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Consequences of Frozen Storage for Amino Acids and Unsaturated Fatty Acids of Tuna (*Thunnus tonggol*) Roe

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Abstract: Amino acid and Unsaturated Fatty Acid (UFA) contents of long tail tuna (*Thunnus tonggol*) roe and their changes were investigated during 9 months of frozen storage at -18°C. These analyses were performed immediately after the freezing, then after 3rd, 6th and 9th months of cold storage at -18°C. Numbers 10 poly unsaturated fatty acids (PUFAs) and 8 monounsaturated fatty acids (MUFAs) were identified. After 9 months of cold storage, these compounds reduced from 34.55 and 27.78% to 24.62 and 25.62%, respectively. The most abundant UFAs in the fresh and frozen roes were C16:1, C18:1, C20:5(n-3), C22:5(n-3) and C22:6(n-3). The results showed that n-3 and n-6 fatty acids in fresh roe were 32.75 and 1.61%, respectively, which decreased to 23.41 and 1.09%, respectively, after 9 months of cold storage. The most abundant amino acids in long tail tuna roe were lysine, histidine, aspartic acid and leucine. According to the results, the amounts of Essential Amino Acids (EAA) and nonessential amino acids (NE) were 104.78 and 75.61 mg g^{-1}, respectively that reduced to 78.25 and 61.40 mg g^{-1} at the end of storage period. Moreover, the amino acid compositions for fresh roe showed a relatively higher ratio of EAA/NE in comparison to frozen samples after 9 months of cold storage. These ratios were 1.38 and 1.27, respectively. These findings are showing that *Thunnus tonggol* can be a rich source of n-3 fatty acids and Essential Amino Acids (EAA) for human health.

Key words: Essential amino acids, n-3 fatty acids, frozen time, *Thunnus tonggol* roe

INTRODUCTION

Long tail tuna (*Thunnus tonggol*) is an economically important fish species in Iran. Its annual catch is considerably high, from which 50% is usually female. So, noticeable amounts of roe are obtained. On the other hand, the profile of UFAs and amino acids of long tail tuna roe were unknown.

Nowadays, it is known that fish lipids are well known to be rich in long chain n-3 Polyunsaturated Fatty Acids (PUFA), especially Eicosapantaenoic Acid (EPA) and Docosahexaenoic Acid (DHA). These fatty acids play an important role in human nutrition, diseases prevention and health promotion (Sidhu, 2003).

Researches indicate that meat and roe of different species of fish have a lot of unsaturated fatty acids and essential amino acids (Shirai *et al*., 2006). Also, there are some reports about fatty acids and amino acids in some invertebrate aquatic animals including sea urchin (Yokota *et al*., 2002).

Further more, these compounds of fish roe are influenced by species, diet and environment. Hence, the fatty acid and amino acid compositions of marine water fish are characterized by high amounts of n-3 PUFA and EAA, respectively. The research show that marine water fish

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generally have higher levels of n-3 PUFA and EAA than fresh water fish (Rahman *et al*., 1995; Bledsoe *et al*., 2003). The most research on long tail tuna in Iran has focused on its distribution, catching method and reproduction. However, no information is available on the unsaturated fatty acid and amino acid compositions of long tail tuna of Iran, particularly about roe that can be a good edible food for human consumption. There are some investigations about fatty acids and amino acids of fish roe such as in mirror dory, mullet and some fish roe products (De Silva *et al*., 2001; Itoh *et al*., 2006; Shirai *et al*., 2006).

Frozen storage is a commonly used method for preserving fish. But, the fish components such as unsaturated fatty acids and amino acids change during period of frozen storage. Little information is available about changes in these compositions of fish roe, especially on long tail tuna roe. Storage of long tail tuna roe at -18°C is of great importance for the amino acid and UFA requirements of human, because this method helps to keep the changes of the roe in the minimum and to increase its shelf life. So, the main objective of this study was to identify UFAs and amino acids profile of *Thunnus tonggol* roe and also to estimate the changes in these compositions of frozen (-18°C) roes over nine months.

MATERIALS AND METHODS

Sample Preparation

Thunnus tonggol roes used in this study were obtained from fish were caught from Persian Gulf in June 2007. After collecting the roes, they were packed into a Styrofoam box with ice and then dispatched for the analysis to the processing laboratory. After that, 3 fresh roes were randomly chosen, mixed and their unsaturated fatty acids and amino acids profile were determined. The rest was frozen at -30°C and then stored at -18°C for nine months. Analysis of these parameters of the frozen roes was carried out immediately after the freezing, then after 3rd, 6th and 9th month of storage. For each experiment, 3 roes were taken out randomly, defrosted at the ambient temperature and homogenized.

