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AMINO ACID CONTENT IN MUSCLE TISSUE OF THE BROWN MEAGRE, *SCIAENA UMBRA* LINNAEUS, 1758 (PISCES: SCIAENIDAE) AS CANDIDATE SPECIES FOR AQUACULTURE IN THE BLACK SEA, TURKEY

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ABSTRACT: In order to understand the nutritive quality focusing on amino acid profiles in the muscle of the brown meagre, *Sciaena umbra*, and to evaluate the potential of the species as a diet source for both human consumption and as candidate species for aquaculture, the main amino acid components in the muscle of this species were analyzed. Amino acid compositions were determined with the LC-MS/MS (Liquid Chromatography Tandem Mass Spectrometer) instrument. Although a total of thirty-nine amino acids were detected / searched by LC-MS/MS instrument, only twenty-four amino acid values were determined. Seven of these amino acids are essential amino acids (EAAs), two are semi-essential amino acids (SEAAs) and fifteen are non-essential amino acids (NEAAs). The most abundant amino acids in brown meagre meat were found as glutamic acid (13.88%), lysine (10.43%), aspartic acid (9.62%), leucine (8.32%), and glycine (6.87%), respectively. These five amino acids accounted for more than 49% of the total amount of amino acids. The levels of the total amino acids (ΣAA = 24.17 g/100g), total essential amino acids (ΣEAA = 9.42 g/100g), total non-essential amino acids (ΣNEAA = 12.39 g/100g), total semi-essential amino acids (ΣSEAA = 2.36 g/100g) for brown meagre in the Black Sea were higher than those reported in the other areas. This study shows that the brown meagre has high nutritional qualities and the results are also presented as an important criterion for obtaining information on amino acid nutritional requirements for aquaculture and healthy human food source for dietitians.

KEYWORDS: seafood; aquaculture; amino acid; nutritional value; human health; Black Sea

INTRODUCTION

Brown meagre, *Sciaena umbra* Linnaeus, 1758, shows distribution in the eastern Atlantic: Southern Bay of Biscay to Mauritania, occasionally southwards to Senegal and also throughout the Mediterranean and Black seas (Froese and Pauly, 2019). This demersal fish species lives up to a depth of 200 m and occurs in shallow coastal waters mainly on rocky and sandy bottoms, often entering estuaries and more active at night (Froese and Pauly, 2019). The brown meagre feeds mainly on crustaceans, and then switches to fish as they grow (Engin and Seyhan, 2009). The maximum age was reported as 19 years in the north-western Adriatic Sea (La Mesa *et al*., 2008) and 18 years in the

Black Sea (Engin and Seyhan, 2009). In the Black Sea, size at sexual maturity was also reported as 19.5±0.16 cm for males and 21.97±0.52 cm for females (Engin and Seyhan, 2009) corresponding to 1or 2 years old.

Nowadays, amount of fishery products provided by different fishing methods is decreasing day by day due to overexploitation of fish stocks, global climate change and water pollution, etc. On the other hand, researches are carried out on the aquaculture of many wild fish species. One of the wild fish species studied in terms of taking it into culture for its cultivation is brown meagre (Çalık, 2015). The first data on growth of cultured brown meagre using diets with different protein and fat contents was reported by Chatzifotis *et al.* (2006). Moreover, in some aquaculture/ hatchery facilities, research studies are carried out for the production of new species such as brown meagre for trial purposes in Turkey (Çalık, 2015). According to Kouroupakis *et al*. (2019) and Kaushik (1998), one of the main aspects of ensuring the production of new species is to determine the quality characteristics of wild individuals belonging to the same species. In this way, useful information about the nutritional needs of the species and the desired product quality can be obtained. Amino acid composition provides useful information about the nutritional value of fish species that determine the fish quality desired by the consumer (Grigorakis, 2017). Moreover, information on fillet amino acids of fish species can be considered as an important criterion for obtaining information on amino acid nutritional requirements (Kaushik, 1998). In addition to these, knowing the amino acid composition of fish meat is also very important in human nutrition in terms of healthy food. Significant levels of essential amino acids (isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan and valine), semi- essential amino acids (arginine and histidine) and non- essential amino acids for the human body have been reported in fish meat for many both in aquacultured and hunted fish species (Kaushik, 1998; Kim and Lall, 2000; Limin *et al*., 2006; Özden and Erkan, 2011; Doğan and Ertan, 2017; Erkan *et al*., 2010; Bilgin *et al*., 2018; Bilgin *et al*., 2019ab; Oztekin *et al*., 2020).

