Taste activity value, free amino acid content and proximate composition of Mountain trout (*Salmo trutta macrostigma* Dumeril, 1858) muscles

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Abstract

In the present study, we identified free amino acid (FAA) contents and chemical composition in four populations of S*almo trutta macrostigma* living in Mediterranean region of Turkey. In addition, taste impacts of FAAs were evaluated by taste active values. Moisture, protein, fat and ash content were found in the ranges of 75.49 - 79.59 %, 16.94 - 19.97 %, 1.58 - 3.75 % and, 1.39 - 1.56 %, respectively. While the significant difference ($p<.05$) were found among the different populations in moisture, protein and lipid content, no significant ($p>.05$) differences in ash content was determined. Thirteen FAAs were identified from the muscle tissue of *S. trutta macrostigma* by HPLC. The Glycine was found predominant as followed by methionine, proline, and glutamic acid. Also, the amount of non-essential free amino acids in *S. trutta macrostigma* muscle were significantly ($p<.05$) higher than the essential amino acids. Glutamic acid, methionin, glycine, aspartic acid and lysine were of high taste activity values (greater than one), they had strong taste impacts on the mountain trout meat flavour.

Keywords: Mountain trout, *Salmo trutta macrostigma*, Free amino acid, Taste activity

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Introduction

Free amino acids (FAAs) are among the most important fractions of non-protein nitrogen in crustaceans and fish (Ikeda, 1980; Yamanaka and Shimada, 1996). Non-protein nitrogenous compounds have been widely used as indicators for evaluation of the taste of fish and shellfish (Konosu and Yamaguchi, 1982; Saito and Kunisaki, 1998). Some peptides and FAAs are very important taste substances (Kato et al., 1989), many of the latter -such as alanine, glutamic acid and glycine- are responsible for flavour and taste in foods. Alanine, glycine and glutamic acid have the ‘‘umami’’ taste typical of crustaceans (Yamanaka and Shimada, 1996).

Non-protein nitrogenous compounds comprise between 0.5 and 1% of total weight of the fish muscle (Spinelli and Dassow, 1982). Free amino acids found in relatively high amounts in fish are glycine, taurine, alanine and lysine. Migratory fishes such as mackerel and tuna contain high amounts of histamine and white-fleshed fishes are high in taurine content (Konosu and Yamaguchi, 1982). Chiou et al. (1990) found that histidine was the most prominent and accounted for about 80% of the total FAA in milkfish. On the other hand, tilapia was rich in taurine and glycine, but contained a small amount of histidine. Antoine et al. (2001) also repoted that histidine levels were higher in white tissue of mahi-mahi and tuna than in red tissue. Red snapper was found higher levels of lysine than histidine. The free amino acid composition in the fish muscles have been previously reported for other fish species, including salmon (Carter et al., 2000; Lund and Nielsen, 2001; Hultman et al., 2004), Taiwanese puffer (Hwang et al., 2000), rainbow trout (Yamamoto et al., 2000), milkfish (Shiau et al., 2001), sardine (Shirai et al., 2002), tuna (Ruiz-Capillas and Moral, 2004), garfish (Dalgaar et al., 2006), and goby (Karanova, 2009).

The mountain trout, *S. trutta macrostigma* (Duméril, 1858), is distributed North Africa, South Europe and West Asia including Anatolia. Occurring in the upper parts of streams and rivers, it has been reported from many running waters in Turkey (Alp et al., 2005). Considered a delicacy in many parts of Turkey, the mountain trout is a preferred catch in sportive fishing (Geldiay and Balık, 1996). Due to its economical potential, several experimental studies were carried out to adapt the mountain trout into culture conditions (Quillet et al.,1991; Krieg et al., 1992; Quillet et al.,1992; Demir et al., 2010). There are a large number of studies on *S. trutta macrostigma* from Turkey concerning reproduction, growth, biomass, processing, and fatty acids (Alp et al., 2003; Kocaman et al., 2004; Alp et al., 2005; Bilgin et al., 2007a,b; Akpınar et al., 2009; Bayır et al., 2010). However, the non-volatile taste active compounds, such as free amino acids, in *S. trutta macrostigma* muscle were not investigated so far. The free amino acid profile and proximate composition are among the important parameters that may be affected by the origin of fish. There is no study about the concentration of free amino acids in muscle of *S. trutta macrostigma*. Thus, the main objective of this study is to establish basic data of proximate composition, free amino acid profile of muscle in natural populations of *S. trutta macrostigma*. Also, it was aimed to

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evaluate the taste impacts of free amino acids present in the mountain trout muscle by taste activity value method.

