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Research Article
Effect of Different Freezing Processes on the Quality and Histological Changes of Red Tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*)

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Abstract

Background and Objective: Tilapia fish has been a favorable food and has a long history of farming. The present study was designed to investigate the effects of freezing speed and time on the chemical quality indices as well as histological changes. **Materials and Methods:** The fillets were frozen and packaged using too slow and quick-freezing methods and stored at -18°C for 6 months. Then fillet changes including drip, total volatile basic nitrogen (TVB-N), peroxide value (PV), thiobarbituric acid value (TBA) were evaluated on a monthly basis. Finally, histological changes were studied every two months by a scanning electron microscope (SEM). **Results:** All studied qualities changed in the course of different months in both quick and slow-freezing methods, though changes were more noticeable in slow-freezing. Drip percentage (4.8-11.4), PV (0.02-0.93 mEq kg^{-1}), TBA (0.03-1.2 mEq kg^{-1}) and TVB-N (12.63-21.93 mg/100 g) levels all showed greater variations with slow freezing. On the other hand, it detected less degradation in fillets by the freezing method. **Conclusion:** The changes in Drip, TVB-N, PV and TBA as well as histological were less dramatic in the samples treated by quick freezing, compared to those of slow-freezing samples.

Key words: Tilapia, quick freezing, slow freezing, drip, TVB-N, chemical indices, SEM and thiobarbituric acid value

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Tilapia fish farming has a long history of about 4,000 years[1]. Currently, over 100 countries are farming Tilapia[2]. The culture of Tilapia normally takes place in ponds with extensive, semi-intensive and intensive production methods[3].

The main reasons for the high level of Tilapia production include rapid growth, high tolerance for changes, adaptation to a wide range of environments with different temperatures, salinity and dissolved oxygen, stress and disease resistance, captive reproduction, short reproduction periods and feeding from low nutrition levels, as well as accepting artificial food immediately after absorbing the yolk sac[1]. In 2008, the supply of fresh products (56 million t) ranked first for fishery production, followed by the supply of frozen fisheries (29 million t). Further, Canning and other processes such as drying and smoking claimed the next ranks[2].

Freezing fish and fisheries can contribute to preserving the quality, increasing shelf-life, delivering fish to high-consumption markets and supplying surplus fisheries during the year[4]. However, there are some drawbacks such as loss of quality, weight loss, fat oxidation especially in fatty fishes and high freezing cost[5]. So far, a large body of research has been conducted on the indices of fatty acids as well as chemical and microbial characteristics in different fishes such as different species of Tilapia. Osibona *et al.*[6] examined the nutritional value and profile of amino acids and fatty acids in *Tilapia zillii*. Based on the results, oleic acid (C18: 1) claimed the highest fatty acid content by 26%. Also, the maximum amount of omega-3 was related to clupanodonic acid (C22: 5) at 3.7% and the ratio of omega-3 to omega-6 was calculated to be 2.7%. In another study, Pirestani *et al.*[7] evaluated the changes in the fatty acid content of several Caspian Sea fish species such as freshwater white fish, golden grey mullet, common Carp, Zander and common Kilka. The results indicated some changes in the fatty acid contents during storage in freezer. A significant decrease also occurred in the content of unsaturated fatty acids with multiple double bonds, while the percentage of saturated fatty acids rose ($p<0.05$). The highest reduction in unsaturated fatty acids with multiple double bonds was related to Zander, which decreased from 23-15%. On the other hand, the highest increase in saturated fatty acids was observed in the same species growing from 35-45% at the end of the storage time.

Usydus *et al.*[8] examined the nutritional value and profile of fatty acids in some fish species available in the Polish market including Nile Tilapia. According to their findings, the omega-6 content was reported to be higher than omega-3's. In another study, Vieira *et al.*[9] compared fatty acids of Nile Tilapia and Red Tilapia. They reported that the percentages of monounsaturated fatty acids were higher than those of saturated fatty acids and poly unsaturated fatty acids. In their study, the ratio of omega-3 to omega-6 was 0.3 in both samples.

