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Research Article

Effect of Frozen Storage on Changes in Lipids and Fatty Acids in Fish

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ABSTRACT

Freezing preservation of fish has been used for thousands of years because of high product quality. 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. The freezing point is often referred to as 'the equilibrium freezing point', and can be defined as the temperature at which a minute ice crystal is about to dissolve in melting. Due to lipid hydrolysis, FFA accumulates in the tissue during frozen storage, especially at high temperatures around -10 to -20 °C Slow freezing rates or fluctuating s temperatures may result in the lysis of lysosomes and thereby increased activity of some endogenous lipases resulting in increased rates of FFA accumulation. Accumulation of FFA does not in itself affect quality attributes of the product but have been shown to interrelate with lipid oxidation and have been proposed to have a pro-oxidant effect on lipids.

Key words: Oil, Modified atmosphere, Fatty acids

INTRODUCTION

Ice crystal nucleation and formation

The formation of ice crystals is proceeded by nucleation, which can be homo- or heterogeneous. Supercooling is the driving force for ice nucleation and is defined as the mdifference between the actual temperature and that of the solid-liquid equilibrium. In a supercooled liquid, homogenous nucleation only occurs if the diffusing molecules spontaneously form a nucleus with a similar structure as ice and with a critical size making it energetically favourable for other water molecules to join. In foods, heterogeneous nucleation is most likely to occur, as a nucleus can form around suspended particles or a cell wall during supercooling. The number of nuclei formed in homo- as well as heterogeneous nucleation increases with increasing degree of supercooling and is crucial for the number and size of ice crystals formed. Apart from the degree of supercooling, the probability of nucleation also depends on the size or volume of the samples because of the statistical nature of the process (Love, 1970; Martino *et al.*, 1998; Wolfe and Bryant, 2001).

Freezing preservation

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).

The role of fish oil in human health

The role of fish oil in human health promotion and disease risk reduction with respect to the vascular system has been well studied (Shahidi and Alasalvar, 2011). Omega-3 fatty acids are not synthesized in the human body, thus the inclusion of fish oil rich in those fatty acids in food products is essential (Jabeen and Chaudhry, 2011). Due to the decline of wild fish stocks as a result of over-fishing and habitat alternations, the consumption of cultured fish could provide or even more omega-3 essential fatty acids such as EPA and DHA than wild fish for the human body (Cahu *et al.*, 2004).

Freezing point depression

The freezing point of food is a critical factor for the determination of many physical properties such as freezing time (Planck's equation), water activity, water distribution, amount of frozen water and thawing time (Rahman and Driscoll, 1994). In fish muscle the freezing point is depressed below that of pure water because of small solutes present in the muscle water. The extent of this depression is approximately proportional to the osmotic pressure of the solution and results in a freezing

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point depression of about one degree Celsius in bulk muscle water (Ross, 1978; Roos, 1986; Wolfe *et al.*, 2002). The freezing point is often referred to as 'the equilibrium freezing point', and can be defined as the temperature at which a minute ice crystal is about to dissolve in melting (Sei and Gonda, 2006). Others use the term 'initial freezing point (James *et al.*, 2005), which is the temperature at which ice crystallisation begins. The ice crystallisation temperature is always below the equilibrium freezing point because supercooling is the driving force for nucleation and ice crystallisation. As described in 4.5 ice crystallisation is followed by the release of latent heat resulting in a rise in temperature to the equilibrium freezing point (Rahman and Driscoll, 1994; Fernandez *et al.*, 2008). The equilibrium freezing point is often estimated from DSC thermograms using either the inflexion point at the left part of the endothermic melting peak (Sablani *et al.*, 2007) or the so-called 'onset temperature' which is the intercept between the tangent at this inflexion point and the baseline. The cooling/freezing curve method is also used to determine the equilibrium freezing point (Rahman, 1995; Kasapis *et al.*, 2000; Sablani *et al.*, 2004). Reported equilibrium freezing points of fish muscle and seafood are: -0.68°C for king fish (Sablani *et al.*, 2007), -1.4°C for tuna (Rahman *et al.*, 2003), -0.9°C for abalone (Sablani *et. al.*, 2004) values between -0.5 and -2.1 °C for squid, calamari, scallop, cuttle, mussel, octopus, and king prawn (Rahman and Driscoll, 1994), -0.83, -0.91, -0.83°C for haddock, cod and sea perch respectively (Fikiin, 1998) and -5°C for tuna (Agustini *et al.*, 2001).