Materials and methods

HPLC grade water, obtained with a Milli-Q water purification system (Human Power II., KOREA) was used throughout the study. All the chemicals were of analytical grade (Sigma, St. Louis, MO). 9-fluorenylmethyl chloroformate (FMOC-Cl) was prepared dissolving in acetonitrile as 4 mg/ml. Borate buffer was prepared from a 250 mM boric acid solution adjusted to pH 8.5 with 1 M sodium hydroxide solution prepared from sodium pellets. The alkaline cleavage reagent was prepared daily in 1 ml batches by mixing 680 µl of 850 mM sodium hydroxide solution with 300 µl of 500 mM hydroxylamine hydrochoride solution and 20 µl of 2-(methylthio)ethanol. The quench reagent was acetonitrile–acetic acid (8:2).

Thirteen amino acids were detected in the samples: aspartic acid, glutamic acid, glycine, proline, arginine, alanine, tyrosine, lysine, methionine, valine, isoleucine, leucine and phenylalanine. Valine and isoleucine were determined together, as their peaks merged. The identity and quantity of the amino acids were determined by comparison with the retention times and peak areas of each amino acid standard.

S.trutta macrostigma specimens were caught from Eşen (Population I), Aksu (Köprüçay) (Population II), Alara (Population III) and Alakır (Population IV) streams in Turkey (Fig1). Demir et al. (2010) reported that some physical and chemical parameters of the water in these localities were similar. Two specimens from each locality were used for analyses. Average weights of the specimens were 96.49±2.20, 69.38±23.99, 108.92±18.74 and 59.59±8.18g, respectively. Specimens were carried in polystrene boxes filled with ice cubes and brought to food laboratory in Süleyman Demirel University, Fisheries Faculty within 5 h. After weighing, the specimens were gutted and washed with tap water to clean the blood and mucus then filleted. Fillets were stored under -80°C untill chemical content and free amino acids analyses. All analyses were performed using the muscle between the gills and the dorsal fin.

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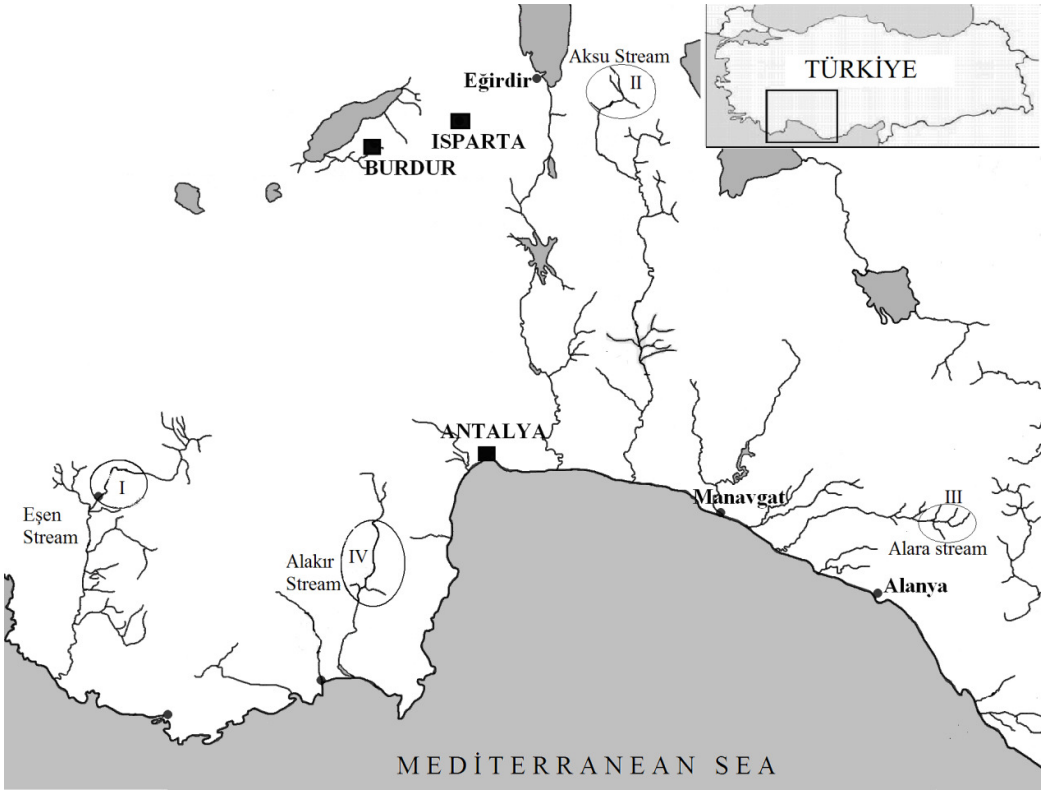


