Scientific Papers. Series D. Animal Science. Vol. LXII, No. 2, 2019
ISSN 2285-5750; ISSN CD-ROM 2285-5769; ISSN Online 2393-2260; ISSN-L 2285-5750

IDENTIFICATION OF AMINOACID PROFILE AND PROXIMATE COMPOSITION OF THREE TROUT BREEDS REARED IN THE NORTH EASTERN REGION OF ROMANIA

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Abstract

Trout meat has sensory features and high nutritional value. The aim of the present study was to identify differences in the proximate composition, amino acid composition of three breeds (brown, brook and rainbow trout) reared under the same conditions in north eastern region of Romania. Chemical samples composition were determined using Association of Official Analytical Chemists (AOAC) methods. The moisture content recorded oscillated between 72.98 and 76.14 g, protein content of trout meat ranged between 17.37 and 19.31 g, the determined lipid content was between 4.41 and 5.82 g, and measured ash was between 1.11 and 1.21gfrom trout's flesh. The amount of essential aminoacids ranged between 26.49 and 28.08%. Among amino acids, the glutamic acid, aspartic acid, arginine, leucine and lysine were predominant. The measured essential to nonessential (E/NS) ratio for the trout flesh ranged between 71.73 and 75.87%. The present study demonstrate that trout meat is a highly source of protein and contains essential amino acids for promoting good health, prevention and healing of diseases in humans.

***Key words**: proximate composition, amino acid, meat, trout.*

INTRODUCTION

Fish meat has outstanding sensory features and high nutritional value. In addition, fish meat also has a strong quantitative valence and plays an important role in ensuring daily protein requirements (Pariser and Wallerstein, 1980; Novikov et al., 1997; Tocher et al., 2003; Segal, 2002; Osibona et al., 2009; Sabetian et al., 2012).

The quality of fish protein depends on their digestibility and the content of essential amino acids such as lysine, methionine, leucine (Tuan et al., 1999; Oliva-Teles, 2000; Usydus et al., 2009; Robbins et al., 2010; Sabetian et al., 2012; Sarma et al., 2015).

Certain amino acids such as aspartic acid, glutamic acid and glycine are known for the important role they play in the process of wound healing. Amino acids, not only have high nutritional value, they also offer many benefits to human health such as reducing blood cholesterol and coronary heart disease (Gibbs et al., 2004).

Saito et al. (2003) finds that certain amino acids, such as tyrosine, methionine, histidine, lysine and tryptophan, have antioxidant action in the human body. Additionally, Kim et al. (1999) mentions that aspartic acid, glutamine, proline, glycine and leucine have strong cytotoxic activity against cancer cells, while Jones et al. (1999) notes the particular importance of glutamine in the proper functioning of many systems in the human body.

According to the literature, chemical composition of trout meat and the amino acids level is influenced by a number of factors such as breed, age, nutrition, fishing season, environment, salinity and water temperature (Shirai et al., 2002; Solvik and Rustad, 2005; Ionescu et al., 2006, Toppe et al., 2007; Erdem et al., 2009, Sabetian et al., 2012, Kaya et al., 2014).

Especially regarding brook trout, data from local and foreign literature consulted are less conclusive in terms of physical-chemical composition and amino acid profile, so our research represents a novelty for Romanian

literature and aims to bring new information to enrich it.
The aim of the present study was to identify differences in the proximate composition, amino acid composition of three breeds (brown, brook and rainbow trout) reared under the same conditions in north eastern region of Romania.

