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Investigation on the Shelf Life and Changes of Lipid and Fatty Acid Composition of Sturgeon (*Acipenser stellatus*) in Frozen Storage

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A study was carried out on the shelf life, fatty acid composition and changes in lipid profile of Sturgeon (*Acipenser stellatus*) from the Southern Caspian Sea for twelve months in frozen storage at -22°C. Fresh fish tissue had 84.41% unsaturated fatty acids whereas in the case of twelve month frozen samples it was 77.45%. Significant amount of polyunsaturated fatty acids (PUFA) was present in the samples. The amount of tissue lipid (9.71%) and ω-3 fatty acids (16.64%) has come down to 8.03 and 8.98%, respectively during frozen storage. The peroxide value increased to 20.11 meq/kg by twelve months in frozen storage from the initial level of 7.24 meq/kg. There were significant variations in the amount of some fatty acids like myristic, α – linolenic and arachidonic acids. Significant difference was also observed in ω-3 and ω-6 fatty acids series during frozen storage. The results of this study indicate that the maximum shelf life for Sturgeon is up to six-months in frozen storage.

Key words: ***Acipenser stellatus*, Fatty acids, Lipid, Frozen storage**

Sturgeon (*Acipenser stellatus*) is a highly valued species in terms of its nutritive value and a source of high quality caviar. Generally, the tissue of this fish is composed of 16.46% protein, 10.12% lipid, 73.56% moisture and 1.5% ash (Hedayatifard & Moeini, 2003). The abundance of the unsaturated fatty acids in the. fat is one of the most significant features of these fishes. Exhaustive studies have been carried out by several workers on the fish fatty acids (Hilditch & Williams, 1964; Exler, 1987; Stansby, 1990; Hedayatifard *et al.*, 2002; Hedayatifard & Moeini 2003; Ackman, 2004; Chan *et al.*, 2004, Ackman, 2005 & Oh, 2005). However little work has been carried out on the qualitative and quantitative composition of fatty acids of sturgeon fish species.

Improper preservation and storage practices can cause oxidation and spoilage of fat in fish and fishery products. Moeini (1989) & Huss (1994) described the process of the deterioration and oxidation of the lipids. Schultz (1994) also described the pathway of Beta-oxidation in the fatty acids with several double bonds. Oxidative spoilage or auto-oxidation process is a reaction between oxygen and unsaturated fat. This process result in the formation of hydroperoxides which are flavorless, but can change the fish color into yellow and brown (Huss, 1994). The deterioration and breaking down of hydroperoxides leads to the formation of aldehydes and ketones. Huss (1994) states that oxidation process can start and accelerates with the increase of the light (particularly ultraviolet ray) and temperature. Most of the fatty fishes are naturally subjected to this type of spoilage. Hultin *et al.*, (1992) described the oxidation process and the factors in fatty fishes. Peroxide value is one of the most important indicators in the fat oxidation. If unsaturation degree of oils is

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high, peroxide formation and spoilage will be accelerated. When the amount of peroxide reaches to a certain value, unpleasant smell and flavor will be indicated by accomplished changes and the production of aldehydes volatile materials and also the free fatty acids with a short chain. As mentioned previously, peroxide value does not cause this smell and flavor, but it can be an acceptable indicator to determine the tissue oxidization degree. The time taken for the production of peroxide will vary, but it will be involved in the spoilage process as a fat oxidation catalyst after attaining a threshold level.

Freezing increases the shelf life of the fish and maintenance of fish in cold condition is called "increase of the high quality life period" (Hedayatifard, 2001). In the present study, the changes of lipid and fatty acid composition of Sturgeon *Acipenser stellatus* in frozen storage and its effect on shelf life are investigated.

Material and Methods:

Acipenser stellatus of average length and weight 126 cm and 8.75 kg caught from the Southern Caspian Sea and transferred to Fishery Company of Mazandaran center (Iran). After doing the biometry and sampling, the whole fish was immediately frozen and stored in an air blast freezer at a temperature of -22 ±0.2 °C.