Storage time

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).

Changes in lipids and fatty acids

Changes in lipids during frozen storage of fish can, directly or indirectly, lead to quality deterioration. Fish and other seafood have a high content of PUFA, which are very susceptible to oxidation during frozen storage, and lipid oxidation is the main reason for quality deterioration in frozen stored fatty fish. Furthermore whole lipids, free fatty acids (FFA) and oxidised lipids or their products can interact with proteins, in some cases resulting in quality deterioration of especially lean species (Shenouda, 1980; Hultin, 1992; Mackie, 1993). Due to lipid hydrolysis, FFA accumulate in the tissue during frozen storage, especially at high temperatures around -10 to -20°C (Aubourg, 1999; Aubourg *et al.*, 2004; Rodriguez *et al.*, 2007). Slow freezing rates or fluctuating storage temperatures may result in the lysis of lysosomes and thereby increased activity of some endogenous lipases resulting in increased rates of FFA accumulation (Geromel and Montgomery, 1980). Accumulation of FFA does not in itself affect quality attributes of the product but have been shown to interrelate with lipid oxidation and have been proposed to have a pro-oxidant effect on lipids (Miyashita and Takagi, 1986; Han and Liston, 1987; Yoshida *et al.*, 1992; Aubourg and Medina, 1997; Rodriguez *et al.*, 2007). Furthermore accumulation of FFA may lead to reactions between FFA and proteins resulting in decreased protein extractability. The exact mechanism of this interaction has not been shown, but is likely to be through electrostatic, Van der Waals, hydrogen or hydrophobic forces rather than covalent binding (Mackie, 1993). The role of whole lipids on the stability of proteins is unclear as they have been suggested to have a protective as well as a detrimental effect (Mackie, 1993). Oxidation of unsaturated fatty acids or triglycerides in fish results in the formation of free radicals produced through decomposition of lipid hydroperoxides via a free-radical mechanism. Free radicals can react with other molecules to form secondary products such as aldehydes, ketones, alcohols, short-chain fatty acids and hydrocarbons. Volatile carbonyl compounds are thought to be responsible for off-flavours and odours in oxidised seafood (Khayat and Schwall, 1983; Sikorski, 1994). Phospholipids undergo faster hydrolysis and oxidation than neutral lipids and though lean species only contain up to 2% lipids, most of these are phospholipids, making them prone to oxidation despite the low lipid content (Han and Liston, 1987). Free radicals can also contribute to protein denaturation and aggregation. Radicals may extract hydrogen from protein side chains such as SH groups resulting in protein radicals, which can react with other proteins or lipids to form aggregates. Malonaldehyde, propanal, and hexanal, which are the end products of lipid oxidation, may also react covalently with side chain groups of proteins (Mackie, 1993). Whether lipid and protein oxidation are concomitant processes or if one precedes the other is still unclear, though (Baron *et al.*, 2007).

Lipid oxidation

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).

Fish transportation

Fresh fish fillets have a short shelf life even at refrigeration temperatures. The limited shelf life is a large hurdle for the export of fresh fillets from Iceland to mainland Europe or USA. Transport by sea to major cities in Europe takes about 4-6 days and even longer to the States. For this reason the transport of choice has been air freight. Recent work has shown that storage of superchilled fillets can extend the freshness period (Martinsdóttir *et al*., 2005). Further, combined use of modified atmosphere packaging (MAP) and super chilling can provide further freshness and shelf life extension for both bulk (Lauzon and Martinsdóttir, 2005) and retail (Wang *et al*., 2008) cod products. These findings may contribute to changes required for fish transportation to

foreign markets as lower costs, increased stability of the cold chain, environmentally-friendly packaging and shipping methods are among the main driving forces for improvement in the field of logistics. It is also anticipated that these changes may lead to decreased losses of fresh food products.