Figure 1: Natural mountain trout specimens were caught in locality (Eşen I: 36.771914°N - 29.407618°E, Aksu II: 37.826410°N - 31.112606°E, Alara III: 36.762420°N - 32.144814°E, and Alakır IV:36.546430°N - 30.288160°E streams)

Moisture content was measured with automatic moisture analyzer (AND MX-50, Japon) (Nielsen, 2003). Nitrogen is determined using Kjeldahl method and protein is calculated from the nitrogen content (AOAC, 1997a). Crude fat was determined according to Standard procedures (Lovell, 1981). Ash contents were determined according to AOAC (1997b) .

The isolation of FAAs was performed by accordance with the technique described by Lopez-Cervates vd., (2006). Fish muscle (20 mg) was extracted with 0.1 M HCl (5 ml) in a homogenizator (Heidolph Diax 900, GERMANY) at 24.000 rpm for 1 min and centrifuged in cold (4°C) at 10.000 rpm for 10 min (Sigma 2-16K, GERMANY). To derivatize, 300 µl of the amino acid standard solution or the prepared sample was deposited in 1.5 ml vials, which were vortexed for 90 s after adding 300 µl of FMOC reagent. The cleavage reagent was added (180µl) and the tubes were vortexed for 15s, then left for 5 min at room temperature, and 420 µl of quench reagent was then added. The resulting solution was vortexed for 15s and filtered with a membrane 0.45 µm. A 10 µl sample of this solution was injected onto the column of the HPLC system.

Detection and quantification were carried out with a Shimadzu LC-20AT prominence System controller (Kyoto, Japan),

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SIL-20AC prominence Autosampler, LC-20AT prominence pump and RF-10AXL Fluorescence Detector (Ex 270 nm, Em 316 nm) for free amino acids. The YMC-Pack ODS-AM (250x4.6 mm, 5µ) column was used. HPLC conditions were as follows: mobile phase A: %1.2 $(NH_4)_2HPO_4$ in 15:85 (v/v) methanol: water; mobile phase B: 15:85 (v/v) methanol: water; mobile phase C: 90:10 (v/v) acetonitrile: water. The flow rate was constant at 1.0 ml/min and the column maintained at 43 °C. The detection was by fluorescence using the wavelengths of excitation and emission at 270 and 316 nm, respectively. The total time between injections was 43 min. The gradient program used is shown in Table 1. Figure 1 shows the HPLC profile of a mixture of amino acids standarts. An example of an HPLC separation of the amino acids from a *S. trutta macrostigma* muscle (Fig. 2).

