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Deterioration Indices and Histological Changes in the Nile Tilapia (*Oreochromis niloticus*) by Different Freezing Methods

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ABSTRACT: The present study aimed to investigate the effects of freezing speed and time on the deterioration indices such as Total Volatile Basic Nitrogen (TVB-N), Peroxide Value (PV), Thiobarbituric acid value (TBA), and pH, as well as the histological changes. Eighty Nile tilapias (*Oreochromis niloticus*) with approximate weight of 700 ± 50 g were caught from the saline water fish in research centre of Yazd, Iran in 2012**.** Then, the fillets were harvested and exposed to slow and quick freezing at 0.2 cm/h speed in the freezer at -18 °C during 18 hours, and 0.8 cm/h speed at -30 °C for 25 minutes, respectively. Finally, the fillets were transferred to -18 °C freezer where they were kept for 180 days. Based on the results, the changes in TVB-N, PV, TBA, pH and histological changes were less dramatic in the samples treated by quick freezing, compared to the slow freezing samples ($p \leq 0.5$).

Keywords: Chemical Quality, Freezing, Histological Change, SEM, Tilapia.

Introduction

Fish begins to spoil immediately after death, which is reflected in developing undesirable flavours gradually, softening the flesh, and losing the fluid containing protein and fat dramatically. Spoilage can be retarded by lowering the temperature of the dead fish and spoilage can be almost stopped if the temperature is kept low enough (Anon, 2010).

Freezing is considered as the most popular means of preserving the quality of fish and fish products with the highest quality. Among the advantages of freezing, we can refer to quality maintenance, the appearance, and the nutritional value of the products. The time of passage from the critical point is considered as one of the most important factors in freezing and decreasing the time leads to better quality of frozen fish after thawing by increasing the freezing speed (Hall, 2011). Preserving the quality, increasing shelf-life, delivering fish to high-consumption markets, and supplying surplus fisheries throughout the year are some other positive aspects of freezing the fish and fisheries (Karami *et al.*, 2013).

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However, the freezing process alone is not a method for preservation. It is merely the means of preparing the fish for storage at a suitably low temperature. In addition, freezing should be accomplished quickly in order to produce a good product. A freezer should be particularly designed for this purpose and freezing is a separate process from low temperature storage (Anon, 2010).

Freezing speed plays a critical role on the nature of the changes taking place during the freezing process in the cell. In addition, the amount of the ice crystals formed has an apparent effect on damaging the cell, contracting cytoplasmic osmosis and cell cellular, and losing the water storage capacity (Sacks *et al.*, 1993).

There are four methods for freezing fish and fish products including slow freezing, quick freezing, rapid freezing, and ultra-rapid freezing. The freezing process is slow, quick, rapid, and ultra-rapid if the freezing speed is 0.2, 0.5-3, 5-10, and 10-100 cm/h, respectively (Hall, 2011).

Nile tilapia (*Oreochromis niloticus*) like other tilapia species belongs to domestic African species and one of the most important species which is reared in tropical and subtropical areas in the world. Further, it is considered as one of the species which is interesting for fish farmers, due to rapid growth, adaptation to different environments, market, low fat and absence of Y-shaped bones in the muscles and simplicity of rearing (Pacheco *et al.*, 2000).

Materials and Methods

- *Sample preparation*

Eighty Nile tilapias with approximate weight of 700 ± 50 g were caught from Salt Water Fish in National Research Institute in Yazd, Iran in 2012. The fish were first washed and degutted. Then, they were kept in chilled seawater (CSW) (16 ppt) and sent for processing in the Fisheries Research Institute in Anzali. In the next stage, the fillets were prepared manually, washed, and kept in polyamide bags at -18 °C for 180 days. In addition, quick and slow freezing was used in the present study. Slow freezing was carried out by inserting the fillets in polyamide bags and freezing the sample**s** at 0.2 cm/h speed in the freezer at -18 °C during 18 hours. Quick freezing was accomplished by freezing the samples at 0.8 cm/h speed at -30 °C during 25 min. Then, the temperature of the fillets at the centre reached to -5 °C when passed through the spiral tunnel. Further, the fillets were transferred to -18 °C freezer where they were kept for 180 days. In order to study TVB-N, PV, TBA, pH and histological changes during the freezing storage, the frozen samples were transferred to a fridge at 4 °C for 18 hours. The above-mentioned parameters were evaluated during the post-harvesting days of 0, 30, 60, 90, 120, and 180.