Modified atmosphere (MA)

The use of modified atmosphere (MA) to affect the shelf life of fresh fish is well documented (Tiffney & Mills, 1982; Farber, 1991, Lampila, 1991; Reddy *et al*., 1992; Davis, 1993). Most of the research has focused on MAP of fish products for the retail market. Considerable research has also been carried out on MA storage of whole white fish (Stansby & Griffiths, 1935; Villemure *et al*., 1986; Einarsson & Valdimarsson, 1990) and salmon (Veranth & Robe, 1979; Barnett *et al*., 1982; Trondsen, 1989; Sörensen *et al*., 1990; Bergslien & Meling, 1991). Retail and bulk packaging ("bag in box" system) of fish fillets in modified atmosphere was the subject of several trials at the Icelandic Fisheries Laboratories (IFL) and Matís since 1980.

MATERIALS AND METHODS

This article is review and the aims of Influence of frozen storage of fish on changes in lipids and fatty acids. The experiment 1 was conducted by Karami *et al.* (2013). 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 (Karami *et al.*, 2013). Skin-off and deboned fillets were produced by the worker. The fillets were washed by tape 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 indices and sensory evaluation were determined on the fresh and frozen fillets monthly (Karami *et al*., 2013).All the analyses were performed in triplicate. The fatty acids methyl esters were analyzed by gas chromatography using a GC Hewlett Packard, Agilent 6890 with 120 m long × 0.25 mm internal diameter silica capillary column (BPX – 70 SGE, HP, USA) that equipped with a flame ionization detector and split injector (Karami *et al*., 2013).. Nitrogen was used as the carrier gas at 20 cm3 /min, the temperature program was: an initial column temperature of 140°C held for 5 min, then increased at 4°C / min until it reached 170 and held for 3 min and then increased again at 2°C/min 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. Peroxide value (PV) expressed as milliequivalents of oxygen/kilogram of lipid were determined according to American Oil Chemist Society (Karami *et al*., 2013).. 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 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) (Karami *et al*., 2013).

The experiment 2 was conducted by Pirestani *et al* (2010). The fish species were studied Caspian kutum (*Rutilus frisii kutum*), golden grey mullet (*Liza aurata*), common carp (*Caprinus carpio*), pike perch (*Sander lucioperca*) and common kilka (*Clupeonella cultiventris caspia*). These species (in the same genus, weight and size; November 2006; 25-30 specimens) were purchased from three different harbors (Anzalii, Babolsar and Torkaman located in the Northern parts of Iran, representing the West, South and East of South Caspian sea, respectively) (Pirestani *et al* 2010). The weights and lengths of these species were 60±5 g and 10±2 cm, 840±10 g and 62±3 cm, 760±10 g and 48±2 cm, 830±15 g and 40±3 cm, and 430±15 g and 30±2 cm, respectively (Pirestani *et al* 2010). Fish specimens were then transported on ice to the laboratory (Department of Food Technology, College of Agriculture, Tarbiat Modares University) during the first 5 hour after having been caught. Upon arrival in the laboratory, the fish specimens were neither headed nor gutted, rather, they were cut into pieces and the edible sections of each and any species from each harbor mixed. The specimens were, then, packaged in individually celled polyethylene bags in term to be frozen at -30ºC (Pirestani *et al.*, 2010). The specimens were stored under a freezing temperature of -24±2ºC. The Analysis of the frozen fish specimens was carried out after a lapse of 1, 2, 3, 4, 5 and 6 months of the storage. The lipids were saponified and esterified for the fatty acid analysis (Pirestani *et al.* 2010). The fatty acid methyl esters (FAMEs) were analyzed on a Unicom model 4600 gas chromatograph (GC) with a flame ionization detector (FID). The esters were separated on a 30 m×0.22 mm i.d. wall-coated open tubular fused-silica capillary column (30 m×0.25 mm×0.22 µm film thickness, BPX70; SGE, Melbourne, Australia) at isothermal temperature of 190°C with helium as the carrier gas (50 psi) is used to separate the fatty acids. A splitless injector (1.2 µL injection) was also used at 240°C and a FID at 250°C during the separation process. The peaks were identified based on their retention times using fatty acid methyl ester standards and all samples run in triplicate. An internal standard method (C15:0) was employed to calculate the fatty acid composition (Pirestani *et al*., 2010).

RESULTS AND DISCUSSION

In the experiment 1 was conducted by Karami *et al*. (2013). Changes in fatty acids profile (g/100g of total fatty acids) of fresh and frozen samples are shown in Table1. 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.