To our knowledge, there are no studies reported for the amino acid composition of brown meagre except for one reported from the Aegean Sea (Kouroupakis *et al*. 2019). As is known, the amino acid component of fish meat is affected by many factors such as the environment the fish lives in and the amount of nutrients, etc. (Kim and Lall, 2000; Erkan *et al*., 2010; Özden and Erkan, 2011). The Black Sea differs from other seas due to its unique ecological characteristics (Bogatova *et al*., 2018). Therefore, the aim of this study was to determine the fillet quality of wild brown meagre obtained from the Black Sea in terms of their amino acid content and to compare them with the results reported from the Aegean Sea.

MATERIALS AND METHOD

Samples: Five brown meagre, *Sciaena umbra* in total were obtained from local fishermen in December 2018 off the coasts of Sinop in the Black Sea, Turkey. The brown meagre specimens just caught with bottom set gill nets were brought to the laboratory in ice. The total length of each specimen was measured with a sensitivity of 1 mm and weighed using a balance with a sensitivity of 0.01 g. After the length and weight

determination, the whole edible muscle was minced and homogenized using homogenizer and then stored in a freezer at -20ºC for one week.

Determination of amino acids: The amino acids analysis of the samples were made in duplicate using the SUBITAM's (Sinop University Scientific and Technological Researches Application and Research Center) Agilent Infinity 1260 HPLC system consisting of a binary pump, a degasser and autosampler coupled with 6460 triple quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). The procedure described below was followed by Bilgin *et al*. (2018) and (2019ab) for amino acid analysis by using LC-MS/MS (Liquid chromatography coupled with tandem mass spectrometry) instrument.

Samples were prepared by using CE-IVD certified and validated Jasem Quantitative Amino Acids LC-MS/MS Analysis Kit (Sem Laboratuar Cihazları Pazarlama San. ve Tic. Inc., Istanbul, Turkey) which included five standards for calibration, labelled stable isotope mixtures of amino acids as internal standard (IS), mobile phases, reagents, chromatographic and mass detection parameters of the method with modified sample preparation procedure comprising an acidic hydrolysis process. The concentrations of the targeted amino acids were determined using both electrospray ionization (ESI) and multiple reactions monitoring (MRM). The general process is specified in this section. Detailed MS/MS settings are explained at below including Table 1a and Table 1b.

The samples were hydrolyzed as follows: 0.5 g of sample was hydrolyzed with 4 ml of acidic hydrolysis reagent-2 in a screw capped glass tube for 24 h at 110ºC. When the sample was cooled to room temperature (~24^{o}C), the hydrolyzed sample was centrifuged (Hettich Universal 320 desktop air cooled centrifuge) at 4000 rpm (g force: 3756.48) for 5 min. (No loss of sample solid phase remains at the bottom and necessary part is supernatant) After that, 10 µl of supernatant was transferred into a sample vial and completed to 1 ml with distilled water in order to obtain 800 fold diluted hydrolysates. Subsequent to the hydrolysis step, kit sample preparation procedures for calibration standards and samples were as follows: 50 µL of the standard or diluted hydrolysate was transferred into a sample vial. Next, 50 µL of the labeled stable isotopes mixture was added as an internal standard and 700 µL of reagent-1 were added to the sample vial before swirling for 5 sec. HPLC system was operated to inject 3 µL of prepared sample into the Jasem analytical column specified for amino acid analysis maintained at 30^{o}C. Chromatographic separation was carried out using Jasem's mobile phase A and B with gradient elution at a flow rate of 0.7 ml/min. The HPLC elution was as follows: the initial LC gradient of 22% A was held for 1 min. Then, the gradient was ramped to 78% A in 3 min. and held for 0.5 min. Finally the column was equilibrated at 22% A for 3 min. The total running time was 7.5 min. Mass spectrometric detection was performed on Agilent 6460 triple quadrupole MS equipped with an ESI source in the positive ion mode. The optimal MS detector settings were as follows: drying gas temperature 150^{o}C, drying gas flow 10 L/min, nebulizer pressure 40 psi (Gauge-Nebulizing takes place in a chamber in which is under the atmospheric pressure not in the vacuum) and capillary voltage of 2000 V (+). The positive ESI mode was operated for the detection of amino acid and IS as protonated form (m/z = $[M+1]^{+}$). Collision-induced dissociation (CID) of this precursor ion produced one major product ion for each amino acid and IS. MRM transitions of the amino acid and corresponding IS (precursor ion to product ion) were monitored at optimum fragmentation voltages (FV) and optimum collision energies (CE) (Table 1a,

