ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(3): 531-537
doi: 10.25083/rbl/24.3/531.537

Received for publication, October, 10, 2018
Accepted, February, 14, 2019

Original paper

Correlation between infrared spectra of fish scales collagen, phosphorus and fish morphology

MARIUS-IONEL NĂDEJDE[1*], ELENA-PETRONELA BRAN[1], IULIANA CARAMAN[1], KAMILOU OURO-SAMA[2], IULIANA-MIHAELA LAZĂR[1]

[1]Doctoral School of Vasile Alecsandri University of Bacau, Calea Marasesti 157, 600115, Bacau, Romania
[2]Laboratoire de Gestion, Traitement et Valorisation des Déchets, Département de Géosciences et Environnement, Université de Lomé, BP : 1515, Lomé, Togo

Abstract Collagen, as the chemical compound is wildly used today in food, cosmetics and medicine. Traditionally in Romania the collagen from bovines is used, but all the people known that the fish contains significant quantities of collagen. The scales resulted from the fishing industry are considered as waste. They can be used as the collagen source. The aim of this paper is to study the content of collagen in six species of fish from the Siret River Basin. The samples were analyzed by Attenuated Total Reflection (FTIR-ATR). The measurements were recorded in the wavelength range of 550-4000 cm^{-1}. For the better results on the collagen content, the both dorsal and ventral sides of scales were analyzed. Also, the correlation between collagen content of fish scales and their external morphology (length, weight) were done. Our experiments have led to the conclusion that from the analysis of scales (dorsal and ventral) through ATR spectra for two species of fish common for Bistrita river water – common bleak (*Alburnus alburnus*) and chub (*Squalius cephalus*), we can identify the particularities of collagen content for each species by FTIR instrument.

Keywords Collagen, fish morphology, fish scale, FTIR

To cite this article: NĂDEJDE MI, BRAN EP, CARAMAN I, OURO-SAMA K, LAZĂR IM. Correlation between infrared spectra of fish scales collagen, phosphorus and fish morphology. *Rom Biotechnol Lett*. 2019; 24(3): 531-537. DOI: 10.25083/rbl/24.3/531.537

*Corresponding author: MARIUS-IONEL NADEJDE, Doctoral School of Vasile Alecsandri University of Bacau, Calea Marasesti 157, 600115, Bacau, Romania
E-mail: nadejdemariusionel@yahoo.ro

Introduction

Fish fauna is used to assess water quality less than macroinvertebrates, because fish have much higher mobility than macroinvertebrates, especially when it comes to feeding and breeding, especially because fish fauna is much more difficult to collect, especially in deep rivers. However, there are many authors who argue that the study of fish fauna, in addition to being a rapid analytical method that provides preliminary results, show a variety of other benefits, such as: the fish are good indicators for the long-term effects (relative longevity, 3-10 years), they are easily identified in the field, present in all aquatic habitats (even in very heavily polluted waters), fish fauna form stable populations with low seasonal fluctuations, environmental requirements, history and distribution of most fish species are well known , are located at the top of the food chain, in aquatic ecosystems, and are food for humans, they are used as biomarkers and are relatively easy to collect and identify.

Scales of the fish are considered waste in the fishing industry. Considering the problem of waste, the scales can be used to obtain collagen. In Romania, the collagen is traditionally extracted frombovine. Because the fish industry is well developed, instead of incineration, the residues of this industry can be used to extract collagen.

Collagen as a chemical compound that has various uses in nowadays - nutrition, beauty etc. Also, this was used to obtain an artificial matrix mixed with elastin or a porous composite of tricalcium phosphate and collagen that can offer an interesting alternative with bone composition (OANA CRACIUNESCU & al. [3], LUCIA MOLDOVAN & al. [11]). There are reported 29 types of collagen genetically distinguishable in animal samples (L.T.M. THUY & al. [6]). From the literature, it is known that different species of fish have different collagen content, and it is proved that marine fish contains a higher concentration of collagen than freshwater fish (W.T. LIU & al. [8]).

Studies on collagen content and characteristics of this compound are based on collagen extraction using chemical methods (S. KRISHNAN & al. [14], Z. MOVASAGHI & al. [16]). These methods require large amounts of materials submitted to the study. The vast majority of studies on fish using fish bought on the market, but if we want to use the fish as an indicator of quality, it is necessary to collect them from the area under investigation.

