Article

Inorganic Powders Prepared from Fish Scales

Tatiana Safronova[1,2,*], Viktor Vorobyov[3], Natalia Kildeeva[4], Tatiana Shatalova[1,2], Otabek Toshev[2], Yaroslav Filippov[2,5], Artem Dmitrienko[1], Olga Gavlina[1], Olga Chernega[6], Elena Nizhnikova [3], Marat Akhmedov[4], and Konstantin Lysenko[1]

[1] Department of Chemistry, Lomonosov Moscow State University, Building, 3, Leninskie Gory, 1, 119991 Moscow, Russia; shatalovatb@my.msu.ru, dmitrienkoao@my.msu.ru, gavlinaot@my.msu.ru, klysenko@gmail.com
[2] Department of Materials Science, Lomonosov Moscow State University, Building, 73, Leninskie Gory, 1, 119991 Moscow, Russia; toshevou@my.msu.ru
[3] Department of Chemistry, Kaliningrad State Technical University, Sovietsky prospect, 1, 236022 Kaliningrad, Russia; viktor.vorobev@klgtu.ru, elena.nizhnikova@klgtu.ru
[4] Kosygin Russian State University, Malaya Kaluzhskaya 1, 119071, Moscow, Russia; kildeeva@mail.ru, akhmedov.mm@yandex.ru
[5] Research Institute of Mechanics, Lomonosov Moscow State University, Michurinsky pr., 1, 119192 Moscow, Russia; filippovyy@my.msu.ru
[6] Department of Food Products Technology, Kaliningrad State Technical University, Sovietsky prospect, 1, 236022 Kaliningrad, Russia; olga.chernega.@klgtu.ru
* Correspondence: safronovatv@my.msu.ru; Tel.: +7(916)3470641

Abstract: Mixture of *abramis brama* (freshwater bream), *carassius carassius* (crucian carp), and *sander lucioperca* (pike perch) scales was used for the preparation of fish scale powder containing about 26.5 wt. % of removed when heating components preferably of organic nature, and 63.5 wt. % of mineral components. Fish scale powder enriched with inorganic components was prepared from washed, dried, and ground fish scale mixture using vibration sieving. Inorganic powders consisting of hydroxyapatite and magnesium whitlockite were obtained via heat treatment of this fish scale powder at 800-1000 °C. Particles of these inorganic powders consisted of sintered grains with dimensions less than 100 nm after heat treatment at 800 °C, less than 200 nm after heat treatment at 900 °C, and 100-500 nm after heat treatment at 1000 °C. Fish scale powder enriched with inorganic components as well as heat-treated inorganic powders consisting of hydroxyapatite and magnesium whitlockite can be recommended for the production of different materials such as ceramics or composites.

Keywords: fish scale powder; high speed grinding; heat treatment; hydroxyapatite; magnesium whitlockite; nanosized grain

1. Introduction

Fish scales as wastes of fishing and food industry are a serious environmental and economic problem [1, 2]. Some quantity of produced fish scales is used for the production of collagen [3], hydroxyapatite [4, 5], guanine [6], animal feed [2, 7], fertilizers [8], food [9], cosmetics [10], adsorbents [11], biomaterials [12] etc.

The main constituents of fish scales are proteins (mainly a collagen) and minerals [13, 14]. The main component of the inorganic mineral constituent of fish scales is hydroxyapatite [15]. Most of the known methods of separating constituents of organic and inorganic nature from each other consisted in transferring of the fish scale components to a soluble state; using heating; treating with enzymes, acids, alkalis, or organic solvents [16, 17, 18, 19]. All these methods are rather long processes (up to two weeks) accompanied by a significant consumption of washing liquids and high energy.

Taking into account the difference between the collagen (1.33 g/cm^3) and hydroxyapatite (3.16 g/cm^3) densities an economically efficient method was developed for obtaining powder enriched with inorganic component and fluff enriched with collagen from fish scales [20, 21, 22]. The main feature of the preparation of this fish scale powder consisted in

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 July 2022 doi:10.20944/preprints202207.0013.v1

using high-speed mechanical grinding of clean dry fish scales mixture and separation of ground cotton-like product via vibration sieving.

The aim of this work consisted in the investigation of properties of inorganic powders prepared using heat treatment at temperatures 400-1000 °C from fish scale powder enriched with an inorganic component.

2. Materials and Methods

2.1. Preparation of fish scale powder enriched with the inorganic component

Preparation of fish scale powder enriched with the inorganic component was performed according to the method of complex processing of fish scales described previously [20, 21, 22]. Fish scales of *abramis brama* (freshwater bream), *carassius carassius* (crucian carp), and *sander lucioperca* (pike perch) (Figure 1) were obtained using an electric fish cleaning machine (RF-0.1, Uralspetsmash LLC, Miass, Russia).

(a) (b) (c)

Figure 1. Camera photos of fish scales of *abramis brama* (a), *carassius carassius* (b) and *sander lucioperca* (c).

A mixture containing 20 wt. % of *abramis brama* fish scales, 20 wt. % of *carassius carassius* fish scales, and 60 wt. % of *sander lucioperca* fish scales were placed in a mesh bag with the mesh size 3x3 mm and loaded into a perforated centrifuge of automatic **wash**ing machine LG model F2WN2S6S3E (LG, Wroclaw, Poland) for cleaning off the organic impurities by stirring in water at a temperature of 20°C for 20 minutes. Then the liquid was drained, and the fish scales were **wash**ed with water and wrung out at 800 rpm.

400 g of the fish scales after treatment in a washing centrifuge, 20 g of sodium chloride NaCl, 4 g of sodium hydro carbonate $NaHCO_3$, and 80 g of crushed ice were loaded into a two-speed mixer Moulinex Delico FP203 (500 W, SEB Group, London, UK) and processed for 10 minutes at 1000 rpm. Then the fish scales were **wash**ed with water to remove salts and organic impurities.

The washed fish scales and water at the weight ratio of 1:4 were placed into the container and kept there at room temperature for 30 minutes with the addition of 5% of sodium chloride NaCl and 1% sodium hydro carbonate $NaHCO_3$ to the mass of fish scales. After separation of a sodium salts water solution containing impurities of organic nature using a sieve (mesh dimension 2x2 mm) the fish scales were **wash**ed with water until the smell disappeared.

