Identification of Fatty Acid Content, Amino Acid Profile and Proximate Composition in Rainbow Trout (*Oncorhynchus mykiss*)

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Abstract: Rainbow trout (*Oncorhynchus mykiss*) was analyzed for proximate composition, amino acid and fatty acid profile. Chemical samples composition were determined using Association of Official Analytical Chemists (AOAC) methods. The protein content of rainbow trout was 19.65±1.2 %. The total lipid content was determined 4.46±0.2 %. The ash was measured 1.33±0.1 % rainbow trout flesh. The moisture content was recorded 71.7±1.9 %. Fatty acid composition were determined by gas chromatography, The total saturated fatty acid (SFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) content were 26.3%, 33.8% and 24.62% of total fatty acids, respectively. Finally, eicosapentaenoic acid (C20: 5 n-3) and docosahexaenoic acid C22: 6 n-3) were the dominant PUFAs. The ratio of n3/n6 fatty acids was 2. 06. Furthermore, The amount of essential amino acids was 35.74±0.1 %. Among amino acids, the glutamic acid, the aspartic acid and lysine were the predominant Amino acids by having 11.1 ±0.4 %, 9.53±0.1 %, and 7.72 ±0.1 % values respectively. The measured essential to nonessential (E/NS) ratio for the rainbow trout flesh was 0. 75. The present results demonstrate that rainbow trout fillet contains essential fatty acids particularly eicosapentaenoic acids and docosahexaenoic acids and essential amino acids for promoting good health, prevention and healing of diseases in humans.
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1. Introduction

Edible fish because of high nutritional quality and easily digestibility are the most important nourishments in human Diet (Gladyshev et al., 2006). lipids are well known as a rich source of long-chain n-3 polyunsaturated fatty acids (LC n-3 PUFA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which cannot be synthesized by humans and commonly obtained from the diet (Alasalvar et al., 2002). It is known that polyunsaturated fatty acids can regulate prostaglandin synthesis and hence induce wound healing (Jia and Turek, 2005; Kryzhanovskii and Vititnova, 2009; Zhang et al., 2010). The n-3 and n-6n-3 and n-6 polyunsaturated fatty acids (PUFAs) have been shown positive effects on cardiovascular diseases and cancers (Marchioli et al., 2009; Jiang et al., 2012). Therefore, increase of fish and other aquatic product consumption is important for human health (McNinch et al., 2009; Choi et al., 2010). Unsaturated fatty acid composition varies among different fishes, especially marine and freshwater species. However, the fish fatty acid is influenced by several factors such as fishing season, food accessibility, fish size, and maturity stage (Erdem et al., 2009; Hazra et al., 1998; Gámez-Meza et al., 1999; Driscoll et al., 2009).

Besides, fish meat plays an important role in supplement of human protein necessity (Novikov et al. 1997; Pariser and Wallerstein 1980; Tocher et al. 2003; Osibona et al., 2009). The protein quality of food depends on their digestibility and content of essential amino acids like leucine, Lysine and Phenylalanine (Tuan et al., 1999; Oliva-Teles, 2000; Usydus et al., 2009; Robbins et al., 2010). However, the composition of fish amino acids as one of the main protein component is inflounced by various factors such as species, size, food resource, fishing season, water salinity and temperature) (Shirai et al., 2002; Sovik and Rustad, 2005; Toppe et al., 2007; E.I, 2009; Erdem et al., 2009).

Rainbow trout (*O. mykiss*) is a salmonidae membrane which lives in cold, clear and well-

oxygenated reservoirs by average water temperature of 16-18 ºC, although it could survive in a wide range of temperature conditions (Pankhurst et al., 1996; Nynca et al., 2012). Rainbow trout is originally from the North America which has been extremely introduced to the other countries (Celik et al., 2008). Because of rapid growth rate and high nutritional value, rainbow trout is now cultured in USA, Japan, Europe, Russia, Canada and Iran (Rasmussen and Ostenfeld, 2000; Dobly et al., 2004; Yasmin et al., 2004; Skinner et al., 2010). Therefore, the Present study was conducted to identify proximate composition of rainbow trout as a main species which has been broadly cultured worldwide. Besides, fatty acid content followed by amino acid composition of rainbow trout was also determined to assess its nutritional value for human consumption.

2. Materials and Methods

Sample preparation

In total 30 rainbow trout samples (511.75±6.92 g weight and 35.2±0.18 cm length) were obtained from a local farm in Jajrood, Mazandaran province, Iran and transferred alive to the Fisheries laboratory of Islamic Azad University, Science and Research Branch, Tehran, Iran. All of the fish samples were healthy reared for eight months in race-way ponds and showed no clinical symptom during the culture period. Feeding was done by Biomar commercial feed. A explanation of diet components can be consulted at http://www.biomar.com/en/BioMar-Denmark/Species--products/Fish-species/Orred/.