For a detailed study of the influence of environmental factors on fish quality, respectively collagen, the samples were collected from the area under study. For this, the research team obtained an approval to achieve fishing in scientific aim, in the Siret River basin. The novelty of this work is the fact that we extracted 2-3 scales from each, from the lateral line, after which the fish was reinstated in the water. In order to investigate proteins in scales, it was used the method of Attenuated total reflection, this method being used with success in the pharmaceutical and food industry (M. HERNÁNDEZ-MARTÍNEZ & al. [9], P. PINKERNEIL & al. [13], T. NAGAI & N. SUZUKI [15]).

Materials and Methods

Description of the area under study

The area where the samples have been taken is a section of the Siret River, downstream Galbeni Accumulation, which has the following GPS coordinates: Lat. N: 46° 26' 7.44" and Long. E: 26° 56' 9.32". The ecosystem is characterized by the following characteristics: average width is 4 m with a maximum depth of 105 m and a minimum of 15 m, and a water flow rate of 0.6 m/s. The watercourse is linear, the substrate being composed of sand, silt and clay. Aquatic vegetation is composed of emerged and submersed macrophytes and the riparian consisting of reedbeds and meadows. The physico-chemical characteristics of the water are: the temperature of 16°C, pH 7.90 and a conductivity of 828 $\mu S/cm^2$. This area was chosen for investigation because it is downstream from the town of Bacau, thus being exposed to contaminants that arrive here with the waste waters discharge from the city. The area is interesting to be studied also because it is the meeting point of the Bistrita river with the Siret river.

Fish species

The six species of fish were studied: *Squalius cephalus* (chub), *Carassius gibelio* (prussian carp), *Gobio obtusirostris* (common pig), *Romanogobio kesslerii* (pig sandy), *Alburnus alburnus* (common bleak) and *Scardinius erythrophthalmus* (common rudd). These species were recorded since 1964 in the studied area by Banarescu P. (P. BĂNĂRESCU [18]) and later, in 2008, by Ureche D. (D. URECHE [19]).

Sampling

The sampling was made after an analysis of weather conditions in the days before departure, so in that interval was not raining, the weather being thermally stable. These analyses were necessary because rainy weather before and during collections influence the results concerning the biological and physico-chemical condition of the area that will be investigated.

The biological material was sampled in October of 2012 was sampled the biological material, using a harmless electrofishing appliance with double insulation, FEG 5000 (D. URECHE [19]). For each sample, after collection, were carried the necessary biometric measurements were carried (length, thickness, weight, etc.), after which were sampled two fish scales from the lateral line were sampled of each species collected. All sampled fishes were then reinstated in water to be able to continue their life cycle.

Two species *Squalius cephalus* (chub) and *Alburnus alburnus* (common bleak) were chosen for further studies. The reason for choosing these two species was that they are common in studied areal and they are less demanding to environmental conditions as well as the number of fished samples was quite enough for analyzes. The water samples were collected to make a correlation between the quality of fish and water. On the spot, were measured the following parameters were measured: pH, water and the environmental temperature and the conductivity. All these physico-chemical characteristics were made using a multiparameter portable device (MultiLab 350i).

Preparation of material for analysis

After being brought to the laboratory the fish scales have been subjected to a heat treatment in an oven at 45°C for 2 hours. Drying of the scales has been required to reduce the amount of water present in the samples. The dry scales were examined at the microscope to determine the dorsal and ventral side of the analysed samples. The samples were labelled as follows: the first two letters indicate the species name (*Squalius cephalus,* CL), followed by two digits for the number of exemplary and a letter for the dorsal, D, and the ventral side, V (CL01D).

ATR Analysis (Attenuated Total Reflection)

The ATR spectroscopy is a very useful, reliable and very well-recognized fingerprinting method. With this method can be analyzed and quantified a lot of substances. One of the advantages of Infrared spectroscopy (IR spectroscopy) is represented by its ability as an analytical technique which allows being obtained spectra from a very wide range of materials. In the literature, have been used IR spectrometers in order have been used to analyze solids, liquids and gases by means of transmitting the infrared radiation directly through the sample (Y.S. LIN & al. [7]).