The cleaned as it was described above fish scales were dried with a hot air fan at the temperature ≤ 50 °C, up to a residual humidity of less than 10% using an electric dryer Spectr-Pribor ESOF-2-0,6/220 Veterok-2 (6 pallets) (Factory of household electrical products Spectr-Pribor LLC, Kursk, Russia).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 July 2022 doi:10.20944/preprints202207.0013.v1

Dried fish scales in portions of 50 g were placed in a container with a capacity of 0.8 liter of high-speed multifunctional grinder (Zhejiang Winki Plastic Co. Ltd., Wuyi, Zhejiang, China) and crushed at 36000 for 5 minutes.

Ground fish scale cotton-like product was separated for several fractions using an electric vibrating flour sifter model PS-300B (Yonkang WD Industry and Trade Ltd., Yongkang, Zhejiang, China). The fraction of ground fish scale powder enriched with the inorganic component was separated using a 170-mesh sieve when passing through the grid cells with the size of 0.088 mm.

The scheme of preparation of fish scale powder enriched with an inorganic component is shown in Figure 2.

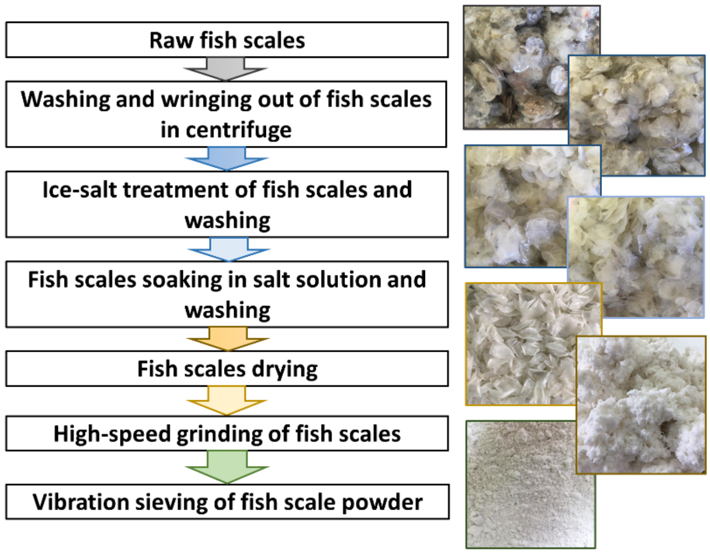


Figure 2. Scheme of preparation of fish scale powder enriched with an inorganic component.

2.2. Thermal evolution of fish scales powder enriched with the inorganic component

Heat treatment of the powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving were conducted at 400 °C, 700 °C, 800 °C, 900 °C, 1000 °C for 2 hours at specified temperatures to investigate the thermal evolution of its microstructure and phase composition, the heating rate was 5 °C/min.

2.3. Characterization Methods

The phase composition of the fish scale powder enriched with the inorganic component, and powders after heat treatment at 400 °C, 700 °C, 800 °C, 900 °C and 1000 °C, was determined by X-ray powder diffraction (XRD) analysis using Rigaku Miniflex 600 diffractometer (CuKα radiation, Kβ filter, D/teX Ultra detector) in the Bragg–Brentano geometry (Rigaku Corporation, Tokyo, Japan), angle interval 2Θ: from 3° to 70°, step 2Θ – 0.02°). Phase analysis was performed using the ICDD PDF2 database [[23]] and Match! software (https://www.crystalimpact.com/, 25 June 2022).

Determination of the total content of potassium, sodium, calcium, magnesium, and phosphorus was done for (sample 1) fish scale powder after heat treatment at 1000 °C and for (sample 2) fish scale powder enriched with the inorganic component. To determine the total content of potassium, sodium, calcium, magnesium, and phosphorus in the samples, 0.6-2.5 g of materials were weighed with a deviation ≤ 0.001g and placed into a conical flask with a capacity of 250 ml. Then, 10 ml of distilled water and 50 ml of nitric acid (GOST 4461-77) were added to each sample. The flasks, containing powders and a solution of nitric acid were boiled for ~ 10 minutes until the material was completely dissolved. Then the solutions were transferred quantitatively into measuring flasks with a capacity of 250 ml and the distilled water was to reach the specified volume. The solutions prepared from reference sample 1 (fish scale powder after heat treatment at 1000 °C) turned out to be transparent and colorless, and sample 2 (fish scale powder enriched with inorganic component) were transparent but light yellow. The prepared solutions were used to determine the named mineral components of the powders under investigation.

The content of sodium and potassium in the samples was determined by the flame photometric method earlier described [24]. The concentration of K^+ and Na^+ ions in solutions was determined by emission flame photometry on a FLAPHO 4 device (VEB Carl Zeiss Jena, Dresden, DDR). The measurements were carried out at a fixed wavelength of 589 nm. Calibration solutions 0.1, 0.25, 0.50, 0.75 mmol/l were used for determination. The error in determining concentrations was 7%.

The content of phosphorus was determined by differential photometric method according to GOST 20851.2-75 consisted in photometry of a colored solution of a phosphor vanadium-molybdenum complex via comparison with solutions containing a known amount of phosphorus. Measurements were carried out at a wavelength of 440 nm in cuvettes with a light-absorbing layer thickness of 10 mm on a KFK-2 photovoltaic concentration colorimeter (ZOMZ, Sergiev Posad, Russia). The error in determining the content calculated for P_2O_5 was 0.5%.

The content of calcium and magnesium ions was determined by complexometric titration according to [25]. The essence of the method consists in the formation in a highly alkaline medium of a poorly dissociated complex compound of both calcium and magnesium with the disodium salt of ethylenediamine -N', N', N', N' – tetraacetic acid (trilon B) and the determination of the equivalent point during titration using a metal indicator. The stability constants of calcium and magnesium ethylenediamine tetraacetates are close in values so the separate determination of calcium and magnesium in their joint presence in the solution is based on the preliminary determination of their total content by titration of the aliquot part of the analyzed solution in an ammonium buffer medium with a pH of 10.5 and with an indicator acid chromium dark blue. In the other aliquot part, pH > 12 was created by introducing 4h KOH, while magnesium was precipitated as hydroxide, it was not filtered out, and calcium was determined complexometrically in the solution. In our case, the indicator was fluorexone. Titration was conducted on a dark background. The error in determining the content calculated for Ca was 2%.