Sampling of frozen stored fish was carried once in every two months. For sample preparation a single fish was filleted and the fillets were homogenised. The homogenised meat sample was then transferred to the Biotechnology department of the Ecology Research Center of Caspian Sea for analyses. The lipid was extracted by the cold method (AOAC, 1984). Modified methods, Chan *et al.*, (2004) and Acman (2002) have been used for providing methyl esters and identifying the fatty acids respectively. The planning and conditions of the injection to gas chromatography machinery (GC-FID) have been adjusted by detector, flame ionization, detector temperature (210°C), column temperature (190°C), injection temperature (200°C), the amount of injection (0.5 micro-liter), paced column: DEGS 15%. The carrier gas was helium with a purity of 99.99% and the current rate was 45 ml min^{-1}. The temperature of the oven was isothermal. The rate of lipid oxidation was determined by Peroxide value described by Hasegawa (1987).

The data was compared using analysis of variance (ANOVA). If significant difference was found, the data was further compared using Duncan test and "SPSS for window, XP".

Results and Discussion

The average fatty acids of *Acipenser stellatus* tissues and the changes of peroxide values have shown in Tables 1 and 2 respectively. The composition of kinds of fatty acid series of *Acipenser stellatus* has also shown in Table 3. The average lipid of *Acipenser stellatus* is about 9.71%, which has 84.41% unsaturated fatty acids and 10.66% saturated fatty acids. But the average of tissue lipid in one year frozen fish decreased to 8.03% that have 74.45% unsaturation fatty acids. Saturated fatty acids content was 9.93% after 12 months frozen storage.

In the tissue of fresh fish, unsaturated fatty acids was 20.54% of the whole-identified fatty acids. This amount is 13.09% for one year frozen fish. In the tissue of a fresh *Acipenser stellatus*, total average ω -3 and ω – 6 fatty acids were 16.64% and 3.90% of the whole-identified fatty acids, respectively. Total average ω -3 and ω – 6 fatty acids were 8.98% and 4.11%, respectively in frozen tissue after one-year (Table 3). The ratio of ω-3/ω-6 fatty acids is 4.26% in the fresh fish and 2.18% in one-year frozen fish. Linoleic and Arachidonic fatty acids included ω-6 series and α–linolenic, Icosapentaenoic and Docosahexaenoic fatty acids include ω-3 series which have also been studied in the present study.

Table 1. The fatty acids profile of Acipenser *stellatus*, in fresh and frozen conditions (g/ 100 g lipid)

Months	C14:0 Miristic	C16:0 Palmitic	C16:1 Palmi	C18:0 Stearic tileic	C18:1 Oleic	C18:2 Linoleic	C18:3 Linolenic	C20:4 Arachi	C20:5 EPA	C22:6 DHA donic
0	1.83± 1.19	7.39± 1.81	20.16± 0.35	1.44± 1.02	43.71± 1.62	3.39± 0.25	7.75± 3.27	0.51± 0.73	5.36± 0.48	3.53± 2.41
1	2.70± 0.40	7.31± 1.54	24.15± 5.46	1.92± 1.01	43.3± 0.87	3.86± 0.46	4.65± 3.02	0.94± 0.45	4.00± 1.63	0.98± 1.14
2	2.60± 0.51	8.10± 1.36	21.42± 3.86	1.63± 1.25	40.98± 1.06	3.44± 1.34	3.22± 3.39	1.20± 0.14	4.70± 0.86	1.04± 1.25
4	1.35± 0.23	5.85± 1.54	18.66± 1.18	0.89± 0.08	47.04± 4.71	3.66± 0.93	0.72± 0.41	1.20± 0.79	4.70± 0.68	1.04± 0.92
6	1.93± 0.11	6.45± 1.30	20.65± 1.61	0.98± 0.04	48.03± 1.41	3.68± 1.10	0.65± 0.08	1.97± 0.42	3.86± 0.50	1.04± 0.14
8	1.95± 0.11	5.35± 1.02	25.22± 3.16	1.05± 0.21	50.20± 0.12	4.17± 1.64	0.64± 0.57	1.02± 0.12	5.66± 1.60	0.80± 0.80
10	1.91± 0.12	6.24± 1.97	19.83± 0.25	1.23± 0.35	46.43± 0.72	4.11± 1.52	1.50± 0.14	1.03± 1.09	4.78± 0.36	3.01± 3.11
12	2.03± 0.11	6.72± 0.86	19.97± 1.05	1.18± 0.11	41.39± 2.18	3.05± 0.12	1.06± 0.16	1.07± 1.59	5.46± 1.76	2.46± 0.45

