fat content (6-8%), white and boneless flesh, which render it a fishery product of export value. Little information is available concerning quality changes undergone by this fish species as a result of storage or processing (Fauconneau & Laroche, 1996; Benjakul & Bauer, 2001).

This study was conducted to determine the effect of citric acid on the lipid stability of catfish fillets during frozen storage by means of a soaking in citric acid solution as the most practical pretreatment.

Materials and methods

Catfish (*S. glanis*) of 63cm and 1400g average length and weight, respectively, were collected in March 2006 from Anzali wetland (24 individuals). The ratio

between male and female of samples was 5:1. The fish were carefully gutted, dressed, and filleted by hand (room temperature ~20-22°C), divided in two groups and kept on ice. Individuals of the first group were left untreated (blank control), directly packaged in polyethylene bags and immediately frozen at -40°C. The other group was immersed in 0.5% CA aqueous solution (water temperature ~15°C). The fillets were removed after 5 min, packaged in polyethylene bags and frozen at -40°C. After 12 h at -40°C all fillets were moved to a -18°C freezer. Sampling was undertaken after 1, 3 and 6 months of frozen storage at -18°C and on the starting material (raw values). For each treatment, three different fish fillet were considered for each time and three samples were taken of each fillet and studied separately. The procedure was followed regardless of sex and stage of maturity.

The water content was determined by calculation of weight difference of the homogenized muscle (1-2g) before and after 24 h drying at 105°C; the results were expressed as g of water/100g muscle (Perez-Alonso *et al.*, 2003). Total lipid was determined according to Cunniff (1998). Quantity results were calculated as g lipid/100g wet muscle.

A piece of flesh (1cm×1cm) was weighed (A), placed between 2 pieces of weighed Whatman No. 42 filter paper, and put on a pyrex watch glass. Initial load of 500g was applied on the top of the sample for 5 min, followed with another 500g loading for an additional 20 min. After pressing with the loads samples were weighed (B). The drip under pressure was determined as (A)-(B) and calculated against sample weight (A) as a percentage (Suvanich *et al.*, 2000).

Lipid hydrolysis was determined according free fatty acid (FFA) assessment. FFA content was determined by adding 25ml of neutralized 96% alcohol (with a few drop of 0.1 N NaOH and adding of phenolphthalein) to 25ml of chloroform extract and it was titrated by 0.1 N NaOH until pink color. Results were expressed as % oleic acid in total lipid (Egan *et al.*, 1997).

Peroxide value (PV) was expressed as meq oxygen/kg lipids. 25ml of chloroform extract was mixed with 37ml of glacial acetic acid and 1ml of freshly prepared saturated potassium iodide solution. 30ml of water was added after 1 min and then titrated with 0.01 N sodium thiosulphate (starch as indicator) (Egan *et al.*, 1997).

The thiobarbituric acid index (TBA) was determined by macerating 10g of sample with 50ml of water (2 min) and washed with 47.5ml of water, also 2ml of 4 M hydrochloric acid was added. The mixture was heated until 50ml of its distillate collected at 10 min from the boiling time. 5ml of distillate and 5ml of TBA reagent were pipetted in a glass stoppered tube and heated for 35 min, then cooled in water for 10 min and the absorbency (D) was measured at 538nm with spectrophotometer (HACH, DR/2000, USA). TBA (mg malondialdehyde/kg fish sample) = 7.8 D. (Egan *et al.*, 1997).

Sensory analyses were conducted by a taste panel according of five to seven experienced judges, according to the guidelines presented in Table 1 (Stodolnik *et al.*, 2005). Four categories were ranked: highest quality (A), good quality (B), fair quality (C) and rejectable quality (D). For each time of experiments three fillets of each treatment thawed and assessed by individual members of taste panel. Scores among panelists were averaged.

Sensory assessment of fillets included the following parameters: flesh appearance, odor, and color. Scores were given: A = 4, B = 3, C = 2 and D = 1.

Data from the different quality measurement were subjected to the one-way ANOVA method to assess significant differences ($P < 0.05$) and comparison of mean was performed using a least-squares difference (LSD) method.