Thermal analysis (TA) was performed to determine the total mass loss of fish scale powder enriched with the inorganic component at heating up to 1000 °C in the air using NETZSCH STA 449 F3 Jupiter thermal analyzer (NETZSCH, Selb, Germany). The gas-phase composition was monitored by the quadrupole mass spectrometer QMS 403 Quadro (NETZSCH, Selb, Germany) combined with a thermal analyzer NETZSCH STA 449 F3 Jupiter. The mass spectra (MS) were registered for the following m/Z values: 15 (NH); 16 (CH_4); 17 (NH_3, HO); 18 (H_2O); 44 (CO_2); 30 (NO); the heating rate was 10 °C/min.

Fish scale powders before and after heat treatment at 400 °C, 700 °C, 800 °C, 900 °C, and 1000 °C were examined by scanning electron microscopy (SEM) on a LEO SUPRA 50VP electron microscope (Carl Zeiss, Jena, Germany; auto-emission source). This investigation was carried out at an accelerating voltage of 3–20 kV using SE2 detectors. The surface of the powders was coated with a layer of chromium (up to 10 nm).

3. Results and Discussion

A micrograph of fish scale powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving is presented in Figure 3. According to this micrograph, powder consists of quite big particles of unregular forms and includes elongated, fibrous fragments inherited collagen fiber structure. The reason for the enrichment of ground fish scale powder with inorganic component during vibration sieving consisted in the remarkable difference between densities of organic (collagen, density about 0,6 g/cm^3) and inorganic (calcium phosphates, density about 3,16 g/cm^3) constituents of fish scales.

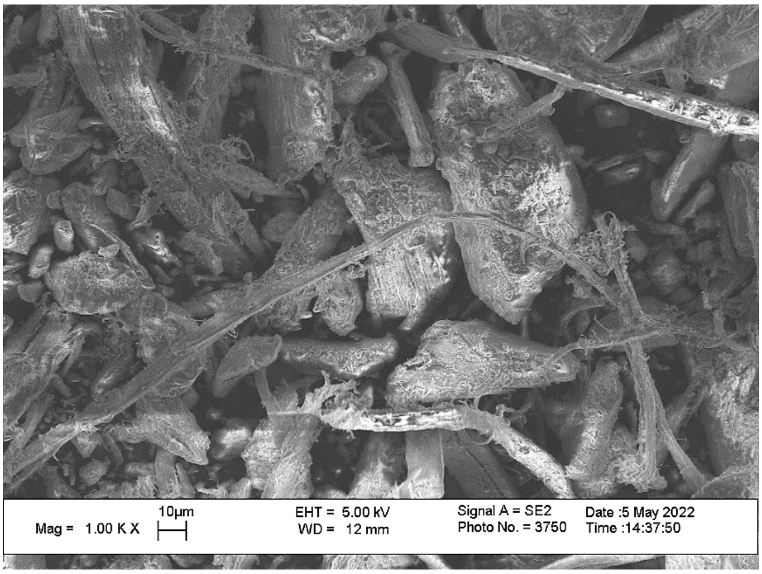


Figure 3. Micrograph of fish scale powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving.

Data of TA of fish scale powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving is presented in **Figure 4**.

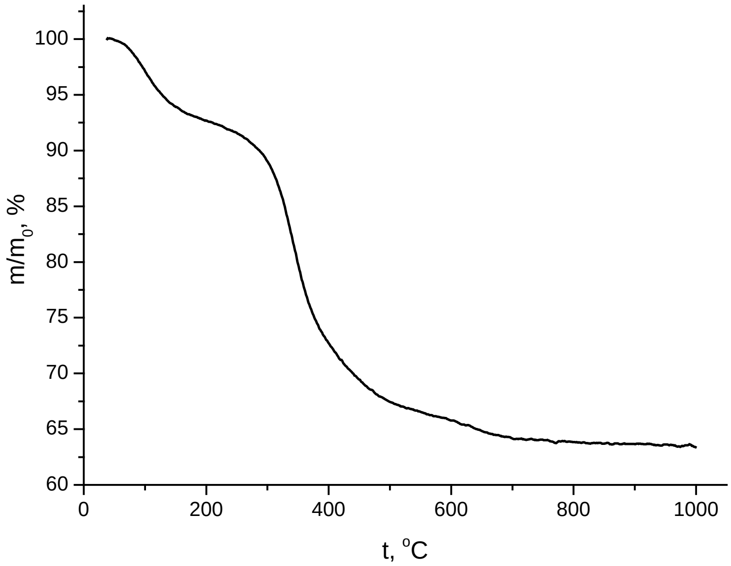


Figure 4. TA of fish scale powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving.

The total mass loss of the fish scale powder enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving when heated up to 1000 °C was 36.5%. There are 3 noticeable steps on the curve of mass loss. The mass loss at the first step (40 – 200 °C, with the maximum at 100 °C) is estimated as 7.5% and according to MS data can be attributed to both adsorbed H_2O (m/Z=18) and NH_3 (m/Z=15) evolving. The mass loss at the second step (200-550 °C, with the maximum at 345 °C) is estimated as 26.0% and at the third step (550-850 °C, no maximum) is estimated as 3.0 %. The mass loss at the second and third steps can be explained by the decomposition of organic component (preferably collagen) presented in fish scale powder. According MS data maximum values of ion current for CH_4 (m/Z=16) was $1.1x10^{-8}$A, for H_2O (m/Z=18) was $4.7x10^{-10}$A, for HO/NH_3 (m/Z=17) was 2,6 $x10^{-10}$A for CO_2 (m/Z=44) was

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 July 2022 doi:10.20944/preprints202207.0013.v1

$1.8x10^{-10}$A, for NH (m/Z=15) was $1.6x10^{-10}$A, and for NO (m/Z=30) was 2.8 $x10^{-11}$A. The choice of such values of m/Z for the mass spectra determination was connected with known from the literature [26, 27] element composition of collagen including C, H, O, and N. As it was underlined in the article [28] during heating many chemical reactions could take place in collagen: dehydration, denaturation, and decomposition. All recorded MS graphs (especially for m/Z=18, m/Z=17, and m/Z=15) had sequences of multiple peaks. Thus recorded MS graphs reflect a combination of complex processes occurring the organic part of fish scale powder enriched with an inorganic component when heating. It should be noted that appearance of TG curve of fish scale powder enriched with inorganic component is in good correlation with TG curve for samples of bone materials with low organic content in investigation reflected in article [29].