Table 1b). The peak area ratio of the amino acid to the assigned IS was evaluated for quantification of targeted amino acid concentration.

Table 1a. (MRM) transitions of amino acids and conditions.

Compound name	Precursor ion (m/z)	Product ion (m/z)	Fragmentation voltage (v)	Collision energy (v)
Taurine	126.1	44.3	110	14
Phenylalanine	166.1	120.1	80	6
Tyrosine	182.1	165.0	80	1
Methionine	150.1	104.1	80	4
Aspartic acid	134.1	74.1	90	10
Threonine	120.2	74.2	80	4
Serine	106.2	60.2	80	4
Alanine	90.2	44.2	80	4
Glycine	76.2	30.1	80	1
Proline	116.2	70.2	90	12
Cystine	241.1	74.2	100	24
Arginine	175.2	70.2	110	20
Histidine	156.1	110.1	100	8
Ornithine	133.2	70.3	80	14
Lysine	147.1	84.2	80	12
Glutamic acid	148.1	84.2	80	12
Leucine	132.2	43.3	100	24
Isoleucine	132.2	69.2	100	14
Valine	118.2	72.2	80	4
1-Methylhistidine	170.1	124.1	100	10
3-Amino isobutyric acid	104.1	86.2	100	2
3-Methylhistidine	170.1	126.2	120	10
Beta-Alanine	90.1	72.1	80	2
5-Hydroxy lysine	163.1	128.1	90	6
Homocystine	269.0	136.0	100	4
Ethanolamine	62.1	44.2	80	4
4-Aminobutyric acid	104.0	87.1	80	6
2-Aminobutyric acid	104.2	58.3	80	4
Anserine	241.1	109.1	130	22
Asparagine	133.1	74.2	70	10
Carnosine	227.1	110.1	110	22
Citrulline	176.2	159.3	80	3
Cystathionine	223.0	134.0	100	8
Glutamine	147.1	84.2	80	12
Homocitrulline	190.0	173.1	80	5
Norvaline	118.1	72.1	90	5
Tryptophan	205.1	188.1	80	1
Sarcosine	90.1	44.2	90	8
Trans-4-Hydroxyproline	132.2	68.2	90	20

Table 1b. MRM transitions of amino acids and conditions.

Compound name	Precursor ion (m/z)	Product ion (m/z)	Fragmentation voltage (v)	Collision energy (v)
Phenylalanine IS	175.1	129.1	100	8
Tyrosine IS	192.1	145.2	80	8
Methionine IS	153.1	107.2	80	6
Aspartic acid IS	137.1	91.2	90	5
Threonine IS	121.1	75.0	80	6
Serine IS	109.1	63.0	90	8
Alanine IS	94.1	48.2	90	6
Glycine IS	78.2	31.3	90	4
Proline IS	122.1	75.2	90	14
Cystine IS	244.9	153.9	90	8
Arginine IS	177.2	70.2	110	20
3-Methyl histidine IS	173.2	127.2	80	10
Ornithine IS	138.2	74.2	80	16
Lysine IS	151.1	88.1	90	16
Glutamic acid IS	150.1	85.2	80	12
Leucine IS	142.2	96.3	120	6
Valine IS	126.1	80.2	80	8
Tryptophan IS	210.1	192.1	90	4
Homocystine IS	277.0	140.0	100	4
Asparagine IS	135.0	89.1	80	4
Glutamine IS	148.1	85.1	80	14
Citrulline IS	177.2	160.2	90	4
Ornithine IS	138.2	74.2	80	16