Results

Water content in the catfish white muscle ranged between 80.9-82.7% in CA treatment, and 83.5-80.77% in control samples, which showed significant changes in months 0 and 3 between two treatments. Lipid content ranged between 4.1-2.5% in CA treatment, and 3.59-2.2% in control treatment, that there was significant change between samples, in month 1.

Amounts of expressible moisture increased with the storage period (Fig. 1). Comparison between two treatments showed that percentages of expressible moisture were significant at months 1 and 6 and was lower in treated fillets at month 6 (41.73%).

Actual mean values of lipid hydrolysis increased ($P < 0.05$) in both kinds of samples during frozen storage, as FFA (Fig. 2). The citric acid treatment led to lower value along the whole storage except at month 1; however, differences were

significant ($P<0.05$) at months 1, 3 and 6. This increase indicates that hydrolytic activity of lipid constituents was occurring over time. Comparison among treatments showed significant differences ($P<0.05$) at 1, 3 months.

Primary lipid oxidation was followed by the PV. Its assessment in both treatments showed that it had a tendency to increase with time, which was especially sharp at the end of the experiments (month 6), (Fig. 3). Significant differences were obtained between untreated and treated fillets at month 6.

Formation of secondary oxidation products followed the gradual increase in both kinds of treatment with storage time (Fig. 4). There were significant differences at months 0, 1 and 6; inhibitory effect of citric acid ($P<0.05$) was detected at 0 and 6. At the end of storage period (month 6), the lowest TBA was observed in samples that were treated with citric acid.

In sensory assessment different attributes were analysed (Table 1). Treated samples provided a better quality score on flesh odor at month 6. Between all the scores, only flesh odor of treated samples had significant difference ($P<0.05$) at month 6 (Figs. 5, 6, 7).

Table 1: Scale employed for evaluating quality of frozen catfish fillets

Attribute	A (Highest quality)	B (Good quality)	C (Fair quality)	D (Rejectable quality)
Flesh Odor	Sharp seaweed and shellfish	Weak seaweed and shellfish	Slightly sour and incipient rancidity	Sharply sour and rancid
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 due to mechanical factors
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

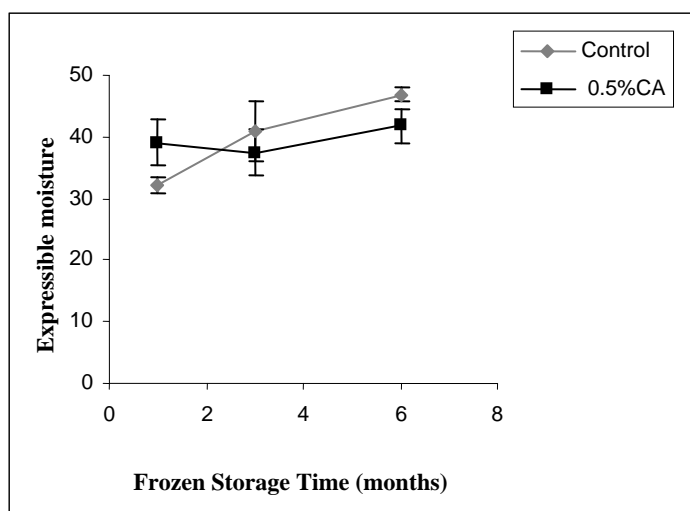


Figure 1: Expressible moisture (%) determination during frozen storage of untreated and treated catfish fillet

* Bars denote standard deviation of the means.

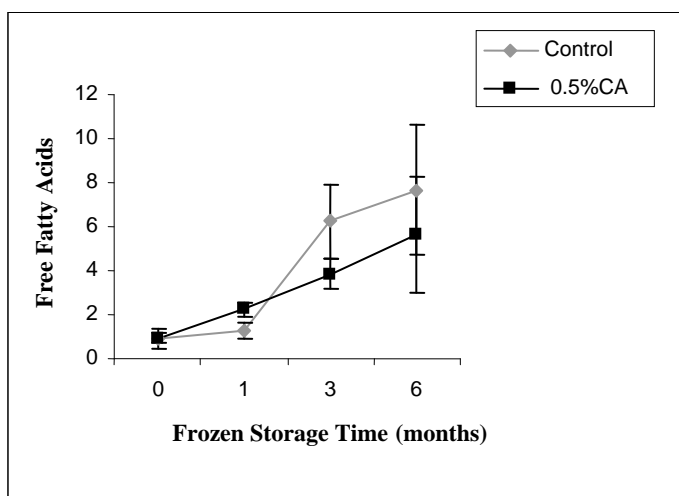


Figure 2: FFA determination during frozen storage of untreated and treated catfish fillet

*Bars denote standard deviation of the means.