Camera photo of powders after heat treatment at 400 °C, 700 °C, 900 °C, and 1000 °C of fish scale powder enriched with the inorganic component is presented in Figure 5.

400 °C (a) 700 °C (b) 900 °C (c) 1000 °C (d)

Figure 5. Camera photo of powders after heat treatment at 400 °C (a), 700 °C (b), 900 °C (c), and 1000 °C (d)

Powders after heat treatment at 400 °C, 700 °C, 900 °C, and 1000 °C had different colors (Figure 5). Powder after heat treatment at 400 °C was colored orange-gray. Powder after heat treatment at 700 °C was colored in dark-gray. Powder after heat treatment at 900 °C was colored light-gray. And powder after heat treatment at 1000 °C was colored white. The reason for powders coloring can be explained by the presence of organic component thermally destructed to a different extent. The deeper is organic component destruction the less coloring is. The colors of heat-treated at 400 °C, 700 °C, 900 °C, and 1000 °C powders in the camera photo (Figure 5) are in good correlation with the temperature dependence of powder mass during heating (Figure 4).

XRD data of fish scale powder before heat treatment and powder after heat treatment at 400 °C is presented in Figure 6. XRD graph of fish scale powder enriched with inorganic component shows the evident presence of quasi-amorphous hydroxyapatite (PDF-card #9-432). XRD graph of powder after heat treatment at 400 °C is not very much different from starting fish scale powder. Except for broad reflexes of quasi-amorphous hydroxyapatite (PDF-card #9-432) there are a couple of reflexes that can be attributed to magnesium whitlockite $Ca_{2,82}Mg_{0,19}(PO_4)_2$ (PDF card #70-682) or $Ca_{18}Mg_2H_2(PO_4)_{14}$ (PDF card #70-2064).

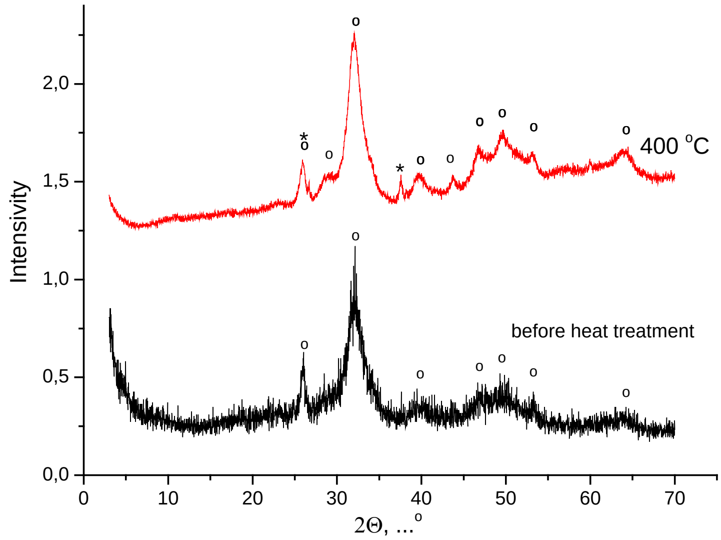


Figure 6. XRD data of powders enriched with the inorganic component prepared from the mixture of fish scales using high-speed grinding and vibration sieving before and after heat treatment at 400 °C: o – $Ca_{10}(PO_4)_6(OH)_2$ (PDF card #9-432; * - $Ca_{2,82}Mg_{0,19}(PO_4)_2$ (PDF card #70-682) or $Ca_{18}Mg_2H_2(PO_4)_{14}$ (PDF card #70-2064).

The phase composition of fish scale powders after heat treatment at 700 °C - 1000 °C (Figure 7) consisted of hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (PDF-card #9-432) and magnesium whitlockite $Ca_{2,82}Mg_{0,19}(PO_4)_2$ (PDF card #70-682) or $Ca_{18}Mg_2H_2(PO_4)_{14}$ (PDF card #70-2064) according to ICDD database [16]. According Match! software database the phase composition of fish scale powder after heat treatment at 1000 °C can be described as containing: hydroxyapatite $Ca_{10}(PO_4)_6(OH)_2$ (card 96-900-2215) or hydroxyapatite-dental $Ca_{4.7}H_{0.46}Mg_{0.05}Na_{0.1}O_{12.51}P_{1.61}$ (card 96-900-2220) and magnesium whitlockite $Ca_{10.115}Mg_{0.385}(PO_4)_7$ (card 96-901-2137) or $Ca_{9.5}Mg(PO_4)_7$ (card 96-901-2138). According Match! the phase composition was determined as 71.8-73.4 wt.% of hydroxyapatite and 26.6-28.2 wt.% of magnesium whitlockite.

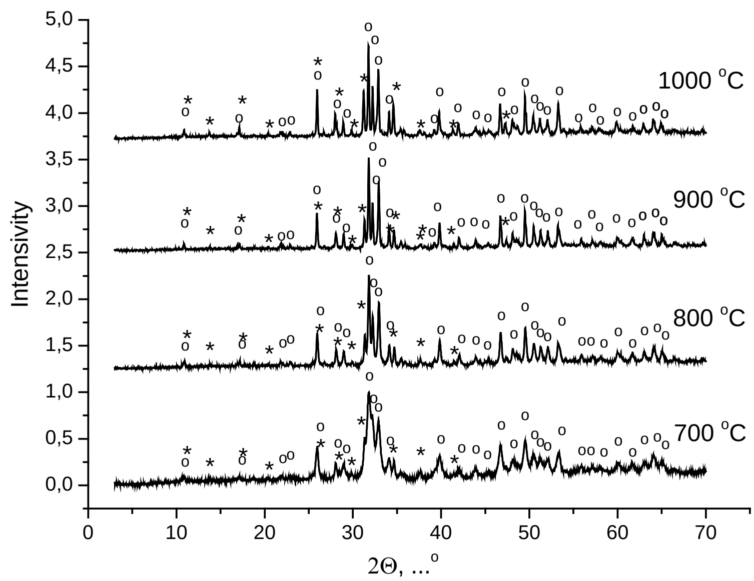


Figure 7. XRD data of powders enriched with the inorganic component prepared from the fish scales mixture using high-speed grinding and vibration sieving after heat treatment in the interval of 700 °C - 1000 °C: o – $Ca_{10}(PO_4)_6(OH)_2$ (PDF card #9-432; * - $Ca_{2,82}Mg_{0,19}(PO_4)_2$ (PDF card #70-682) or $Ca_{18}Mg_2H_2(PO_4)_{14}$ (PDF card #70-2064).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 July 2022 doi:10.20944/preprints202207.0013.v1

Figures 6 and 7 show the expected phenomena of transformation of quasi-amorphous inorganic constituents of fish scales into the crystalline phases due to heat treatment. Similar processes have been observed before [30, 31] when preparing hydroxyapatite from fish scales.

