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Amino Acid and Fatty Acid Composition of Freshwater Mussels, *Anodonta pseudodopsis* and *Unio tigridis*

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HS collected the samples and did field studies. BEA carried out the laboratory work and analysis and wrote the article

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ABSTRACT

Proximate composition, fatty acid and amino acid levels were measured in *A. pseudodopsis* and *U. tigridis* from the Lake Gölbaşı. Crude protein and lipid contents were higher in *U. tigridis* (10.75%, 0.96%) than in *A. pseudodopsis* (8.63%, 0.77%, respectively), whereas the situation was vise versa for fatty acid compositions. The proportions of Omega-3 (*n3)* were higher than those of Omega-6 (*n6)* in both of the mussels. *n6*/*n3* ratio, which was 0.90 for *A. pseudodopsis* and 0.99 for *U. tigridis,* is an index for comparing the relative nutritional value of seafood oil.Leucine and lysine were the most important proportions of the essential amino acids, and tryptophane was the minor one. The ratio of essential/non essential (E/NE) amino acids was determined as 0.37 for *A. pseudodopsis* and 0.34 for *U. tigridis*. Hence, these mussels are reliable sources of nutrition to cover amino acid requirements of human.

INTRODUCTION

Mussels are commercially valuable aquatic organisms, which are easy to cultivate or harvest in coastal areas. They are very important as human diet since they are an inexpensive source of protein with a high biological value, essential minerals and vitamins (Karakoltsidis *et al*., 1995; Astorga-Espana *et al*., 2007). Both the amount of fatty acid and the proportions of saturated, monounsaturated, and polyunsaturated fatty acids in mussel species contribute to a healthy diet and also contain significant amounts of omega-3 and omega-6 fatty acids. For this reason, mussels should be considered a low-fat, high-protein food-one that can be included in a low-fat diet (King *et al*., 1990). Factors such as water temperature, nutrient availability and reproductive cycle can influence biochemical composition of mussels (Fernandez-Reiriz *et al*., 1996; Okumuş and Stirling, 1998). The overall quality of mussels is the result of biological, chemical and organoleptic characteristics such as the shells, flesh and yield typical taste and flavor as well as absence of undesirable components. In previous studies, biochemical indices such as fatty acid, amino acid profiles and nutritive value of mussel tissues have been reported for populations in various parts of the world (Slabyj and Carpenter, 1977; Pranal *et al*., 1995; Uno *et al*., 1999; Freites *et al*., 2002; Orban *et al*., 2002; Mclean and Bulling, 2005; Babarro *et al*., 2006; Şengör *et al*., 2008; Ersoy and Şereflişan, 2010; Oliveira *et al*., 2014; Padidela and Thummala, 2015). It is well known fact that the fatty acid and amino acid profiles may vary from species to species due to the differences in biochemical properties among mussels. The sea and freshwaters of Turkey has potentially rich sources of aquatic foodstuffs in terms of variety. The Lake Gölbaşı is a natural lake in the south eastern Mediterranean region of Turkey and it is one of the most important freshwater reservoirs in the region. Total lake area is about 1200 ha which bares 400 ha of marshes. The economically valuable fish and mussel species of the lake are *Clarias gariepinus, Cyprinus carpio, Leuciscus lepidus, Anguilla anguilla, Tilapia* sp., *Carasobarbus luteus, Mugil saliens, U. terminalis, Potamida littoralis, U. tigridis* and *A. pseudodopsis*. Although the consumption of mussels is considerably low in the region and the products are usually exported to Europe and the USA. Low consumption of shellfish is primarily habitual, and may be promoted with the delivery of sufficient information about their biochemical composition and nutritional value as well as providing governmental support for the seafood processing industry.

The determination of the proximate, fatty acid and amino acid profiles of mussel tissues would definitely build up information for consumers about the nutritional value of mussels. The primary aim of this work was to identify how the proximate compositions, total fatty acid and amino acid profiles varied between the two mussel species, *A. pseudodopsis* and *U. tigridis*.

