

## Evaluation of Nutritional Quality of Caspian Shad Muscle, *Alosa caspia caspia* (Eichwald, 1838) in Southern Caspian Coast

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**Abstract:** The main aim of this research is to identify fatty acids and nutritional quality of Caspian Shad muscle. Caspian shad muscle contains 17.1% crude protein and 2.82% crude fat. Caspian shad muscle dedicated, appropriate amino acid, they included, Glutamic acid (13.66%), Aspartic acid (10.65%), Valine (9.04%), Leucine (8.7%) and Arginine (6.11%). Caspian Shad muscle tissue amino acids had high nutritional value compared to other fish. The obtained results of UFA and SFA show they were, 74.14% and 36.87 %, respectively, in fresh tissue, so that, DHA (C22:6n-3) and cis vassenic (C18:1n-9) have high amounts (12.16, 22.41) of UFA and palmitic acid (23.57 %) was the most SFA. The ratio of n-3 to n-6 poly unsaturated fatty acid (PUFAs) was 2.50%, thus Caspian shad muscle is rich in n-3 PUFA. Therefore the fish can be recommended as a good source of poly unsaturated fatty acid for the health of the consumers.

**Key words:** Amino acid • Fatty acid • Proximate composition • n-3/n-6.

### INTRODUCTION

The shads or river herring make up the genus *Alosa*, this fish related to herring in the family of *clupeidae*. Several species can be found on both sides of the Atlantic Ocean and Mediterranean Sea. Genus *Alosa* can also be found throughout the Caspian Sea. Many are found in freshwater during spawning and some are only found in landlocked freshwater. *Alosa* is generally pelagic. They are mostly anadromous or semi anadromous with the exception of strictly freshwater landlocked species. *Alosa* is generally migratory and schooling fish. There are species native to the Black Sea and Caspian Sea, as well as the Persian Gulf. *Alosa* species of the Caspian are systemically characterized by the number of rakers on the first gill arch by some scientists.

Fats supply energy and contain essential fatty acids and serve as a carrier for the absorption of the fat-soluble vitamins A, D, E and K and carotenoids [1, 2]. Fats are a source of antioxidants and numerous bioactive compounds and serve as building blocks of membranes and play a regulatory role in numerous biological

functions. Dietary fat is found in foods derived from both plants and animals [3]. Proteins are major components of all muscles, tissues and organs and are necessary and crucial in every process that occurs within the body such as metabolism, digestion and transportation of nutrients and oxygen in the blood. They are also necessary for the production of antibodies, which fight against microbial infections and are the main nutrients that keep our hair shiny, nails strong, our skin fresh and are important to growing our bones strong and healthy [4]. The best sources of complete protein are found in animal foods such as meat, fish and shellfish. Muscle tissue of fish is an important source of protein for humans [5]. Fish is safer and healthier to be consumed compare with goat, mutton, buffalo and chicken meat [6]. Generally, fish has been widely accepted as a good source of protein and other elements necessary for the maintenance of healthy body [7, 8]. The nutritional quality of fish is highly associated with its content of essential fatty acids (EPAS).  $\omega$ -3 polyunsaturated fatty acids cannot be synthesized effectively by human and must be obtained by diet [9,10]. Generally the main part of fish used for human nutrition is muscles.

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The long chain polyunsaturated fatty acid has gained global attention because of its role in human coronary artery disease prevention, development of retina and brain and also decrease incidence of breast cancer, rheumatoid arthritis, multiple sclerosis, psoriasis and inflammation. Omega3 fatty acids seem to have small, dose-dependent hypotensive effect, the extent of which seems to be dependent on the degree of hypertension. DHA seems to be more effective than EPA in lowering blood pressure [11]. Thus analysis of fatty acid profiles of the tissues, such as muscles from fish living in their natural ecosystem, can yield valuable information [12, 13]. There are also differences in terms of fatty acid composition between fresh water and marine fish. Fresh water fish is generally recognized to contain lower levels of  $\omega$ -3 PUFA than marine fish. However, there is a big variation in fatty acid composition of different individual fish of the some species. Diet, location and season are the major factors affecting the fatty acid composition [14 - 16].

Several previous studies papers have reported the composition of fresh and marine water fish. Determining the amino acid and fatty acid profiles of Shad river muscle (anadromous fish) will improve the nutritional information available to researchers. The objectives of this study were to analyze the amino acid and fatty acid composition of *Alosa caspia caspia* muscles and determine its nutritional quality.