- *Chemical analyses*

First, TVB-N value was measured by the micro-diffusion method described by Goulas and Kontominas (2005). The PV was determined according to the AOCS official method (1989), expressed as milliequivalents (mEq) of O_2/kg of lipid. Regarding TBA (mg malondialdehyde/kg), the method proposed by Kirk and Sawyer (1991) was implemented. Further, the pH was measured for the homogeneous mixture of fish and distilled water (1:10, w: v), by using a digital pH meter (Hernandez *et al.*, 2009).

In the next stage, 1 mm^3 of epaxial muscle from dorsal area was cross-sectioned to study the histological structure of the fillets by using electron microscopy (SEM). According to Liorca *et al.* (2003), the samples were cut into 12

mm^3 cubes and fixed by using glutaraldehyde (2% in 0.025 M phosphate buffer, pH 6.8) at 4 °C for 48 h, and accordingly through osmium tetroxide (2% in 0.025 M phosphate buffer, pH 6.8) for 1.5 h. In addition, they were dehydrated in a series of ethanol dilutions of increasing concentration (10, 20, 40, 60, 80 and 100%). The samples were then exposed to the critical point technique in a SEM (LEO 440i). Further, they were coated with gold (10^{-2} Pa) in a Bal-Tec SCD 005 with an ionization current of 40 MA for 90s. Finally, the observation was conducted in a scanning electron microscope (Jeol JSM-6300) at 15 KV and at a working distance of 15 mm.

- ***Statistical analyses***

ANOVA and Tukey's test were used for comparing the means of replicates by using Minitab software (Version 16, USA). The level of significance was considered as 0.05.

Results and Discussion

- ***TVB-N***

Based on TVB-N results, an increase in the storage time of the samples in the cold room caused a significant increase ($p<0.05$) in TVB-N contents of the samples. In this regard, as shown in Table 1, the parameter increased from 11.50 ± 0.01 in the fresh samples to 23.80 ± 0.08 mgN/100 in the slow-freeze treatment and 21.00 ± 0.12 mgN/100 in the quick-freeze treatment. The value was higher in the slow-freeze treatment which can be related to the deterioration of proteins and oxidative decay resulted in the higher production of TVB-N (Chevalier *et al.*, 2000). The results are consistent with those of Liu *et al.* (2010) and Farag (2012) on Nile Tilapia and Ali (2012) on *Liza aurata*. In addition, the results indicated a significant difference ($p<0.05$) in TVB-N contents of the samples during the days 0 and 30 with the selected parameters during the days 60, 90, 120, 150, and 180 in both slow and quick-frozen samples. Further, the deterioration progress was observed in the samples during 180 days storage at -18 ºC and the extent of progress in slow-freeze treatment was faster than the amount in the quick-freeze treatment ($p<0.05$). In a study by Farag (2012), it has been mentioned that microbial activity including the decomposition of lipids, proteins and non-protein compounds results in releasing the undesirable nitrogenous compounds such as TVB-N, TMAO-B, DMA-B, TMA-B and H_2S and accordingly inducing the unwanted changes in odour and flavour of fish (Farag, 2012). Further, according to Coban (2013), the TVB-N value of 30-35 mg/100g is considered as the maximum acceptable value in fish flesh (Coban, 2013). Regarding the present study, the value was in the acceptable range at the end of storage period (Table 1).

- ***PV***

As shown in Table 2, the mean peroxide value in the fresh samples of Nile tilapia was 0.01 ± 0.01 mEq/kg which indicated a significant increase ($p<0.01$) during the storage period. In addition, the PV value at the end of 180 days was 0.86 ± 0.01 and 0.049 ± 0.05 meq/kg, in the slow and quick freeze samples, respectively. Compared to the fresh samples, an increase in the PV in the slow and quick-freezed samples could improve the rancidity during the frozen storage (Nazemroaya *et al.*, 2009). In this regard, lipid oxidation played the most significant role in the nutritional value.

Further, a significant difference was observed at the PV values during 0 and 30 days, compared to the days 60, 90, 120, 150 and 180 in both slow and quick-freeze samples ($p<0.05$).

Table 1. Changes in TVB-N (on wet weight basis) during frozen storage at -18 ºC

Time (month)	*Oreochromis niloticus*	
0 (Control group)	**Slow freezing**	**Quick freezing**
1	11.50 ± 0.01^{aA}	11.50 ± 0.01^{aA}
2	11.20 ± 0.14^{aA}	11.60 ± 0.15^{aA}
3	15.40 ± 0.19^{bA}	17.90 ± 0.10^{bB}
4	16.80 ± 0.24^{bA}	20.52 ± 0.14^{cB}
5	19.01 ± 0.11^{cA}	21.00 ± 0.11^{dB}
6	21.00 ± 0.26^{dA}	22.02 ± 0.10^{dB}

Different small letters in a column show a significant difference ($p<0.05$) between the different storage times in the cold room and the capital letters in a row show a significant difference ($p<0.05$) between the different freezing methods.