Chemical analysis data of fish scale powder enriched with inorganic component before (Sample 1) and after (Sample 2) heat treatment at 1000°C are presented in Table 1.

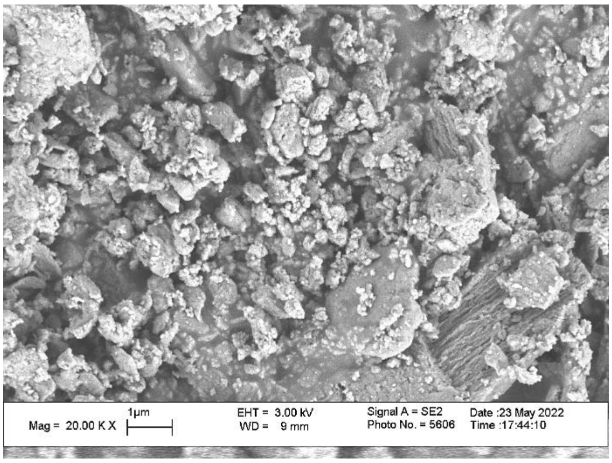
Table 1. Chemical analysis data of fish scale powder enriched with inorganic component before (Sample 1) and after (Sample 2) heat treatment at 1000 °C powder

Number of sample	Description of sample	Content, wt.%				
		Na	Ca	Mg	P	Sum
Sample 1	Fish scale powder enriched with inorganic component	0,4	21,5	1,7	10,9	34,5
Sample 2	Fish scale powder after heat treatment at 1000 °C	0,2	35,7	2,2	19,4	57,5
	According formula $Ca_{10}(PO_4)_6(OH)_2$	0	39,9	0	18,5	58,4

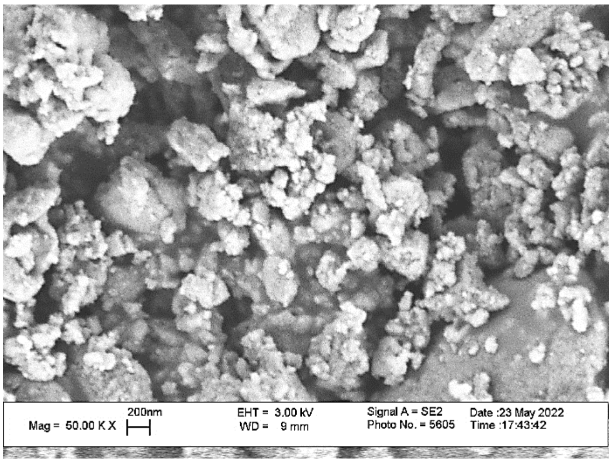
In all samples, potassium was determined in trace amounts. The sodium Na, calcium Ca, and magnesium Mg ions were found in the samples under investigation. Chemical analysis data allow supposing that calcium ions in fish scale hydroxyapatite and whitlockite can be substituted by cations detected. Both sodium Na and magnesium Mg could be integrated into the calcium phosphate structures of scales during the fish life cycle in an aqua medium. Using of sodium salts (NaCl and $NaHCO_3$) at stages of fish scales purification also can be a reason for the presence of sodium Na both in fish scale powder enriched with inorganic component and in fish scale powder after heat treatment at 1000 °C. Lower content of sodium in fish scale powder after heat treatment at 1000 °C can be explained for example by the possibility of NaCl evacuating from sample 2 at temperatures higher than its melting point (801 °C) [32].

Micrographs of powders prepared from the mixture of fish scales using high-speed grinding and vibration sieving after heat treatment at 400 °C (a, b), 700 °C (c, d), 800 °C (e, f) 900 °C (g, h) and 1000 °C (i, j) using magnification x20000 (a, c, e, g, i) and x50000 (b, d, f, h, j) are presented in Figure 8. After heat treatment at 400 °C and 700 °C particles of powder still are in the environment of the thermally destructed organic matrix of dark-orange (400 °C) or dark-grey (700 °C) color as it was shown in Figure 5. The arrangement of inorganic particles after heat treatment is determined by their places in the collagen matrix before heat treatment. Dimensions of inorganic particles after heat treatment at 400 °C (a, b) and 700 °C (c, d) were less than 50 nm. Due to the very small dimensions of inorganic crystals naturally formed in the organic (collagen) matrix one can see in SEM photos of heat-treated at 800-1000 °C powders the signs of sintering. Particles of inorganic powders prepared from fish scale powder via heat treatment at 800-1000 °C consisted of sintered grains. Dimensions of grains in these particles after treatment at 800 were less than 100 nm; after heat treatment at 900 °C they were 50-200 nm and after heat treatment at 1000 °C they were 100-500 nm.