The objective of this work was to evaluate the effects of slow and quick freezing on chemical quality indices and histological changes in Red tilapia (*Oreochromis niloticus*× *Tilapia mosambicus*) meat. This is the first study on Tilapia after importing and culturing it in Iran. The ultimate goal of this work is to assess the changes of ice-crystal sizes during different types of freezing and storage and to offer the best freezing process to relevant factories in Iran.

MATERIALS AND METHODS

Sample preparation: In order to conduct this study, 80 Red tilapias with a mean weight of 700±50 g were caught from the pools in Saltwater Fish Research Station located in Bafq, Yazd in May 2011. After gutting, ice powder and fish with the ratio of 1:1 were placed in insulated tanks and transferred to National Fisheries Processing Research Center in Anzali, affiliated with the Iranian Fisheries Research Organization. In this center, the fish weight was calculated in gram and the tilapia fillets without skin and bones were prepared by hand. Then, the fillets were weighed by a digital scale with an accuracy of 0.01 g, where the mean weight of fillets was 100±5 g. The average diameter of the fillets was also determined by caliper which was 36±2 mm.

The samples were divided into slow-freezing, quick-freezing and control groups. Regarding the slow-freezing group, each fillet was placed inside a polyamide bag, with the fillets kept in a freezer at -18°C to be frozen immediately after the labeling. In the freezer, the fillets were then frozen after 18 hrs. The freezing speed was 2 mm h^{-1} for these samples and they were stored at -18°C for 6 months.

Concerning the quick-freezing method, a spiral freezing tunnel (Coppens Corporation of the Netherlands) was used[5] in which the samples were frozen for 25 min at -30°C. The temperature at the center of the fillets reached -5°C after passing through the spiral tunnel. The freezing speed for these samples was 8 mm h^{-1}. Once labeled, each fillet was placed in a polyamide bag and transferred to the freezer at -18°C. They reached the final temperature of the freezer after freezing for 4.5 h. Later, the samples were stored for 6 months which were then removed from the freezer and defrosted in a refrigerator at 4°C for 18 h. The tests related to chemical factors and deterioration indices including peroxide, thiobarbituric acid and the total volatile basic nitrogen were conducted with

three replications for each sample on specific dates. Finally, histological changes were evaluated using scanning electron microscopy (SEM).

Chemical analyses: First, the drip was measured according to Ng and Bahurmiz[10] method. The frozen samples were first weighed and then placed in plastic bags in a refrigerator at 4°C for 24 h. Once defrosted in the refrigerator, some water dripped from the samples.

The peroxide value (PV) expressed as the milli-equivalents of oxygen/kilogram of lipid was determined according to American Oil Chemist Society (AOCS)[11]. Next, the thiobarbituric acid value (TBA, mg malondialdehyde/kg) was measured based on the method proposed by Kirk and Sawyer[12]. In the next stage, the total volatile basic nitrogen (TVB-N) value were estimated by the micro-diffusion method as previously introduced by Goulas and Kontominas[13].

Histological changes: The SEM imaging was first conducted from the cross section of the samples[14]. In this regard, a cubic millimeter of epaxial Tilapia muscle tissue was cross-sectioned, along the spine and near the dorsal fin. It was then fixed for 24 h in a solution containing glutaraldehyde 4% (Merck Co., Germany). Subsequently, the samples were washed with Sodium Carboxylate Buffer 0.1 M for 20 min and were further fixed at 4°C for 2 h in Osmium tetroxide solution (Merck Co., Germany). The samples were rewashed with buffer and dewatered by acetone (Merck Co., Germany) and placed in a desiccator. Finally, the samples were coated with gold powder and prepared for SEM imaging at 15 kV (LEO 440i model 1995, UK).

