
Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of Red Tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*) fillets

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Abstract

The aim of the investigation was to determine the changes in fatty acids profile and chemical quality indexes of Red Tilapia fillets during frozen storage at -18°C. The fish were filleted by hand. The prepared fillets were then placed to the polyamide pouches and stored at -18°C for 150 days. Fatty acid profile, sensory properties and chemical quality indices were determined for a five month period. Results showed that 29 fatty acids were identified in the fresh and frozen samples. Polyunsaturated fatty acids (PUFA) were found higher than saturated fatty acids (SFA) in fresh samples but after 150 days of frozen storage this ratio became reverse. Oleic acid and Linoleic acid were the major MUFA (mono unsaturated fatty acids) and PUFA in fresh and frozen samples, respectively. The ratio of n3/n6 decreased from 0.59 to 0.49. The thiobarbituric acid value (TBA. Mg malondialdehyde/kg) increased significantly ($p < 0.05$) throughout the storage time from 0.03 to 1.26. Peroxide value (PV), Total volatile bases (TVB-N) and pH value also increased but were well within the limit of acceptability.

Keywords: Frozen storage, Fatty acids profile, Sensory evaluation, Tilapia

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Introduction

Fish is widely acknowledged for its nutritious value and is a major food source in many countries. Fish lipids have a high content of polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acids (EPA; 20:5n-3) and docosahexaenoic acids (DHA; 22:6n-3) (Pazos et al., 2005; Bayir et al., 2006). Freezing preservation of fish has been used for thousands of years because of high product quality Persson and Londahl (1993).

The concept of frozen storage relies on the lowering of the products temperature to slow down spoilage so that the thawed fish can retain the freshness (Kolbe et al., 2004).

However, fish and fishery products can undergo undesirable changes during storage and deterioration may limit the storage time. These undesirable changes result from protein denaturation (Fijuwara et al., 1998; Benjakul et al., 2005) and lipid oxidation (Sarma et al., 2000; Richards, 2002). The muscle proteins undergo a number of changes (causing insolubility and formation of aggregates) which modify their structural and functional properties Badii and Howell (2002).

Degradation of PUFA by lipid oxidation during storage leads to formation of volatiles associated with rancidity (Pazos et al., 2005). The high degree of unsaturated lipids makes fish tissues highly susceptible to peroxidation and rapid deterioration. Oxidative changes are

mainly related to taste and texture of the fish. In later stages of lipid peroxidation, changes in color and nutritional value are observed Dragoev et al. (1998).

Tilapia is the second most important group of farmed fish after carp and the most widely grown of any farmed fish (El-Sayed, 2006). It is farmed in at least 85 countries, with most production coming from Asia (China) and Latin America (Ecuador, Honduras and Costa Rica). Tilapia, as a freshwater fish species, has been one of the most widely cultured species all over the world due to its fast growth rate, easy cultivation and high feed efficiency ratio (El-Sayed, 2006). Many types of the tilapia products are available in the world markets. Today, fresh or frozen Tilapia fillets are available in different sizes and packages, as skin-on, skin-off, deep skinned, individually quick frozen, smoked and sashimi grade, and are treated by carbon monoxide or ozone dipped. Interesting byproducts have emerged such as leather goods for clothing and accessories, gelatin from skins for time-released medicines and flower ornaments made from dried and colored fish scales. Recently, tilapia was imported by the Iranian Fisheries Research Organization (IFRO) for its aquaculture adaptation and introducing the fish as new aquaculture specie. The current study was the first research on the Tilapia processing in the country. The aim of this study was to investigate the effects of frozen storage time on fatty acid composition (FAs), quality chemical indexes and sensory properties of the Red tilapia fillets.

Materials and methods

Sample preparation

Eighty Red tilapia (700 and 800 g in weight), which used in this study, was supplied by saline water fish research center of Yazd in May of 2011. The fish were gutted, beheaded and washed. The prepared samples were then covered with ice in the CSW boxes and transferred to the laboratory of the National Fish Processing Research Center in Anzali city. Skin-off and deboned fillets were produced by the worker. The fillets were washed by tap water and packed by Polyamide pouches and stored at -18°C for 150 days. Air-blast freezing was carried out at -18°C using an air speed of 3 m/s. Fatty acids composition, chemical quality indexes and sensory evaluation were determined on the fresh and frozen fillets monthly. All the analyses were performed in triplicate.

