LIFE STYLE AND STRUCTURE DIFFERENCES BETWEEN SPECIES OF FRESHWATER FISH

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Article submited: 15.12.2022; accepted: 05.01.2023

Abstract

It has been recommended that people eat fish rich in unsaturated fats at least twice a week to reduce the risk of heart disease. Fish consumption is significant, mainly from fish living in ocean saltwater. However, in countries without sea like Hungary, the richness of freshwater fish has developed a wide range of cooking techniques for fish with different nutrition. We suspect that muscle structure differences have not yet been investigated. The difference in fatty acid composition of African catfish and Siberian sturgeon is known, but no morphological studies have been performed on their muscle structure. The aim of this study was to compare the structure differences between freshwater fish with different lifestyles. The organization of muscle structure was monitored in meat by means of cytochemistry combined with scanning electron microscopic studies on tissues of two different species, and the techno-functional parameters measured. The filleted muscles of African catfish (Clarias gariepinus) and Siberian sturgeon (Acipenser baerii) were compared after fresh and fast freeze. The associated complex structure of muscle in both species appeared different. One is a tightly closed muscle mass, while the other is a soft structure, which shows a different degree of softness of the meat after baking. In both species, the right muscle structure is beneficial under extreme environmental conditions. The different skeletal structure in fish needs altered processing, which we wish to continue with further testing and to prepare tasty food for consumers and use in dietetics.

Keywords: freshwater fish, muscle tissue structure, ingredients, scanning electron microscope

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Introduction

Fish consumption on Earth is significant, mainly from saltwater fish living in ocean important source of nutrients. In the EU, the fish consumption per capita is the second highest in the world around 2 kg/capita/year but about 6.7 kg/capita/year in Hungary [1], and some individual EU Member States have among the highest rates in the world. However, in countries without sea, such as Hungary, the richness of freshwater fish has developed a wide range of cooking techniques for fish with different nutrition.

One of the popular and well-breeding fish is the Siberian sturgeon or Lena Hungary is a native fish as it migrated from the Black sea after the ice age (Figure1) [2]. It is cold-blooded species. Today, its main stock is found in the rivers of Eastern Europe and western Siberia. The meat of the sturgeon is extremely valuable because its taste is excellent and is threaded with cartilage. They moved to Hungary in 1981 and they have been settled in our fishing lakes these days. The Siberian sturgeon weighs about 65 kg, they are long-lived, up to 60 years, and they reach sexual maturity rather late [3].

Another favorite fish is the African catfish (*Clarias gariepinus* Burchell). This species is native to Africa and Asia Minor, and it can be farmed and found everywhere. Its adaptability is high, not picky about food and can develop properly in polluted environments [4]. It is native to Inland waters of Africa, but it is living in Netherland and Hungary too. The catfish is perfectly adapting to high stocking densities, thanks to its auxiliary respiratory system it can withstand even persistently low oxygen levels. Furthermore, it is a heat tolerant fish but it dies at temperatures below 15 oC or in a microbiologically infected environment [2]. The meat of the catfish is fiber-free, low-fat, it has an excellent taste and is well transportable due to its elongated body, similar to that of eel or ling.

Although these are well-transportable fish, their meat, and the quality of the meat products made from them has several factors. The age, gender, posture and feed of the animal primarily determine the quality of the meat. It plays an outstanding role in the chemical composition of raw meats, physical properties such as color, texture, and techno-functional properties. In addition, it is important how we prepare, how we store to preserve the quality for a long time.

Millions of people live with minor or major disorders, of which metabolic diseases are the most serious, the number of those is currently on the rise. Many people prefer white and easily digestible meat, which is an inexpensive source of protein from chicken but we forget about fish. Freshwater fish is barely available for purchase high and therefore consumption is especially low in countries where sea fishing is not possible.