Statistical data analysis: All of the above-mentioned experiments were performed by three replicates from three separate samples. In order to analyze the data, one-way ANOVA and Tukey's test were used in Minitab 16 software. The results were considered as significant at p-values less than 0.05.

RESULTS

Drip: The results for the magnitude of drip in the Tilapia samples are presented in Table 1. According to this Table 1, the growth of the drip for slow-freezing samples was higher than that of quick freezing ($p<0.05$). In slow freezing of Red Tilapia samples, the drip percentage during 6 months storage increased from 4.8-11.4%, while it rose from 2.1-6.1% in the quick-freezing samples. As can be observed, the increase in the drip volume was directly related to the refrigerator storage time ($p<0.05$).

Table 1: Changes in the drip percentage of samples during the storage period at -18°C

	Red tilapia	
Time (month)	Slow freezing	Quick freezing
0 (Control group)	-	-
1	4.8 ± 0.2^{aB}	2.1 ± 0.1^{aA}
2	5.6 ± 0.2^{aB}	2.0 ± 0.1^{aA}
3	7.1 ± 0.1^{bB}	2.6 ± 0.2^{aA}
4	9.3 ± 0.1^{cB}	3.1 ± 0.4^{bA}
5	10.8 ± 0.2^{dB}	5.4 ± 0.3^{cA}
6	11.4 ± 0.1^{eB}	6.1 ± 0.2^{cA}

The different lowercase letters in a column represent a significant difference between the refrigerator storage time and the different uppercase letters in a row represent a significant difference between the freezing methods

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

	Red tilapia	
Time (month)	Slow freezing	Quick freezing
0 (Control group)	0.02 ± 0.01^{aA}	$0.02\pm0.01^{aA*}$
1	0.05 ± 0.01^{aA}	0.05 ± 0.01^{aA}
2	0.15 ± 0.12^{bA}	0.11 ± 0.14^{bA}
3	0.26 ± 0.11^{cB}	0.17 ± 0.09^{bA}
4	0.53 ± 0.11^{dB}	0.32 ± 0.08^{cA}
5	0.76 ± 0.05^{eB}	0.45 ± 0.01^{dA}
6	0.93 ± 0.11^{fB}	0.69 ± 0.04^{eA}

*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

Peroxide: Data in Table 2 indicated the extent of peroxide production during the freezing at -18°C for the Red Tilapia fillets. The peroxide level in fresh Red tilapia samples was 0.02, which reached 0.93 and 0.69 mEq/kg in 6-month samples with slow and quick-freezing treatments, respectively.

In addition, the value of peroxide for quick-freezing samples was less than rather than slow-freezing samples. Specifically, a significant difference was observed between slow-and quick-freezing samples by increasing the peroxide level ($p<0.05$).

TBA: Based on the results reported in Table 3, the thiobarbituric acid significantly increased during slow and quick freezing, which was higher than the amount produced for slow-freezing samples ($p<0.05$). The value was 0.03 mg kg^{-1} for fresh Red tilapia, growing to 1.26 and 1.00 mg kg^{-1} at the end of the measurement time (6 months) in the slow and quick-freezing treatments, respectively.