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MATERIALS AND METHODS

Mussels of commercial size that is 8-10 cm long were collected contemporaneously from the Lake Gölbaşı located in the south eastern Mediterranean region of Turkey. Samples were immediately transported to the laboratory. Total length and weight of the samples were measured to the centimeter and gram. Then mussels were rapidly washed and manually shucked by cutting the adductor muscle with a knife. The samples were quickly frozen in polyethylene bags and stored at -18 °C prior to analysis. Proximate analyses were carried out using properly homogenized samples. The protein was determined by the Kjeldahl procedure (AOAC, 1984). Moisture was determined by oven drying at 105°C to constant weight (AOAC, 1990). Total lipid was extracted from the muscle tissues using Bligh and Dyer (1959) method. The lipid content was gravimetrically determined. Ash was determined gravimetrically in a muffle furnace by heating at 550°C to constant weights (AOAC, 1990). The analyses were done in triplicates for protein, moisture, lipid and ash contents.

The lipids were esterified according to Metcalfe *et al*. (1966). The fatty acid methyl esters were analyzed on a Thermo quest trace gas chromatograph equipped with SP-2330 fused silica capillary column, 30x0.25 mm ID 0.20 mm film thickness. Column injector and detector temperatures were 240 and 250°C, respectively. Carrier gas, helium; split ratio 1/150; column flow 75 ml/min; make-up 30 ml/min (He) range 1; sample injection 0.5 ml. The fatty acid methyl mixture No. 189-19 was used for standards (Sigma).

Amino acid analysis of 0.1-mg samples hydrolyzed with 1 mL 6 N HCl for 24 h was made with an Eppendorf Biotronik LC 3000 microprocessor controlled amino acid analyzer.

Analysis of variance was used to evaluate the analysis data and significant differences among means were determined by Independent Samples-T Test (p=0.05). Statistical calculation was performed with SPSS 15.0 for windows.

Table I.- Proximate composition (%) of *A. pseudodopsis* and *U. tigridis*.

Component	*A. pseudodopsis*	*U. tigridis*
Moisture	87.47±0.87*	82.75±0.36
Protein	8.63±0.25	10.75±0.24*
Lipid	0.77±0.03	0.96±0.06*
Ash	0.29±0.02	1.73±0.04*

*The values are significantly different at p<0.05.

RESULTS AND DISCUSSION

Proximate composition

The values for the proximate composition of the mussels, *A. pseudodopsis* and *U. tigridis*, cultivated in Gölbaşı Lake, with respective mean and standard-deviations are given in Table I. Protein, lipid and ash contents of *U. tigridis* were found lower than those of *A. pseudodopsis*. While the percentage of crude protein, lipid, ash and moisture in the flesh of *A. pseudodopsis* were 8.63, 0.77, 0.29 and 87.47%, those values in *U. tigridis* were 10.75, 0.96, 1.73 and 82.75%, respectively. Özdemir *et al*. (1999) and Carvalho *et al*. (2007) reported similar results in other mussel species. Slabyj and Carpenter (1977) stated that raw mussel meat contained 81.2% moisture, 3.29% protein, 0.81% lipid, 0.41% ash. Furthermore, Muller and Tobin (1980) reported that the edible part of mussels is 80% water, 10% protein, 1.5% lipid, 3% carbohydrate, and 2% ash, with 275 kj of energy.

Fatty acid (FA) composition

Investigation of fatty acids revealed that 85.02% of them could be identified and consisted of 27 FAs in total in the flesh of *A. pseudodopsis*. The rate of identification of FAs in *U. tigridis* was higher (89.51%). The number of identified saturated fatty acids (SFAs) was 11 and they were representing 29.05% and 28.01% of total FAs, repectively. Thus, Both MUFA and PUFA, together with unidentified fatty acids combines around 70% of the fatty acids. Similar values were found for other mussel species (Freites *et al*., 2002; Vernocchi *et al*., 2007). There were eight of each of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) extracted from both of the species. the remaining fatty acids found in both species (about 70%) were mono and polyunsaturated fatty acids (MUFA+PUFA).