Prior to the analysis, all Specimens were decapitated and filleted after removing the tails, Fins, and viscera by a clean scalpel. The samples divided to four groups each containing double fillets of 10 specimens. The fillets of each group were then homogenized with a mixture and frozen in a fridge under -18 °C before being used for analysis.

Proximate Analysis

The samples were homogenized and their proximate analyses were determined based on the Association of Official Analytical Chemists (AOAC) methods. Moisture and ash content were analyzed by AOAC (2005) code 39.1.01 and 935.47, respectively. Lipid content was determined according to the Bligh and Dyer (1959) method. Crude protein content was also measured by converting the nitrogen content based on the Kjeldahl's method AOAC (2005) code 39. 1. 15. All analysis were done in triplicates (n=3) and results were expressed as g/100g edible part of the fish.

Lipid extraction and Fatty acid analysis

Lipids from rainbow trout flesh were extracted based on Acid Hydrolysis method of AOAC (2005) method 948. 15 Fat (crude) in sea food. Briefly, the fatty acids of rainbow trout fillet were converted to fatty acid methyl esters (FAME) by acetyl chlorine. Fatty acid composition was then determined by gas chromatography on a HEWLETT-PACKARD 5890 series II, equipped with a column capillary BPX70 (30 m × 0.25 mm × 0.25 µm) and a Flame – Ionization Detector (F.I.D). Column injector and detector temperature were held at 250ºC and 260ºC, respectively. Carrier gas was flowed by nitrogen in current of 0.6 ml/min. Unsaturated fatty acids were identified by comparing the retention time of each fatty acid versus its respective authentic standard. The results expressed as percentage (%) of total fatty acid compositions.

Amino acid Analysis

Amino acid composition was determined in triplicates after hydrolysis of the fillet samples in 6 M HCl at 110 °C for 24 h. The hydrolyzed samples were analyzed in triplicates using an amino acid analyzer (Eppendorf LC 3000, Germany) equipped as follows: analytic column of PEEK-Separation Column 'LUFA' L 00 282 98 07; precolumn of PEEK-Pre Column 'LUFA' VL 00 286 998 07; injection volume of 30 µl; flow rate of 0.2ml/min; reactor temperature of 125 °C; excitation of 440 nm; emission of 570 nm. The results were stated as mg/g fillet

Statistical Analysis

Data were presented as mean ± standard deviation (SD) and subjected to analysis of variance (ANOVA). Significant means were compared by one-way procedure tests at the 5% confidence level using Duncan´s multiple range test.

3. Results and Discussion

Proximate Composition

The results of proximate analysis of the rainbow trout fillet are shown in Table 1. The present findings were in accordance with those previously reported by Özden (2005). However, González-Fandos et al. (2004) reported higher lipid content (6. 55 %) and lower protein content (16.04 %) of rainbow trout when the results compared to the present study. Slight differences among separate researches could be explicated by different culture conditions and genetically characteristics of experimental strains. Higher protein content of the applied feed may be caused to greater fillet protein content. In general, the proximate composition of the fish analyzed is in agreement with the data available on the proximate

composition of similar fish species as reported in previous work species (Kinsella et al., 1977; Ünlüsayin et al., 2001; USDA, 2005; Testi et al., 2006).

Table 1. Proximate composition of rainbow trout (*O. mykiss*) fillet. Data are expressed as mean±SE.

Proximate Composition	Fresh Fish
Moisture (g/100 g)	71.70±1.9
Ash (%g/100 g)	1.33±0.1
Protein (g/100 g)	19.65±1.2
Lipid (g/100 g)	4.46±0.2