Table 2. Changes in PV (on wet weight basis) during frozen storage at -18 °C

Time (month)	*Oreochromis niloticus*	
	Slow freezing	**Quick freezing**
0 (Control group)	0.01 ± 0.01^{aA}	0.01 ± 0.01^{aA}
1	0.04 ± 0.01^{aA}	0.03 ± 0.01^{aA}
2	0.09 ± 0.06^{bA}	0.08 ± 0.02^{bA}
3	0.20 ± 0.05^{cB}	0.14 ± 0.09^{cA}
4	0.36 ± 0.09^{dB}	0.22 ± 0.08^{dA}
5	0.64 ± 0.06^{eB}	0.31 ± 0.08^{eA}
6	0.86 ± 0.01^{fB}	0.49 ± 0.05^{fA}

Different small letters in a column show a significant difference ($p<0.05$) between the different storage times in the cold room and the capital letters in a row show a significant difference ($p<0.05$) between the different freezing methods

Finally, the PV values in quick-freeze samples were more acceptable than those of slow-freeze samples. A significant difference was reported for PV values in the two samples under study ($p<0.05$). According to Jasour *et al.* (2011), peroxide value, as a key factor in reducing fish flesh quality, is responsible for deterioration during storage. The results of the present study are in line with those of Jasour *et al.* (2011), Olele *et al.* (2013), Ali (2012), which reported a significant increase in the peroxide value content during frozen storage in *Oncorhynchus mykiss*, *Chrysichthys furcatus*, and *Liza aurata*, respectively.

- ***TBA***

TBA index is used as a tool for MDA (malonaldehyde) measurement, which is regarded as one of the products resulting from lipid hydro peroxidation. TBA is formed during the PUFA oxidation and is considered as a secondary product of lipid oxidation and a suitable marker for evaluating the fish freshness (Ali, 2012).

Based on the results of the present study, the TBA value was 0.01 ± 0.00 mg/kg in the control samples selected from Nile Tilapia (Table 3). The values increased significantly ($p <0.05$) as the TBA value reached to 1.20 ± 0.06 mg/kg in the slow-freeze and 1.00 ± 0.09 mg/kg in the quick-freeze samples at the end of storage time. This change may be due to the effect of freezing on decreasing water activity, and increasing oxidation, free radical and fatty acid formation, which made the product susceptible for oxidation (Seifzadeh *et al.*, 2012). Further, no significant difference was observed in the TBA values during the days 0, 30 and 60 ($p>0.05$) although a significant difference was reported in the values during the days

90, 120, 150 and 180 in both slow and quick-freeze treatments ($p<0.05$).

Lakshmisha *et al.* (2008) reported that the 5 mg of MDA/kg can be considered as a suitable marker for measuring the quality of the frozen fish while fish meat including MDA of 8 mg/kg is consumable. In the present study, the TBA concentration was acceptable at the end of storage period (Table 3). Our results indicated that the deterioration of the samples during the storage time and the progression of the process was faster in the slow-freeze treatment, compared to that of the quick-freeze treatment.

- *pH*

The pH value in the control group was 6.16 ± 0.05, which increased slightly during the storage period as the value reached to 6.61 ± 0.02 and 6.53 ± 0.05 in the slow and quick-freeze samples, respectively, at the end of storage period at -18 °C for 6 months (Table 4). This increase can be due to the formation of volatile substances and NH_3 due to the decomposition of nitrogenous compounds by microbial activities (Farag, 2012). Microbial enzymes released before freezing can increase the pH during the freezing period, leading to the loss of quality (Farag, 2012).

Based on the results, no significant difference was observed in the pH values between the days 0, 30, and 60 and the days 90, 120, and 150 ($p>0.05$), while the difference was significant between the days 0, 30 and 60 and the days 90, 120, 150 and 180 ($p<0.05$).