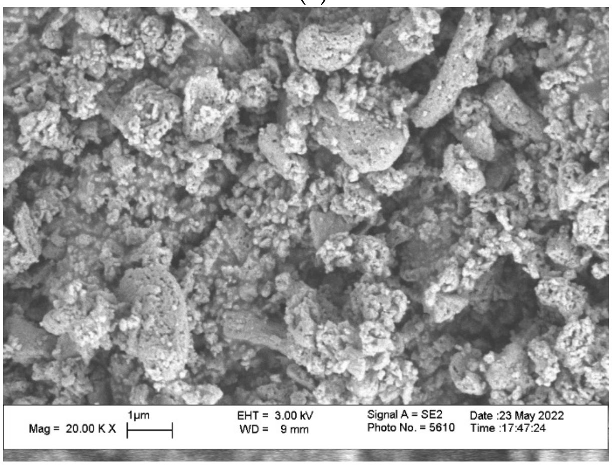
doi:10.20944/preprints202207.0013.v1



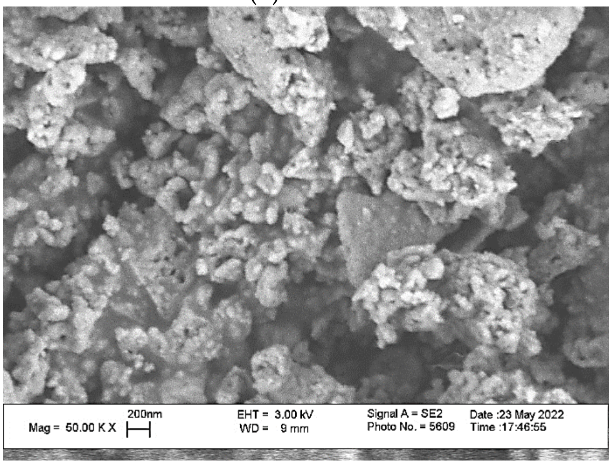
(a) 400 °C



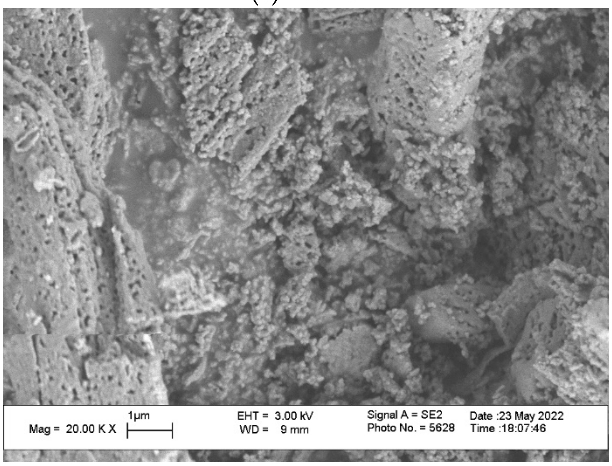
(b) 400 °C



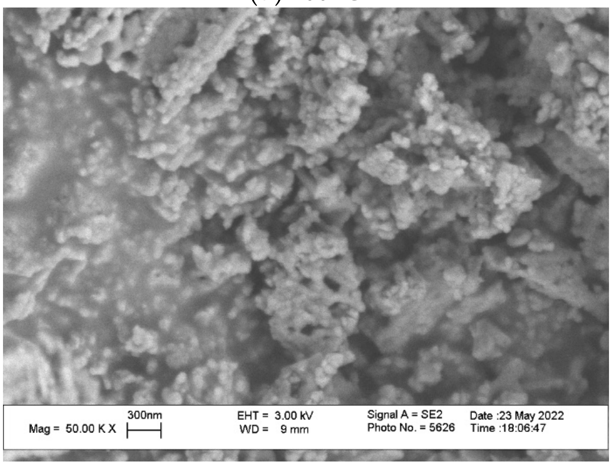
(c) 700 °C



(d) 700 °C

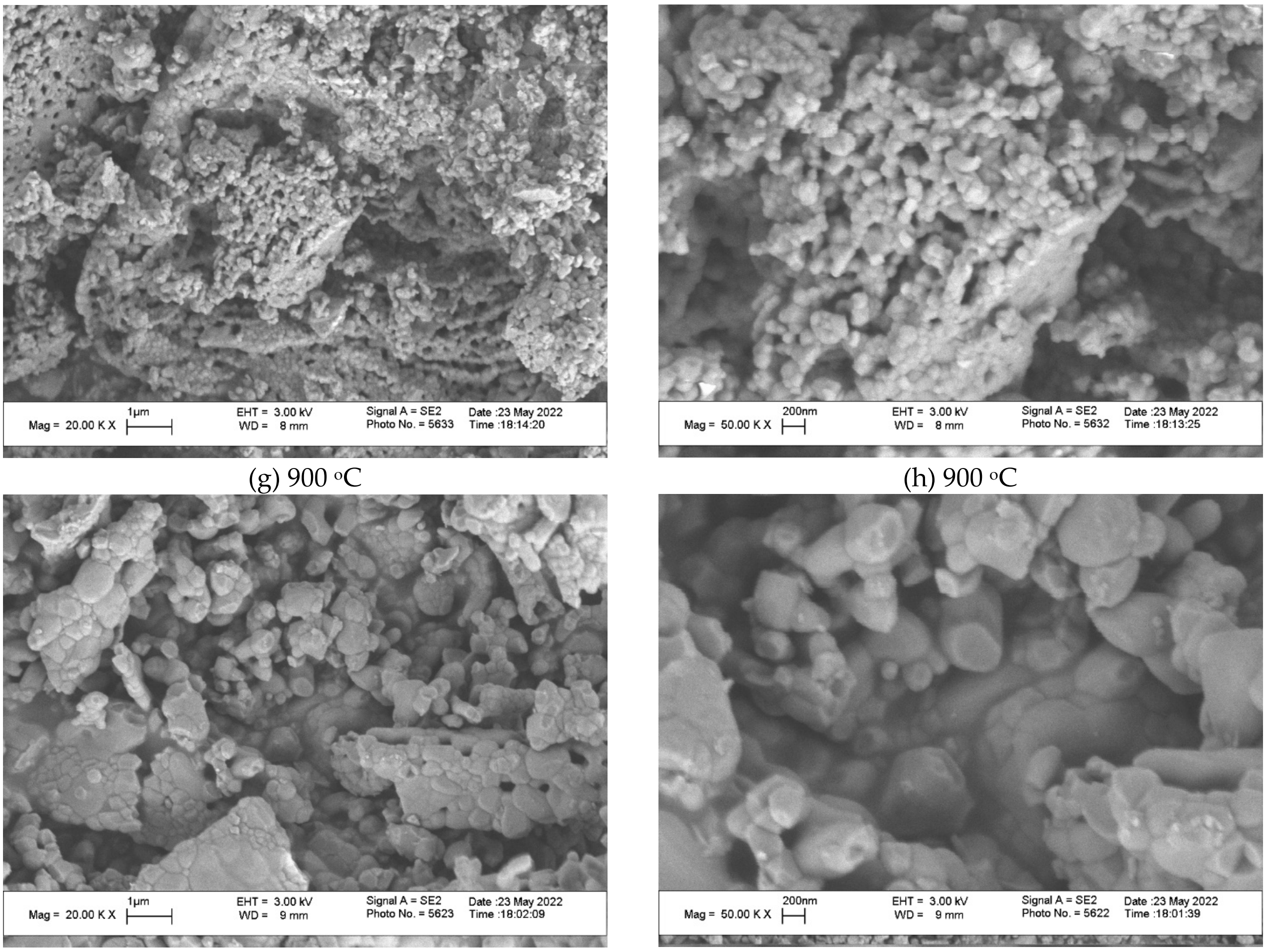


(e) 800 °C



(f) 800 °C

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 1 July 2022 doi:10.20944/preprints202207.0013.v1



(g) 900 °C (h) 900 °C

(i) 1000 °C (j) 1000 °C

Figure 8. Micrographs of powders prepared from the mixture of fish scales using high-speed grinding and vibration sieving after heat treatment at 400 °C (a, b), 700 °C (c, d), 800 °C (e, f) 900 °C (g, h) and 1000 °C (i, j) using magnification x20000 (a, c, e, g, i) and x50000 (b, d, f, h, j).