The changes of chemical quality indices (PV, TBA, TVB-N and pH) of samples during frozen storage are shown in Table 1. As indicated in Table 1, 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.

Table 1: Changes in TBA, PV, TVB-N and pH values of samples during frozen storage at -18c0 (Karami *et al*., 2013).

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

a,b,c,d,e Means in the same column followed by different superscripts are significantly different (P<0.05).

Table 2: Sensory evaluation scores of samples during frozen storage at -18c0 (Karami *et al*., 2013).

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

a,b,c,d,e Means in the same column followed by different superscripts are significantly different (P<0.05).

Table 3: Lipid content of five fish species from South Caspian Sea during frozen storage (-24°C) a, b.

Month of storage	Caspian kutum	Golden grey mullet	Common carp	Pike perch	Common kilka
0	0 6.71±0.01a	4.93±0.03a	3.61±0.03a	1.97±0.05a	10.23±0.09a
1	1 6.39±0.07b	3.66±0.09b	3.27±0.08b	1.24±0.03c	10.46±0.08a
2	2 4.78±0.07c	3.74±0.08b	3.26±0.07b	1.36±0.03b	9.17±0.18bc
3	3 4.81±0.04c	3.34±0.17c	2.92±0.06c	1.13±0.02d	9.42±0.09b
4	4 3.81±0.10d	2.68±0.06d	3.08±0.18bc	1.13±0.02d	8.97±0.21c
5	5 3.74±0.08d	2.45±0.08d	2.49±0.03d	1.17±0.17cd	8.57±0.24d
6	6 2.96±0.01e	2.19±0.14e	1.73±0.05e	1.15±0.04cd	7.25±0.03e

aData is expressed as Mean±SD (n= 3).

Table 4: Changes in fatty acids contenta of five fish species from South Caspian Sea during frozen storage (-24°C) h, i. (Pirestani *et al*., 2010).

Species	ST a	SFA b	MUFA c	PUFA d	EPA+DHA/C16 e	n3/n6	PUFA/SFA g
Caspian kutum	0	28.99±0.23f	56.25±0.62a	14.76±0.38a	0.57±0.02a	4.54±0.39ab	0.51±0.01a
	1	29.71±0.55ef	56.33±0.58a	13.94±0.13c	0.52±0.02b	4.96±0.39a	0.47±0.01b
	2	30.75±0.47e	56.07±0.23a	13.21±0.31c	0.47±0.04c	4.44±0.42abc	0.43±0.01c
	3	31.84±0.32d	55.48±0.45ab	12.71±0.06c	0.42±0.01d	3.72±0.54bcd	0.40±0.00d
	4	33.73±0.20c	55.25±0.40ab	10.94±0.87d	0.33±0.01e	3.15±0.54d	0.32±0.01e
	5	36.02±0.53b	54.34±0.52bc	9.72±0.21e	0.28±0.02f	3.36±0.21d	0.27±0.01f
	6	37.13±0.57a	53.55±0.49c	9.32±0.29e	0.25±0.01f	3.46±0.40cd	0.25±0.01f
Golden grey mullet	0	41.06±0.80e	44.72±0.78a	14.22±0.67a	0.41±0.03a	4.72±0.27a	0.35±0.02a
	1	42.21±0.32de	44.17±0.09a	13.6±0.24ab	0.38±0.01ab	4.39±0.42a	0.32±0.01ab
	2	43.09±0.14d	43.96±0.16ab	13.03±0.20bc	0.36±0.01bc	4.44±0.46a	0.30±0.00bc
	3	44.54±0.71c	43.01±0.31b	12.28±0.46c	0.33±0.01cd	4.28±0.25ab	0.29±0.01c
	4	45.61±0.07c	42.94±0.32b	11.45±0.44d	0.31±0.01d	4.24±0.39ab	0.25±0.01d
	5	48.05±0.08b	41.65±0.14c	10.08±0.25e	0.25±0.01e	3.54±0.20bc	0.21±0.00e
	6	49.89±0.66a	41.05±0.66c	9.25±0.02e	0.21±0.00f	3.05±0.17c	0.18±0.00e

a Storage time in month (s); b Saturated fatty acid, c Monounsaturated fatty acid; d Polyun saturated fatty acid, e Ecosapentaenoic acid+docosahexaenoic acid/palmitic acid; f n3 PUFA/n6 PUFA, g Polyunsaturated fatty acid/saturated fatty acid. h Value in the same column with different letters within a same strain are significantly different at a level of 0.01. i Data is expressed as Mean±SD (n= 3).