Fatty acid determination

Lipid of the samples was extracted according to Folch et al. (1957). Lipid extracts were then saponified with 0.5 N methanolic NaOH and further transesterified with BF_3 in methanol (AOAC, 2000). The fatty acids methyl esters were analyzed by gas chromatography using a GC Hewlett Packard, Agilent 6890 with 120 m long \times 0.25 mm internal diameter silica capillary column (BPX – 70 SGE, HP, USA) that equipped with a flame ionization detector and split injector. Nitrogen was used as the carrier gas at $20\text{ cm}^3\text{ min}^{-1}$, the temperature program was: an initial column

temperature of 140°C held for 5 min, then increased at $4^{\circ}\text{C min}^{-1}$ until it reached 170 and held for 3 min and then increased again at $2^{\circ}\text{C min}^{-1}$ until 200°C and maintained at 250°C . Fatty acid peaks in the samples were identified by comparing the retention times of the samples with that of the standard mixture of FAME (Supleco TM, 37 component FAME MIX) which contained from C4:0 to C22:6n-3.

Chemical analyses

Peroxide value (PV) expressed as milliequivalents of oxygen/kilogram of lipid were determined according to American Oil Chemist Society, (AOCS) (1994). Thiobarbituric acid value (TBA, mg malondialdehyde/Kg) was determined according to the method proposed by Kirk, 1991. Total Volatile basic Nitrogen (TVB-N) value was estimated by the micro-diffusion method Goulas and Kontominas (2005). pH was determined for the homogeneous mixture of fish and distilled water (1:10, w:v), using a digital Mettler Toledo pH meter Hernandez et al. (2009).

Sensory evaluation

Eight trained persons conducted sensory evaluation of the cooked Red Tilapia fillets. Panelists scored the fillets for color, odor, flavor, texture and general acceptability using a nine-point hedonic scale (1, dislike extremely to 9, like extremely) Lin and Morrissey (1994).

Statistical analyses

Statistical software Minitab ® Release 16, (Minitab Inc, Pennsylvania) was used to analyze the data in two and one way analysis of variance (ANOVA) for fat, moisture and fatty acids composition in samples.

Results

Changes in fatty acids profile (g/100g of total fatty acids) of fresh and frozen samples are shown in Table 1. Twenty nine fatty acids were identified in the samples. The fat content and fatty acid composition of fish vary according to the species, seasons and environmental conditions. The amounts of SFA, MUFA and PUFA in the fresh fillets were 27.12%, 39.01% and 33.52%, respectively. Comparison to the fresh sample, a significant ($P < 0.05$) decrease of PUFA was observed during the frozen storage, but the SFA and MUFA of the samples were found to increase. In the fresh samples, the highest amount of the SFA, MUFA and PUFA were C16:0 (16.87%), C18:1c (29.75%) and C18:2 n-6 (18.15%), respectively. The C22:6 n-3 and C20:5 n-3 fatty acids which are the most important of the fish lipid in nutrition. These two fatty acids decreased dramatically after 150 days of frozen storage. The total amount of n-3 (12.40%) fatty acids of the fresh fillets was less than the n-6 (20.83%) fatty acids. The ratio of n-3/n-6 was 0.59 of the fresh samples and this ratio decreased to 0.49 after 150 days of frozen storage.

Table 1: Changes in fatty acid composition of the fresh and frozen samples (means \pm SD), ($p < 0.05$; % of total lipids)