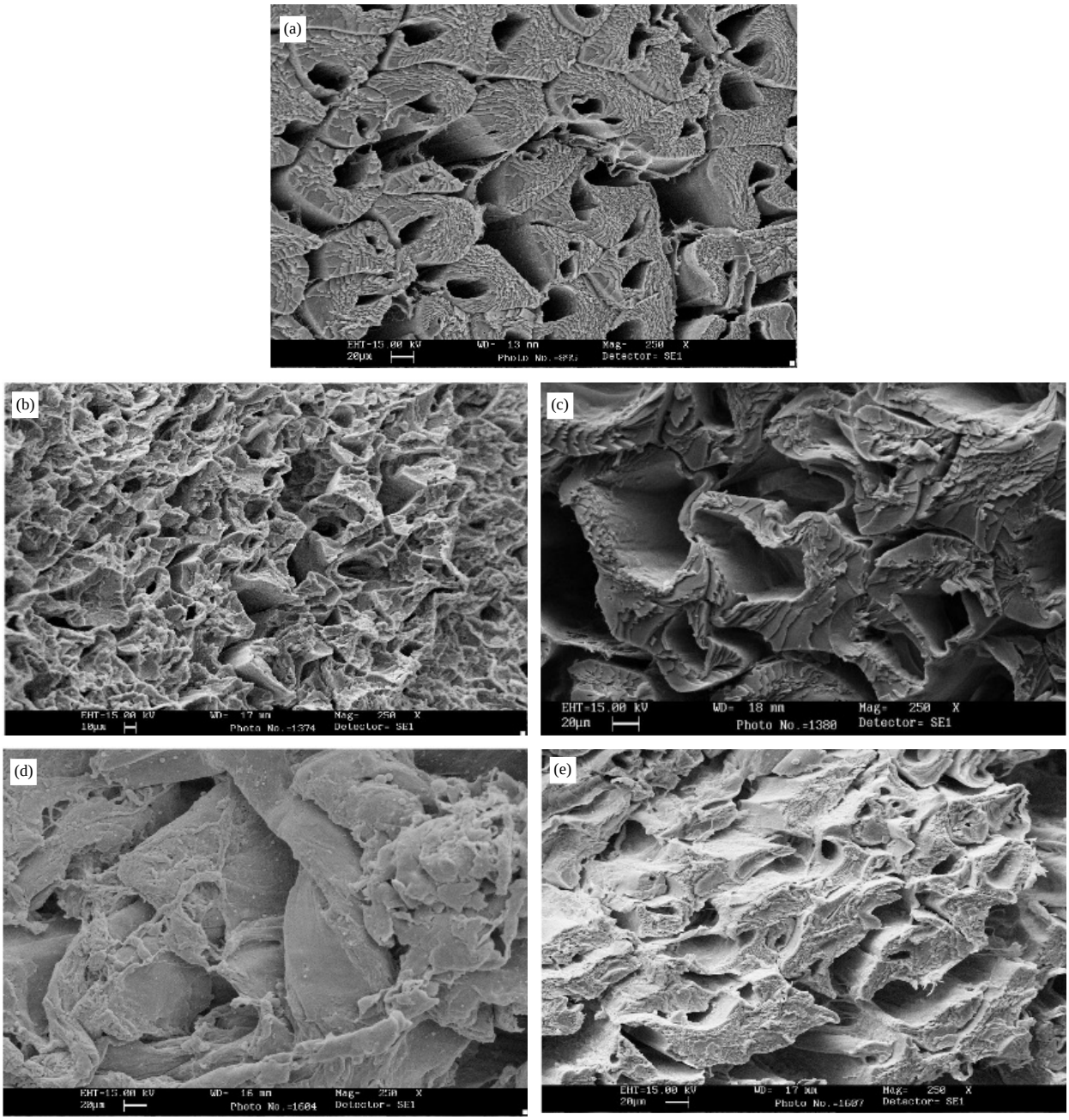

Fig. 1(a-e): SEM images of changes in the microstructures of Red Tilapia fillets during 6 months (250x), (a) Fresh sample of Red tilapia (control group), (b) Slow freezing of Red tilapia after 3 months, (c) Quick freezing of red tilapia after 3 months, (d) Slow freezing of red tilapia after 6 months and (e) Quick freezing of red tilapia after 3 months

TVB-N: The total volatile basic nitrogen (TVB-N) as a series of ammonia compounds, dim ethylamine oxid-trimethylamine oxide was considered another deterioration indicator in the present study. The production of these bases was directly related to the time and level of deterioration, which can be relatively used as a deterioration indicator of fish. According to the results in Table 4, the TVB-N in fresh Red Tilapias was 12.63 mg/100 g of muscle, rising to 21.93 and 20.40 mg/100 g of muscle in the slow and quick-freezing samples, respectively ($p<0.05$).