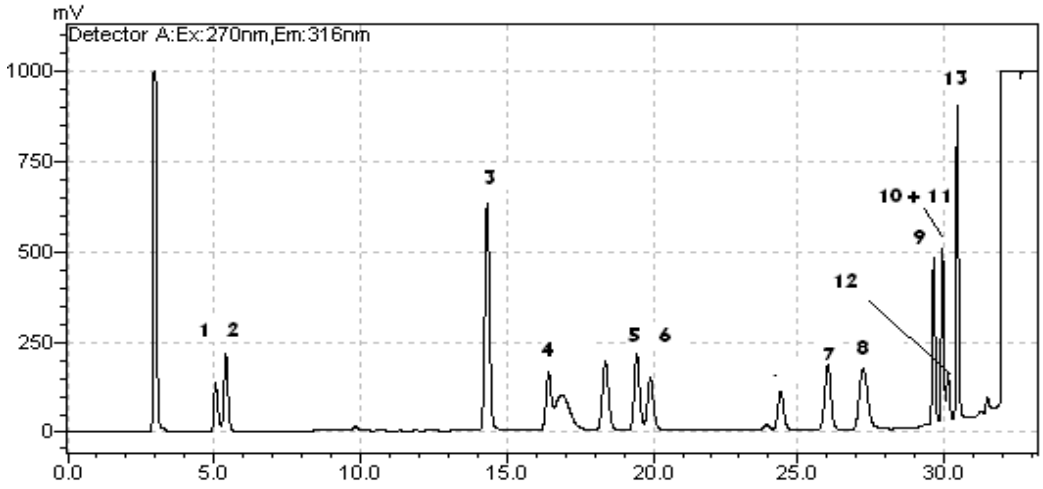


Figure 2: HPLC profile of a mixture of amino acids standards (1: aspartic acid, 2: glutamic acid, 3: glycine, 4: proline, 5: arginine, 6: alanine, 7: tyrosine, 8: leucine, 9: methionine, 10+11: valine+ isoleucine, 12: leucin, 13: phenylalanine)

Table 1: Gradient program employed for the separation of FMOC-amino acids

Time (min)	Eluent A (%)	Eluent B (%)	Eluent C (%)
0.01	11	68	21
25.00	11	59	30
26.00	11	49	40
35.00	0	0	100
40.00	0	0	100
40.10	16.5	69	14.5
43.00	16.5	69	14,5

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Taste values each free amino acid were calculated with concentration divided of taste threshold (Cha and Cadwallader 1998; Chen and Zhang 2007; Fuentes et al., 2009).

The compounds whose taste activity value was greater than 1 were considered as active in food taste (Chen and Zhang, 2007).

This experiment was conducted in duplicate, and the mean values were obtained from 3 measurements (n = 3 × 2). Data are reported as means ± standard deviation. Statistical treatment of the data was performed using the software SPSS 16.0 Windows program (SPSS, Chicago, USA). The statistical significance of differences between mean values was set as $P \leq .05$ with the Duncan test.

Results

The proximate compositions of *S. trutta macrostigma* muscles in natural populations are shown in Table 2. Moisture, ranging from 75.49 to 79.59 %, was the major constituent of the muscle. Protein, fat and ash contents were found within the ranges of 16.94–19.97, 1.58–3.75 and, 1.39–1.56 %, respectively. Significant differences in moisture, protein and lipid content among the samples were found ($p<.05$). However, no significant differences in ash content were found among the samples ($p>.05$). The protein content was significantly higher in *S. trutta macrostigma* from populations I and III when compared to the other two populations.

Table 2: Chemical compositions of *S. trutta macrostigma* muscle (%, wet basis)

	I	II	III	IV	Means
Moisture	75.49±0.18^{b}	79.59±1.44^{a}	76.61±1.84^{b}	79.57±1.15^{a}	77.82
Protein	19.97±0.26^{a}	16.94±0.98b	19.08±0.87^{a}	16.95±0.81^{b}	18.24
Lipid	3.75±0.72^{a}	1.98±0.17b^{c}	2.70±0.73^{b}	1.58±0.35^{c}	2.50
Ash	1.39±0.06^{a}	1.39±0.05^{a}	1.44±0.11^{a}	1.56±0.29^{a}	1.44

Data are means ± Standard Deviation (n=2x3).

Means followed by the same letter within each row are not significantly different ($p>.05$).

The content of FAAs in muscles of *S. t. macrostigma* from the 4 natural populations in Turkey is shown in Table 3. Thirteen FAA were identified from the muscle tissue. Glycine was the predominant FAA in all the samples, accounting for 44.71, 43.12, 44.63 and 40.86 % of the total FAA respectively in the population I, populatiın II, population III and population IV samples. The differences between amounts of this FAA were not statistically significant ($p>.05$). Glycine abundance was followed by methionine, glutamic acid and lysine in population I. Proline is the second major FAA in population II, followed by lysine and glutamic acid. Methionine was the FAA present at the second highest level in populations III, followed by tyrosine and lysine. Glycine abundance was followed by proline, methionine and glutamic acid in population IV (Table 3). The ANOVA showed significant differences in the FAA profile according to origin, except for glycine, lysine, valine+isoleucine and leucine contents. Glutamic acid concentration of the populations

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I, II and IV were significantly ($p<.05$) higher than population III. Aspartic acid, on the other hand, showed significant variations among populations. Tyrosine concentration of population IV was significantly ($p>.05$) lower than the populations I, II and III. Also, the amount of non-essential free amino acids in *S. trutta macrostigma* muscle were significantly ($p<.05$) higher than the essential amino acids.