The living systems, animal and plant cells are highly complex colloidal systems. In the cytosol containing carbohydrates and salts due to this, the cell sap behaves like a colloidal solution during freezing. During slow freezing, extracellularly crystals form from the water and some of the cellular fluid diffuses into the extracellular space and freezes into the crystal nodule formed there. In medium-fast freezing speed changes the size of the water crystal during quick freezing, heat removal is faster than cell liquid diffusion, resulting in intracellular freezing inside the cell. During quick freezing, heat removal is faster than cell sap diffusion, resulting in intracellular freezing of the cell liquid [5, 6]. The ice crystals form at temperatures between -0.6 -2.2 oC. This rapid crossing of the temperature range eliminates the formation of large ice crystals. This reduces tissue and cellular destruction.

The biological fats are solid in texture differ from oils which are liquid at ambient temperatures. But chemically there is a little difference since the substances are composed predominantly of esters of glycerol with fatty acids, so called triacylglycerol (TG). Biologically in the living we used synonymously the lipid term that those are including substances all important fat-soluble soluble in food and nutrition. Their function is distinguished as structural fats, metabolic fats, storage fats or fats in transit in the body [7, 8 and 9]. The fatty components can be made within the body from carbohydrates consumed in the diet. Exceptions are the essential fatty acids (EFAs) that those fatty acids the human and other animals must ingest because the body requires for good health but cannot synthetize them [10] which two of the fatty acids are only known to be essential for humans, the alpha-linolenic acid (omega-3 fatty acid) and the linolenic acid (an omega-6 fatty acid) [11, 12].

The value of fish meat with lipids is for preventing, protecting and improving many diseases, it is also our natural source of proteins, minerals and ions. Atrial fibrillation is a dangerous condition that tends to strike the elderly and can lead to stroke or heart failure. Omega-3 fatty acid intake related to other dietary factors known to reduce coronary heart disease risk assuming moderate consumption.

Western diets are deficient in omega-3 fatty acids, and need to add excessive amounts of omega-6 fatty acids compared with the diet on which human beings evolved and their genetic patterns were established [13, 14, 15]. The omega-6 polyunsaturated fatty acids (PUFA) and a very high omega-6/omega-3 ratio, as found in today's Western diets, promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory as well as autoimmune diseases, whereas increased levels of omega-3 PUFA (a low omega-6/omega-3 ratio) exert suppressive effects.

Hungary is rich with freshwater fish in rivers but the consumption of fish meat lower than the European average, in 2007: 3.8 kg/capita/year, in 2016 increased to 6.7 kg/capita/year. There are some popular freshwater farms in Hungary but different in feeding and they are living in different ecological area with altered genetic background and lifestyle. These two fish, sturgeon and catfish, are also relatively easy to breed in our country, despite the fact that they come from a very different habitat in terms of their ancient origin, however, they retain their genetic and phenotype well.

Based on it, we hypothesized, that the right muscle structure is depending from processing of freezing technology of the meat before cooking and the results being beneficial under extreme environmental conditions.

The aim of this study was to compare the techno-functional parameters and structure differences of muscle between freshwater fish with different lifestyles. We would like to show the differences of molecular components from farmed freshwater fish in Hungary, whose original habitat is alive but popular among consumers.

Material and methods

Studied species

African catfish (*Clarias gariepinus*) and Siberian sturgeon (*Acipenser baerii*) were compared after fresh- (precooled), slow- and rapid- freezing (Figure 2).

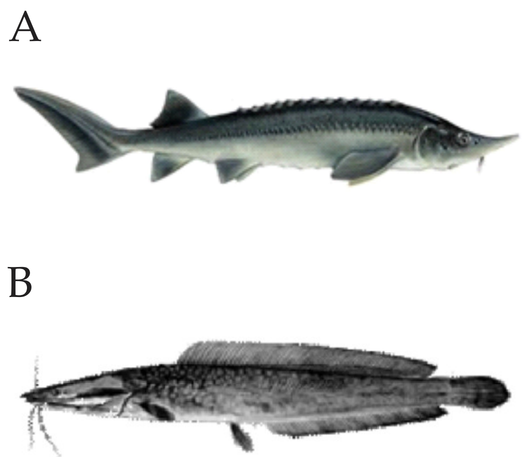

Fig.1. The Siberian sturgeon (*Acipenser Baerii*) (A) and African catfish (*Clarias gariepinus Burchell*) (B) are very popular and well breeding in Hungary.