The studies were provided in the Spectrometry Laboratory of Vasile Alecsandri University of Bacau, Engineering Department. The ATR-FTIR measurements (Fourier Transform Infrared Spectroscopy) have been performed using the spectrometer Bruker Tensor 27 equipped with an attenuated total reflectance accessory (ATR) with a zinc selenide crystal. The big size scales were chosen for analyses in order to cover entire ATR crystal. The sample was pressed with a press accessory for better contact between the scales and the crystal. Each scale was examined both ventral and dorsal side. The spectra were recorded at the room temperature. The scanning range was 550 cm^{-1} -4000 cm^{-1} at the resolution of 16 cm^{-1}. The background air spectrum was scanned before each scan. Spectra were processed with the OriginLab version 7.5 software.

Results and Discussions

The ATR spectra for the six species of fish have been studied to identify the characteristic bands of collagen and phosphorus. The ATR spectra were recorded for each scale for both ventral and dorsal side. In Figure 1 are presented the ATR spectra for the six species of fish that have been studied. From these spectra we selected according to (M.S. HEU & al. [10], T. NAGAI & N. SUZUKI [15]) the characteristic bands of: collagen (Amide I and Amide II) and phosphorus that were frequently identified in fish samples by other authors (C. CEYLAN & al. [1], C. CEYLAN & al. [2], G. M. CUGNO & al. [4], M. HERNÁNDEZ-MARTÍNEZ & al. [9], F. PATI & al. [12], P. PINKERNEIL & al. [13], T. NAGAI & N. SUZUKI [15], Z. FANG & al. [17]).

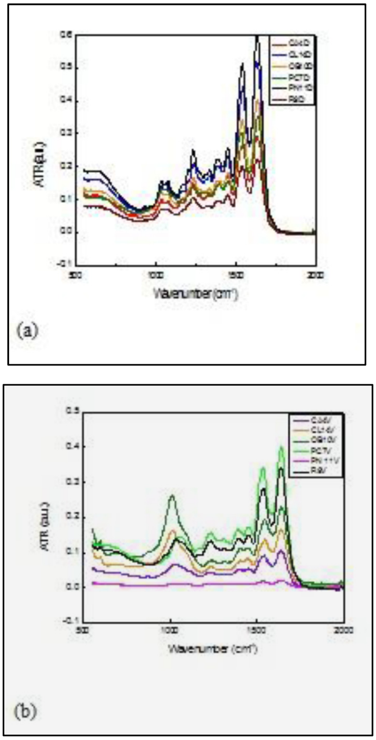

Figure 1. The ATR Spectra characteristic for the fish species studied: **a**- dorsal side, **b**- ventral side.

After analysing the ATR spectra we noted a relationship between the characteristic bands of phosphorus oxide and collagen. While the intensity of the collagen bands decreases, the intensity of the phosphorus oxide band increases (Fig. 1). Furthermore, for the species *Carassius gibelio* (prussian carp), *Gobio obtusirostris* (common pig), *Romanogobio kesslerii* (pig sandy), *Scardinius erythrophthalmus* (common rudd) we can notice that the ratio between phosphorus oxide bands compared to the collagen bands, remains constant. We did some additional investigation of the phosphorus oxide and collagen bands for common bleak (*Alburnus alburnus*) and chub (*Squaliuscephalus*).

In Figure 2 and Figure 3 there are presented the ATR spectra for common bleak (OB11 and OB17) and chub (CL09 and CL17) for both ventral and dorsal side, in the spectral range of 550 cm^{-1} – 2000 cm^{-1}.

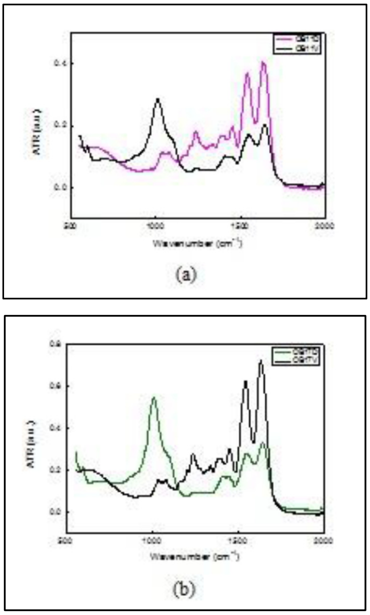

Figure 2. The ATR spectrum for Common bleak scales: **a**- OB11 dorsal and ventral; **b-** OB17 dorsal and ventral.