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Table 3: Free amino acid (FAA) concentrations (mg/g in wet basis), taste thresholds for each amino acid and the taste values in four populations of *S. trutta macrostigma*

Free Amino Acids						Taste threshold (mg/ml)[1]	Taste values[2]				
	I	II	III	IV	Means		I	II	III	IV	Means
Aspartic acid	0.00±0.00^{d}	0.21±0.13^{a}	0.08±0.03^{bc}	0.10±0.07^{b}	0.10±0.11	0.03	0.00	7.00	2.58	3.33	3.23
Glutamic acid	1.11±0.34^{a}	1.00±0.47^{a}	0.37±0.25^{b}	0.81±0.30^{a}	0.82±0.39	0.05	22.17	20.07	7.42	16.14	16.45
Glycine	5.15±0.97^{a}	5.59±0.85^{a}	4.68±1.94^{a}	4.19±1.10^{a}	4.90±1.20	1.3	3.96	4.30	3.60	3.23	3.77
Proline	0.33±0.05^{c}	2.17±0.39^{a}	0.34±0.05^{c}	1.70±0.44^{b}	1.13±0.79	3	0.11	0.72	0.11	0.57	0.37
Alanine	0.10±0.04^{b}	0.46±0.25^{a}	0.05±0.04^{b}	0.30±0.14^{a}	0.23±0.20	0.6	0.16	0.77	0.08	0.49	0.38
Tyrosine	0.68±0.24^{b}	0.51±0.17^{b}	0.93±0.17^{a}	0.14±0.06^{c}	0.56±0.30	-	-	-	-	-	-
Arginine *	0.06±0.02^{b}	0.07±0.03^{b}	0.03±0.01^{b}	0.11±0.05^{a}	0.06±0.04	0.5	0.11	0.13	0.05	0.21	0.12
Lysine*	0.85±0.25^{a}	1.02±0.64^{a}	0.73±0.27^{a}	0.59±0.13^{a}	0.80±0,35	0.5	1.70	2.04	1.46	1.17	1.60
Methionine*	2.13±0.82^{a}	0.73±0.18^{c}	1.74±0.60^{ab}	1.11±0.69^{bc}	1.43±0.72	0.3	7.08	2.44	5.81	3.71	4.77
Valine+ Isoleucine*	0.30±0.20^{a}	0.37±0.05^{a}	0.37±0.06^{a}	0.49±0.06^{a}	0.38±0.11	-	-	-	-	-	-
Leucine*	0.48±0.09^{a}	0.69±0.29^{a}	0.62±0.15^{a}	0.43±0.13^{a}	0.55±0.18	1.9	0.25	0.36	0.32	0.22	0.29
Phenylalanine*	0.32±0.14^{a}	0.12±0.04^{c}	0.30±0.07^{ab}	0.20±0.06^{bc}	0,23±0.10	0.9	0.35	0.14	0.33	0.22	0.25
Non-essential FAAs	7.36±1.37^{b}	9.95±1.27^{a}	6.44±1.84^{b}	7.24±1.45^{b}	7.75±1.85						
Essential FAAs	4.13±0.55^{a}	3.00±0.32^{b}	3.78±0.53^{a}	2.93±0.72^{b}	3.46±0.69						
Total FAAs	11.49±1.75^{a}	12.95±1.43^{a}	10.22±1.98^{a}	10.17±1.92^{a}	11.21±1.96						

*Essential free amino acids.

[1] Data from Kato et al. (1989).

[2] Taste value= compound concentration divided by taste threshold (Cha and Cadwallader 1998; Chen and Zhang 2007;Fuentes et al., 2009)

- Not available for taste threshold data.

Data are means ± Standard Deviation (n=6). Means followed by the same letter within each row are not significantly different ($p>.05$)

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The taste activity values in muscles of *S. trutta macrostigma* from the natural populations in Turkey is given in Table 3. The taste activity values of glutamic acid (sour/umami), methionine (bitter/sweet), glycine (sweet) aspartic acid (sour/umami) and lysine (bitter/sweet) in all samples were 16.45, 4.77, 3.77, 3.23, and 1.60, respectively (Table 3). The glutamic and aspartic acids showed the highest taste value and lowest threshold according to origin, except for population I for aspartic acid (Table 3). It is suggested that free amino acids such as aspartic acid and glutamic acid are the most effective FAAs on the taste of *S. trutta macrostigma*. Methionine, glicine and lysine are the other FAAs that have the taste activity values higher than 1 in all specimens (Fig. 3).