MATERIALS AND METHODS

Biological material was represented by 30 individuals of brook, rainbow and brown trout of both sexes, weighing about 250 grams, reared under the same condition in a trout farm from Suceava County. To achieve the proposed goals, from the biological material which was studied during 2017-2018, were made up three experimental batches, each of 10 individuals per batch, for the three studied breeds.
The fish samples were kept on ice in isothermal box, until they arrived to the laboratory and immediately frozen and stored at -21ºC until analyses.
To determine the physical-chemical composition of trout meat were gathered samples from side musculature of fishes from all three batches. All the analyses were conducted in duplicate replicate.
Determination of water and dry matter was realized through the method of drying in oven, which is the most used indirect method and suppose the drying of sample in oven at +105°C, till reaching a constant weight, in according to SR ISO 1442:2010. For this purpose, were used the Sartorius analytical balance (Gottingen, Germany) and the ECOCELL Blueline Comfort drying oven (Neuremberg, Germany).
Protein determination consists in decomposing the analyzed sample by heating with sulfuric acid in the presence of catalysts to reduce organic nitrogen to ammonium ions which can be determined by distillation / titration.The equipment used is Kjeltec Auto 2300 - Tecator, Sweden, which is a semi-automatic version of the crude protein Kjeldahl determination.
Determination of lipids content was realized using Soxhlet method, which consists in fat extraction from the analyzed sample using petrol ether using Velp Scientifica - SER 148 device (method specified by the manufacturer, AOAC Official methods o analysis 2005 and compatible with SR ISO 1443:2008.
Ash was determined by calcinations at 550ºC in calcinations oven according to SR ISO 936:2009.
Quantitative determination of amino acids was carried out in two stages in agreement with SR EN ISO 13903/2005 and AOAC Official Methods of Analysis (2005). The first stage was the hydrolysis of the peptide bonds existing between the constituent amino acids of the sample protein. In the case of determination of sulphur amino acids, the hydrolysis step is preceded by oxidation. The second stage was the determination (dosing) of the amino acids released following hydrolysis of the peptide bonds. In turn, this stage requires several steps: the individual separation of the amino acids from the existing mixture in the protein hydrolyzed, their detection and quantification.
Derivatization of pre-column sample was performed with OPA (ortho phthaldehyde), AMP (mercaptopropionic acid), FMOC (9-fluorenyl methyl chloroformate).
Amino acids were separated by high performance liquid chromatography using the HPLC Surveyor Plus Thermo Electron chromatographic system on a reversed-phase Hypersil BDS C18 (Thermo Electron) column, the reading being in the ultra-violet (338 nm). A Hypersil BDS (Base Deactivated Silica) C18 column with silica gel, with dimensions of 4.6 mm and a particle size of 5 μm. A gradient elution method is used, the chromatographic conditions being the following flow rate: 1 mL/min; injected volume: 25 μL; wavelength to be read: 336 nm; column temperature: 25°C.
Cysteine is determined as cystic acid in the hydrolysis of oxidized samples, but is calculated as cysteine using its molar mass. Also, methionine is determined as methionine sulfone from the oxidized and hydrolyzed samples, but is converted to methionine by the use of methionine moles.
The software used for statistical analysis was SPSS. We calculated the average, standard deviation, coefficient of variation and statistical significance of differences between samples.

RESULTS AND DISCUSSIONS

The chemical composition of trout meat, in addition to the genetic factors, is also influenced by environmental factors such as water quality, its pH and temperature, oxygen content, technological factors, feeding, type of food used, season of the year, age and size of the fish (Fauconneau et al., 1993, 1995; Buchtova et al., 2007; Menoyo et al., 2007; Fallah et al., 2011; Vranić et al., 2011; Sabetian et al.; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Water content of fillet (side muscles) obtained from the studied trout breeds had close values (Table 1) ranking between 72.98 g for brook trout samples and 76.14 g for brown trout samples, values which fall within the limits from literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macíaset al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Dinović et al., 2011; Fallah et al., 2011; Mocanuet al., 2012; Vranićet al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Table 1. Proximate composition of brook, rainbow and brown trout meat (g/100 g)

Specification		*Brook trout*	*Rainbow trout*	*Brown trout*
Water	$\bar{X} \pm s_{\bar{x}}$	72.98 ± 0.84^{bc}	74.25 ± 0.43^{ac}	76.14 ± 0.27^{ab}
	V%	2.08	1.74	1.44
Dry matter	$\bar{X} \pm s_{\bar{x}}$	27.02 ± 0.84^{bc}	25.75 ± 0.43^{ac}	23.86 ± 0.27^{ab}
	V%	5.61	4.52	3.36
Proteins	$\bar{X} \pm s_{\bar{x}}$	19.31 ± 0.43^{bc}	17.87 ± 0.61^{a}	17.37 ± 0.24^{a}
	V%	5.69	2.68	4.35
Fats	$\bar{X} \pm s_{\bar{x}}$	5.82 ± 0.23^{bc}	5.02 ± 0.22^{ac}	4.41 ± 0.21^{ab}
	V%	8.80	4.92	6.16
Ash	$\bar{X} \pm s_{\bar{x}}$	1.19 ± 0.01^{bc}	1.11 ± 0.01^{ac}	1.21 ± 0.07^{a}
	V%	3.81	4.20	2.81

Mean values in rows marked with different letters differ significantly at $p<0.05$

As it can be observed from these data the highest dry matter content is registered at brook trout specimens 27.02 g, while in the case of brow brown trout samples were registered the lowest values of 23.86 g.

