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Amino Acid Composition of Roe from Wild and Farmed Beluga Sturgeon (*Huso huso*)

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ABSTRACT

Caviar is one of the valuable and most popular fish products all over the world that are obtained from sturgeons. Nowadays, the wild resources of these fishes are diminished, therefore, to meet the demand for the product, farming sturgeons has been considered. The chemical composition and the amino acids profiles of the wild and farmed roe obtained from beluga (*Huso huso*) were compared and the results have indicated that the amount of glutamine, serine, alanine, methionine, and lysine in the wild roe were higher than the farmed one (P< 0.05). The total amino acids (TAAs) and the ratio of essential amino acids (EAAs) to TAAs and EAAs to non-essential amino acids (N-EAAs) in the samples from the wild and farmed roe were similar (P> 0.05). The protein efficiency ratio (PER) and chemical score in farmed and wild roe were also similar (P> 0.05). The results showed that the farmed roe was similar to the wild one based on chemical composition, chemical score, PER, EAAs/TAAs and EAAs/N-EAAs. According to the results, farmed roe can be a good substitution for wild beluga roe (*Huso huso*).

Keywords: Caviar, Beluga, Amino acid, Chemical score.

INTRODUCTION

Amino acids, which are the building blocks for protein synthesis, are important energy substrates and are involved in specific physiological functions (Aragão *et al*., 2004). Seafood is considered as nutritious food mainly due to its readily digested proteins, which are an excellent source of essential amino acid (EAA) (Sanchez-Alonso *et al*., 2007). However, the composition of amino acids of aquatic animals used for food, such as fish, is strictly influenced by intrinsic (species, size, and sexual maturity) and extrinsic factors (food resources, fishing season, water salinity, and temperature) (Özyurt and Polat, 2006).

Sturgeons are the largest (Şener *et al*., 2005) and most valuable freshwater fish in the world. In recent years, the intensive culture of certain sturgeon species has been developed similar to the more traditional cultured species, namely, salmonids and cyprinids (Garcia-Gallego *et al*., 1999) to meet the demand for flesh and caviar. Caviar

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is one of the valuable seafood, which is obtained from sturgeon fish. Demand for caviar on export markets is estimated at 500 tons annually (Gessner *et al*., 2002; Bledsoe *et al*., 2003) and it is increasing (Ovissipour and Rasco, 2011), but loss of wild harvest is reflected in capture figures from all countries around the Caspian Sea (Azerbaijan, Iran, the Russian Federation, Kazakhstan and Turkmenistan). In Iran, the country that has one of the most reliable harvest projections, the total sturgeon catches and caviar production in 1993 was 1,710 and 106 tons, while in 2009 it decreased to 178 and less than 10 tons, respectively (IFO, 2009; Ovissipour and Rasco, 2011). For this reason, farming of different species of sturgeon fish has been considered to compensate this loss of production.

The Beluga sturgeon (*Huso huso*) is distributed within the basins of the Caspian, Black and Adriatic seas, where it once supported major commercial fisheries, but it is now considered as endangered species by the International Union for the Conservation of Nature and Natural Resources (Sturgeon Specialist Group, 1996). With the aim of rehabilitating threatened wild stocks and meeting commercial demands, some countries around the Caspian Sea, especially Iran, now use this species for aquaculture purposes because of the decrease in their amounts and supplying the caviar and meat to the market. On the other hand, Beluga is one of the most preferred fish species in the countries around the Caspian Sea owing to its desirable aroma and nutritional value. Beluga is suitable for aquaculture (both meat and caviar production) because of its fairly fast growth, ease of reproduction in captivity, high food conversion ratio, and the tolerance against variation in farming conditions (Abedian-Kenari *et al*., 2009; Mohseni *et al*., 2006; Vaccaro *et al*., 2005). Intensive aquaculture of this species for human consumption has only expanded in the more recent years. Unlike for the other sturgeon species (Mohseni *et al*., 2006; Zareh *et al*., 2006), there is little published information currently available on the nutritional value of caviar of *H. huso* (Ovissipour and Rasco, 2011). Furthermore, important issues for sturgeon culture are that biochemical and gross composition of the fish and caviar, as well as the texture and taste of the product, should match that of wild sturgeon (Ovissipour and Rasco, 2011).