RESULTS AND DISCUSSION

The mean total length and wet weight of brown meagre used in the analysis ranged between 35.2 - 37.1 cm (mean: 36.2±0.33 cm) and 562.9 - 640.7 g (mean: 617.4±18.43 g), respectively. In the Black Sea, brown meagre reaches the age of 2, 3, 4, 5 when they are 26.87±3.1 cm, 32.14±2.9 cm, 37.61± 2.0 cm and 40.92±3.5 cm, respectively (Engin and Seyhan, 2009). According to these values, the examined fish can be considered as 3 or 4-year-old that has already reached size at sexual maturity (>2 years). According to the regulation for commercial fishing (communication numbered 4/1) between the years of 2016-2020 in Turkey, the minimum legal size (MLS) is 25 cm for brown meagre (Anonymous, 2016). For the regulation between the years of 2020-2024, this MLS ban will be applied as 35 cm (Anonymous, 2020). Coincidentally, the fish used for analysis in the present study were above the MLS applied in both regulations.

In previous studies (Bilgin *et al*., 2018, 2019ab), the amino acid composition of fish living in the Black Sea was reported for only nineteen amino acids using the LC-MS/MS

instrument. Unlike previous studies, in the present study, the LC-MS/MS instrument was commanded for the first time to detect thirty nine amino acids for a fish species in the Black Sea. The amino acid patterns, groups and per cent ratios of brown meagre are given in Table 2. Of the thirty nine amino acids detected in total, seven were essential amino acids (EAA: isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine), two semi-essential amino acids (SEAA: arginine and histidine) and twenty nine non-essential amino acids (NEAA: 1-methylhistidine, 2-aminobutyric acid, 3-aminoisobutyric acid, 3-methylhistidine, 5-hydroxy lysine, alanine, anserine, asparagine, aspartic acid, beta-alanine, carnosine, citrulline, cystathionine, cystine, ethanolamine, 4-aminobutyric acid, glutamic acid, glutamine, glycin, homocitrulline, homocystine, norvaline, ornithine, proline, sarcosine, serine, taurine, trans-4-hydroxyproline and tyrosine).

Although twelve of the twenty nine NEAAs (2-aminobutyric acid, 3-aminoisobutyric acid, 5-hydroxy lysine, anserine, beta-alanine, carnosine, cystathionine, 4-aminobutyric acid, homocitrulline, homocystine, norvaline and sarcosine) were detected by the LC-MS/MS instrument, the values were less than method detection limits. However, under acidic hydrolysis conditions, glutamine and asparagine are converted to glutamic acid and aspartic acid, respectively, on the other hand, tryptophan disappears (Table 2). This is the result of the analysis method applied for amino acid detection.

In our study, the most abundant amino acids in brown meagre meat were found as glutamic acid (13.88%), lysine (10.43%), aspartic acid (9.62%), leucine (8.32%), glycin (6.87%), arginine (6.50%), alanine (6.42%), valine (5.16%), phenylalanine (4.51%), threonine (4.16%), tyrosine (3.54%), proline (3.34%), serine (3.33%), isoleucine (3.28%), histidine (3.27%), methionine (3.12%), respectively. This composition was reported as glutamic acid (17.32%), aspartic acid (11.58%), lysine (10.19%), leucine (8.34%), alanine (6.35%), arginine (5.51%), valine (4.88%), threonine (4.65%), isoleucine (4.52%), glycin (4.36%), phenylalanine (4.26%), serine (4.02%), tyrosine (3.51%), proline (3.32%), methionine (3.31%), taurine (2.41%) and histidine (1.46%) by Kouroupakis et al. (2019) in the Aegean Sea (Table 2). The most abundant amino acids in both studies were glutamic acid, lysine, aspartic acid and leucine, respectively, and these four amino acids accounted for more than 42% of the total amount of amino acids in brown meagre meat/muscle both in the Black Sea (42.24%) and the Aegean Sea (47.43%). In addition to these, the four most abundant amino acids in fish meat were reported as glutamic acid > phenylalanine > aspartic acid > lysine for *Trigla lucerna*, glutamic acid > lysine > aspartic acid > arginine for *Scorpaena scrofa*, proline > phenylalanine > glutamic acid > lysine for *Scorpaena porcus*, proline > phenylalanine > glutamic acid > lysine for *Merluccius merluccius*, proline > glutamic acid > phenylalanine > lysine for *Lophius piscotorius* and proline > phenylalanine > glutamic acid > lysine for *Trachinus draco* by Özden and Erkan (2011) in the Marmara Sea. These classification were also reported as phenylalanine > glutamic acid > aspartic acid > lysine for *Psetta maxima* (Özden and Erkan, 2011), as glutamic acid > aspartic acid > lysine > leucine for *Merlangus m. euxinus*, *Engraulis encrasicolus* and *Spicara smaris* (Bilgin *et al*., 2018; Bilgin *et al*., 2019ab) in the Black Sea and as lysine > leucine > aspartic acid > glutamic acid for *Upeneus moluccensis* in the Antalya Gulf of Turkey, Mediterranean Sea (Doğan and Ertan, 2017). As can be deduced from the results of the above-mentioned