Photo by https://www.dreamstime.com/photos-images/siberian-sturgeon.htmlandhttps://stock.adobe.com/search?k=%22 clarias+gariepinus%22

Fig.2. Meat of African catfish (1, 2 and 3) and Siberian sturgeon (4, 5 and 6). Photo by Zoltán Répás

After the processing of cleaning and filleting of precooled fish, small part of muscle was fresh-freezing (precooled) at 4 oC (Figure 2: 1, 4) or fast-freezing at -40 oC (Figure 2: 2, 5) or slow-freezing at -20 oC (Figure 2: 3, 6). After freezing the pieces of tissues were washed in phosphate buffer containing 4.0 % saccharose first.

Techno-functional parameters

Measurement of water holding capacity

The test was done by Grau and Hamm' filter press method [16], (n = 10 /fish). From the samples, the weight of tiny pea-grain tissues was determined, wrapped in filter paper, then placed between two glass sheets and pressed for 5 minutes with 1 kg weight. After that, the mass of fish meat was back and calculated from the data.

Determination of the water-binding ability

The determination of the water-binding ability was performed using a cooking probe. The size of the samples was cut into pieces of 3x5 cm (n = 10 /fish), measured its weight and placed it in a heat-treatable bag and sealed it with vacuum and foil baking. The heat treatment at 75 oC lasted 10 minutes which we brought back to room temperature. The weight was measured before packaging and after heat treatment. After heat treatment, the water was carefully soaked on the surface of the samples and then was calculated the water-binding ability [8].

Scanning electron microscopy analysis

The skeletal muscles of two types of fish were collected and the anatomy analyzed with a stereo microscopy. Here the main aim was to study high resolution ultrastructure with scanning electron microscopy (SEM). The tissues were treated in phosphate buffer (PBS, pH 6.7) containing 4.0% saccharose to keep the physiological condition of cells, and continued with 30% saccharose at 4 oC during 2 hours in PBS (pH 6.7). Small pieces of fish samples were fixed in 2.5% glutaraldehyde containing PBS (pH 6.7) supplemented with 30% saccharose during 2 hours. After post-fixation in 1% OsO_4 for 1 hour, the samples were dehydrated in aqueous solutions of increasing ethanol concentrations, critical point dried, mounted on specimen stubs, covered with 15 nm chromium by a Quorum Q150T ES sputter and observed in a JEOL JSM-

7100F/LV scanning electron microscope by using 3 kV accelerating voltage.

Analysis and documentation of the samples were carried out with a program of scanning electron microscope and Microsoft programs.

Organoleptic analysis

The sensory examination was carried out by 17 independent students from the University of Szeged Faculty of Engineering. The fish products examined were different types of frozen and cold smoked fish. A total of six samples were evaluated: the pre-chilled (EH), slow (SF) and fast frozen (FF) fish of the two tested species. The evaluation criteria are texture, taste, and smell. The evaluation was based on a textual assessment and a scoring method, which ranged from 0 to 10 points. The higher the score a sample received, the soft it tasted and smelled better. Text opinions were evaluated according to their content as positive or rather negative.

Results

Techno-functional parameters

Techno-functional property includes water holding capacity, water-binding ability and emulsification of the pH.

We measured the proximal techno-functional parameters of muscle in Siberian sturgeon and African catfish (Figure 1 and 2; Table1). The water holding capacity, was highest in precooled muscle in both fish has well as the water-binding ability. However, the boiling loss was high after fast freezing in sturgeon and catfish too. Mass of meat was highest after cooling back (Table1), it is opposite what was expected, with slow freezing method in both fish.

In the African catfish the techno-functional parameters are rather positive characters but in Siberian sturgeon the physiological taste, consistency, flavors and essence were much more pronounced in organoleptic analysis. This was preliminary experiments (not shown).