## METHODS AND MATERIALS

**Sample Preparation:** The experimental fish were obtained from natural conditions. 50 pieces of Caspian Shad (*Alosa caspia caspia*) were caught by Beach Seine from Caspian southern coast line, Mahmoodabad city, Mazandaran Province, Iran. Wet body weight was  $600 \pm 15$ g and body length was  $30 \pm 5$ cm. Samples were transported to the laboratory under the ice at a ratio of 1 to 1.

**Proximate Composition Analysis:** Moisture content was measured by drying sample in an oven at  $105^\circ\text{C}$  until a constant weight was obtained [17]. Crude protein content was determined by kjeldahl method [17] and a conversion factor of 6.25 was used to convert total nitrogen to crude

protein. Fats were determined colorimetrically according to Blight and Dyer [18]. Ash was determined by mineralization at a temperature  $550$ - $600^\circ\text{C}$  [17].

**Amino Acid Analyses:** Amino acid composition of samples was measured according to high performance liquid chromatography (HPLC) and fluorescence detector (USA, CA. San Clara, series 1100, A gilet). The 0.2 gram of sample was hydrolyzed for 20 hours at  $110^\circ\text{C}$  with 6 N HCl in sealed glass tubes filled with nitrogen gas, without oxygen. Fluorescence detector at wavelengths of 250 and 395 nm and a temperature of  $40^\circ\text{C}$  was activated. Fluorescence detector using a combination of specific amino acids from 12.5 to 75 Pykvmvl was calibrated. The content of tryptophan was determined by colorimetric method [19] after alkaline hydrolysis of each sample. All measurement was carried out in triplicate.

**Determination of Fatty Acid Profile:** Total lipid was extracted by chloroform-methanol (1:1v/v) and estimated gravimetrically [14]. The fatty acids in the total lipids were esterified into methyl esters by saponification with 0.5 N methanolic NaOH and transesterified with  $\text{BF}_3^1$  (w/v) methanol [17].

The FAMES<sup>2</sup> were analyzed on HP5890 Series II GC<sup>3</sup> equipped with gas chromatograph with a FID<sup>4</sup> and a 30m capillary column with an internal diameter of 0.25 mm, liquid phase Supelco wax 10, film thickness 0.25  $\mu\text{m}$ . Separation conditions: nitrogen carrier gas, flow rate of 1  $\mu\text{L}/\text{min}$ . Temperature of the detector was  $260^\circ\text{C}$ ; of the injector  $250^\circ\text{C}$  and of the column,  $155^\circ\text{C}$ . The individual fatty acids were identified by comparing their retention times with the standards of Supelco.

**Statistical Analysis:** Calculations made were the mean, standard deviation and coefficients of variation in percent. Statistical comparisons among data were made using statistical software SPSS version 13 for windows.

## RESULTS AND DISCUSSION

**Proximate Composition:** The proximate composition of Caspian Shad is shown in Table1. Crude protein, crude fat and crude ash content of the Caspian Shad muscles were 17.1, 2.82 and 1.7%, respectively. The crude fat content

<sup>1</sup>: Tri Fluoride Bore

<sup>2</sup>: Fatty Acids Methyl Esters

<sup>3</sup>: Gas Chromatograph

<sup>4</sup>: Flame Ionizing Detector

Table 1: Proximate composition of Caspian Shad muscle

Component	Moisture (%) <sup>a</sup>	Crude protein (%) <sup>a</sup>	Crude Fat (%) <sup>a</sup>	Crude Ash (%) <sup>a</sup>
Mean	77.35	17.1	2.82	1.7
SD	0.03	0.03	0.01	0.03

Table 2: Essential Amino acid composition of Caspian Shad muscle

Non Essential amino acid	Content (DW%) Mean±SD
Arginine	6.1±0.02
Histidine	1.15±0.01
Isoleucine	6.84±0.02
Leucine	8.7±0.05
Lysine	2.98±0.05
Phenylalanine	3.42±0.04
Taurine	5.89 ±0.01
Valine	9.04±0.02
Metionine	4.06±0.05
Total	48.18±0.02