Table 3. Changes in TBA (on wet weight basis) during frozen storage at -18 °C

Time (month)	*Oreochromis niloticus*	
	Slow freezing	Quick freezing
0 (Control group)	0.01 ± 0.00 ^{aA}	0.01 ± 0.00 ^{aA}
1	0.01± 0.12 ^{aA}	0.02 ± 0.07 ^{aA}
2	0.07 ± 0.08 ^{aA}	0.07 ± 0.09 ^{aA}
3	0.10 ± 0.10 ^{bA}	0.14 ±0.08^{bB}
4	0.40± 0.10 ^{cA}	0.66±0.01^{cB}
5	0.68± 0.11 ^{dA}	0.80 ± 0.09 ^{dB}
6	1.00 ± 0.09 ^{eAA}	1.20± 0.06 ^{eB}

Table 4. Changes in pH (on wet weight basis) during frozen storage at -18 °C

Time (month)	*Oreochromis niloticus*	
0 (Control group)	Slow freezing	Quick freezing
1	6.16 ± 0.05 ^{aA}	6.16 ± 0.05 ^{aA}
2	6.21 ± 0.05 ^{aA}	6.26 ± 0.05 ^{aA}
3	6.29 ± 0.05 ^{aA}	6.33 ± 0.07 ^{aA}
4	6.35 ± 0.05 ^{bA}	6.39 ± 0.01 ^{bA}
5	6.43 ± 0.05 ^{bA}	6.46 ± 0.00 ^{bA}
6	6.48 ± 0.00 ^{bA}	6.53 ± 0.06 ^{bA}

Different small letters in a column show a significant difference ($p<0.05$) between the different storage times in the cold room and the capital letters in a row show a significant difference ($p<0.05$) between the different freezing methods

The change in pH can be used as an index for post-mortem changes including the transformation of glycogen to lactic acid and the deterioration of muscles including proteins and nucleotides during the storage period (Wang *et al.*, 2011). The quick-freeze samples had more acceptable pH values, compared to those in the slow-freeze samples. In fact, the difference was different between the pH values in the two above-mentioned samples ($p<0.05$). The results are consistent with those of Pacheco *et al.* (2000), Erkan and Ozden (2008), Tsironi *et al.* (2009) and Moawad *et al.* (2013).

- ***Histological changes***

Figures 1-5 illustrate the SEM graphs of the fresh and frozen samples by using the two studied methods.

As shown, histological deterioration increased through increasing the storage time and the most substantial change was observed during the 6th month. In addition, the structure changed during freezing and accordingly the muscular fibres were destroyed. The changes were more significant in the slow-freeze samples, compared to those in the quick-freeze samples. The results indicated that the quick freeze samples were more acceptable during the storage, compared to the slow freeze samples. In addition, the rate of changes in deterioration indices and histological changes in quick freeze samples were lower than those of the slow freeze samples.

Given the results of the previous studies on the constitutional changes of tissues and denaturation of fish muscles, as well as the effects of speed and duration of freezing, the changes are minimized by increasing speed and decreasing the duration of freezing (Bello *et al.*, 1982; Chen and Pan, 1997; Alizade *et al.*, 2007).

Actomyosin indicates a distinctive pattern although the decomposition of the structure and the aggregation of interlaced filaments happen after a few months. Compared to the slow freezing samples, the denaturation of proteins was lower in the quick freezing samples, as the myofibrils maintained their structure in the former samples after six months of storage (Figs 1d and e)

Denaturation occurs due to freezing since the bonding interactions are responsible for the secondary structure (hydrogen bonds to amides) and the tertiary structure are disrupted. Therefore, a variety of reagents and conditions can pave the way for denaturation. The precipitation or coagulation of the protein is regarded as the most common observation in the denaturation process (Tanford, 1968). Matsumoto (1979) described denaturation as the aggregation formed by the substantial increase in cross linkages among the molecules like hydrogen, ionic, hydrophobic and disulfide bonds. The formation of ice crystals during the storage period results in decomposing the cell membrane and destructing the tissue. In addition, the lower freezing speed leads to the faster critical point of freezing, as well as the lower and more unified ice crystals. Further, the ice crystals in the above-mentioned way are formed within the cells extracellularly and the resultant damage becomes less (Alizadeh *et al.*, 2007).

The results of the present study suggested that the fillets of Nile tilapia can be stored for a longer period of time and the changes are in the acceptable range in the chemical indices.

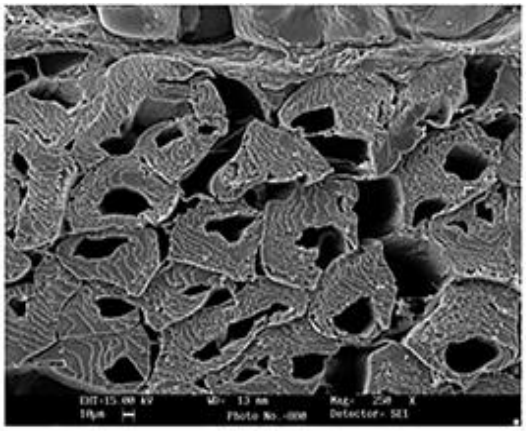

Fig. 1. The fresh sample of *Oreochromis niloticus* (control group) (250x)