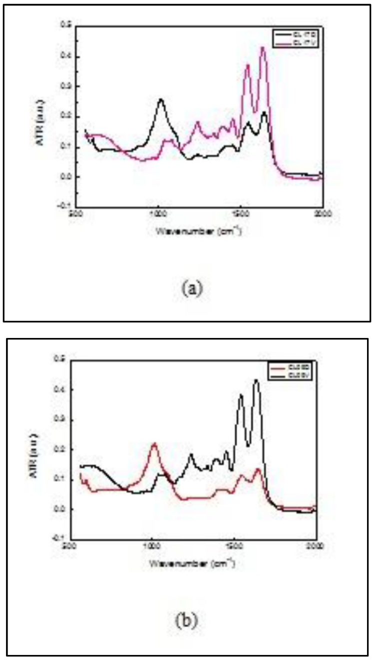

Figure 3. The ATR spectrum for Chub: **a**- CL17 dorsal and ventral; **b**- CL09 dorsal and ventral.

In this spectral range, it can be noticed two bands for chub and for common bleak which have the maximum at 1000 cm^{-1} and 600 cm^{-1}. These bands change their contour depending on the age of the analyzed fish (Figure 2 and Figure 3). The size of the analyzed sample was associated with the relative age of the studied specimen. According to (W.T. LIU & al. [8], M.S. HEU & al. [10], P. PINKERNEIL & al. [13], Z. MOVASAGHI & al. [16]) the doublet from 1000 cm^{-1} and 600 cm^{-1} corresponds to the vibration of PO_4^{3-} molecule.

It can be observed from Figure 2 and Figure 3 that for large specimens (corresponding to older samples) the band at 1000 cm^{-1} moves to higher wavenumbers and it becomes a broadband with two picks at 1036 cm^{-1} and 1080 cm^{-1} and the band at 600 cm^{-1} disappears. It can be concluded that with the age of fish the modification in the composition of the scale takes place, namely a redistribution of the phosphorus oxide molecules.

According to W.T. Liu, the band with maximum at 1542 cm^{-1} is attributed to Amide II and the band with maximum at 1629 cm^{-1} is attributed to Amide I. The researchers E. H. Green and R. W. Tower (Y.S. LIN & al. [7]) after analyzing the structure and composition of scales from 40 species of American fish from 25 families, have determined that the scale contains about 76% collagen and 24% of Ichthylepidin.

It was calculated the ratio between Amide I and Amide II bands to see how the intensity of these bands varies depending on samples age. Table 1 shows the average values of the ratio band with a maximum at 1542 cm^{-1} and 1629 cm^{-1}.

Table 1. The mean value of the band intensity ratio Amide I/Amide II

Species / Scale	Dorsal (un.rel.)	Ventral (un.rel.)
Chub	0,862	0,859
Common bleak	0,869	0,857

The value of the ratio Amide I/Amide II for the dorsal side of the scale is higher in comparison with the ventral side of the scale for both studied species (chub and common bleak).

Figure 4 shows that the ratio Amide I/Amide II for both dorsal and ventral side of scale increases with increasing the sample size in case of chub and common bleak.

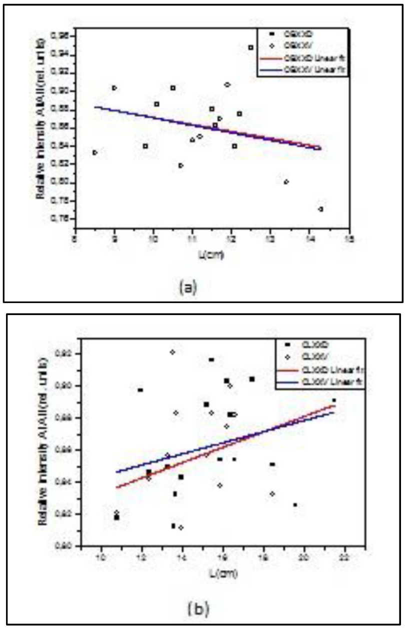

Figure 4. The ratio Amide I/Amide II depending on the size of the sample: (**a**) – Common bleak, (**b**) – Chub.