Histological changes: Figure 1 illustrated the SEM graphs of the fresh and frozen samples using the two aforementioned methods.

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

	Red tilapia	
Time (month)	Slow freezing	Quick freezing
0 (Control group)	$0.03\pm0.04^{aA*}$	$0.03\pm0.04^{aA*}$
1	0.03 ± 0.01^{aA}	0.05 ± 0.12^{aA}
2	0.08 ± 0.11^{aA}	0.08 ± 0.10^{aA}
3	0.16 ± 0.08^{bB}	0.09 ± 0.09^{aA}
4	0.59 ± 0.05^{cA}	0.61 ± 0.11^{bA}
5	0.83 ± 0.10^{dB}	0.68 ± 0.07^{bA}
6	1.26 ± 0.10^{eB}	1.00 ± 0.04^{cA}

*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 4: Changes in TVB-N (on wet weight basis) during frozen storage at -18°C

	Red tilapia	
Time (month)	Slow freezing	Quick freezing
0 (Control group)	12.63 ± 0.05^{aA}	12.63 ± 0.05^{aA}
1	12.66 ± 0.11^{aA}	12.66 ± 0.11^{aA}
2	18.36 ± 0.15^{bB}	15.86 ± 0.14^{bA}
3	19.86 ± 0.21^{cB}	16.08 ± 0.12^{bA}
4	20.73 ± 0.06^{dB}	19.06 ± 0.20^{cA}
5	21.60 ± 0.12^{eB}	19.06 ± 0.10^{cA}
6	21.93 ± 0.22^{eB}	20.40 ± 0.20^{dA}

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

As displayed, arise in the storage time has led to increased histological deterioration with the most considerable change observed after the 6th months. The changes in slow-freezing samples were greater than those of the quick-freezing counterparts.

DISCUSSION

According to Table 1, the drip percentage was directly related to the increased refrigerator storage time; this increase has been higher in slow-freezing samples compared their quick-freezing counterparts ($p<0.05$). Several studies have suggested that drip increase and the fish weight loss occur after defrosting[15-17]. With raising the freezing speed and time, the drip percentage diminished. It was because of the difference in the location of the ice crystals, their size and shape and consequently the physical damages to the muscle fibers. The extracellular large and irregular ice crystals damage the cellular wall, explaining the higher drip percentage observed in the slow freezing. In the study of Alizadeh *et al.*[18], which compared the effect of slow and quick freezing on salmon, the drip percentage of the slow-freezing samples reached 11.25% after one month while it increased to 7.75% in the quick-freezing method ($p<0.05$).

Hydroperoxides, odorless and flavorless compounds are regarded as the primary products of fat oxidation which are relatively unstable. Note that no organoleptic changes occur in fish. Some hydroperoxides produce ketones without breaking the carbon chain, while others produce ketones after breaking the carbon chain and produce flavorful and aromatic compounds.

According to the results in Table 2, in the present study the production of peroxide in Red Tilapia grew where its content reached from 0.02 in fresh Red tilapia samples to 0.93 and 0.69 mEq/kg in slow and quick-freezing samples, respectively ($p<0.05$). In addition, a significant difference was observed at 95% confidence level in quick and slow-freezing treatments due to their different oxidation rates. In other studies conducted on a variety of fishes, the same values as those considered in the present study were obtained. For example, in the study of Karakam and Boran[19], the amount of peroxide increased from 1.8 -8.2 mEq/kg in Anchovy fish after six months of storage in a freezer at -18°C.