n = 3, ± values indicate standard deviation

Regarding lipid percentage and the changes in Myristic acids, α–linolenic and Arachidonic, there is significant difference between the fresh fish and 12 months frozen fish ($P<0.05$). The difference between ω-3 and ω-6 unsaturated fatty acids was also significant ($P<0.05$).

As given in Table 2, peroxide value in a fish tissue attained to 10.11 meq kg^{-1} from the 6th month and reached to 20.01 in the 12th months. The extracted lipid from the marine animals exists as liquid under usual conditions which also includes a lot of unsaturated fatty acids with several double bonds.

Table 2. Peroxide value of *Acipenser stellatus* tissue during frozen storage (meq kg^{-1})

Months	Peroxide
4	7.24 ± 0.51
6	10.11 ± 0.21
8	7.16.20 ± 0.31
10	19.81 ± 0.18
12	20.01 ± 0.39

n = 3, ± values indicate standard deviation

The compounds also existed with Triglycerides (Moeini, 1989). The fatty acid profile (Table 3) of *Acipenser stellatus* indicate considerable abundance of the unsaturated fatty acids in it. The tissue of *Acipenser stellatus* contain significant of unsaturated fatty acids with long chain like ω-3 (with 16.64%) and ω-6 series (with 3.9%). The content of the ω-3 series in this fish tissue is more than ω-6 series (Table 3).

Table 3. Fatty Acid Composition in *Acipencer stellatus* (g /100 g lipid)

Fatty acid series	In fresh fat tissue	In 12 months frozen condition
Saturated fatty acids	*10.66*	*9.93*
Unsaturated fatty acids	84.41	74.45
Omega-3 series (ω-3)	16.64	8.98
Omega-6 series (ω-6)	3.90	4.11
Monoenoic fatty acids	63.87	61.36
ω-3 + ω-6	20.31	13.09
EPA + DHA	8.89	7.92
Polyenoic fatty acids	20.52	13.09
High Unsaturated fatty acids	9.40	8.98

This characterization can be seen in the other sturgeon species as well. It was observed that the amount of the unsaturated fatty acids with several double bonds in the tissues of the marine fishes is more than fresh water fishes (Isuyev and Musayev, 1989, Xu, *et al.*, 1993 Chen *et al.*, 1995). As it can be seen in Table 4, *Acipenser stellatus* is one of the most valuable fish due to its lipid amount and unsaturated fatty acids which can be recognized as an important species amongst other marine animals. Comparison of the fatty acids in *Acipenser stellatus* and the other marine animals defines the relation of its changes with the fish species, age and also their life conditions is given in Table 4.

Table 4 showed that the freezing in suitable conditions can cause high quality life (HQL), during the fish storage. Moeini (1989) stated that the fish nutrient compositions such as vitamins, fat, protein and minerals could be saved using the suitable freezing for a long time without any changes. Johnston *et.al*,(1995) also expressed that the cold does not change and decompose the nutrient composition in the fish, but prevents the micro-organisms growth and stops their activity and destroys a lot of parasites. The cold will affect on the food products and its compositions (Hedayatifard and Moeini, 2003, Johnston *et.al*, 1995). The percentage of the total fatty acids in the tissue of *Acipenser stellatus* decreased during one year frozen storage. The decreasing trend of α-linolenic (from 7.75% to 1.06%), Docosahexaenoic (from 3.53% to 2.25%), and Oleic acid (from 43.7% to 41.39%) are shown in Table 1. In the case of Arachidonic and Myristic acid the values showed an increase (from 0.51% to 1.06% and from 1.83% to 2.03%) respectively (Table 1). The total lipid of the fresh fish tissue was 9.71% which decreased to 8.03% in one-year frozen storage. The amount of fatty acid (g / 100 g lipid) showed an increase, but when compared to tissue weight (g/ 100 g tissue) it decreased (Stansby, 1990, Ackman, 2005). Myristic acid is a saturated fatty acid and its increase shows the increase of saturation in fat composition. Stansby (1990) had also reported the increase of the fat saturation in unsuitable storage conditions. There was significant difference between the changes of Myristic, α-linolenic and Arachidonic acids statistically in the level of 95%., during one-year frozen storage.