Table 3: Non Essential Amino acid composition of Caspian Shad muscle

NonEssential amino acid	Content (DW%) Mean±SD
Glutamic acid	13.66±0.03
Serine	7.03±0.01
Aspartic acid	10.65±0.04
Glycine	5.48±0.07
Alanine	9.31±0.09
Tyrosine	2.61±0.04
Total	48.74±0.04

was higher than the amount found in *Aspius aspius*, *Exoux lacius*, *Mugil cephalus* and *Zander luciperca* [20 - 22]. The crude fat content was lower than the amount found in *Rutilus frisii kutum* (anadromous fish Caspian Sea) [23]. The varied content of fat was compensated by the content of water. Low fat fish have higher water contents and as a result, their flesh is in color [24]. Based on fat and moisture Caspian Shad is a lean fat fish, with a fat content 2-5% by weight. Fat content is influenced by species, seasons, geographical regions, age and maturity [25].

**Amino Acid Composition:** The essential amino acid composition of Caspian Shad muscle is shown in Table 2. Caspian Shad protein has a high amount of essential amino acid; they include Valine (9.04%), Leucine (8.70%), Isoleucine (6.84%) and Arginine (6.01%). Caspian Shad is also reach in valine and leucine.

They are not produced by the body and must be extracted from food. Valine is an essential amino acid and is required for muscle metabolism, repair and growth of tissue and maintaining the nitrogen balance in the body.

Valine also assists to regulate blood sugar and energy levels. Leucine is an essential amino acid which assists to regulate blood sugar and energy levels, production of the human growth hormone, wound healing as well as the growth and repair of muscle tissue. Leucine can also assist in the prevention of the breakdown of muscle proteins that may occur after severe stress or trauma. Valine is part of the three branched chain amino acids (BCAA) - the other two are Leucine and Isoleucine [26].

Low amount of essential amino acid have contained Metionine (4.06%), Phenylalanine (3.42%), Lysine (2.98%) and Histidine (1.15%). Histidine is an essential amino acid that your body needs during periods of growth, stress and recovery from illness and injury, but minimum amount of an essential amino acid in Caspian Shad muscle was Histidine (1.15%).

Histidine is one of the basic amino acids due to its aromatic nitrogen-heterocyclic imidazole side chain. This amino acid is metabolized into the neurotransmitter histamine and the set of genes that produce the enzymes responsible for histidine synthesis. Histidine is also a precursor of histamine, a compound released by immune system cells during an allergic reaction. It is needed for growth and for the repair of tissue, as well as the maintenance of the myelin sheaths that act as protector for nerve cells. The non essential amino acid composition of Caspian Shad muscle is shown in Table 3.

Caspian Shad proteins have contained a high amount of non essential amino acid, they are included Glutamic acid (13.66%), Aspartic acid (10.65%), Alanine (9.31%) and Arginine (6.01%). Caspian Shad proteins have contained a high amount Glutamic acid. Glutamic acid, a non-essential amino acid is synthesized from a number of amino acids including Ornithine and Arginine, it helps with the transportation of potassium across the blood-brain barrier, although it does not pass this barrier that easily. Glutamic acid can be used as fuel in the brain and can attach itself to nitrogen atoms in the process of forming glutamine and this action also detoxifies the body of ammonia. This action is the only way in which the brain can be detoxified from ammonia [20]. Low amount of non essential amino acids have contained Glycine (5.48%) and tyrosine (2.61%).

**Fatty Acid Composition:** Fatty acid composition is shown in Table 4. The fatty acid profile of the Caspian Shad was dominated by saturated fatty acid (SFAs), which comprised about 36.87% of total fatty acid. Among, the SFAs, palmitic acid (16:0) was the dominated saturated

Table 4: Fatty acid composition of Caspian Shad muscle

	Fatty acid	Mean±SD
Saturated fatty acid (%) <sup>a</sup>	C14:0	2.85 ±0.05
	C16:0	23.57±0.05
	C17:0	4.11±0.05
	C18:0	5.76±0.10
	C24:0	0.58±0.07
	ΣFA	36.87±0.06
Mono saturated fatty acid (%) <sup>a</sup>	C18:1n-9	22.41±0.07
	C18:1n-7	3.27 ±0.09
	ΣMUFA	25.68±0.08
Poly unsaturated fatty acid (%) <sup>a</sup>	C18:2n-6	5.72 ±0.06
	C18:3n-3	1.01±0.04
	C20:4n-6	1.05±0.06
	C20:5n-3	3.11±0.05
	C22:6n-3	12.16±0.07
	ΣPUFA	48.73±0.06
Other fatty acid (%) <sup>a</sup>	ΣPUFAn-3	16.76
	ΣPUFAn-6	6.73
	PUFAn-3/ ΣPUFAn-6	2.50

<sup>a</sup>Percentage of total fatty acid

fatty acid, according for 23.57 of total fatty acid. These results are in agreement with previous studies on SFAs of other species [5,20, 27- 29].