Fatty Acid Analysis

Total lipid was extracted by chloroform-methanol (1:1, v/v) and estimated gravimetrically (Bligh and Dyer, 1959). The fatty acids in the total lipids were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with 14% BF3 (Tri Fluoride Bore) (w/v) in methanol (AOAC, 1995). The fatty acid methyl esters (FAMEs) were analyzed on a Hewlett-Packard 6890 Gas Chromatograph (GC) equipped with a Flame Ionization Detector (FID). The esters were separated on a BPX-70 column (120 m×0.25 mm i.d.). Column injector and detector temperatures were 285 and 320°C, respectively. The carrier gas was nitrogen (flow 0.6 mL min^{-1}). Identification of unsaturated fatty acids was performed by comparison to retention time of authentic standards.

Amino Acid Analysis

Total amino acid content of roe samples was determined on three sub-samples, each in triplicate. L-Hydroxyproline was used as an internal standard and the amino acids were analyzed by a pre-column fluorescence derivative method using a fully automated Younglin HPLC 6300. Tryptophan was not determined in this study.

Statistical Analysis

All experiments were carried out in triplicate for statistical treatments. The data on amino acid and unsaturated fatty acid compositions were subjected to analyses of variance (one-way ANOVA), at the 5% confidence level using Duncan's multiple range test.

RESULTS AND DISCUSSION

Changes in Unsaturated Fatty Acids (UFA) profile (% total fatty acids) of long tail tuna roe during frozen storage are shown in Table 1. Table 1 showed that the fatty acids C14:1, C15:1, C20:4(n-6), C18:3(n-6) in fresh and frozen samples are in lower concentrations than the other UFAs. The major UFAs identified in the roe were C16:1, C18:1(n-9), C20:5(n-3) (EPA) and C22:6(n-3) (DHA). Most researchers such as De Silva *et al*. (2001) and Shirai *et al*. (2006) have reported that the main unsaturated fatty acids in fish roe are C18:1(n-9), C20:5(n-3) (EPA) and C22:6(n-3) (DHA). Some reports indicate that the fatty acid composition of fish roe is influenced by diet but does not depend on the degree of maturation (Kaitaranta and Ackman, 1981; Shirai *et al*., 2001).

When fatty acids were compared to the other species, it was observed that the percent composition of PUFA in long tail tuna roe (34.55) was lower than the reported values (48.7, 49.0) in herring and capelin roe, respectively. On the other hand, the MUFA in the tuna roe (27.78) was higher in comparison to the reported values (14.5, 18.1) for these species, respectively (Tocher and Sargent, 1984). So, tuna roe is good source of MUFAs. According to Wang *et al*. (1990), marine fish are rich in n-3 fatty acids, especially EPA and DHA. The results of this study showed that total n-3 fatty acid was higher than n-6 PUFA (Table 2).

Table 1 showed that during the frozen storage, the differences in unsaturated fatty acids, except for C15:1, C18:3(n-3) and C20:4(n-6), were significant ($p<0.05$). On the other hand, the initial MUFA and PUFA compositions were 27.22 and 33.94% that decreased to 25.62 and 24.62% at the end of

Table 1: Changes in unsaturated fatty acid composition in *T. tonggol* during frozen storage at -18°C (% total fatty acids)