Although there are some researches on the flesh proximate composition and amino and fatty acids in wild and cultured forms of sturgeon fishes (Abedian-Kenari *et al*., 2009; Badiani *et al*., 1996; Chen *et al*., 1995; Paleari *et al*., 1997; Vaccaro *et al*., 2005; Zareh *et al*., 2006), none of these was focused on the caviar of the *H. huso*. Thus, the main objectives of this study were to analyze and compare the amino acid profiles of wild and farmed roe obtained from the *Huso houso*.

MATERIALS AND METHODS

Sample Collection

All of the female wild Beluga sturgeon, *Huso huso*, (nine samples with mean weight of 73.2±8.2 kg) were provided in March 2012 by the Iranian government's sturgeon hatchery, Shahid Marjani Hatchery Center (Gorgan, Iran, Lat 36°37´ N, Long 53°05´ E). The experimental fish were captured during their spawning season by legal fisherman of the Faridpak Fishing Station, on the south eastern coast of the Caspian Sea. The specimens were caught using Gill nets with the standardized mesh and dimensions set by the Iranian Fisheries Research Organization. On the basis of the recruitment program set by the government, sturgeon which were not suitable for reproduction were considered for caviar production. Individual samples were slaughtered and processed (washed and gutted) on site under high hygienic conditions. Then, 65 g of roe from each of the nine females were collected, placed into separate plastic containers, and immediately transported in an insulated box with a

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suitable quantity of flaked ice (completely covered by ice) to the laboratory and kept in the frozen condition until analyzed. Simultaneously, roe (3-5 mm in diameter) from farmed female *H. huso* (Mean weight= 64.1±6.8 kg) were also collected from a private sturgeon rearing center (Talesh city, Iran). Farmed *H. huso* were reared in concrete tanks with freshwater constantly overflowing and during their farming key parameters were kept relatively constant (temperature: 18–23.5°C, oxygen saturation: 76–81%, pH: 6.9–7.6, and Salinity≤ 1 ppt).

Proximate Composition

The proximate analysis of the samples was done according to the procedures of the Association of Official Analytical Chemists (AOAC, 2005). Moisture, ash, protein, and fat contents were assayed by methods 934.01, 920.153, 954.01, and 991.36, respectively. Moisture content was determined by oven drying of the samples (Heraeus, D-63450, Hanau, Germany) at 105°C to get a constant weight (approximately 20 hours); lipid content were extracted with petroleum ether by using an automatic Soxtec system (FOSS, SoxtecTM 2050, Höganäs, Sweden); crude ash was determined by incineration in a muffle furnace (Isuzu, Tokyo, Japan) at 600°C for 3 hours; crude protein was determined by the Kjeldahl method (N×6.25) using an automatic Kjeldahl system (230-Hjeltec Analyzer, Foss Tecator, Höganäs, Sweden). Before analysis, samples were thawed (in the original pot) in a refrigerator (Yakhsaran, Tehran, Iran) at 2±1°C for approximately 5 hours.