Table 1. Proximal techno-functional parameters of muscle in Siberian sturgeon and African catfish. Measured by Zoltán Répás

Parameters	Sample	Siberian sturgeon	SEM ±	African catfish	SEM
Water holding capacity, %	Precooled	88,90	4,5	82,37	3,95
	Slow-freezing	76,01	3,47	81,98	4.60
	Fast-freezing	74,63	4,89	81,65	4.58
Water-binding ability, %	Precooled	84,42	2,74	88,98	5,84
	Slow-freezing	87,62	1,86	88,33	3.95
	Fast-freezing	80,72	1,02	88,70	3,76
Boiling loss, %	Precooled	15,58	0,97	11,02	0,49
	Slow-freezing	12,37	0,83	11,67	1,49
	Fast-freezing	19,28	1,42	11,30	1,58
Mass changed, %	Precooled	98,83	-	99,55	-
	Slow-freezing	98,27	-	99,58	-
	Fast-freezing	98,42	-	99,42	-
Mass change, after smoking, %	Precooled	92,99	-	97,31	-
	Slow-freezing	94,99	-	96,67	-
	Fast-freezing	92,24	-	97,51	-
Mass after cooling back, %	Precooled	84,40	-	91,17	-
	Slow-freezing	88,00	-	90,79	-
	Fast-freezing	85,33	-	90,02	-

Structure of skeletal muscle in freshwater fish using scanning electron microscopy

Methods were developed for studying with scanning electron microscope (SEM) to investigate and compare the muscle structure by molecular scale between two different originated freshwater fish.

The structures of skeletal muscle are differing between Siberian sturgeon (Acipenser sp.) and African catfish (Clarias sp.) in Figure1. The stiffness and the color of muscle appeared to be different in both species (Figure 2) of freshwater fish.

Earlier, the scoring assessment of organoleptic analysis gave a high score in both species. In this investigation, we determined the molecular structure of muscle of Siberian sturgeon and African catfish by SEM method.

African catfish has a tightly closed muscle mass with darker red color, while the Siberian sturgeon has a light rose color (Figure 2, 1-3). Muscle of the sturgeon shows more softness (Figure 2, 4-6). The precooled and slow-freeze muscle structure was very similar comparing to the own controls (precooled) in species. But in the sturgeon samples there are several small drops of fat comparing to catfish (Figure 3 left side comparing to the catfish in precooled samples). The bars on SEM photos of Siberian sturgeon are 10 µm on the left column and 100 µm on right side, but opposite on photos of African catfish, bars are100 µm on the left column and 10 µm on right side in Figure 3. The precooled and slow-freeze patterns were not different in photos of the sturgeon as well as of catfish.

The molecular structure appeared to be altered after fast-freeze variously depending on the molecular structure (Figure 3).

We have some knowledge that the slow-freeze can change the muscle structure because ice crystals come into being and this process can influence the tissues structure but not in freshwater fish [17]. This work is first to demonstrate the difference between freshwater fish can keep the original muscle structure in altered habitats.