The fatty acid profile generally exhibits a dominance of the two classes, SFAs and PUFAs (Table II). SFAs in *A. pseudodopsis* represented 29.05% of the total FAs. The least of them was MUFAs with 23.06% and the most abundant group was PUFAs representing 32.91% of the total. Similar situation was observed for *U. tigridis,* as well, and the values were 28.10%, 21.80% and 39.61%, respectively. Analyses have shown that the major SFA was palmitic acid (C16:0) for both of the species and constituted about the half of the SFAs. Similar results for *M. galloprovincialis* have also been reported in the literature (Freites *et al*., 2002; Vernocchi *et al*., 2007). Another highly abundant fatty acid in mussels was stearic acid. They, together, made up of ¾ of total SFAs. However, the least of the SFAs were varied depending on the species and it was C21:0 for *A. pseudodopsis* and C22:0 for *U. tigridis*.

Table II.- Fatty acid profiles (% total fatty acids) of *A. pseudodopsis* and *U. tigridis*.

Fatty acids (%)	*A. pseudodopsis*	*U. tigridis*
C12:0	0.06±0.01	0.20±0.03*
C14:0	0.89±0.01	1.16±0.01*
C15:0	0.77±0.03	1.13±0.06
C16:0	15.05±0.07	15.04±0.11
C17:0	1.50±0.02	1.81±0.01*
C18:0	6.98±0.05	6.37±0.28
C20:0	1.13±0.08*	0.74±0.05
C21:0	0.05±0.01	0.07±0.01
C22:0	1.08±0.01*	0.05±0.01
C23:0	1.37±0.01	1.41±0.02
C24:0	0.17±0.02	0.12±0.02
∑SFA	29.05	28.10
C14:1	0.27±0.02	1.20±0.02*
C15:1	0.89±0.01	2.90±0.02*
C16:1	4.04±0.04	6.79±0.07*
C17:1	1.26±0.00	1.54±0.01*
C18:1	14.15±0.17*	6.09±0.04
C20:1	0.71±0.02	0.78±0.01
C22:1	0.08±0.01	0.96±0.01*
C24:1	1.66±0.01*	1.54±0.02
∑MUFA	23.06	21.80
C18:2*n*6	7.52±0.48*	4.68±0.09
C18:3*n*6	2.00±0.01	3.94±0.18*
C20:2*n*6	1.70±0.02	1.66±0.01
C20:4*n*6	0.40±0.01	1.52±0.01*
C22:2*n*6	3.96±0.08	7.93±0.11*
C18:3*n*3	2.68±0.04	3.15±0.01*
C20:5*n*3	8.10±0.03	9.58±0.05*
C22:6*n*3	6.55±0.02	7.15±0.05*
∑PUFA	32.91	39.61
∑*n*6	15.58	19.73
∑*n*3	17.33	19.88
*n*3/*n*6	1.11	1.01
Unidentified	14.98	10.49

*The values are significantly different at $p<0.05$.

Each of these fatty acids represented 0.05% of the total. The most and the least abundant constituent of MUFAs was different for both species. Oleic acid (C18:1) constituted more than half (14.15% of the total) of the MUFAs in *A. pseudodopsis* whereas it was palmitoleic acid (C16:1) representing one third of MUFAs (6.79% of the total) in *U. tigridis*. Both of these fatty acids, together, constituted more than half of the total MUFAs in both species. The least abundant components of MUFAs were C22:1 (0.08%) and C20:1 (0.78%), respectively. As for PUFAs predominant fatty acid component was EPA (C20:5n3) for both species. Although C18:2*n*6 was in the second rank in the PUFAs of *A. pseudodopsis,* it was C22:2*n*6 for *U. tigridis*. DHA (C22:6n3) was in the third place amongst PUFAs. The lowest value within PUFAs obtained for C20:4*n*6.

The proportions of PUFAs-*n3* (17.33 for *A. pseudodopsis,* 19.88 for *U. tigridis*) were higher than those of PUFAs-*n6* (15.58 for *A. pseudodopsis* to 19.73 for *U. tigridis*). The *n6*/*n3* ratio is a good index for comparing the relative nutritional value of fish oil (Pigott and Tucker, 1990). The UK Department of Health recommends an ideal ratio of *n6*/*n3* of 4.0 at maximum (HMSO, 1994). A higher ratio is of great importance in order to diminish coronary heart diseases, plasma lipid levels and cancer risks (Kinsella *et al*., 1990). In this study, the *n6*/*n3* ratio of *A. pseudodopsis* and *U. tigridis* was 0.90 and 0.99, respectively.