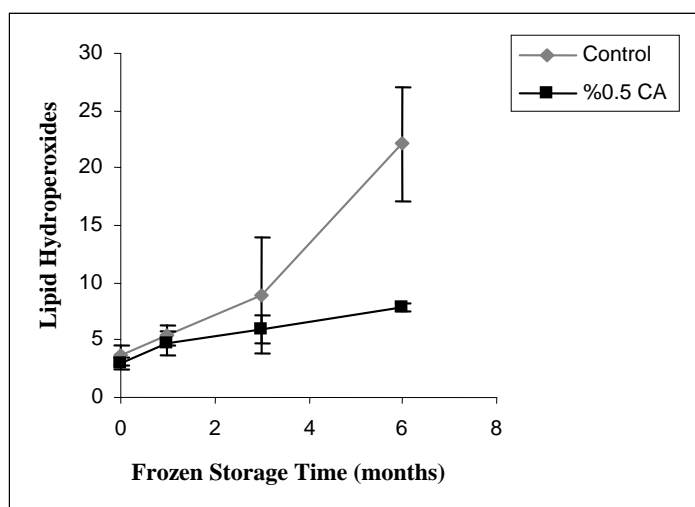


Figure 3: Peroxide value determination during frozen storage of untreated and treated catfish fillet.

* Bars denote standard deviation of the means.

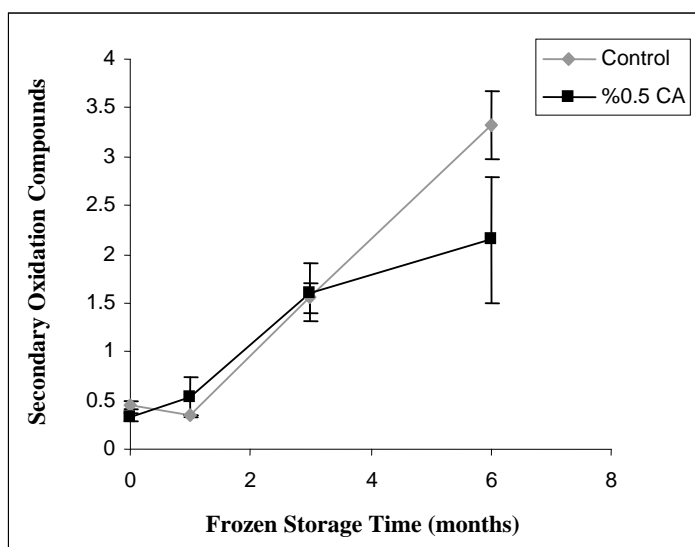


Figure 4: TBA determination during frozen storage of untreated and treated catfish fillet

* Bars denote standard deviation of the means.