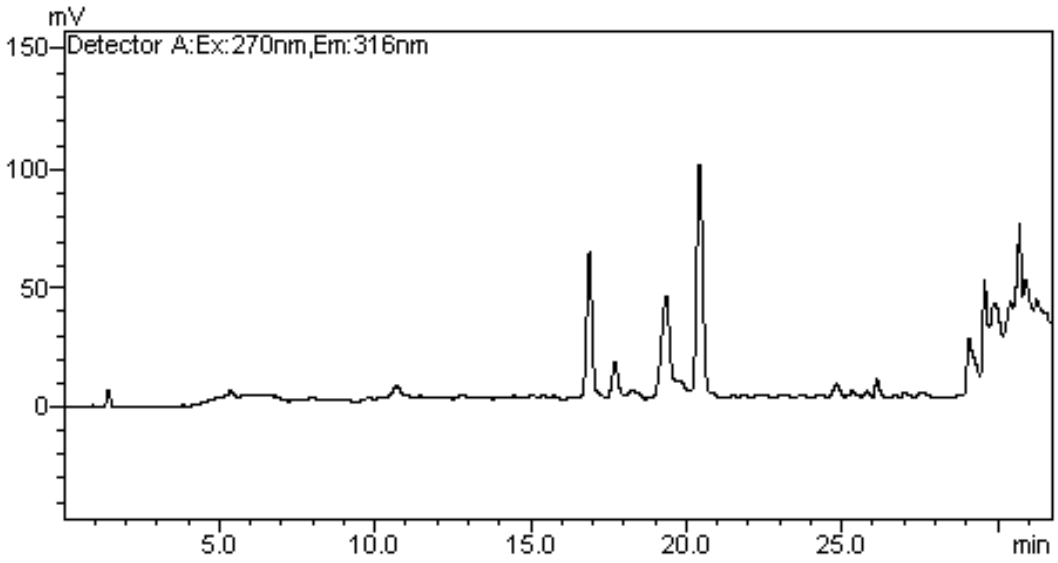


Figure 3: Example HPLC profile of amino acids in *S. trutta macrostigma* muscle (population III)

Discussion

Proximate biochemical analysis provides information on the nutritional value of a particular organism used as a source of food. The chemical composition of fish flesh varies not only between species, but also between individuals depending on sex, age, feed, stage of maturity, environment, season and also muscle location (Sikorski et al., 1990). Moisture, ranging from 75.49 to 79.59 %, was the major constituent of the *S. trutta macrostigma* muscle (Table 2). Protein, fat and ash contents were found within the ranges of 16.94–19.97, 1.58–3.75 and, 1.39–1.56 %, respectively. According to the Duman et al. (2011), the chemical composition of *S. trutta macrostigma* meat was found to be 75.55 – 77.86 % moisture, 18.35 -18.57 % protein, 1.70 – 3.71 % lipid, and 1.12 – 1.17 % ash. In addition, Bilgin et al. (2007) also reported similar results (78.901 % moisture, 16.218 % protein, 2.551 % lipid, and 1.330 % ash). These results are in agreement with our values

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(Table 2). Wide variation in moisture content between species was observed in raw freshwater fish, ranging from 65 to 80 g/100 g (Puwastien et al., 1999). Crude protein content in fish flesh varies depending on the species, the nutritional condition, the state of nutrition, and the productive cycle of animal (Sikorski et al., 1990). Puwastien et al. (1999) also reported the protein content range for raw freshwater fish as 17 to 20 g/100 g.

Fish can be grouped into four categories according to their fat content lean fish (<2%), low (2-4 %), medium (4-8 %) and high fat (>8 %) (Ackman, 1989). In terms of the lipid content, *S. trutta macrostigma* can be considered to be about in the low fat fish category. As a whole the results showed that *S. trutta macrostigma* is a high protein content fish with the lowest moisture and lipid content. The ash content was high in *S. trutta macrostigma* which is about 1.44%. Principal composition of fish is 16-21 % protein, 0.2-5% fat, 1.2-1.5% mineral and 66-81% water (Love, 1970).

As reported from other fish species (Konosu and Yamaguchi 1982), glycine was the predominant FAA in all the samples. Glycine abundance was followed by methionine, glutamic acid and lysine in population I. Proline is the second major FAA in population II, followed by lysine and glutamic acid. Methionine was the FAA present at the second highest level in populations III, followed by tyrosine and lysine. Glycine abundance was followed by proline, methionine and glutamic acid in population IV (Table 3). These differences might be due to different environmental and nutritional conditions, as has been established in previous studies (Hwang et al., 2000; Fuentes et al., 2009). Fuentes et al., (2009) claimed that the differences between the FAAs profile could be the result of proteolysis. We are assuming that the differences between mentioned values could be the result of transportation times of specimens.