Proteins are the basic substances that offer products their nutritional value. Therefore, the quality of food is assessed primarily by their content of protein.

Within chemical composition of muscle tissue after water, proteins are the major constituents of animal bodies; proteins perform extremely varied functions and growth, maintenance and repair off all cells are dependent upon them, reflecting a high degree of structural organization and specialization (Sabetian et al., 2012; Wang and Hun, 2017; Simeanu et al., 2017).

Protein content of trout breeds fillet from the experimental batches ranged between 17.27 g for brown trout, and 19.31 g for brook trout, values similar to those mentioned in the specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al. 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanu et al.,2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Lipids are among the most important biochemical constituents of fish (Aras et al., 2003), are found in sarcoplasm (in the form of fine droplets) under the skin, in the muscular tissue (Haliloglu and Aras, 2002) and blood plasma (Booth et al., 1999), but also in various organs such as the liver, spleen or gonads (Hatano et al., 1989; Hederson, 1996; Tocher, 2003; Segal, 2006), muscle fiber structures (mitochondria, microsomes, nuclei).

Lipids from trout meat vary within wide limits, ranging between 1.7 gand 9 gas it was found in the specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanu et al., 2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

Fish meat presents a good palatability when the lipid content ranges from 3.5% - 4.5% (Liu, 2002).

The fat content of the analyzed trout's fillet ranged between 4.41 g, in case of brown trout and 5.82 g for brook trout, values that place them in the category of fish medium lipid content (4–8%). And this time data obtained were within the limits mentioned in the consulted specialty literature (Fauconneau et al., 1993b; Corser et al., 1999; Plavša et al., 2000; Savić et al., 2004; García-Macías et al., 2004; Bud and Mireşan, 2008; Celik et al., 2008; Alçiçek et al., 2010; Fallah et al., 2011; Dinović et al., 2011; Mocanuet al., 2012; Vranić et al., 2010, 2011; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

According to the literature, the ash content varies from 1 to 2 g (Plavśa et al., 2000; García-Macías et al., 2004; Bud and Mireşan, 2008; Fallah et al., 2011; Vranić et al., 2011; Mocanu et al., 2012; Sabetian et al., 2012; Kaya et al., 2014; Sirakov, 2015; Wang and Hun, 2017).

The ash content registered values ranging from 1.11 g for rainbow trout meat to 1.21 g for brown trout, values similar to those mentioned in literature. For all the constituents of chemical composition, there are, significant statistical differences between breeds ($p<0.05$).

Amino acid composition of the three trout meat is given in Table 2.

The protein compositions of the examined trout breeds contained the highest levels of glutamic acid (8.75% - 9.16%), followed by aspartic acid, leucine and lysine observations supported by Iwasaki and Harada (1985), Farmanfarmaian and Sun (1999), Beklevik et al. (2005), Sabetian et al. (2012), Kaya et al., (2014), Sirakov (2015), Wang and Hun (2017), tryptophan was not determined.

Wesselinova (2000) reported that the amounts and types of amino acids in fish muscle is affected by catching time and location, and Green et al. (2002) mentioned that the ratio of essential to non-essential amino acids (EAA/NEAA ratio) in dietary protein has important effects on protein utilization by fish.

The lowest levels in decreasing amounts were registered in the case of tyrosine (2.80–2.88%) and histidine (1.56%-1.63%), observations in according with those reported by Sabetian et al. (2012) (only in the histidine level), Kaya et al. (2014), Wang and Hun (2017), but in contradiction with the results mentioned by Sirakov (2015) which mention methionine and serine as limiting amino acids.