studies, although it is not a general phenomenon and varies according to the fish species and regions, the most amino acids in fish meat are glutamic acid, lysine, aspartic acid and leucine and these results were also consistent with the our results.

In the present study, the levels of the total amino acids (ΣAA = 24.17 g/100g), total essential amino acids (ΣEAA = 9.42 g/100g), total non-essential amino acids (ΣNEAA = 12.39 g/100g), total semi-essential amino acids (ΣSEAA = 2.36 g/100g) for brown meagre in the Black Sea were higher than those, reported by Kouroupakis *et al*. (2019) in the Aegean Sea (Table 2). These differences between the two studies may be due to the reasons explained below.

i) Differences in examined fish size. In this study, fish of 617.4±2.4g in weight and 36.2±0.3 cm in length were used, which had already reached sexual maturity, while smaller size (25.0±1.7cm) and weight (200.8±5.0g) fish were used in the Aegean Sea study. Fish size is an important factor in terms of being more advantageous as prey in reaching nutrients.

ii) The difference in sampling period which effect to both reproduction time and food quality and abundance: in this study, samples were provided at the beginning of winter (December), in the other study, sampling was carried out in the middle of spring (April) and in the beginning of summer (June). Sampling time is a factor that directly affects the amount and quality of nutrients and may have affected the nutritional composition (food quality and abundance).

iii) The differences of seas (different geographic region) where sampling is conducted. The Black Sea and Aegean Sea differ especially in terms of the amount of salinity (the salinity rate is about 33% in the north of the Aegean Sea and 37% in the south, this rate is around 18% in the Black Sea) and consequently the food quality and abundance.

iv) The differences in method and instrument used for analysis. Differences in the number and amount of amino acids of the same species or different species sampled from the same region or different region at the same time or at different time may also be due to the difference in the method used in the studies. Namely, both derivatization and hydrolisation procedures applied during the preparation of the sample and amino acid analysis may cause changes in the number and amount of amino acids. (Note: as applied in this study, there is no derivatization procedure in the method applied using the LC-MS / MS instrument for determining the number and amount of amino acids and the samples are directly hydrolyzed). According to Kouroupakis *et al*. (2019), tryptophan was not quantified due to its susceptibility to acid hydrolysis, while cystein reacts with cysteine to form cystine. Moreover, during the acid hydrolysis procedure, asparagine is converted to aspartate and glutamine to glutamate, so to be reported values for these amino acids (Asx: aspartate or asparagine and Glx: glutamate or glutamine) should be represented the sum of both amino acids.

The ratio of ΣEAA to ΣAA was recorded as 38.98% in the Black Sea and 40.15% in the Aegean Sea. Further, slightly higher levels ratio of ΣEAA to ΣNEAA has been recorded in our study (76.05%) in compared with the Aegean Sea population (75.90%). The reason for this may be the high number of non-essential amino acids detected in our method using liquid chromatography coupled with tandem mass spectrometry instrument (see: Table 2).