Fatty Acid Profile

Fatty acid composition of the rainbow trout is presented in Table 2. The results showed that fatty acids are grouped as saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs). For the case of saturated fatty acids, myristic (C14:0), palmitic (C16:0) and stearic acid (C18:0) were found to be the most predominant components for rainbow trout. The same findings were also observed by Osman et al. (2007) and Celik et al. (2008). The most abundant of PUFA were Linoleic (C18:2), linolenic (C18:3), eicosapentaenoic acid (EPA) (C20:5 n-3) and docosahexaenoic acid (DHA) (C22:6 n-3). Among MUFAs, oleic and palmitoleic acids were the predominant fatty acids, accounting for almost 65 % and 25 % of total MUFA, respectively. In the present study, MUFA was the highest followed by SFA and PUFA. Özden (2005) and Testi et al. (2006) differently found that PUFA was the highest in rainbow trout followed by MUFA and SFA. Haliloglu et al. (2004) and Yanar et al. (2006) about fatty acid composition and distribution in rainbow trout are in agreement with the data available in the present study. In the present study, EPA and DHA were accounted for 6.98 % and 9.10 % of the total fatty acids in the flesh of the rainbow trout. According to Piggott and tucker (1990), the n-3/n-6 ratio is a better index in identifying nutritional value of fish oils of different species. Table 2 also shows the ratio of n-3/n-6 in fish studied. In the present study, the n-3/n-6 ratio was found to range 2.06 % in rainbow trout meat, while the ratio of higher than 1:1 is considered to be optimal for nutritional purpose (Simopoulos, 1989; Burghardt et al., 2010).

Table 2. Fatty acid profile of rainbow trout (*O. mykiss*) fillet as % of total fatty acid. Data are expressed as mean±SE.

Variable	Mean	Standard Deviation	Variance	Range	Min	Max
C14:0	4.29^{d}	0.47	0.22	1.38	3.54	4.92
C15:0	0.49^{g}	0.18	0.03	0.61	0.23	0.84
C16:0	19.09^{a}	2.03	4.41	6.16	15.03	21.19
C17:0	0.71^{fg}	0.20	0.04	0.58	0.42	1.00
C18:0	4.97^{d}	0.80	0.64	2.44	3.70	6.14
C20:0	0.76^{fg}	0.10	0.01	0.41	0.63	1.04
C22:0	0.81^{fg}	0.29	0.08	0.82	0.36	1.18
C24:0	0.70^{fg}	0.09	0.009	0.36	0.60	0.96

∑SFA[1]	**31.85**	**3.97**	**15.77**	**11.97**	**25.04**	**37.01**
C14:1	0.17^{gh}	0.03	0.001	0.11	0.12	0.23
C15:1	0.07^{h}	0.04	0.002	0.12	0.01	0.13
C16:1	8.47^{b}	0.19	0.038	0.74	8.10	8.84
C17:1	0.74f^{g}	0.11	0.011	0.46	0.50	0.96
C18:1 n-9t	0.06^{h}	0.02	0.0004	0.08	0.03	0.11
C18:1 n-9c	19.81^{a}	1.39	1.92	4.66	18.44	23.10
C20:1 n-9	1.28^{f}	0.06	0.004	0.25	1.17	1.42
C22:1 n-9	0.13^{gh}	0.07	0.005	0.24	0.04	0.28
C24:1 n-9	0.16^{gh}	0.08	0.006	0.30	0.06	0.36
∑MUFA[2]	**30.89**	**1.81**	**3.27**	**6.55**	**28.60**	**35.15**
C18:2 n-6t	0.04^{h}	0.02	0.0004	0.07	0.01	0.08
C18:2 n-6c	3.27^{e}	0.17	0.03	0.80	3.00	3.80
C18:3 n-6	0.31^{g}	0.09	0.008	0.30	0.15	0.45
C20:2	0.68^{g}	0.10	0.01	0.36	0.50	0.85
C20:4 n-6	0.31^{g}	0.06	0.004	0.22	0.23	0.45
C22:2	2.16^{ef}	0.31	0.098	1.08	1.78	2.86
C18:3 n-3	2.70^{ef}	0.12	0.015	0.47	2.43	2.90
C20:3 n-3	0.07^{h}	0.03	0.001	0.10	0.03	0.13
C20:5 n-3 (EPA)	4.07^{d}	1.45	2.09	3.76	2.25	6.01
C22:6 n-3 (DHA)	6.93^{c}	0.51	0.255	1.52	6.33	7.85
∑PUFA[3]	**20.55**	**2.76**	**7.60**	**8.66**	**16.73**	**25.39**
∑ n-3	13.78	2.06	4.25	5.54	11.22	16.76
∑ n-6	6.77	0.72	0.52	2.81	5.69	8.50
n-3/n-6	2.02	0.13	0.016	0.41	1.82	2.23
EPA + DHA/C:16	0.59	0.17	0.031	0.49	0.37	0.86
PUFA/SFA	0.66	0.18	0.033	0.56	0.42	0.98
Fatty Acids	**83.29**	**1.61**	**2.60**	**6.79**	**80.35**	**87.14**

Different superscripts in a same column are significantly different ($p<0.05$)
[1] SFA means total saturated fatty acids
[2] MuFA means total monounsaturated fatty acids
[3] PuFA means total polyunsaturated fatty acids.