The sensory qualities of the samples were evaluated in terms of color, odor, taste, texture and general acceptability (Table 2). The sensory scores decreased progressively with the storage time in the fillets (P<0.05).

In the experiment 2 was conducted by Pirestani *et al* (2010). Table 3 shows the lipid content of the fish species which ranged from 1.97% for pike perch to 10.23% for common kilka, also classified as lean or high fat fish (< 2% lean, 2-4% medium, 4-8% fat and >10% high-fat) Pirestani *et al*. (2010). Based on the lipid content, golden grey mullet, common carp and pike perch were categorized as lean and medium fat fish with lipid content less than 5%SFAs, MUFAs, PUFAs, EPA+DHA/C16 (polyene index) and *n*3/*n*6 changes during the frozen storage are summarized in Tabl 4. In all the fish species, the distribution of fatty acids was as SFAs> MUFAs> PUFAs Pirestani *et al.* (2010).

Furthermore, PUFAs were more than SFAs (SFAs< PUFAs+ MUFAs), while during frozen storage polyunsaturated fatty acids decreased as compared with the saturated fatty acids. Among SFAs, those occurring in the highest proportions during the storage period were palmitic (C16:0) and stearic (C18:0) acids. However, a significant difference was observed among the SFAs content during frozen storage except in the case of the first month of storing. As for carp this exception lasted for two months Pirestani *et al.* (2010). Oleic acid (C18:1*n*9) was the main fatty acid among the MUFAs in all the fish species. Except for months 5 and 6 in kutum and kilka, and months 3, 4, 5 and 6 in mullet, there was no significant difference among the MUFAs content during the storage priod (Pirestani *et al*., 2010). In addition, no significant difference could be observed among the MUFAs content in carp and pike perch. It seems that the MUFAs content in all the species are approximately fixed. The flesh of the five fish species contained high concentrations of *n*3 PUFAs including ecosapentaenoic acid (EPA, C20:5*n*3), docosahexaenoic acid (DHA, C22:6*n*3) as the major components (Pirestani *et al*., 2010).

EPA is the most important essential fatty acid of the *n*3 series in human diet because it is the precursor to the 3-series eicosanoids (Pirestani *et al*., 2010). The highest EPA was found in mullet, accounting for 7.53% of its total fatty acids. pike perch (11.36% of the total fatty acids); whereas mullet showed lower DHA content among the studied fish species. The DHA/EPA (C22:6*n*3/ C20:5*n*3) ratio of studied species were 1.4, 0.53, 1.18, 3.28 and 1.71 in Caspian kutum, golden grey mullet, common carp, pike perch and common kilka, respectively. C20:5*n*3 has been recognized as beneficial for fir human health by reducing the risk of cardiovascular disease Table 3 shows the lipid content of the fis species which ranged from 1.97% for pike perch to 10.23% for common kilka, also classified as lean or high fat fish (< 2% lean, 2-4% medium, 4-8% fat and >10% high-fat) (Pirestani *et al.*, 2010). Based on the lipid content, golden grey mullet, common carp and pike perch were categorized as lean and medium fat fish with lipid content less than 5% (Bennion, 1997) while Caspian kutum and common kilka were classified as fat and high-fat fish. According to Feeley *et al.* (1972) low-fat fish species have higher water content and, as a result, their flesh is broghter in color. Lipid deterioration is the main cause of low shelf life of fatty fish due to progressive oxidation and enzymatic hydrolysis of unsaturated fatty acids in them (Sarma *et al.,* 2000). PUFAs in pike perch were the highest among the fish species with a significant decrease in the amount of these fatty acids during the frozen storage priod (37, 35, 28, 33 and 29% in kutum, mullet, carp, pike perch and kilka, respectively (Pirestani *et al*., 2010). The oxidative changes in the frozen fish lipids may be caused by the occurrence of radical indicators of the process. These types of radicals are easily formed in pike perch, because of its lipid content of a higher PUFA (Pirestani *et al*., 2010).

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