Fatty acid	Fresh	2th day	30thday	60th day	90th day	120th day	150th day
C12:0	0.04 \pm 0.00*	0.05 \pm 0.00*	0.05 \pm 0.00*	0.08 \pm 0.00*	0.09 \pm 0.00*	0.09 \pm 0.00*	0.11 \pm 0.01*
C14:0	2.74 \pm 0.13*	2.49 \pm 0.02*	2.55 \pm 0.03*	2.55 \pm 0.01*	2.69 \pm 0.08*	3.21 \pm 0.02*	3.11 \pm 0.00*
C15:0	0.32 \pm 0.01*	0.37 \pm 0.01*	0.37 \pm 0.00*	0.34 \pm 0.01*	0.42 \pm 0.00*	0.40 \pm 0.01*	0.42 \pm 0.03*
C16:0	16.87 \pm 0.14*	17.38 \pm 0.06*	17.88 \pm 0.03*	17.98 \pm 0.03*	17.80 \pm 0.00*	18.25 \pm 0.03*	18.74 \pm 0.03*
C17:0	0.76 \pm 0.01*	0.71 \pm 0.01*	0.82 \pm 0.01*	0.67 \pm 0.03*	0.71 \pm 0.01*	0.68 \pm 0.00*	0.27 \pm 0.01*
C18:0	5.65 \pm 0.03*	5.94 \pm 0.01*	6.28 \pm 0.10*	6.45 \pm 0.03*	6.43 \pm 0.01*	6.38 \pm 0.01*	6.85 \pm 0.01*
C20:0	0.29 \pm 0.01*	0.28 \pm 0.01*	0.27 \pm 0.01*	0.25 \pm 0.01*	0.40 \pm 0.01*	0.22 \pm 0.01*	0.17 \pm 0.03*
C22:0	0.13 \pm 0.01*	0.13 \pm 0.01*	0.12 \pm 0.01*	0.12 \pm 0.01*	0.16 \pm 0.01*	0.10 \pm 0.01*	0.07 \pm 0.01*
C24:0	0.32 \pm 0.01*	0.38 \pm 0.01*	0.32 \pm 0.01*	0.29 \pm 0.03*	0.65 \pm 0.01*	0.32 \pm 0.01*	0.36 \pm 0.01*
Σ SFA	27.12 \pm 0.22*	27.73 \pm 0.011*	28.66 \pm 0.28*	28.73 \pm 0.18*	29.35 \pm 0.09*	29.65 \pm 0.22*	30.10 \pm 0.31*
C14:1	0.32 \pm 0.02*	0.33 \pm 0.03*	0.33 \pm 0.01*	0.48 \pm 0.01*	0.29 \pm 0.01*	0.54 \pm 0.01*	0.59 \pm 0.01*
C15:1	0.11 \pm 0.00*	0.12 \pm 0.01*	0.11 \pm 0.01*	0.10 \pm 0.01*	0.11 \pm 0.00*	0.13 \pm 0.01*	0.11 \pm 0.01*
C16:1	6.72 \pm 0.08*	5.58 \pm 0.12*	6.03 \pm 0.05*	6.35 \pm 0.02*	7.01 \pm 0.06*	6.82 \pm 0.03*	7.75 \pm 0.03*
C17:1	0.61 \pm 0.01*	0.69 \pm 0.02*	0.62 \pm 0.01*	0.68 \pm 0.01*	0.76 \pm 0.02*	0.87 \pm 0.01*	0.66 \pm 0.03*
C18:1	0.29 \pm 0.11*	0.32 \pm 0.01*	0.31 \pm 0.01*	0.36 \pm 0.01*	0.33 \pm 0.01*	0.38 \pm 0.03*	0.43 \pm 0.00*
C18:1 α n-9	29.75 \pm 0.20*	31.56 \pm 0.04*	31.99 \pm 0.05*	32.10 \pm 0.01*	32.96 \pm 0.03*	33.44 \pm 0.06*	33.45 \pm 0.03*
C20:1	0.24 \pm 0.02*	0.28 \pm 0.02*	0.24 \pm 0.03*	0.29 \pm 0.01*	0.19 \pm 0.01*	0.34 \pm 0.01*	0.55 \pm 0.03*
C22:1	0.24 \pm 0.03*	0.22 \pm 0.00*	0.37 \pm 0.01*	0.27 \pm 0.01*	0.31 \pm 0.01*	0.39 \pm 0.03*	0.43 \pm 0.01*
C24:1	0.73 \pm 0.02*	0.82 \pm 0.02*	0.61 \pm 0.01*	0.49 \pm 0.01*	0.56 \pm 0.01*	0.51 \pm 0.01*	0.53 \pm 0.01*
Σ MUFA	39.01 \pm 0.19*	39.82 \pm 0.12*	40.61 \pm 0.30*	41.12 \pm 0.11*	42.52 \pm 0.14*	43.42 \pm 0.19*	44.50 \pm 0.30*
C18:2 α	0.29 \pm 0.01*	0.29 \pm 0.01*	0.25 \pm 0.02*	0.32 \pm 0.01*	0.30 \pm 0.01*	0.25 \pm 0.04*	0.34 \pm 0.03*
C18:2 α n-6	18.15 \pm 0.06*	17.55 \pm 0.03*	18.12 \pm 0.01*	18.01 \pm 0.05*	15.62 \pm 0.03*	15.67 \pm 0.03*	14.16 \pm 0.02*
C20:2 n-6	0.81 \pm 0.13*	0.69 \pm 0.02*	0.62 \pm 0.01*	0.71 \pm 0.01*	0.70 \pm 0.01*	0.69 \pm 0.03*	0.65 \pm 0.01*
C18:3 n-6	0.23 \pm 0.01*	0.34 \pm 0.01*	0.29 \pm 0.01*	0.35 \pm 0.01*	0.35 \pm 0.01*	0.34 \pm 0.01*	0.29 \pm 0.01*
C18:3 n-3	1.98 \pm 0.00*	1.92 \pm 0.03*	2.08 \pm 0.01*	2.27 \pm 0.01*	1.87 \pm 0.02*	1.90 \pm 0.01*	1.91 \pm 0.08*
C20:3 n-6	0.31 \pm 0.01*	0.33 \pm 0.01*	0.29 \pm 0.01*	0.32 \pm 0.01*	0.45 \pm 0.01*	0.32 \pm 0.01*	0.28 \pm 0.01*