It chose TBA and TVB-N as indicators of chemical changes in fillets. Based on Table 3 and 4, both these indicators increased due to freezing and their changes were more significant in the slow-freezing method. In another study on Turbot fish, Chevalier *et al.*[16] reported an increase in TBA in the samples. The value rose from 0.41-0.49 and 0.45 mg kg^{-1}, respectively during 75 days of storage in the freezer in slow and quick-freezing samples ($p<0.05$). In addition, in the study of Karakam and Boran[19], the level of TBA increased from 0.3-1.3 mg kg^{-1} in Anchovy fish after 6 months of storage in a freezer at -18°C. Our results can support the findings of these studies.

Denaturation and aggregation of myofibrillar proteins are regarded as two unpleasant drawbacks during freezing. They usually result in loss of biological activity and significant changes in physical performance and structure. The rate and time of freezing were among the main reasons for diminished denaturation during freezing. In addition, a reduction in the freezing rate and time led to increased protein degradation. As observed in the SEM images (Fig. 1), lower denaturation and aggregation of the protein occurred in quick-freezing samples in Red Tilapias compared to the slow-freezing treatment. Therefore, myofibrillar proteins had considerably retained their structure after six months in the quick-freezing treatment. Nevertheless, denaturation and aggregation of proteins, as well as degradation of their structure were

observed in quick-freezing treatment. According to Charoenrein[20], denaturation after freezing occurs due to the aggregation of proteins caused by the formation of molecular transverse bonds such as disulfide bonding. Matsumoto *et al*.[21] considered denaturation as a result of the aggregation caused by the progressive increase in intermolecular transverse bonds. Several methods are available for detecting and measuring the denaturation of fillets such as myofibrillar solubility, viscosity of actomyosin, adenosine triphosphate enzyme activity and microscopic observation of the internal tissue. In the present study, observation by electron microscopy was used to monitor histological changes and denaturation. As observed, actomyosin indicated an entirely recognizable structure before freezing, but their natural structure was degraded and the aggregation of complex filaments could be observed after several weeks of storage in the freezer. A large number of studies have been conducted on histological changes and denaturation of fish muscle tissue as well as on the effect of freezing time and rate. Based on the results, these changes could be minimized by increasing the freezing speed and shortening the freezing time[18,22,23].

The ice crystals formed during freezing, leading to cell wall and ultimately tissue damage are regarded as another important factor degrading the structure of the tissue. Larger and more uniform crystals result in diminished freezing rate and accelerated critical freezing phase. Thus, they are formed inside the cell instead of its outside and incurs less damage to the tissue accordingly. The present study indicated that there was less degradation in the tissue in quick-freezing treatments, which could be related to smaller ice crystals. The same results were found in the study of Alizadeh *et al*.[18] and Bello *et al*.[23].