As reported in previous studies (Kinm and Lall, 2000; Limin *et al*., 2006; Erkan *et al*., 2010; Özden and Erkan, 2011; Doğan and Ertan, 2017), the above values demonstrated that considerable variations in amino acid levels can be obtained from the different and/or same fish species. Such variations are probably as a result of several factors such as species, size, differences in food quality and abundance, environmental condition, etc. as well as methods used for amino acid determination.

Table 2. Comparison of amino acid profile (g/100 g in wet weight) of the Brown meager, *Sciaena umbra*, obtained from the Black Sea and the Aegean Sea.

AA groups	Amino acids (AAs)	Black Sea (Present study) (36.2±0.3cm, 617.4±2.4g)			Aegean Sea (Kouroupakis *et al*. 2019) (25.0±1.7cm, 200.8±5.0g)		
		Mean	S.E.	%	Mean	S.E.	%[#]
EAA	Isoleucine	0.79	0.062	3.28	0.92	0.063	4.52
	Leucine	2.01	0.056	8.32	1.70	0.096	8.34
	Lysine	2.52	0.015	10.43	2.08	0.116	10.19
	Methionine	0.75	0.033	3.12	0.68	0.044	3.31
	Phenylalanine	1.09	0.024	4.51	0.87	0.056	4.26
	Threonine	1.01	0.092	4.16	0.95	0.063	4.65
	Tryptophan*	0.00	0.000	0.00			
	Valine	1.25	0.049	5.16	1.00	0.062	4.88
	∑EAA	**9.42**	**0.042**	**38.98**	***8.21***		***40.15***
NEAA	1-Methylhistidine	0.02	0.001	0.07			
	2-Aminobutyric acid**	0.00	0.000	0.00			
	3-Aminoisobutyric acid**	0.00	0.000	0.00			
	3-Methylhistidine	0.13	0.000	0.55			
	5-Hydroxy lysine**	0.00	0.000	0.00			
	Alanine	1.55	0.018	6.42	1.30	0.072	6.35
	Anserine**	0.00	0.000	0.00			
	Asparagine***	0.00	0.000	0.00			
	Aspartic acid	2.32	0.311	9.62	2.37	0.127	11.58
	Beta-Alanine**	0.00	0.000	0.00			
	Carnosine**	0.00	0.000	0.00			
	Citrulline	0.20	0.020	0.84			
	Cystathionine**	0.00	0.000	0.00			
	Cystine	0.20	0.021	0.81			
	Ethanolamine	0.01	0.002	0.05			
	4-Aminobutyric acid**	0.00	0.000	0.02			
	Glutamic acid	3.35	0.100	13.88	3.54	0.188	17.32
	Glutamine***	0.00	0.000	0.00			

Continued……

	Glycin	1.66	0.282	6.87	0.89	0.105	4.36
	Homocitrulline**	0.00	0.000	0.00			
	Homocystine**	0.00	0.000	0.00			
	Norvaline**	0.00	0.000	0.00			
	Ornithine	0.14	0.002	0.57			
	Proline	0.81	0.009	3.34	0.68	0.049	3.32
	Sarcosine**	0.00	0.000	0.00			
	Serine	0.81	0.002	3.33	0.82	0.074	4.02
	Taurine	0.25	0.000	1.04	0.49	0.102	2.41
	Trans-4-Hydroxyproline	0.07	0.003	0.30			
	Tyrosine	0.86	0.071	3.54	0.72	0.087	3.51
	∑NEAA	**12.39**	**0.029**	**51.26**	***10.81***		***52.87***
SEAA	Arginine	1.57	0.049	6.50	1.13	0.095	5.51
	Histidine	0.79	0.039	3.27	0.30	0.107	1.46
	∑SEAA	**2.36**	**0.044**	9.77	***1.43***		***6.98***
	∑AA	**24.17**	**0.033**	**100**	***20.44***		***100***
	∑EAA/∑NEAA	***76.05***			***75.90***		

*****Tryptophan disappears under acidic hydrolysis, ******Not detected, ******* Glutamine converts to glutamic acid, *******asparagine converts to aspartic acid, ♯percentage values in the study of Kouroupakis *et al*. (2019) were calculated by us.

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