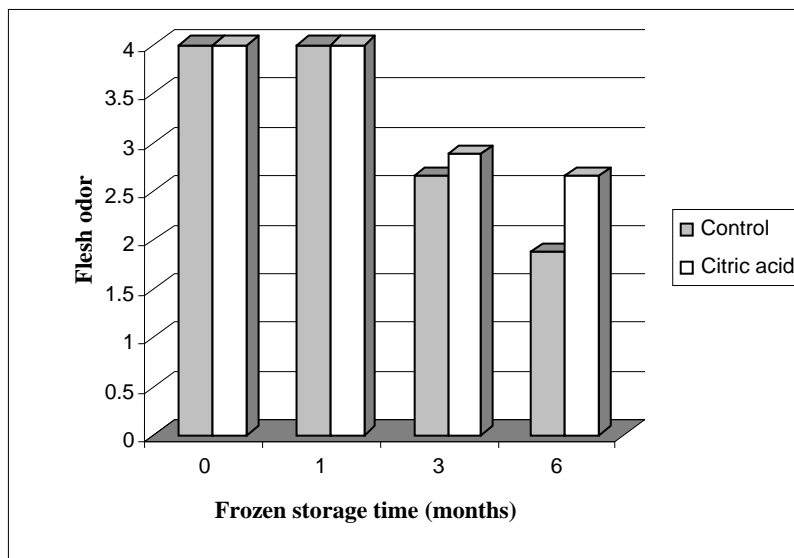


Figure 5: Changes in rancid odor value in frozen catfish fillet at -18°C.

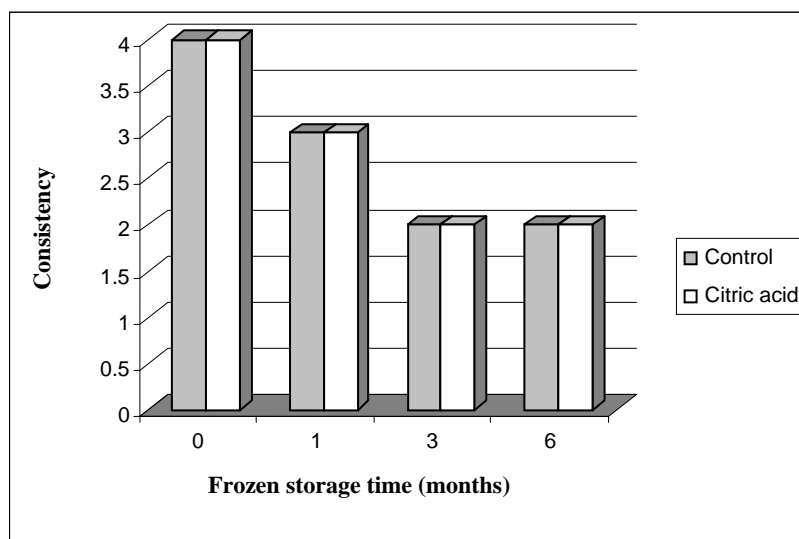


Figure 6: Changes in rancid consistency value in frozen catfish fillet at -18°C.

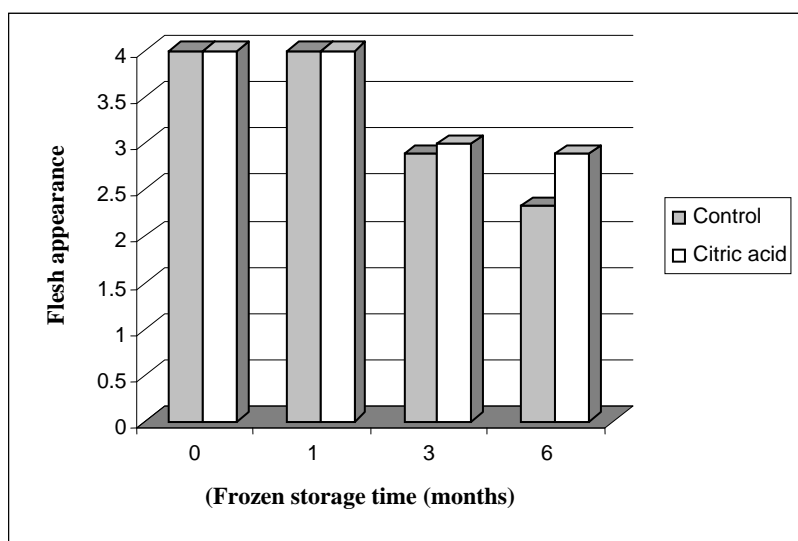


Figure 7: Changes in rancid appearance value in frozen catfish fillet at -18°C.

Discussion

During storage, variations in water and lipid contents were observed. The variations may be explained as a result of individual fish variations, according to previous researches on other frozen fish species (Craig *et al.*, 1996; Aubourg *et al.*, 2004; Stodolnik *et al.*, 2005).