C18:4 n-3	1.35±0.01 ^a	1.40±0.04 ^a	1.14±0.02 ^a	1.50±0.02 ^a	1.23±0.01 ^a	1.27±0.03 ^a	1.28±0.03 ^a
C20:4 n-6	1.33±0.02 ^a	1.36±0.03 ^a	1.11±0.01 ^a	0.99±0.03 ^a	1.01±0.01 ^a	0.82±0.01 ^a	0.83±0.03 ^a
C20:5 n-3	0.63±0.03 ^a	0.67±0.01 ^a	0.63±0.02 ^a	0.53±0.01 ^a	0.33±0.01 ^a	0.62±0.01 ^a	0.30±0.03 ^a
C22:5 n-3	1.49±0.02 ^a	1.47±0.05 ^a	1.50±0.03 ^a	1.28±0.01 ^a	1.06±0.01 ^a	1.01±0.11 ^a	1.02±0.01 ^a
C22:6 n-3	6.95±0.12 ^a	6.05±0.03 ^a	5.04±0.14 ^a	4.39±0.12 ^a	3.88±0.08 ^a	3.60±0.21 ^a	3.42±0.18 ^a
Σ PUFA	33.52±0.21 ^a	32.07±0.22 ^a	31.07±0.24 ^a	30.67±0.29 ^a	26.80±0.15 ^a	26.49±0.32 ^a	24.8±0.41 ^a
Other	0.83±0.06	0.90±0.01	0.49±0.06	0.33±0.03	0.21±0.01	0.25±0.03	0.16±0.01
Σ n-3	12.40±0.12 ^a	11.51±0.17 ^a	10.39±0.11 ^a	9.97±0.15 ^a	8.37±0.21 ^a	8.41±0.20 ^a	7.93±0.12 ^a
Σ n-6	20.83±0.16 ^a	20.27±0.12 ^a	20.43±0.24 ^a	20.38±0.11 ^a	18.13±0.19 ^a	17.84±0.09 ^a	16.21±0.11 ^a
n-3/n-6	0.59 ^a	0.57 ^a	0.51 ^a	0.49 ^a	0.46 ^a	0.47 ^a	0.49 ^a
PUFA/SFA	1.23 ^a	1.16 ^a	1.08 ^a	1.07 ^a	0.91 ^a	0.89 ^a	0.82 ^a
EPA+DHA/C16:0	0.45 ^a	0.39 ^a	0.32 ^a	0.27 ^a	0.24 ^a	0.23 ^a	0.20 ^a

□

^{a,b,c,d,e,f} Means in the same column followed by different superscripts are significantly different ($p < 0.05$).