A special distribution of the band Amide I/Amide II is found in common bleak. The ratio of these bands for the dorsal and ventral side of the scale decreases. We suppose that this is a specific characteristic of common bleak.

In order to identify the characteristic features of each given species of fish, *chub* and *common bleak* in our case, we identified the bands corresponding to P-O molecule (Table 2).

In Table 2, can be observed that for the *chub* the band at 1000 cm^{-1} appears only on the ventral side of the scale. The band at 600 cm^{-1} is accompanied by the band at 1035 cm^{-1} and the bands with a maximum at 1035 cm^{-1} and 1080 cm^{-1} can be found just on the dorsal side of *chub* scales and the bands at 600 cm^{-1} and 1000 cm^{-1} disappear. In case of *common bleak,* the presence of the band at 1000 cm^{-1} is not accompanied by the other characteristic bands of P-O bond. The bands with maxima at 1035 cm^{-1} and 1080 cm^{-1} appear in tandem especially on the ventral side of the scale and can be assigned as a characteristic of *common bleak*. It was concluded that more accurate data can be extracted from the ventral side of the scales according to the analyses of ATR spectra.

The content of phosphorus (P-O bond) in scales was analyzed depending on the size of the sample. The value of the intensity of the bands at 1035 cm^{-1} and 1080 cm^{-1} has been reported to the intensity of the band which corresponds to Amide I (W.T. LIU & al. [8]).

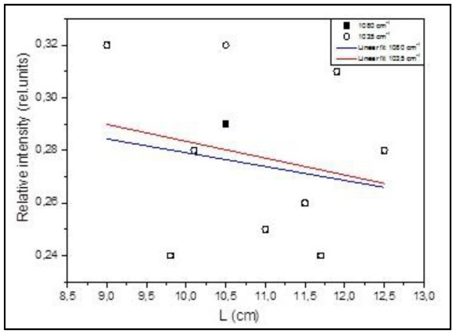

Figure 5. The relative intensity of the bands corresponding to P-O bond compared to the intensity of the band corresponding to Amide I on the ventral side of the scales of common bleak.

As it was mentioned above, the appearance of these two bands (1035 cm^{-1} and 1080 cm^{-1}) leads to the disappearance of the band at 1000 cm^{-1} for the *common bleak*. It can be observed that the intensity of the bands presents a decrease in the size of fish (Figure 6).

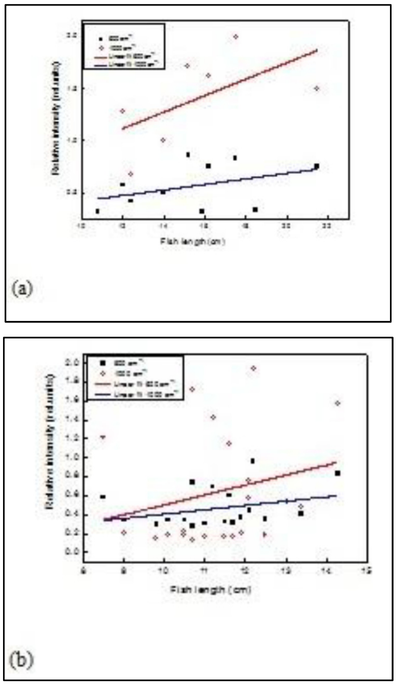

Figure 6. The relative intensity of the bands corresponding to P-O bond compared to the intensity of the band corresponding to Amide I for the ventral side of the chub scales (**a**) and common bleak scales (**b**).

Table 2. The identification of bands corresponding to P-O molecule for chub and common bleak