Among the *n3* series, both mussels are good sources of EPA (8.10–9.58%) and DHA (6.55–7.15%). Similarly, Freites *et al*. (1999) reported that fatty acid profiles in *M. galloprovincialis* were in the range of 8.86–12.70% for DHA. Vernocchi *et al*. (2007) indicated that DHA contents of *M. galloprovincialis* averaged 4.98%-6.29%.

British Nutrition Foundation (1992) has also recommended that people who has balanced and healthy diet consume 0.2 g of EPA+DHA daily. In this respect, Table II provides the researchers with valuable information for preparing diet tables.

Amino acid composition

The amino acid compositions of *A. pseudodopsis* and *U. tigridis* are shown in Table III. Hydroxyproline and glutamic acid was the most abundant amino acids in two mussel species Hydroxyproline is a non-proteinogenic amino acid formed by the post-translational hydroxylation of proline. Hydroxyproline is a major component of protein collagen; it plays key roles for collagen stability. Because hydroxyproline is largely restricted to collagen, the measurement of hydroxylproline levels can be used as an indicator of collagen content (Nelson and Cox, 2005; Brinckman *et al*., 2005; Szpak, 2011). Glutamic acid, which is widely known amino acids, is an important source of nitrogen, although it's a non-essential amino acid which is usually used to improve or balance the taste of monosodium glutamate. However, glutamate exists naturally in many foods, such as meat, milk, fish, poultry,

and vegetables, in varying amounts (Lopez, 1975). The amount of asparagine, tryptophan, ornithine and sarcosine were lower than the other amino acids. *U. tigridis* had higher levels of glutamic acid, serin, glycine, arginine, alanine, lisine, leucine, hydroxyoroline and proline than *A. pseudodopsis*.

Table III.- Amino acid profiles (g/100 g) of *A. pseudodopsis* and *U. tigridis.*

Amino acids (g/100 g)	*A. pseudodopsis*	*U. tigridis*
Aspartic acid	0.643	0.693
Glutamic acid	0.898	1.012*
Asparagine	<0.038	<0.038
Serine	0.346	0.434*
Histidine	0.210	0.224
Glycine	0.384	0.492*
Theronine	0.444	0.466
Citrulline	0.269	0.270
Arginine	0.548	0.689*
Alanine	0.382	0.457*
Tyrosine	0.462	0.499
Cystine	0.438	0.457
Valine	0.304	0.318
Methionine	0.238	0.241
Tryptophan	<0.024	<0.021
Phenynlalanine	0.310	0.342
Isoleucine	0.323	0.368
Ornithine	<0.018	<0.028
Leucine	0.535	0.661*
Lysine	0.534	0.582
Hydroxyoroline	2.221	2.878*
Sarcosine	<0.020	<0.024
Proline	0.466	0.665*
Total	9.987	11.839*

*The values are significantly different at p<0.05.

The nutritional quality of protein is connected to the quantity of the essential amino acids in food (Acton and Rudd, 1987). Leucine and lysine was the most important proportions of the EAAs, and tryptophane is the minor one. Pranal *et al*. (1995) noted that arginine and threonine represented the most important proportions of the essential amino acids and tryptophane and methionine are the minor ones in *M. galloprovincialis*. In the present study, the ratio of essential/non essential (E/NE) amino acids was determined as 0.37 for *A. pseudodopsis* and 0.34 for *U. tigridis* (Table III).

Millward (1997) determined the requirement of amino acid (g/100 g protein) for adult human as follows; histidine: 1.4; isoleucine: 3.1; leucine: 3.5; lysine: 2.1; threonine: 3.0; tryptophane: 7.0 and valine: 3.1. It can be concluded that these mussels can cover amino acid requirements as food for human.

CONCLUSION

The fatty acid and essential amino acid amounts of both species are quite proper for the human diet, and these species are recommended for consumption regularly. This study was expected to shed light on future studies on fatty acid and amino acid compositions of both species, which has lacked the necessary attention so far.

Satement of conflict of interest

All authors have no conflict of interest with any one about this manuscript.

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