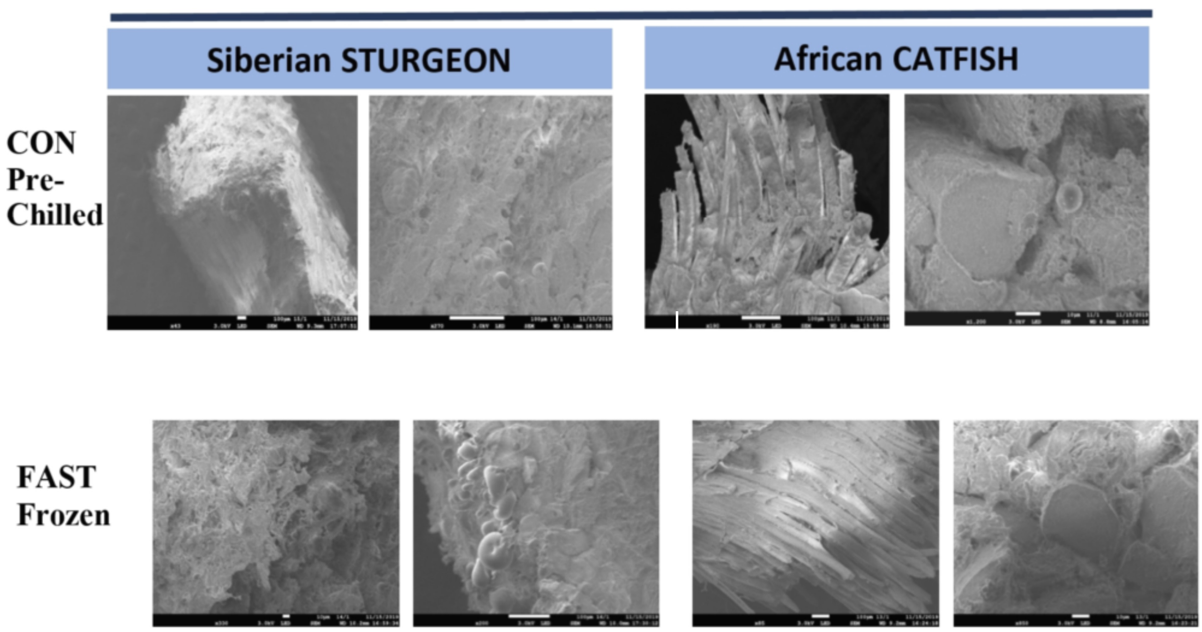

Fig.3. Analysis by scanning electron microscope of muscle tissues of Siberian sturgeon and African catfish after pre-chilled and fast-frozen. CON = control, Pre-Chilled tissues; FAST= fast-frozen tissues. Investigated by Dr. Ildikó Domonkos.

However, we had not so much knowledge about this progress in freshwater fish until now. Our results revealed that both the fast-freeze and slow-freeze can altered the molecular structure but may be the precooled tissues is maintaining only the original structure of myocytes under short intervals.

The scanning electron microscopy method is an advantageous choice for the detection of quality differences in meat because they supported the organoleptic tests, the thousands of years of experience in the processing of meat with visible tissue changes on the sections of Figure 3.

In the case of processing of fast-freezing in Siberian sturgeon, but the slow-freezing process in African catfish was found of the organoleptic characteristics of the final product; they are positively influenced by the freezing process. In the future we are able to carry out deeper analysis at molecular level with high resolution.

Organoleptic analysis

The SEM technique was an advantageous possibility for the deeper analysis of macromolecular systems in the structure of muscle cells and fibers.

The texture of fresh and fried muscle of catfish and sturgeon fillets significantly differ from that of the roasted fillets in meat because they supported the organoleptic tests, the thousands of years of experience in the processing of meat with visible tissue changes on the sections.

In the case of processing of fast-freezing in Siberian sturgeon, but the slow-freezing process in African catfish was found of the organoleptic characteristics of all parameters in the final product, in smoked fish of them; they are positively influence by the right freezing process.

After fried with oil, the techno-functional parameters of the African catfish were rather positive characters with slow freezing, but Siberian sturgeon with fast-freezing was the best in substance, taste and essence.

This study is the first which has an advantage in the industrial freshwater fish meat processing.

Discussion

The purpose was to demonstrate that the (1) muscle tissues can be studied at high resolution in freshwater fish by nanotechnology methods at molecular level, (2) the skeletal muscle structure can be different depending from originated of animal and (3) living style. (4) The organoleptic analysis of freshwater fish can be reflected the connection between these molecular differences in the muscle.

Fiber's, structural elements as well as the lipid content of muscle are different between these species demonstrated Pelvic et al. (2019) [18]. The knowledge of the molecular structure and composition of meat is important for the precise setting of functional parameters. After frying the slices in oil, both the African catfish slow-freeze, and Siberian sturgeon fast-freeze are best in substance, consistency, taste and essence. The sturgeon was better 5% in taste comparing to catfish only, but substance and essence were closed in scoring assessment of organoleptic analysis (n= 17 students, unpublished data). This analysis with SEM needs to be repeated more times.