		Frozen storage period				
Fatty acids	Fresh roe	Initial	3rd month	6th month	9th month	SD*
C14:1	0.08±0.01	0.06±0.00	0.09±0.01	0.07±0.01	0.06±0.02	S
C15:1	0.01±0.00	0.01±0.00	0.01±0.01	0.02±0.02	0.02±0.02	NS
C16:1	3.49±0.03	3.41±0.03	3.07±0.02	3.14±0.02	3.14±0.02	S
C17:1	0.80±0.02	0.76±0.02	0.65±0.02	0.62±0.02	0.58±0.02	S
C18:1t	0.24±0.03	0.23±0.05	0.25±0.02	0.11±0.07	0.18±0.02	S
C18:1c	21.88±0.76	21.41±0.48	18.88±0.08	20.75±0.13	20.44±0.03	S
C18:2t	0.17±0.03	0.21±0.02	0.19±0.02	0.18±0.03	0.12±0.01	S
C18:2c	1.31±0.04	0.95±0.01	0.75±0.03	0.91±0.03	0.90±0.03	S
C18:3 n-3	0.46±0.03	0.41±0.03	0.43±0.02	0.40±0.01	0.36±0.05	NS
C18:3 n-6	0.16±0.02	0.15±0.02	0.15±0.02	0.11±0.02	0.09±0.01	S
C20:1	0.30±0.01	0.36±0.04	0.32±0.02	0.31±0.01	0.27±0.02	S
C18:4 n-3	0.36±0.03	0.31±0.02	0.30±0.03	0.27±0.03	0.25±0.02	S
C20:3 n-3	0.18±0.02	0.15±0.01	0.12±0.01	0.14±0.04	0.10±0.01	S
C20:4 n-6	0.14±0.03	0.14±0.01	0.12±0.01	0.12±0.02	0.10±0.01	NS
C20:5 n-3	5.29±0.02	5.22±0.05	4.31±0.13	4.11±0.04	4.15±0.02	S
C24:1	0.97±0.02	0.96±0.01	1.08±0.06	0.98±0.01	0.89±0.03	S
C22:5 n-3	1.66±0.15	1.68±0.01	1.36±0.02	1.25±0.05	1.27±0.02	S
C22:6 n-3	24.79±0.03	24.70±0.03	21.80±0.11	19.11±0.04	17.28±0.06	S

S: Significant difference; NS: No Significant difference, The values are expressed as mean±SD, n = 3, *Significance of difference

Table 2: Changes in kinds of unsaturated fatty acid series in *T. tonggol* roe (% fatty acids)

Unsaturated fatty acid series	Fresh roe	Frozen storage period: Initial	3rd month	6th month	9th month
Σ MUFA	27.78	27.22	24.41	26.02	25.62
Σ PUFA	34.55	33.94	29.53	26.62	24.62
ΣHUFA	31.75	31.62	27.50	24.49	22.73
Σn-3	32.75	32.47	28.32	25.29	23.41
Σn-6	1.61	1.25	1.02	1.15	1.09

MUFA: Monounsaturated Fatty acids, PUFA: Polyunsaturated Fatty Acids, HUFA: High Unsaturated Fatty Acids storage time, respectively. Decrease in MUFAs and PUFAs maybe due to breaking down of fatty acid chains, especially long chain PUFAs and the compositions with five or six double bonds (HUFA) (Table 2). Similar results were obtained by Castrillon *et al*. (1996), who reported that PUFAs diminished in sardine (*Clupea pilchardus)* during frozen (-30°C) storage.

Table 3 shows the amino acid profile of long tail tuna roe stored as frozen at -18°C. In long tail tuna roe, 9 Essential Amino Acids (EAA) and 8 nonessential amino acids (NE) were identified. The most abundant amino acids in the roe were lysine, histidine, aspartic acid and leucine. The most studies about amino acids in fresh roe showed that these amino acids are important in them. For example, Iwasaki and Harada (1985) reported that lysine and aspartic acid are the most important essential and nonessential amino acids, respectively, in blue fin tuna roe. The contents of them were 8.38 and 8.75 mg g^{-1}, respectively. On the other hand, tyrosine, cysteine and methionine are in lower concentrations than the other amino acids in the most of fish roes. In this study, the amounts of methionine and tyrosine in tuna roe were 4.47 and 4.75 mg g^{-1}, respectively that were not considerable in comparison to the others.

A decrease in the contents of lysine and methionine was observed during frozen storage. Alvarez *et al*. (1990) showed that lysine diminished in hake *(Merluccius* sp.*)* stored at -12°C for 4 months, too. They stated that a decrease in lysine content during storage period can display a reaction of lysine with formaldehyde and lysine can change to formallysine (Alvarez *et al*., 1990). Also, some researchers reported a decrease in content of methionine owing to the transition of methionine to methionine sulfoxide (Wesselinova, 2000). According to Wesselinova (2000), oxidation is only done in methionine, during frozen storage. It was also reported by some scientists that other essential amino acids were decreased at the end of storage period. Castrillon *et al*. (1996) showed that amino acid compositions of sardine (*Clupea pilchardus)* changed during frozen storage at -20°C, too. Especially, S-amino acids have decreased at -20°C. Also, histidine, tyrosine, leucine, lysine and phenylalanine diminished during this time, as the same as long tail tuna roe (Table 3).