Amino Acid Analysis

The Pico Tag method, with slight modifications was used for amino acid analysis, as described previously (Abedian-Kenari *et al*., 2009). Before analysis, 5 g from each sample was homogenized in a Waring blender (Waring, New Hartford, CT) for 1 minute, the homogenized samples were defatted in chilled acetone (Merck, Darmstadt, Germany) and low fat residue were used for AA analysis. Samples were hydrolyzed in evacuated sealed ampoules with 6M hydrochloric acid for 24 hours at 110°C and then derivatization was done by phenylisothiocyanate (PITC). In the precolumn steps, samples were first hydrolyzed with HCl, and then derivatized with PITC to produce phenylthiocarbamyl (PTC) amino acids prior to HPLC analysis. Quantities of the solutions were analyzed by reverse-phase HPLC (Shimadzu, Kyoto, Japan). The HPLC system was a Waters system (Milford, MA, USA) and consisted of a Waters 1525 binary HPCL pump, Waters 2487 Dual l absorbance detector, Rheodyne injector, and Breeze software. The column was a PICO.TAG physiological free AA analysis C_{18} reversed-phase (300×3.9 mm id) also from Waters. The AA content was determined from standard curves (PIERS amino acid standard H; Thermo scientific Co. USA) based on peak area measurements. Samples were run in triplicate and the average areas were calculated. In the present study, tryptophan was not determined, since it was partially destroyed by acid hydrolysis. Methionine and cysteine were underestimated as they were not determined separately. Glutamine was converted to glutamate, and asparagine to aspartate, during acid hydrolysis, thus, the sum of the respective AA in the proteins was reported. Peaks were identified and areas calculated using Breeze software (Version 3.200, Waters, Milford, MA, USA).

Protein Efficiency Ratio (PER)

The following equations were used to calculate the protein efficiency ratio developed by Alsmeyer *et al*. (1974):

$PER= -0.468+0.454[Leu]–0.104[Tyr]$

$PER=1.816+0.435[Met]+0.780[Leu]+0.211[His]–0.944[Tyr]$

$PER= 0.08084[X_7]–0.1094$

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$PER = 0.06320[X_{10}] - 0.1539$

Where:

$X_7 = Thr+Val+Met+Ile+Leu+Phe+Lys$; and $X_{10} = X_7+His+Arg+Tyr$

Chemical Score

The chemical score of the wild and cultural roe were calculated in comparison with the essential amino acid (EAA) profile in a standard protein as described by FAO/WHO (1990) according to the following equation (Bhaskar *et al.*, 2008):

$$\text{Chemical score} = \frac{\text{EAA in test protein (g/100g)}}{\text{EAA in standard protein (g/100g)}}$$

Statistical Analysis

The results are expressed as mean±standard deviation. The data were tested for homogeneity of variances at a significance level of $P< 0.05$ and probability values less than .05 were considered as statistically significant. An unpaired *t*-test and one-way ANOVA (Duncan's multiple tests) was used for mean comparison. Statistical data analysis was performed with SPSS.15 software (SPSS, Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Proximate Composition

The mean (±SD) percentages of chemical compositions of roe obtained from wild and farmed beluga were as follows: 25.43% and 23.81% protein, 14.8% and 15.67% lipid, 1.73% and 1.60% ash, and 56.21 and 57.29% moisture contents, respectively (Table 1). Although there was no significant differences between the samples, the observed non-significant variations were probably due to the fish nutrition, the fish living area and environmental circumstances, and seasonal changes (Hamzeh and Rezaei, 2011; Özyurt and Polat, 2006; Wesselinova, 2000).

Amino Acid Profiles

The content of 17 amino acids (except for tryptophan) of roe from the wild and cultured Beluga is shown in Table 2. According to the results, the total amino acid (TAA) was not different in wild roe (29.93 g 100 g^{-1}) and for the cultured one (28.75 g 100 g^{-1}) ($P > 0.05$). The amounts of glutamine, serine, alanine, methionine and lysine in the cultured samples were lower than the wild samples ($P< 0.05$) and the essential amino acids (EAAs) were 15.37 and 14.61 g 100 g^{-1} in the wild and cultured samples, respectively. The content and composition of amino acids in fish products depend on feeding (Wesselinova, 2000), age, season, and location (Abedian-Kenari *et al.*, 2009), and the storage conditions since, during the storage, chemical reactions between α-amino and aldehyde groups present in amino acids cause a reduction in AA content of a product (Vidotti *et al.*, 2003).The ratios of EAAs to Non-EAAs in the wild and cultured obtained roe were 1.05 and 1.03 and the percentages of EAAs to TAAs were 51.35 and 50.81, respectively ($P> 0.05$). The data have shown that the EAAs in Beluga roe, in both wild and cultured, included more than half of the amino acids contents, which were higher than the reference value proposed by FAO and WHO (1973). Comparing with the other studies on the different fish flesh have also shown that in this study both cultured and wild roes have higher ratio EAAs to Non-EAAs and also EAAs to TAAs than fish flesh reported by