CONCLUSION

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

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REFERENCES

1. El-Sayed, A.F.M., 2006. Tilapia Culture. CABI., UK.
2. FAO., 2010. The State of World Fisheries and Aquaculture. FAO., Rome, Italy, Pages: 218.
3. Pillay, T.V.R. and M.N. Kutty, 2005. Aquaculture: Principles and Practices. 2nd Edn., Blackwell Publishing, London, ISBN-10: 1405105321, Pages: 640.
4. Karami, B., Y. Moradi, A.A. Motallebi, E. Hosseini and M. Soltani, 2013. Effects of frozen storage on fatty acids profile, chemical quality indices and sensory properties of red tilapia (*Oreochromis niloticus* × *Tilapia mosambicus*) fillets. Iran. J. Fish. Sci., 12: 378-388.
5. Hall, G.M., 2010. Fish Processing: Sustainability and New Opportunities. Wiley-Blackwell, New York, USA., ISBN: 978-1-4051-9047-3, Pages: 312.
6. Osibona, A.O., K. Kusemiju and G.R. Akande, 2009. Fatty acid composition and amino acid profile of two freshwater species, African catfish (*Clarias gariepinus*) and tilapia (*Tilapia zilli*). Afr. J. Food Agric. Nutr. Dev., 9: 608-621.
7. Pirestani, S., M.A. Sahari and M. Barzegar, 2010. Fatty acids changes during frozen storage in several fish species from South Caspian sea. J. Agric. Sci. Technol., 12: 321-329.
8. Usydus, Z., J. Szlinder-Richert, M. Adamczyk and U. Szatkowska, 2011. Marine and farmed fish in the Polish market: Comparison of the nutritional value. Food Chem., 126: 78-84.
9. Vieira, V.A.R.O., A.W. Hilsdorf and R.G. Moreira, 2012. The fatty acid profiles and energetic substrates of two Nile tilapia (*Oreochromis niloticus*, Linnaeus) strains, red stirling and chitralada and their hybrid. Aquacult. Res., 43: 565-576.
10. Ng, W.K. and O.M. Bahurmiz, 2009. The impact of dietary oil source and frozen storage on the physical, chemical and sensorial quality of fillets from market-size red hybrid tilapia, *Oreochromis* sp. Food Chem., 113: 1041-1048.
11. AOAS., 1994. Official Methods and Recommended Practices of the American Oil Chemists Society. American Oil Chemists Society, Champaign.
12. Kirk, R.S. and R. Sawyer, 1991. Pearson's Composition and Analysis of Foods. 9th Edn., Longman, London, UK., ISBN-13: 9780582409101, Pages: 708.
13. Goulas, A.E. and M.G. Kontominas, 2005. Effect of salting and smoking-method on the keeping quality of chub mackerel (*Scomber japonicus*): Biochemical and sensory attributes. Food Chem., 93: 511-520.
14. Llorca, E., I. Hernando, I. Perez-Munuera, A. Quiles, S.M. Fiszman and M.A. Lluch, 2003. Effect of batter formulation on lipid uptake during frying and lipid fraction of frozen battered squid. Eur. Food Res. Technol., 216: 297-302.
15. Makri, M., M. Melvin, G. Hotos and X. Doubi, 2007. The biochemical and sensory properties of gilthead sea bream (*Sparus aurata*) frozen at different characteristic freezing times. J. Food Qual., 30: 970-992.

16. Chevalier, D., A. Sequeira-Munoz, A. Le Bail, B.K. Simpson and M. Ghoul, 2000. Effect of freezing conditions and storage on ice crystal and drip volume in turbot (*Scophthalmus maximus*): Evaluation of pressure shift freezing vs. air-blast freezing. Innovat. Food Sci. Emerg. Technol., 1: 193-201.
17. Cao, E., Y. Chen, Z. Cui and P.R. Foster, 2003. Effect of freezing and thawing rates on denaturation of proteins in aqueous solutions. Biotechnol. Bioeng., 82: 684-690.
18. Alizadeh, E., N. Chapleau, M. de Lamballerie and A. Le Bail, 2007. Effects of freezing and thawing processes on the quality of Atlantic salmon (*Salmo salar*) fillets. J. Food Sci., 72: E279-E284.
19. Karacam, H. and M. Boran, 1996. Quality changes in frozen whole and gutted anchovies during storage at-18°C. Int. J. Food Sci. Technol., 31: 527-531.
20. Charoenrein, S., 2017. Microstructural Changes and their Relationship with Quality and Stability of Frozen Foods. In: Food Microstructure and its Relationship with Quality and Stability, Devahastin, S. (Ed.)., Woodhead Publishing, USA., pp: 123-138.
21. Matsumoto, J.J., 1980. Chemical Deterioration of Proteins. American Chemistry Society, Washington, DC.
22. Suzuki, T., 1981. Fish and Krill Protein: Processing Technology. Applied Science, London, ISBN: 0853349541.
23. Bello, R.A., J.H. Luft and G.M. Pigott, 1982. Ultrastructural study of skeletal fish muscle after freezing at different rates. J. Food Sci., 47: 1389-1394.