¹ SFA= Saturated Fatty acid

² MUFA= Mono Unsaturated Fatty acid

³ PUFA= Poly Unsaturated Fatty acid

The changes of chemical quality indexes (PV, TBA, TVB-N and pH) of samples during frozen storage are shown in Table 2.

As indicated in Table 2, PV showed significantly ($P<0.05$) differences after 150 days of storage at -18°C . The highest value of PV was observed in 150th day (0.93 meq/kg).

Secondary lipid oxidation was studied by thiobarbituric acid (TBA) value. TBA records revealed an increased rate of lipid

oxidation during frozen storage of the samples. A significant ($P<0.05$) increase in TBA (from 0.03 in fresh samples to 1.26) was observed at the end of the storage. TVB-N is a commonly used chemical method to determine spoilage of fish. The initial TVB-N content of the samples, used in this study was 12.63 mg/100g flesh. During the storage time, TVB-N was increased to 21.93 mg/100g of flesh. An increase of pH value was also found in fresh samples as compared to the frozen once.

Table2: Changes in TBA, PV, TVB-N and pH values of samples during frozen storage at -18°C

Freezing time (Days)	TBA	PV	TVB-N	pH
Fresh (control)	0.03 ± 0.04^a	0.02 ± 0.01^a	12.63 ± 0.05^a	6.26 ± 0.05^a
2	0.03 ± 0.01^a	0.05 ± 0.01^a	12.66 ± 0.11^a	6.36 ± 0.05^a
30	0.08 ± 0.11^a	0.15 ± 0.12^b	18.36 ± 0.15^b	6.52 ± 0.05^b
60	0.16 ± 0.08^b	0.26 ± 0.11^c	19.86 ± 0.21^c	6.63 ± 0.05^b
90	0.59 ± 0.05^c	0.53 ± 0.11^d	20.73 ± 0.06^d	6.66 ± 0.05^b
120	0.83 ± 0.10^d	0.76 ± 0.05^e	21.60 ± 0.12^e	6.70 ± 0.00^b
150	1.26 ± 0.10^e	0.93 ± 0.11^f	21.93 ± 0.22^e	6.88 ± 0.05^c

^{a, b, c, d, e} Means in the same column followed by different superscripts are significantly different ($p<0.05$).

Each value is a mean \pm SD of triplicate determinations.

The sensory qualities of the samples were evaluated in terms of color, odor, taste, texture and general acceptability (Table 3). The sensory scores decreased progressively with the storage time in the fillets ($p<0.05$).

Table3: Sensory evaluation scores of samples during frozen storage at 18 C

Freezing time (Days)	Color	Odor	Taste	Texture	General acceptability
Fresh	9.00±0.00 ^a	9.00±0.00 ^a	8.75±0.46 ^a	8.75±0.46 ^a	8.75±0.35 ^a
2	8.66±0.51 ^a	8.62±0.51 ^{ab}	8.62±0.51 ^a	8.37±0.51 ^{ab}	8.62±0.51 ^{ab}
30	8.16±0.75 ^{ab}	8.12±0.64 ^{bc}	8.25±0.42 ^{ab}	8.00±0.53 ^{ab}	8.00±0.53 ^{bc}
60	7.33±0.81 ^{bc}	7.50±0.53 ^c	7.62±0.30 ^b	7.62±0.74 ^b	7.57±0.53 ^c
90	6.83±0.75 ^c	6.37±0.51 ^d	6.37±0.21 ^c	6.25±0.70 ^c	6.42±0.56 ^d
120	5.50±0.54 ^d	5.62±0.51 ^{de}	5.25±0.30 ^d	5.37±0.51 ^{cd}	5.42±0.78 ^e
150	5.16±0.40 ^d	5.12±0.64 ^e	4.50±0.53 ^d	4.62±0.74 ^d	4.57±0.50 ^e

^{a, b, c, d, e} Means in the same column followed by different superscripts are significantly different ($p < 0.05$).