As the main amino acid constituent from the trout's meat composition, glutamine is essential for cell proliferation, as a nitrogen donor during purine and pyrimidine synthesis.

The human organism has the biological ability to produce a limited number of amino acids, which is why many of these must be obtained from sources of animal or vegetable protein by daily diets, trout meat being a rich source in essential amino acids needed for growth, and development.

The role of these essential amino acids is very important because, valine is an essential amino acid, needed to maintain the nitrogen balance, it performs its activity in synergy with leucine and isoleucine, participating in synthetic proteins, having anabolic role in muscle cells, also assuring the coordination of movements; leucine is required by ketogenic function, deficiency in leucine prevents normal growth, leading to body weight loss and a negative nitrogen balance; isoleucine participates in the synthesis of hemoglobin, regulates blood glucose levels, speeds recovery of the body after surgery or wounds and has anabolic effects; threonine is a lipotropic agent that prevents the accumulation of fat in the liver, and through degradation substances it participates in the synthesis of porphyrin; lysine is involved in the process of growth of the body, as well as in the formation of red blood cells, being a precursor of carnitine and is the limiting amino acid in cereal based diet; phenylalanine is a tyrosine precursor, with an important role in the synthesis protein, and is involved in mediating the transmission of nerve impulses as a dopamine precursor; methionone interferes with lipid metabolism, preventing fat accumulation in the liver and provides cysteine and S-adenosyl methionine biosynthesis, contributes as a methyl group donorand in the same time is essential in the diet for producing taurine, which exhibits clear antihypertensive effects (Wiley et al., 1986; Simeanu et al., 2015; Wang and Hun, 2017; Simeanu et al., 2017).

However, the contents and proportions of muscle amino acids were basically the same for all the trout breeds displaying a conservative pattern, which was in accord with the observations of Kizak (2013) on tench and Wang and Hun (2017) on rainbow trout.

The differences in the amino acid profiles for the three breeds can be related to different aspects, such as feed utilization and its composition, the retention of amino acids and the amino acid profile of the fish body as well as environmental conditions, breeding system, size of the fish, catching season (Rodehutscord et al., 1997; Wesselinova, 2000; Green et al., 2002; Shirai et al., 2002; Solvik and Rustad, 2005; Toppe et al., 2007; Erdem et al., 2009; Kaya et al., 2014; Sirakov, 2015).

In this study, the total amino acid (TAA), total essential amino acid (EAA), total non-essential amino acid (NEAA) and total delicious amino acid (DAA) contents did not differ significantly ($P > 0.05$) between all the three trout breeds.

The ratio between EAA and TAA had the highest value in the case of brown trout samples of 0.73 and the lowest in the case of brook trout samples of 0.7 while the ratio between EAA and NEAA had higher value forthe rainbow trout samples of 0.75, the lowest value again at brook trout samples.

These results showed that the EAA to TAA and EAA to NEAA ratios for all of breeds were comparable to the reference values of nearly 40% and above 60%, respectively, recommended by the FAO/WHO, which indicates that all trout breeds may be considered high-quality protein food sources.

Each amino acid contributes, in different degrees, to the aroma of foods. Several amino acids taste sweet (glycine and alanine) or delicious (umami) to humans. Aspartic acid and glutamic acid have a sour taste, but they are responsible for the umami taste in the presence of sodium salt (Gunlu and Gunlu, 2014).

Table 2. Amino acid composition of trout meat (% from dry sample)