Therefore, it can be concluded that the fat oxidation during frozen storage took

Table 4. Fatty Acids Profile in the Tissue of Different Fresh and Marine Fish Species (g/ 100 g lipid)

Fish and shellfish	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	C20:4	C20:5	C22:6	Reference
Acipenser stellatus	1.83	7.39	20.16	1.44	43.71	3.39	7.75	0.51	5.36	3.53	*Present study (Table 1)*
Acipenser persicus	1.77	6.73	17.75	1.23	45.11	3.59	2.80	2.16	4.75	2.21	Hedayatifard & Moeini, 2003
A. transmontanus (farmed)	-	-	-	-	-	18.56	1.41	2.34	2.81	5.24	Xu, *et al*, 1993
A. oxyrhynchus	1.43	25.90	-	2.61	35.70	0.40	0.26	1.36	1.78	6.10	Chen *et al*, 1995
A. guldenstaedti (larve)	2.81	21.74	7.46	8.71	21.06	2.00	-	1.97	6.62	*1.46*	Isuyev & Musayev, 1989
Cyprinus carpio	1.42	15.71	5.00	5.71	20.00	5.71	1.42	2.85	5.71	4.28	Aggelousis & Lazos, 1991
Arius spp.	1.90	25.20	10.40	5.90	16.10	0.90	0.80	3.7	5.00	13.2	Eid *et al*, 1992
Salmo salar	2.40	11.20	3.80	4.50	24.00	3.10	5.20	4.7	5.70	19.8	Exler, 1987
Onchorhyncus mykiss	2.72	12.40	2.27	10.59	21.78	2.72	2.42	2.87	3.02	8.01	Exler, 1987
Nemipterus spp.	1.90	33.70	7.60	18.70	15.60	0.6	-	1.80	3.10	6.70	Eid *et al*, 1992
Liza aurata	5.42	14.39	2.14	17.22	17.09	5.96	8.72	1.49	2.44	3.52	Hedayatifard *et al*, 2002
Charcarhynidae	1.77	16.22	2.66	5.55	21.77	1.77	0.66	2.44	7.11	11.77	Exler, 1987
Penaeus aztecus	1.80	10.40	6.40	5.10	7.40	0.40	-	7.50	18.4	17.3	Exler, 1987
Perna viridis	1.95	7.67	7.09	1.45	0.37	0.48	0.32	1.99	12.29	24.38	Chen *et al*, 1995
Loligoidae	3.60	26.6	5.90	1.00	4.70	0.20	-	0.90	14.80	34.60	Exler, 1987

place and the unsaturated fatty acids, especially poly unsaturated fatty acids are more susceptible to oxidation. Thus, the lipid in marine products can be protected from oxidation by controlling temperature of refrigerating room. Peroxide value is identified as an indicator of the fat oxidation. It has been accepted that peroxide value should be less than 5 meq. kg^{-1} or less than 10 in international units (AOAC, 1984), in fresh foods. If the peroxide value ranges between 10 to 20, an unpleasant smell and flavor will appear, and if it is higher, the oil or the fat material will become rancid.

In the present study, after six months in frozen storage of *Acipenser stellatus*, peroxide value reached 10.11 meq. kg^{-1} and by 12^{th} month it increased the critical level of 20.01 meq kg^{-1}. *Acipenser stellatus* is a fatty fish and high fat in the tissue causes the increase of peroxide value which can progress during long-term frozen storage. Frozen storage of the sturgeon fish do not affect the amount and composition of fatty acid up to six months due to its thick, leather–like skin. The results showed that this species is suitable for human consumption for a period of six months in frozen storage.

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