According to Table 3, the differences in amino acids were significant ($p<0.05$), during frozen storage. The amount of EAA in tuna roe (104.78) is higher than NE (75.61) (Table 4), because long tail tuna is a marine fish. Therefore, the ratio of EAA/NE in fresh water fish roe such as common carp (0.8) is lower in comparison to tuna roe (1.38) (De Silva *et al*., 2001). However, at the end of frozen storage,

Table 3: Changes in amino acid composition in *T. tonggol* during frozen storage at -18°C (mg g^{-1})

		Frozen storage period				
Amino acids	Fresh roe	Initial	3rd month	6th month	9th month	SD*
Aspartic acid	19.24±0.03	18.36±0.01	18.37±0.05	14.30±0.02	16.47±0.04	S
Serine	5.82±0.02	5.02±0.06	5.76±0.03	5.42±0.08	5.51±0.04	S
Glutamic acid	10.62±0.01	11.10±0.02	9.86±0.06	10.44±0.05	10.58±0.04	S
Glycine	13.80±0.03	13.06±0.05	11.02±0.07	10.13±0.04	8.90±0.04	S
Histidine	19.48±0.02	18.97±0.03	16.01±0.06	15.77±0.08	15.67±0.06	S
Arginine	5.52±0.04	5.43±0.03	5.64±0.05	4.16±0.04	4.27±0.05	S
Threonine	4.50±0.02	4.37±0.03	4.52±0.04	2.30±0.05	2.23±0.05	S
Alanine	8.80±0.02	8.84±0.03	7.59±0.08	6.67±0.06	5.78±0.05	S
Proline	6.83±0.03	6.71±0.03	6.22±0.06	6.58±0.05	6.13±0.05	S
Cysteine	5.75±0.03	5.80±0.02	4.39±0.07	4.00±0.06	3.77±0.06	S
Tyrosine	4.75±0.03	4.59±0.03	4.08±0.07	3.95±0.05	4.22±0.03	S
Valine	8.93±0.04	8.98±0.04	8.90±0.04	8.25±0.09	8.10±0.06	S
Methionine	4.47±0.03	4.43±0.03	3.98±0.11	4.51±0.04	3.62±0.03	S
Lysine	21.10±0.03	19.54±0.04	16.24±0.07	17.81±0.06	15.55±0.05	S
Isoleucine	7.15±0.02	7.00±0.04	7.23±0.08	6.60±0.09	5.89±0.05	S
Leucine	18.58±0.02	18.00±0.03	15.00±0.08	15.44±0.07	12.14±0.04	S
Phenylalanine	15.04±0.05	15.21±0.03	12.83±0.04	13.25±0.08	10.69±0.08	S

S: Significant difference, The values are expressed as mean±SD, n = 3, *Significance of difference

Table 4: Changes in kinds of amino acid series in *T. tonggol* roe (mg g^{-1})

Amino acid series	Fresh roe	Frozen storage period Initial	3 rd month	6th month	9th month
Σ EAA	104.78	101.85	90.38	88.11	78.25
Σ NE	75.61	73.53	67.31	61.57	61.40
EAA/NE	1.38	1.38	1.34	1.43	1.27

EAA: Essential Amino Acids, NE: Nonessential Amino Acids, EAA/NE: ratio of Essential to Nonessential Amino Acids

this value decreased to 1.27 in tuna roe (Table 4). It was also found that amino acid levels, especially lysine and histidine of tuna roe were relatively close to the values given for amino acid requirements of human, even after 9 months of storage (Bledsoe *et al*., 2003). Therefore, *Thunnus tonggol* roe is rich in EAA and might be considered a useful food source due to these compositions.

CONCLUSION

The results obtained from this study show that the roe of long tail tuna (*Thunnus tonggol*) contains large amounts of PUFA, particularly n-3 fatty acids and also considerable contents of EAA. On the other hand, the amino acid and unsaturated fatty acid contents of frozen tuna roes maintained their quality during 9 months of storage. Therefore, the roe of long tail tuna can be beneficial sea food for human health, especially due to the rich n-3 fatty acids and essential amino acids. But, further research is needed to investigate the effects of frozen storage on the fatty acids and amino acids of other important fish roes.

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