Discussion

The amounts of the fatty acid fractions in the fresh sample were MUFA > PUFA > SFA however, at the end of the storage these amounts were changed to MUFA > SFA > PUFA. Our results were similar to the findings on Red tilapia fatty acids changes during storage at -20°C for 30 weeks which reported by Ng and Bahurmiz (2009). The differences in the fatty acid composition had a decisive role in the formation of hydroperoxide. The oxidative changes in frozen fish lipids may be caused by the occurrence of radicals, which are indicators of this process. During frozen storage tilapia flesh is very susceptible to lipid oxidation due to its high PUFA content Young (2009). In fresh and frozen samples DHA was more than EPA content although both of them were decreased at the end of storage ($p < 0.05$). The same results were found in Red hybrid tilapia and Nile tilapia (Rasoarahona et al., 2005; Ng et al., 2009). The n-3/n-6 ratio has been suggested to be a useful indicator for comparing relative nutritional value of fish oils. It was suggested that a ratio of 1/5 would

constitute a healthy human diet Osman et al. (2001). In the present study, n-3/n-6 had a recommended ratio at fresh samples (0.59) and even at the end of frozen storage (0.49). The PUFA/SFA ratio reveals that fish is a good source of PUFA Andrade et al. (1995). In our study this ratio was 1.23 for the fresh tilapia fillet. The negative relationship occurs between this ratio and storage time Henderson and Tocher (1987). At the end of frozen storage period, this ratio was reduced as 0.82.

The EPA+DHA/ C16:0 ratios (Polyene Index) is a good index to determine lipid oxidation Taheri et al. (2012). In this study, this ratio was decreased (from 0.45 to 0.20) significantly ($p < 0.05$). the same results were found in Mackerel (Sahari et al., 2009) , and Cobia (Taheri et al., 2012). The negative relationship between this ratio and storage time showed that oxidation mechanisms are active during storage. The frozen storage time resulted in the increase ($p < 0.05$) in PV from 0.02 to 0.93 meq O₂/kg lipid. This increase could be related to the lipid oxidative deterioration. Ben-Gigirey et al. (1999) reported that increase of

PV in frozen fish in contrast with fresh fish showed development of rancidity during frozen storage. The increasing of TBA in fillets was observed ($p < 0.05$) during storage time, that indicated lipid oxidation during frozen storage. The increasing of the TBA value has been demonstrated by previous studies (Liu et al., 2010; Tokur et al., 2004). It is also important to note that TBA records may not reveal the actual rate of lipid oxidation, since malonaldehyde can interact with other compounds in the fish body such as amines, nucleosides and nucleic acid, proteins, phospholipids or other aldehydes that are end-products of lipid oxidation, and this interaction may vary greatly with the species of fish (Sarma et al., 2000). Lakshmisha et al. (2008) reported that a maximum level of 5 mg malonaldehyde/kg of fish indicates good quality for fish frozen, chilled or stored with ice, while the fish may be consumed up to the level of 8 mg malonaldehyde/kg. In the present study TBA values of the samples were well within the acceptability at the end of storage. Lipid deterioration is the main cause of reduced shelf life of fatty acid due to progressive oxidation and enzymatic hydrolysis of unsaturated fatty acids (Sarma et al., 2000). In the present study PV and TBA increased significantly ($p < 0.05$) during frozen storage and oxidation of these unsaturated fatty acids causes lipid deterioration. During the storage time, TVB-N increased to 21.93 mg/100g flesh. The levels of 30 to 35 mg/100g muscle are considered the limit of acceptability of the fish (Connel, 1995). In the present study, the TVB-N content was well within the limit of acceptability. This could be attributed to the freezing effect, as it inhibits the bacterial activity. pH value of the samples were increased ($p < 0.05$) with storage time, that indicating alkaline compounds were accumulated through autolytic activities or microbial metabolism (Tokur et al., 2004; Pons-Sanchez-Cascado et al., 2006; Liu et al.,

2010). Decreasing in sensory score indicated the loss of freshness in samples (Table 3). This could be due to lipid oxidation and rate of rancidity (Connel, 1995). The sensory data show that the samples were still in acceptable condition at the end of storage. The results of the investigation indicated that the tilapia fillets had the best quality before frozen at -18°C and after 150 days reduced the quality of fillet but were well within the limit of acceptability.

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