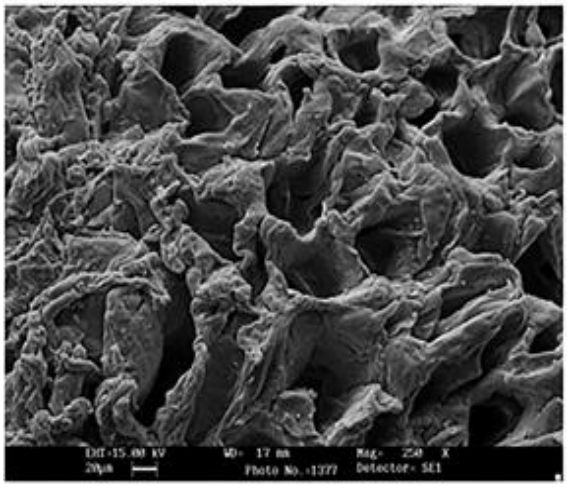

Fig. 2. Slow freezing of *O. niloticus* after 3 months (250x)

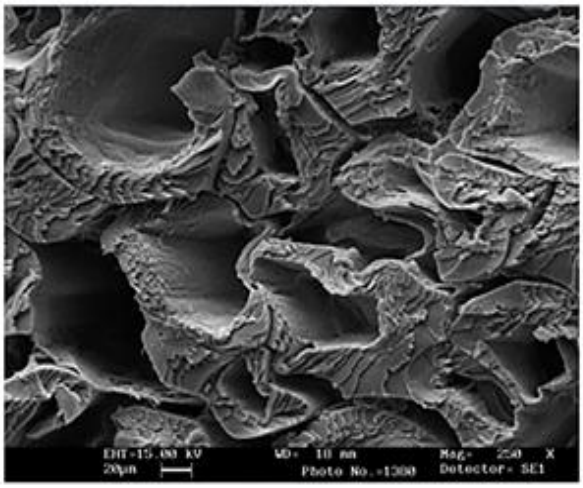

Fig. 3. Quick Freezing of *O. niloticus* after 3 months (250x)

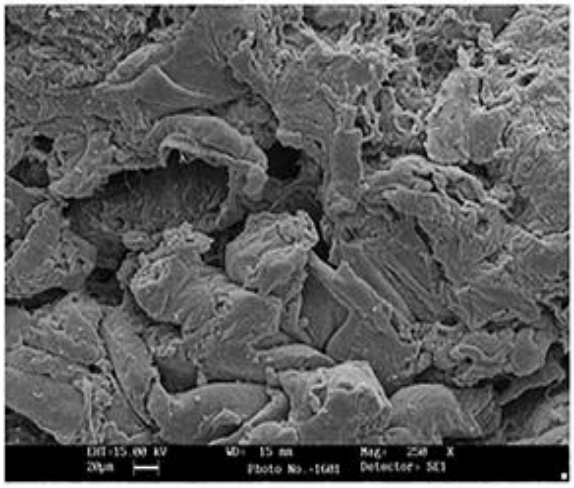

Fig. 4. Slow freezing of *O. niloticus* after 6 months (250x)

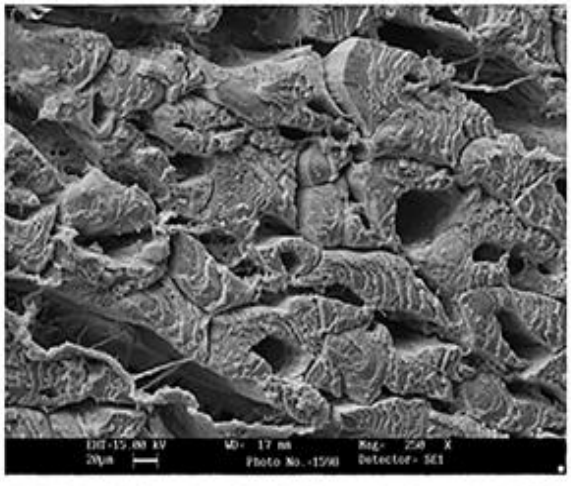

Fig. 5. Quick Freezing of *O. niloticus* after 6 months (250x)

Conclusion

The results of this study indicated that quick freezing method helps to keep the quality of the fillets at a higher standard when freezing in cold room was required. Chemical indices of decay were significantly lower in fillets after quick freezing on compared to those of slow freezing ones. Furthermore, histological properties of the fillets were better preserved after quick freezing.

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