Water-holding capacity of tissues decreased during frozen storage. Decreases in water-holding capacity lead to increased expressible moisture and protein denaturation (Suvanich *et al.*, 2000).

The formation of FFAs itself does not lead to nutritional losses. However, its examination was deemed to be important since it has been proved that the accumulation of FFAs is to some extent related to lipid oxidation enhancement and texture deterioration by interaction with proteins (Losada *et al.*, 2004). Further oxidation produces low molecular weight compounds that are responsible to the rancid off-flavor and taste of fish and fish products (Pacheco-Aguilar *et al.*, 2000).

Our results regarding FFAs formation are in agreement with Aubourg *et al.* (2004), who found significant changes in FFA for mackerel fillets treated with citric acid (0.05, 0.15 and 0.5%). Effects of using rosemary extract and onion juice on minced sardine (*Sardina pilchardus*) also showed that antioxidant treatments were more effective in decreasing lipid hydrolysis than untreated samples (Serdaroglu & Felekoglu, 2005).

Hydroperoxides formation occurred during storage in both untreated and treated samples. Aubourg *et al.* (2004) also found that addition of citric acid resulted in a decrease of PV and TBA in mackerel fillets during frozen storage. Li *et al.* (1998) illustrated that lipid hydroperoxides interacted with pigments and other macromolecules in fish, causing discoloration and off-flavor.

Tang *et al.* (2001) studied the effects of tea catechin and α -tocopherol on rancidity inhibition of raw minced red meat, poultry and fish muscle and found that antioxidants were more effective on fish. In this study, comparison between both kind of samples showed increases in TBA formation in all treatments during storage but untreated samples had most amounts of oxidized lipid.

Sensory evaluations showed that scores decreased with time for the attributes. These results were similar with previous results on fish species under similar conditions (Stodolnik *et al.*, 2005; Aubourg *et al.*, 2005).

Stodolnik *et al.* (2005) studied the effect of flaxseed (*Linum usitatissimum*) extract on mackerel. Their results showed that antioxidant treatment caused improvement of odor and appearance of fish flesh during frozen storage and extended shelf life of products.

Present results indicate that use of citric acid makes fish muscle less prone to oxidation and PV and TBA were lower in treated samples. Further research is enabling to find different kind of antioxidant compounds that are effective and so that have synergic effects will lead to a greater preservative approach and partial inhibition of the quality loss.

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اثر بازدارندگی اسید سیتریک در جلوگیری از رانسیدیتی فیله‌های منجمد اسبله (*Silurus glanis*)

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چکیده

تأثیر محلول اسید سیتریک بر ثبات چربی فیله‌های اسبله (*Silurus glanis*) طی نگهداری به صورت منجمد (تا ۶ ماه) بررسی شد. گسترش رانسیدیتی به کمک تعدادی از شاخص‌های بیوشیمیایی (اسیده‌های چرب آزاد، پراکسیدها و محصولات ثانویه اکسیداسیون) اندازه‌گیری شد و با اندازه‌گیری رطوبت تحت فشار و ارزیابی حسی (بو، استحکام و ظاهر فیله‌ها) کامل گردید. تیمار با اسید سیتریک سبب کاهش تشکیل اسیده‌های چرب آزاد (ماه ششم، ۷/۴۳ درصد اسید اولئیک در چربی کل)، پراکسید (۷/۹۸ میلی‌اکی والان اکسیژن در کیلوگرم چربی در ماه ششم) و ترکیبات ثانویه اکسیداسیون چربی (۲/۱۶ میلی‌گرم مالونالدئید در کیلوگرم ماهی) گردید. فیله‌های تیمار شده با اسید سیتریک در مقایسه با تیمار شاهد حاوی مقادیر کمتر پراکسید، مواد واکنش دهنده با تیوباربتوریک اسید، اسیده‌های چرب آزاد (طی صفر، ۳ و ۶ ماه نگهداری به صورت منجمد) و رطوبت تحت فشار (۶ ماه) بود.

کلمات کلیدی: ماهی اسبله، اکسیداسیون چربی، اسید سیتریک، ماندگاری

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