4. Conclusions

The unique fish scale powder enriched up to 63.5 wt. % with inorganic component were used for the preparation of inorganic powders with phase composition presented by hydroxyapatite and magnesium whitlockite. The main feature of the preparation of this fish scale powder consisted in using high-speed mechanical grinding of clean dry fish scales mixture and separation of ground cotton-like product via vibration sieving. The difference in densities of fish scale constituents, i.e. collagen and calcium/magnesium phosphate was the main reason for the possibility of separation of ground cotton-like product into two parts: powder enriched with inorganic component and fluff enriched with organic (collagen) component. In this work, we presented the investigation aimed at the preparation of inorganic powders using heat treatment of fish scale powder enriched with an inorganic component. It was shown that inorganic constituents (very small inorganic crystals) naturally formed in the organic (collagen) matrix in fish scales can be treated as an excellent precursor of fine inorganic powders. Fish scale powder enriched with inorganic component and powders prepared via heat treatment at temperatures 400-1000 °C can be used for the production of ceramics with the phase compositions belonging to different oxide systems including CaO, P_2O_5, and MgO. Development of new technologies for different new materials production using fish scales as a resource of excellent starting components can diminish the level of planet contamination.

Author Contributions: For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, T.S. (Tatiana Safronova), V.V. and N.K.; methodology, T.S. (Tatiana Safronova), V.V. and N.K.; investigation, T.S. (Tatiana Safronova), V.V., N.K., T.S. (Tatiana Shatalova), O.T., Y.F., A.D., O.G., O.C., E.N., M.A. and K.L.; resources, V.V., K.L., T.S. (Tatiana Shatalova); data curation, T.S. (Tatiana Shatalova), O.G., A.D., M.A.; writing—original draft preparation, T.S. (Tatiana Safronova), V.V. and N.K.; writing—review and editing, T.S. (Tatiana Safronova); visualization, T.S. (Tatiana Safronova), T.S. (Tatiana Shatalova), O.T., Y.F., O.G.; supervision, T.S. (Tatiana Safronova), V.V.; project administration, T.S. (Tatiana Safronova), V.V.; funding acquisition, V.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by project of Federal Agency for Fisheries (Ministry of Agriculture of the Russian Federation) with reg. no. 122030900086-1 dated March 9, 2022, code 01-**32**-05-1 "Development and improvement of food industry production systems".

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The research was carried out using the equipment of MSU Shared Research Equipment Center "Technologies for obtaining new nanostructured materials and their complex study" and purchased by MSU in the frame of the Equipment Renovation Program (National Project "Science") and in the frame of the MSU Program of Development.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Coppola, D.; Lauritano, C.; Palma Esposito, F.; Riccio, G.; Rizzo, C.; de Pascale, D. Fish waste: from problem to valuable resource. *Mar. Drugs*. **2021**, *19* (2), 19020116. https://doi.org/10.3390/md19020116

[2] Arvanitoyannis I. S., Kassaveti A. Fish industry waste: treatments, environmental impacts, current and potential uses. *Int. J. Food Sci. Technol*. **2008**, *43* (4), 726-745. https://doi.org/10.1111/j.1365-2621.2006.01513.x

[3] Subhan, F.; Hussain, Z.; Tauseef, I.; Shehzad, A.; Wahid, F. A review on recent advances and applications of fish collagen. *Crit. Rev. Food Sci. Nutr*. **2021**, *61* (6) 1027-1037. https://doi.org/10.1080/10408398.2020.1751585

[4] Hernández-Ruiz, K.L.; López-Cervantes, J.; Sánchez-Machado, D.I.; del Rosario Martínez-Macias, M.; Correa-Murrieta, M.A.; Sanches-Silva, A. Hydroxyapatite recovery from fish byproducts for biomedical applications. *Sustain. Chem. Pharm*. **2022**, *28*, 100726. https://doi.org/10.1016/j.scp.2022.100726

[5] Mondal, S.; Mahata, S.; Kundu, S.; Mondal, B. Processing of natural resourced hydroxyapatite ceramics from fish scale. *Adv. Appl. Ceram*. **2010**, *109*(4), 234-239. https://doi.org/10.1179/174367613X13789812714425

[6] Chen, F.; Guo, D.; Gao, J.; Ma, Y. Bioinspired crystallization of guanine. *J. Phys. Chem. Lett*. **2021**, *12* (48), 11695-11702.

[7] Vorobyev, V.I. Technology of feed flour based on fish scales. PhD Thesis. Kaliningrad State Technical University (KSTU), Kaliningrad, Russia, 01 June 2018. https://search.rsl.ru/ru/record/01008706647

[8] Basu, B.; Banik, A.K. Production of protein rich organic fertilizer from fish scale by a mutant Aspergillus niger AB100 _ A media optimization study. *J. Sci. Ind. Res. (India)*. **2005,** *64*, 293-298. http://nopr.niscpr.res.in/handle/123456789/5112

[9] Vorobyev V.I., Nizhnikova E.V., Egorova K.V. Fish scales and new directions of its practical application. In Proceedings of the X International Baltic Maritime Forum. Innovations in science, education and entrepreneurship – 2021. Kaliningrad, Russia, 04-09 October 2021. ISBN: 978-5-7481-0485-2, **2022**, *1*, 39-45. https://www.elibrary.ru/item.asp?id=48318018

[10] Vorobyev, V.I.; Nizhnikova, E.V. The use of fish scales for cosmetic purposes. *Izvestiya of KSTU (KSTU News)*. **2020**, *59*, 132-140. (in Russian) https://doi.org/10.46845/1997-3071-2020-59-132-140 https://www.elibrary.ru/item.asp?id=44386612 https://cyberleninka.ru/article/n/ispolzovanie-rybiey-cheshui-v-kosmeticheskih-tselyah