Table 1. The proximate composition of wild and farmed beluga roe (%).(*n*= 9, Mean±SD)

	Protein	Lipid	Ash	Moisture
Wild	25.43±2.83 [a]	14.8±1.74 [a]	1.73±0.26 [a]	56.21±4.26 [a]
Farmed	23.81±3.88 [a]	15.67±2.16 [a]	1.60±0.27 [a]	57.29±3.23 [a]

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Table2. Amino acid composition of the wild and farmed Beluga roe and its chemical score (g 100 g^{-1} wet sample) (Mean±Standard DE; n= 9).

	Roe (g 100 g^{-1})		Chemical score		
AA [a]	Cultured	Wild	Ref [b]	Cultured	Wild
Essential amino acids					
Histidine	0.85±0.06	0.91±0.02	2	0.42	0.45
Isoleucine	1.33±0.02	1.36±0.04	4	0.33	0.34
Leucine	2.35±0.06	2.41±0.02	7	0.33	0.34
Lysine	2.07±0.02	2.32±0.06	5.5	0.38	0.42
Methionine	0.51±0.02	0.66±0.08	3.5	0.14	0.19
Phenyl alanine	1.12±0.04	1.21±0.06	4.29 [c]	0.53 [c]	0.55 [c]
Tyrosine	1.17±0.08	1.14±0.04			
Threonine	1.53±0.03	1.6±0.06	4	0.38	0.4
Tryptophan	ND	ND	1.21	ND	ND
Arginine	2.11±0.06	2.18±0.02	5	0.42	0.43
Valine	1.57±0.06	1.58±0.09	5.42	0.29	0.29
Non-essential amino acids					
Asparagine	2.62±0.11	2.43±0.07			
Glutamine	4.76±0.04	4.95±0.07			
Serine	2.07±0.02	2.18±0.06			
Glycine	0.91±0.11	0.96±0.04			
Alanine	1.71±0.04	1.89±0.04			
Proline	1.34±0.02	1.38±0.04			
Cystine	0.73±0.02	0.77±0.03			
Indices					
TAA [d]	28.75	29.93			
∑EAAs [e]	14.61	15.37			
∑Non-EAAs [f]	14.14	14.56			
∑EAAs/∑Non-EAAs	1.03	1.05			
∑EAAs/TAAs (%)	50.81	51.35			
SaAAs [g]/TAAs (%)	25.66	24.65			
SwAAs [h]/TAAs (%)	9.11	9.52			

[a] Amino Acids; [b] Essential amino acid requirements for adults proposed by FAO/WHO, 1985; [c] Phenyl alanine+Tyrosine; [d] Total Amino Acids; [e] Total Essential Amino Acids; [f] Total Non-Essential Amino Acids; [g] Savory Amino Acids, [h] Sweet Amino Acids.

Jhaveri *et al.* (1984) as follow: 0.71 for cod (*Gadus morhua*), mullet (*Mugil cephalus*); 0.77 for mackerel (*Scomber japonicus*) and sea bream (*Pagrus major*), 0.69 for sardine (*Sardinamelonosticta*), 0.74 for herring (*Clupea pallasi*), 0.75 chum salmon (*Oncorhyn chusketa*) and the others found 0.77 for Pacific flounder (*Paralich thysolivaceus*) by Iwasaki and Harada (1985) and 0.81 for Beluga (*Huso houso*) by Abedian-Kenari *et al*. (2009).The percentage of savory amino acid (including asparagine and glutamic acid that are well known for their fresh tasting) to TAA was 24.65 and 25.66 and the sweet amino acid (including Glycine and Alanine) to TAA was 9.52 and 9.11% for the wild and cultured samples, respectively (P> 0.05). Our data was similar to those measured by Abedian-Kenari *et al.* (2009) on Beluga flesh, which were 23.9-29.2% for savory and 10.8-12.0% for the sweet amino acids.