It was well known that fatty acid composition of different species changed depending on feeding condition, environmental factors, and physiological condition such as maturing stage (Akpinar and Aksoylar 1988; Metin and Akpinar 2000). Hayashi & Takagi (1978) revealed an obvious decline of the muscle lipid content during the spawning period, which concurred by gonad maturation. The specimens used in the present study were all immature and, therefore, higher fatty acid contents could be related to their growing stage and lipid accumulation in muscle tissue.

Amino Acid Profile

Amino acid composition of the rainbow trout is given in Table 3. Aspartic acid, glutamic acid, leucine, and lysine were the main amino acids of rainbow trout fillet which supported by previous studies (Iwasaki and Harada, 1985; Farmanfarmaian and Sun, 1999; Beklevik et al., 2005). Iwasaki and Harada (1985) similarly reported that the main amino acids in fish muscle were aspartic acid, glutamic acid and lysine. Wesselinova (2000) also reported that the amounts and types of amino acids in fish muscle were affected by catching time and location.

Amount of non-essential amino acids in rainbow trout fillet was higher than essential amino acids (Table 3). Therefore, the essential amino acids/non essential amino acids (N/E) ratio of rainbow trout flesh was found to be 0.75. The ratio of E/NE was determined as 0.77 for sea bream (*Pagrus major*), o.77 for mackerel (*Scomber japonicus*), 0.75 chum salmon (*O. keta*), and 0.77 pacific flounder (*Paralichthys olivaceus*) by Iwasaki and Harada (1985). Seventeen different amino acids were obtained in rainbow trout flesh, 8 Essential amino acids and 9 nonessential amino acids were identified. In this study essential amino acids are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, valine. Rainbow trout flesh includes all of the essential amino acids. The results obtained from this study shown that rainbow trout (*O. mykiss*) fillet has well-balanced and high quality protein source in the respect of E/NE ratio in all samples.

Table 3. Amino acid profile of rainbow trout (*O. mykiss*) as mg/g of the fillet. Data are expressed as mean±SE.

Variable	Mean	Standard Deviation	Variance	Range	Min	Max
Aspartic acids	8.96^{ab}	0.93	0.86	3.35	7.03	10.38
Threonine *	4.38^{e}	0.55	0.30	1.74	3.20	4.94
Serine	3.44^{f}	0.81	0.65	2.64	1.90	4.54
Glutamic acid	10.70^{a}	0.64	0.41	2.34	9.45	11.79
Proline	2.60^{g}	0.28	0.08	0.88	2.00	2.88
Glycine	4.35^{e}	0.69	0.48	2.19	3.16	2.35
Alanine	4.77^{e}	1.14	1.30	3.24	3.63	6.87
Valine*	3.78^{ef}	0.72	0.53	1.80	2.66	4.46
Methionine*	3.24^{e}	0.73	0.53	2.05	2.03	4.08
Isoleucine*	3.48^{f}	0.45	0.20	1.31	2.71	4.02
Leucine*	7.52^{bc}	0.66	0.44	1.82	6.77	8.59
Tyrosine	3.10^{f}	0.23	0.05	0.78	2.70	3.48
Phenylalanine*	3.30^{f}	0.29	0.09	1.02	2.70	3.72
Histidine	2.10^{cd}	0.35	0.12	1.01	1.62	2.63
Lysine*	6.93^{cd}	1.28	1.63	3.34	5.20	8.54

Arginine	4.52[e]	0.32	0.10	0.94	4.10	5.04
E/NE	0.73	0.03	0.001	0.11	0.66	0.77
∑E1	32.62	3.54	12.57	11.28	25.40	36.68
∑NE2	44.54	3.81	14.55	12.81	36.44	49.25

Different superscripts in a same column are significantly different ($p<0.05$)
[1] E means essential amino acids
[2] NE means non-essential amino acids.

It was also found that level of amino acids component, particularly lysine, histidine, and tryptophan of rainbow trout fillet was comparatively adjacent to values known for amino acid of human necessities of human (Bledsoe et al., 2003). Accordingly, rainbow trout fillet is wealthy in essential amino acids and could be used as a valuable food source for human eating.

4. Conclusion

The present research shows that rainbow trout fillet is a good product for human consumption in terms of nutritional value. According to the results of research, rainbow trout has a good quality in view of total fatty acids and amino acids. Rainbow trout flesh includes all of the essential amino acids and is richened in fatty acid group's n-3 and n-6. It has also valuable amounts of protein contents. Therefore, the flesh of rainbow trout (*O. mykiss*) could be beneficial sea food for human health.

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