Species	Intensity at 600 cm^{-1}	Intensity at 1000 cm^{-1}	Intensity at 1035 cm^{-1}	Intensity at 1080 cm^{-1}		Species	Intensity at 600 cm^{-1}	Intensity at 1000 cm^{-1}	Intensity at 1035 cm^{-1}	Intensity at 1080 cm^{-1}
CL01D	-	-	X	X		OB01D	X	-	-	-
CL01V	X	X				OB01V	X	X	-	-
CL03D	-	-	X	X		OB02D	-	-	X	X
CL03V	X	-	-	-		OB02V	X	-	X	-
CL05D	-	-	X	X		OB03D	X	X	-	-
CL05V	X	X	-	-		OB03V	-	-	X	X
CL04D	-	-	X	X		OB05D	-	-	X	X
CL04V	X	X	-	-		OB05V	X	X	-	-
CL08D	X	-	-	-		OB04D	X	X	-	-
CL08V	X	-	X	-		OB04V	X	X	-	-
CL09D	X	X	-	-		OB08D	-	-	X	-
CL09V	-	-	X	X		OB08V	X	-	X	X
CL11D	-	-	X	X		OB06D	X	-	X	X
CL11V	X	X	-	-		OB06V	-	-	X	X
CL14D	-	-	X	X		OB10D	X	-	X	X
CL14V	X	X	-	-		OB10V	X	X	-	-
CL12D	X	-	X	-		OB09D	X	X	-	-
CL12V	-	-	X	X		OB09V	-	-	X	X
CL13D	X	-	X	-		OB11D	-	-	X	X
CL13V	-	-	-	X		OB11V	X	X	-	-
CL17D	X	X	-	-		OB13D	X	X	-	-
CL17V	-	-	X	X		OB13V	-	-	X	X
CL19D	X	X	-	-		OB14D	-	-	X	X
CL19V	X	X	-	-		OB14V	X	X	-	-
CL15D	-	-	X	X		OB12D	X	X	-	-
CL15V	X	X	-	-		OB12V	-	-	X	X
CL20D	X	-	X	-		OB15D	X	X	-	-
CL20V	X	-	X	-		OB15V	-	-	X	X
						OB19D	X	X	-	-
						OB19V	-	-	X	X
						OB18D	X	X	-	-
						OB18V	X	-	X	X
						OB20D	-	-	X	X
						OB20V	X	X	-	-

In Figure 6 are presented the relative intensities of the bands with peaks at 600 cm^{-1} and 1000 cm^{-1} reported to the intensity of Amide I for *chub* and *common bleak* (ventral and dorsal side of the scales).

The intensity of the bands corresponding to the P-O bond in the ventral side of scales increases when the samples size increase too (Figure 6). The band with a maximum at 600 cm^{-1} for the twospecies of fish, has a lower rising slope than the band with a maximum at 1000 cm^{-1}. The rising slope for *chub* is 0.120 (600 cm^{-1}) and 0.344 (1000 cm^{-1}) while for the *common bleak* is 0.099 (600 cm^{-1}) and 0.248 (1000 cm^{-1}).

Conclusions

From the analysis of scales (dorsal and ventral) through ATR spectra for two species of fish common for Bistrita river water – common bleak (*Alburnus alburnus*) and chub (*Squalius cephalus*), we identified the particularities of collagen content for each species by FTIR instrument.

Collagen concentration ratio was determined for each species. It was found that on the dorsal side of scales are more pronounced the bands of the Amide I and the bands of Amide II on the ventral side of the scales.

For both studied fish species was noted that more accurate and relevant data can be obtained from the analyses of the ventral side of the scales.

To specify the characteristic features of a given species of fish, in our case chub and *common bleak*, we identified the bands corresponding to P-O molecule.

The intensity of the bands corresponding to the P-O bond, for both species studied decreases with the size of fish.

The ratio Amide II/Amide I for both species of fish is increasing depending on the age of the fish, the band with a maximum at 1000 cm-1 having a sharp slope.