Chemical Score and Protein Efficiency Ratio (PER)

The nutritive value of protein of any ingredient depends mainly on the protein's capacity to fulfill the needs of organisms with respect to essential amino acids and

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also amino acids composition and their digestibility (Vidotti *et al*, 2003; Abedian-Kenari *et al*, 2009). There are some parameters that are used for evaluation of the nutritional values of a protein including chemical score and PER. In chemical score, the AAs in products are compared with the reference protein recommended by FAO/WHO (1991) and in the PER the specific AAs are used by various equations based on certain AAs (Table 3). There is no significant variation between the two groups of roe with respect to chemical score of their amino acids and their PER.

CONCLUSIONS

The result of the present study showed that although there were some differences in the two amino acid profiles of the cultured and wild roe, there was no significant difference between the two kinds of roe based on nutritional values including chemical score and protein efficiency ratio. Therefore, it can be proposed that cultured obtained roe is a good substitution for the wild type.

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Table 3. Protein Efficiency Ratio of the wild and farmed Beluga roe (mg amino acid gr^{-1}).

Protein efficiency ratio	Wild	Cultural
-0.468+0.454[Leu]–0.104[Tyr]	9.29	9.07
-1.816+0.435[Met]+0.780[Leu]+0.211[His]–0.944[Tyr]	28.35	25.82
0.08084[X7]–0.1094 [a]	8.91	8.39
0.06320 [X10]–0.1539	9.57	9.1

[a] X7: Thr+Val+Met+Ile+Leu+Phe+Lys, [b] X10: X7+His+Arg+Tyr.

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بررسی و مقایسه اسیدهای آمینه تخم حاصل از فیل ماهیان (*Huso huso*) پرورشی و وحشی

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چکیده

خاویار از مهمترین و محبوب ترین فرآورده های دریایی در سراسر جهان می باشد که از تاس ماهیان بدست می آید. امروزه تعداد تاس ماهیانی که از طبیعت به منظور استحصال خاویار صید میشوند رو به کاهش است، بنابراین جهت تامین نیازهای موجود، پرورش این ماهیان مورد توجه قرار گرفته است. در این مطالعه ترکیب شیمیایی و پروفیل اسید آمینه ی تخم حاصل از فیل ماهی طبیعی و پرورشی ارزیابی شده و نتایج بدست آمده نشان داده که میزان اسید اُمینه گلوتامین، سرین، آلانین، متیونین و لیزین در فیل ماهی طبیعی بیشتر از فیل ماهی پرورشی بوده است ($P<0/05$). میزان کل اسیدهای آمینه، میزان اسیدهای آمینه ضروری به کل اسیدهای آمینه، و به اسیدهای آمینه غیر ضروری در دو گروه تفاوت معنی داری نداشت. میزان کارائی پرتئین و امتیاز شیمیایی اسیدهای آمینه نیز در دو گروه باهم تقاوت معنی داری نداشت. نتایج بدست آمده نشان داد که به رقم وجود تفاوت در میزان برخی اسیدهای آمینه؛ محتوای ترکیبات شیمیایی، امتیاز شیمیایی اسیدهای آمینه، میزان کارائی پروتئین، نسبت اسیدهای آمینه ضروری به کل اسیدهای آمینه و به اسیدهای آمینه غیرضروری در بین دو گروه اختلاف معنی داری نداشت و بدین ترتیب میتوان عنوان کرد که خاویار حاصل از فیل ماهی پرورشی می تواند جایگزین خوبی برای نمونه های طبیعی باشد.

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