The closed fibrous structure of the African catfish needs the destructive effect of large ice crystals due to slow freezing. This and the lipid content led to the improvement of the tissue structure associated with the beneficial pleasure value.

These two fish are also relatively easy to breed in our country, despite the fact that they come from a very different habitat in terms of their ancient origin, however, they retain their genetic and phenotype well.

The structural changes in Sturgeon due to rapid freezing and higher lipid content proved to be sufficient to increase the enjoyment value of the product. Consumption a fish, as a good meat, will be raised most likely, due to the com-

plete values of fish meat addition to daily diets in different diseases [19, 20, 21].
In the future, we are able to use to developed new methods to compare tissue structure of freshwater fish by SEM technique. The knowledge of the molecular structure and composition of meat is important for the precise setting of functional parameters.

The PUFA ratio of polyunsaturated fatty acids is very high in fish oil, including n-3 fatty acids, which have a primary role in preventing the development of cardiovascular diseases. In the Biological Research Center of Szeged (Hungary) Professor Tibor Farkas MD biochemist was the first to determine the content of carp (Cyprinus carpio L.) n-3 fatty acids in the 1970s. He drew first attention to the physiological significance of this and then began to develop it in medicine. After that, research in this direction was also started in saltwater fish. The unsaturated fatty acids reduce LDL levels and they increase the protective HDL cholesterol levels in our body. Also beneficial for the heart and vascular system [20, 21].

Organoleptic values were excellent both in species but altered freezing parameters.

Consuming freshwater fish helps to maintain some diets with different meals in healthy and non-healthy people to regenerate our body and prevent diseases.

Conflict of interest
The authors declare no conflict of interest.

Acknowledgements
This work was supported by the projects NKFIH-112688, OTKA K112688 and we thanks to Prof. Dr. Monica C. Dragomirescu and Prof. Dr. Győző Garab for supporting the project.

Resumo

Oni rekomendis, ke homoj manĝu fiŝojn riĉaj je nesaturitaj grasoj almenaŭ dufoje semajne por redukti la riskon de kormalsano. Fiŝokonsumo estas signifa, plejparte de fiŝoj vivantaj en oceana salakvo. Tamen, en landoj sen maro kiel Hungario, la riĉeco de dolĉakvaj fiŝoj evoluigis larĝan gamon de kuirteknikoj por fiŝoj kun malsama nutrado. Ni suspektas, ke muskolstrukturaj diferencoj ankoraŭ ne estis esploritaj. La diferenco en grasacida konsisto de afrika anariko kaj siberia sturgo estas konata, sed neniuj morfologiaj studoj estis faritaj pri ilia muskola strukturo. La celo de ĉi tiu studo estis kompari la strukturdiferencojn inter dolĉakvaj fiŝoj kun malsamaj vivstiloj. La organizo de muskola strukturo estis monitorita en viando per citokemio kombinita kun skanaj elektronaj mikroskopaj studoj sur histoj de du malsamaj specioj, kaj la tekno-funkciaj parametroj mezuritaj. La filetaj muskoloj de afrika anariko (Clarias gariepinus) kaj siberia sturgo (Acipenser baerii) estis komparitaj post freŝa kaj rapida frostigo. La rilata kompleksa strukturo de muskolo en ambaŭ specioj ŝajnis malsama. Unu estas forte fermita muskola maso, dum la alia estas mola strukturo, kiu montras malsaman gradon de moleco de la viando post bakado. En ambaŭ specioj, la ĝusta muskola strukturo estas utila sub ekstremaj mediaj kondiĉoj. La malsama skeleta strukturo en fiŝoj bezonas ŝanĝitan prilaboradon, kiun ni deziras daŭrigi kun pliaj provoj kaj prepari bongustajn manĝaĵojn por konsumantoj kaj uzon en dietetiko.

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