[11] Liu, Wen-Kuang, et al. "From waste to health: synthesis of hydroxyapatite scaffolds from fish scales for lead ion removal." Jom 69.4 (2017): 713-718. https://doi.org/10.1007/s11837-017-2270-5

[12] Majhooll, A.A.; Zainol, I.; Jaafar, C.N. A.; Mudhafar, M.; Ha, A.; Asaad, A.; Mezaal, F.W. Preparation of fish scales hydroxyapatite (FsHAp) for potential use as fillers in polymer. *J. Chem. Chem. Eng.* **2019**, *13*, 97-104. https://doi.org/10.17265/1934-7375/2019.03.002

[13] Gil-Duran, S.; Arola, D.; Ossa, E.A. Effect of chemical composition and microstructure on the mechanical behavior of fish scales from Megalops Atlanticus. *J. Mech. Behav. Biomed. Mater.* **2016**, *56*, 134-145. http://dx.doi.org/10.1016/j.jmbbm.2015.11.028

[14] Sionkowska, A.; Kozlowska, J. Fish Scales as a Biocomposite of Collagen and Calcium Salts. *Key Engineering Materials*. **2013**, *587*, 185–190. https://doi.org/10.4028/www.scientific.net/KEM.587.185

[15] Caruso, G.; Floris, R.; Serangeli, C.; Di Paola, L. Fishery wastes as a yet undiscovered treasure from the sea: Biomolecules sources, extraction methods and valorization. *Marine drugs*. **2020**, *18* (12), 18120622. Mar. Drugs 2020, 18, 622; https://doi.org/10.3390/md18120622

[16] Harada O.; Hasegawa Y. Extraction method and extraction apparatus of collagen, production method and production apparatus of hydroxyapatite, and collagen-containing aqueous extract and hydroxyapatite. Patent JP №2008285456, 27.11.2008.

[17] Li L.; Yang Y.; Yao T.; Lin Y.; Chen L.; Xue X. Comprehensive extraction method of collagen, hydroxyapatite and protein in fish scales. Patent CN №107056933. 18.08.2017.

[18] Liu Y.; Ji S.; Zhang L.; Yang F. Method for extracting hydroxyapatite and collagen from fish scales by using deep eutectic solvent Patent CN №111302319. 19.06.2020.

[19] Zhang Y; Tu D; Dai Z. Method for extracting fish scale collagen protein peptide powder and hydroxyapatite. Patent CN №108949882. 07.12.2018.

[20] Vorobyev, V.I.; Nizhnikova, E.V. Obtaining fractions of collagen and hydroxyapatite from fish scales *Izvestiya of KSTU (KSTU News)*. **2021**, *62*, 80-91. (in Russian) https://doi.org/10.46845/1997-3071-2021-62-80-91, https://www.elibrary.ru/item.asp?id=46369968, https://cyberleninka.ru/article/n/poluchenie-fraktsiy-kollagena-i-gidroksiapatita-iz-rybiey-cheshui

[21] Vorobev V.I. Method for production of food collagen-containing products, Patent RU 2734034 C1, 12.10.2020. https://www1.fips.ru/ofpstorage/Doc/IZPM/RUNWC1/000/000/002/734/034/%D0%98%D0%97-02734034-00001/document.pdf

[22] Vorobev V.I. Method of processing fish scales to obtain collagen and hydroxyapatite. Patent Application RU 2021116247. 03.06.2021. https://new.fips.ru/registers-doc-view/fips_servlet?DB=RUPAT&DocNumber=2021116247&TypeFile=html

[23] ICDD. International Centre for Diffraction Data; Kabekkodu, S., Ed.; PDF-4+ 2010 (Database); ICDD: Newtown Square, PA, USA, 2010; Available online: https://www.icdd.com/pdf-2/ (accessed on 21 June 2022).

[24] Poluektov N.S. *Methods of analysis by flame photometry*, 2nd ed.; Chemistry: Mosow, USSR, 1967; 308 p.

[25] Schwarzenbach G., Flaschka G. *Complexometric titration*, Chemistry: Mosow, USSR, 1970; 360 p.

[26] Shoulders, M.D.; Raines, R.T. Collagen structure and stability. *Annu. Rev. Biochem.* **2009**, *78*, 929–958. https://doi.org/10.1146/annurev.biochem.77.032207.120833

[27] Pati, F.; Adhikari, B.; Dhara S. Isolation and characterization of fish scale collagen of higher thermal stability. *Bioresour. Technol.* **2010**, *101*, 3737–3742. https://doi.org/10.1016/j.biortech.2009.12.133

[28] Cucos, A.; Budrugeac, P. Simultaneous TG/DTG–DSC–FTIR characterization of collagen in inert and oxidative atmospheres. *J Therm Anal Calorim*. **2014**, *115* (3), 2079-2087. https://doi.org/10.1007/s10973-013-3116-1

[29] Mkukuma; L.D., Skakle, J.M.S.; Gibson, I.R.; Imrie, C.T.; Aspden, R.M.; Hukins, D.W.L. Effect of the proportion of organic material in bone on thermal decomposition of bone mineral: an investigation of a variety of bones from different species using thermogravimetric analysis coupled to mass spectrometry, high-temperature X-ray diffraction, and Fourier transform infrared spectroscopy. *Calcif. Tissue. Int.* **2004**, *75*(4), 321-328. https://doi.org/10.1007/s00223-004-0199-5

[30] Kongsri, S.; Janpradit, K.; Buapa, K.; Techawongstien, S.; Chanthai, S. Nanocrystalline hydroxyapatite from fish scale waste: Preparation, characterization and application for selenium adsorption in aqueous solution. *Chem. Eng. J.* **2013**, *215*, 522-532. https://doi.org/10.1016/j.cej.2012.11.054

[31] Pon-On, W.; Suntornsaratoon, P.; Charoenphandhu, N.; Thongbunchoo, J.; Krishnamra, N.; Tang, I.M. Hydroxyapatite from fish scale for potential use as bone scaffold or regenerative material. *Mater. Sci. Eng. C.* **2016**, *62*, 183-189. https://doi.org/10.1016/j.msec.2016.01.051

[32] Safronova, T.V.; Steklov, M.Yu.; Putlyaev, V.I.; Shekhirev, M.A. Na-replaced Ca-scarce carbonate-hydroxyapatite for production of ceramic materials. *Composite Materials Constructions.* **2006,** № 4, 34-38. https://www.elibrary.ru/item.asp?id=11846526