According to the Dalgaar et al., (2006), histidine was the major FAA in chilled garfish fillets, followed by alanine, lysine, glysine, glutamic acid and serine. Lund and Nielsen (2001) investigated content in FAAs of fresh salmon. The dominant FAA in all samples was histidine, constituting about 48% (weight %) of total FAA content in salmon. Alanine, glutamic acid and glycine were also important, constituting about 22% of total FAA content. Hultman et al., (2004) also reported that the dominating FAAs in flesh salmon were glycine/arginine (coaliting) and alanine, followed by lysine, threonine and glutamic acid. Ruiz-Capillas and Moral (2004) found that the dominating FAA in fingerling rainbow trout was glutamic acid (13.76 mg/100 g), aspartic acid (9.70 mg/100 g), lysine (8.65 mg/100 g) and leucine (7.83 mg/100 g). Shiau et al. (2001) revealed that predominant FAA of white muscle of milkfish *Chanos chanos* was histidine, followed by taurine and glycine. Our values are partially in agreement with results determined from different fish species (Table 3).

Each amino acid contributes, to differing degrees, to the taste of foods. Glycine and alanine have a pleasant sweet taste, and they are widely presented in large quantity in seafoods (Fuke and Konosu, 1991; Spurvey et al., 1998; Wu and Shiau, 2002). Aspartic acid and glutamic acid have a sour taste, but it is

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responsible than umami taste in the presence of sodium salt. Umami taste is considered to contribute largely to taste of many seafood. Umami was first defined as the characteristic taste elicited by glutamates, and has since also been associated with monosodium glutamate (Yamaguchi and Ninomiya, 2000). Although, some amino acids as aspartic and glutamic acids were present in small amounts in the fish muscle, their taste impacts were strong because of their low threshold values. Taste activity value was a very useful index (Chen and Zhang, 2007). The taste activity values of glutamic acid (sour/umami), methionine (bitter/sweet), glycine (sweet) aspartic acid (sour/umami) and lysine (bitter/sweet) in all samples were 16.45, 4.77, 3.77, 3.23, and 1.60, respectively (Table 3). The glutamic and aspartic acids showed the highest taste value and lowest threshold according to origin, except for population I for aspartic acid (Table 3). It is suggested that free amino acids such as aspartic acid and glutamic acid are the most effective FAAs on the taste of *S. trutta macrostigma*. Fuentes et al. (2009) reported that glutamic and aspartic acid exhibited the highest taste values in mussels. Similarly, previous studies have also explained that the highest taste values of glutamic and aspartic acids were observed for other seafoods, including skipjack tuna sauce (Cha and Cadwallader, 1998), pacific whiting sauce (Tungkawachara et al., 2003), shrimp muscle and shrimp processing by-products (Heu et al., 2003). Methionine, glicine and lysine are the other FAAs that have the taste activity values higher than 1 in all specimens (Table 3). Similar results were reports for mussels (*Mytilus galloprovincialis*) from different Spanish origins (Fuantes et al., 2009), Skipjack Tuna Sauce (Cha and Cadwallader, 1998), pacific whiting sauce (Tungkawachara et al., 2003). Chen and Zwang (2007) also reported that glycine which is responsible the sweet taste in seafoods had high taste activity value in Chinese mitten crab (*Eriocheir sinensis*).

Mountain trout which is preferred by consumers because of interesting nutrition values flesh is one of the most important sportive fishing resources in the Turkey. Mountain trout has been widely accepted as a good source of protein and lipid. Therefore, studies focused on adaptation of aquaculture conditions.

According to our results, significant differences in protein, lipid and moisture content depending on the origin in mountain trout were found. As a result, glutamic acid that is well known flavour enhancer, is primarily responsible FAA followed by methionine (bitter/sweet), glicine (sweet), aspartic acid (sour), lysine (sweet/bitter) on flavour in *S.trutta macrostigma* muscle. In the case of adapting to aquaculture conditions, *S.trutta macrostigma* which is healty and delicious fishery product based on the presence of high taste activity FAAs values could be serve to consumers at retail level.

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