Amino acid	Brook trout	Rainbow trout	Brown trout
Aspartic acid (Asp)*	6.45±0.81^{bc}	7.03±0.46^{ac}	6.84±0.62^{ab}
Glutamic acid (Glu)*	9.16±0.49^{bc}	8.75±0.60^{ac}	9.81±0.66^{ab}
Serine (Ser)	3.18±0.43^{bc}	2.97±0.20^{ac}	3.02±0.13^{ab}
Glycine (Gly)*	4.28±0.16	4.44±0.35	4.41±0.14
Threonine (Thr)^{E}	3.17±0.57	3.79±0.35	3.72±0.42
Arginine (Arg)	5.38±0.31	5.68±0.50	5.74±0.55
Histidine (His)	1.56±0.06	1.65±0.25	1.63±0.08
Alanine (Ala)*	4.54±0.32	4.72±0.26	4.69±0.36
Tyrosine (Tyr)	2.88±0.21	2.88±0.12	2.80±0.08
Valine (Val)^{E}	3.47±0.38^{bc}	3.81±0.18^{a}	3.66±0.38^{b}
Phenylalanine (Phe)^{E}	3.37±0.23	3.31±0.20	3.33±0.15
Isoleucine (Iso)^{E}	3.56±0.28	3.63±0.16	3.60±0.19
Leucine (Leu)^{E}	6.38±0.58	6.85±0.47	6.46±0.76
Lysine (Lys)^{E}	5.82±0.34	5.82±0.32	5.79±0.14
Cysteine (Cys)	3.66±0.01^{c}	3.80±0.13	3.76±0.05^{a}
Methionine (Met)^{E}	3.68±0.04^{c}	3.85±0.12	3.84±0.05^{a}
Essential amino acids $\sum_{EAA}$	29.49±1.47	31.80±1.66	30.41±1.63
Delicious amino acids $\sum_{DAA}$	24.43±0.32	24.94±0.42	25.75±0.57
Non essential amino acids $\sum_{NEAA}$	41.11±1.52	41.91±1.60	41.66±1.21
Total amino acids $\sum_{TAA}$	70.60±1.98	73.02±3.41	73.13±2.96
$\sum_{EAA}/\sum_{TAA}$	41.77±1.72	43.54±2.53	41.58±2.29
$\sum_{EAA}/\sum_{NEAA}$	71.73±1.49	75.87±1.63	72.99±1.42
$\sum_{DAA}/\sum_{TAA}$	34.60±1.11	34.15±1.91	35.21±1.76

EAA – essential amino acids; NEAA – non-essential amino acids; TAA – total amino acids; Tryptophan was not determined;*denotes delicious amino acids (DAA);
Mean values in rows marked with different letters differ significantly at p<0.05

The level of total delicious amino acid (DAA) was higher in the case of brown trout samples than in rainbow and brook trout samples (Table 2), been suggested by Ruiz-Capillas and Moral (2004), that these free delicious amino acids are related to the characteristic fish flavour and that different contents of these amino acids may cause variations in fish flavour.

The ratio of DAA to TAA ranged from 34.15% in the case of rainbow trout to 35.21% for brown trout samples (Table 2). These values were higher to those reported for *Silurus asotus*

L. (31.83-32.33%) by Jiang (2012) but lower than those reported for Masu salmon (37.31-37.46%) by Wang et al. (2015), and for rainbow trout (36.61% to 36.92%) in the diploid fish and (36.01% to 37.68%) in the triploid fish by Wang and Hun (2017).

The results obtained from this study showed that the tested trout breeds have a well-balanced and high-quality protein source in the respect of the EAA/TAA ratio and the obtained data are in confirmation with those mentioned for rainbow trout by Sabetian et al. (2012) and Wang and Hun (2017), but lower for brown trout Kaya et al. (2014) and Sirakov (2015).

According to the obtained data, the sum of the sulphur-containing amino acids (methionine and cysteine) presented the highest score among rainbow trout proteins (Table 2), indicating that rainbow trout is rich in methionine and cysteine, while the lowest values were registered at brook trout proteins, while the sum of the aromatic amino acids (phenylalanine and tyrosine) presented the lowest score among brown trout proteins.

CONCLUSIONS

The values obtained after chemical determinations enlightened the fact that all trout breeds from the experimental batches fall into the limits cited in the literature, highlighted that all trout breeds have a good nutritional value.

After evaluation of chemical composition of trout meat, was observed that brook trout individuals had a higher content in dry matter and protein.

The protein compositions of the examined trout breeds contained the highest levels of glutamic acid, followed by aspartic acid, leucine and lysine.

The ratio between EAA and TAA had the highest value in the case of brown trout samples of 0.73 and the lowest in the case of brook trout samples of 0.7 while the ratio between EAA and NEAA had higher value forthe rainbow trout samples of 0.75, the lowest value again at brook trout samples.

The present study demonstrate that trout meat is a highly source of protein and contains essential amino acids for promoting good health, prevention and healing of diseases in humans.

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