References

1. C. CEYLAN, T. TANRIKUL, H. ÖZGENER, Biophysical evaluation of physiological effects of gilthead sea bream *(Sparus aurata)* farming using FTIR spectroscopy, *Food Chemistry*, 145: 1055 (2014).
2. C. CEYLAN, F. SEVERCAN, A. OZKUL, M. SEVERCAN, F. BOZOGLU, N.TAHERI, Biophysical and microbiological study of high hydrostatic pressure inactivation of Bovine Viral Diarrhea virus type 1 on serum, *Veterinary Microbiology*, 154: 266 (2012).
3. O. CRACIUNESCU, L. MOLDOVAN, W. BUZGARIU, O. ZARNESCU, D. BOJIN, G.L. RADU, Preparation of an Elastin–Collagen Artificial Matrix. Evaluation of Its Structure and Biocompatibility, *Romanian Biotechnological Letters*, Vol. 9, No. 4, pp 1785-1792 (2004).
4. G.M. CUGNO, G. LA ROSA, D. ZHANG, L.-N. NIU, F.R. TAY, H. MAJD, D. AROLA, On the mechanical behavior of scales from *Cyprinus carpio*, *Journal of the mechanical behaviour of biomedical materials* 7: 17 (2012).
5. E.H. GREEN, R.W. TOWER, The organic constituents of the scales of fish, *Bulletin of United States Fish Commission*, 97 (1901).
6. L.T.M. THUY, E. OKAZAKI, K. OSAKO, Isolation and characterization of acid-soluble collagen from the scales of marine fishes from Japan and Vietnam, *Food Chemistry*, 149: 264 (2014).
7. Y.S. LIN, C.T. WEI, E.A. OLEVSKY, A. MARC MEYERS, Mechanical properties and the laminate structure of *Arapaima gigas*scales, *Journal of the mechanical behaviour of biomedical materials,* 4: 1145 (2011).
8. W.T. LIU, Y. ZHANG, G.I. LI, Y.Q. MIAO, X.H. WU, Structure and composition of teleost scales from snakehed *Channaargus (Cantor) (Perciformes: Channidae)*, *Journal of Fish Biology*, 72:1055 (2008).
9. M. HERNÁNDEZ-MARTÍNEZ, T. GALLARDO-VELÁZQUEZ, G. OSORIO-REVILLA, N. ALMARAZ-ABARCA, A. PONCE-MENDOZA, M.S. VÁSQUEZ-MURRIETA, Prediction of total fat, fatty acid composition and nutritional parameters in fish fillets using MID-FTIR spectroscopy and chemometrics, *LWT – Food Science and Technology*, 52: 12 (2013).
10. M.S. HEU, J.H. LEE, H.J. KIM, S.J. JEE, J.S. LEE, Y.J. JEON, F. SHAHIDI, J.S. KIM, Characterization of Acid- and Pepsin-soluble Collagens from Flatfish Skin, *Food Sci. Biotechnol.*, 19(1): 27 (2010).
11. L. MOLDOVAN, E.I. OPRITA, O. CRACIUNESCU, C. TARDEI, D. BOJIN, O. ZARNESCU, Histo-chemical and Scanning Electron Microscopic Characterization of Tricalcium Phosphate – Collagen Conjugated Sponges, *Romanian Biotechnological Letters,* Vol. 9, No. 5, pp. 1887-1893, (2004).
12. F. PATI, P. DATTA, B. ADHIKARI, S. DHARA, K. GHOSH, P.K.D. MOHAPATRA, Collagen scaf-folds derived from fresh water fish origin and their biocompatibility, *J Biomed Mater Res Part A* 100A:1068 (2012).
13. P. PINKERNEIL, J. GÜLDENHAUPT, K. GERWERT, C. KÖTTING, Surface-Attached Polyhistidine-Tag Proteins Characterized by FTIR Difference Spectroscopy, *ChemPhysChem*, 13: 2649 (2012).
14. S. KRISHNAN, S. SEKAR, M.F. KATHEEM, S. KRISHNAKUMAR, T.P. SASTRY, Fish Scale Collagen – A Novel Material for Corneal Tissue Engineering, *Artif Organs*, 36(9):829 (2012).
15. T. NAGAI, N. SUZUKI, Isolation of collagen from fish waste material – skin, bone and fins, *Food Chemistry*, no.68: 277 (2000).
16. Z. MOVASAGHI, S. REHMAN, I. UR REHMAN, Fourier Transform Infrared (FTIR) Spectroscopy of Biological Tissues, *Applied Spectroscopy Reviews*, 43: 134 (2008).
17. Z. FANG, Y. WANG, Q. FENGA, A. KIENZLE, W.E.G. MÜLLER, Hierarchical structure and cyto-compatibility of fish scales from *Carassius auratus*, *Materials Science and Engineering* C 43: 145 (2014).
18. P. BĂNĂRESCU, Fauna R.P.R., Pisces-Osteichthyes, XIII, *Ed. Acad., Bucureşti*, 959 p., (1964).
19. D. URECHE, Studii ecologice asupra ihtiofaunei în Bazinul mijlociu şi inferior al Râului Siret, *Ed. PIM, Iaşi*, (2008).