Mono saturated fatty acids (MUFAs) and poly unsaturated fatty acids (PUFAs) accounted for 25.68% of the total fatty acids and 48.73% of the total fatty acids, respectively. The main mono saturated fatty acid was Oleic acid (C18:1n-9), accounting for 22.41% of the total fatty acids, followed by cis-vaccenic acid (C18:1n-7) (3.27%). 8 fresh water species (*Rutilusfrisi kutum*, *Clupeonella cultiventris*, *Cyprinus carpio*, *Sander luciperca*, *Salmo salar*, *Onchorhynchus mykiss*, *Mugil cephalus* and *Liza aurata*) were investigated and results showed, Oleic acid of MUFAs was dominated fatty acid in species [30].

The dominate PUFA was DHA (C22:6n-3), accounting for 12.60% of the total fatty acids, followed by LA (C18:2n-6), EPA (C20:5n-3), ALA (C18:3n-3) and AA (C20:4n-6). However, except for LA (5.72%) all of these three fatty acids were present in minor (1.01-3.11%).

In last decades, PUFAs of  $\omega_3$  family have been recognized to be essential components of human diet [31]. These acids, particularly EPA (C20:5n-3)  $\omega_3$  & DHA (C22:6n-3)  $\omega_6$ , appeared to play key role in ontogenesis especially neural development Functioning of cardiovascular system and immune system.

Regular consumption of food with appropriate content of EPA and DHA provides prevention and treatment of depression cardiovascular and some other diseases [32- 33]. The benefits effects of fish consumption are depending on both fat content and PUFA composition [34].

Researches on marine fat and oils begun from several decades. The fatty acid of Rainbow trout (*Onchorhynchus mykiss*), Atlantic salmon(*Salmo salar*) and Herings(*Clupeidae heringus*) fish tissue have been identified in this regards [35].

Previous researches have been reported that diet rich in poly unsaturated fatty acid increase the amount of serum glucose, because decline insulin secretion, feeding dietary fish and fish oil/meal to human and animals, decreased blood pressure, glucose content were higher [36].

Sixty percent of heart attacks occur without due to irregular heartbeats which the fatty acid DHA can prevent. Some studies have indicated that that EPA is more effective for lowering blood triglycerides. Both DHA and EPA lower triglycerides by reducing the rate of fatty acid synthesis in the liver [37].

The n-3 and n-6 PUFA of the Caspian Shad accounted for 16.63% and 6.73% of total fatty acids, respectively. The results of this study showed that total n-3 fatty acids were higher than n-6 fatty acid. The n-3/n-6 ratio is good index for comparing relative nutritional value of fish oil of different species and a higher ratio of n-3/n-6 PUFAs has often been cited as an index of nutritional value. The ratio of n-6/n-3 PUFAs found in this study was lower than the value (4.0 at maximum) recommended by the UK Department of Health [38]. Values higher than the maximum are harmful to health and may promote cardiovascular disease [39].

This was confirmed by data obtained by Zmijewski *et al.* [21] who found that, freshwater carnivorous fish can be characterized by greater n-3/n-6 fatty acid ratio than phytophagous and benthophagous cyprinoid fish (tench, ide, crucian carp, grass carp). Differences in fatty acids of marine and freshwater fishes should not only be considered with respect to species habitat but also based on their natural diet, especially whether a species is herbivorous, omnivorous or carnivorous [40]. Apart from that, size, age, reproductive status of fish, environmental conditions and especially water temperature, influence lipid content and fatty acid composition of fish muscle to a lipid content and fatty acid composition of fish muscle to a certain extent [41-42].

## CONCLUSION

Determination of fatty acid composition indicated the palmitic acid (C16:0) and oleic acid (C18:1n-9) more abundant SFA and MUFA in Caspian Shad muscle. Caspian Shad muscle is high quality protein source with

a well-balanced composition of essential amino acids. Caspian Shad fish muscle consumption growth and development of children with cerebral neurons due to the amount of protein and fatty acids is recommended.

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