Comparative Studies on the Physico-chemical Characteristics of Bio-materials with Collagen from Calf and Fish Skins from Black Sea

MELAT CHERIM1, RODICA SIRBU1, ANETA TOMESCU2, MARIUS FLORENTIN POPA3, EMIN CADAR4
1Ovidius University of Constanta, Faculty of Pharmacy, 24 Mamaia Blvd., 900527, Constanta, Romania
2Ovidius University of Constanta, Faculty of Medicine, 24 Mamaia Blvd., 900527, Constanta, Romania

Collagen hydrolysers are three-dimensional polymeric materials with limited cross-linking and high hydrophilicity, having multiple medical applications. The most used collagen is the one extracted from bovine skin, which is now the industrial source of collagen. Due to the outbreak of some threatening diseases such as BSE, transmissible spongiform encephalopathy, foot-and-mouth disease, researchers have sought a safer alternative to collagen. This was the marine resource, which offered multiple opportunities to capitalize on clean sea-water raw material. This paper presents a comparative study of the physico-chemical properties of collagen hydrogels derived from collagen obtained from calf and skin from the Black Sea. Physico-chemical and spectrophotometric analyses were performed to determine the structure. Studies have been conducted to analyze rheological behavior, antioxidant activity and antimicrobial activity. The total antioxidant capacity (ACL) is higher for collagen mixtures with 40% ethyl alcohol and shows higher values for fish collagen compared to calf collagen. Antimicrobial analysis shows that all collagen hydrogels show antimicrobial activity, both gram-positive (Staphylococcus aureus ATCC 6538P) and gram-negative (Escherichia coli ATCC 10536), which increases with increasing collagen concentrations.

Keywords: collagen hydrogels, calf collagen, fish collagen, actioxidant capacity, antimicrobial activity

Collagen in all its characteristic forms is presented as a polymer that is distinguished by increased hydrophilicity, variable ionic character and diverse functionality, and can be involved in a large number of interaction systems with other micro or macromolecular components [1]. To date, at least 29 different types of collagen have been identified, which are composed of 46 distinct polypeptide chains [2-3], differentiated by the structure into: striatum (fibrous), non-fibrous (network formation), microfibrillation (filamentous) and those that are associated with fibrils [4]. All have a triple helix characteristic, but the length and size of the helix and the nature of the non-helical portion vary from one type to another [5-6]. Triple helix molecules have terminal globular domains and are called procollagen. These globular regions are cleaved to different degrees to give a polymerized structure (tropocollagen), which is the base unit of collagen. The amino acid sequence in collagen is generally a repetitive tripeptide unit (Gly-X-Y), where X is frequently Pro and Y is Hyp [7-8]. Collagen possesses the characteristics of a distinct biomaterial in the biopolymer class [9-13]. Collagen has a broad utility as a biomaterial in the treatment of wounds [14-15]. The skin is permanently subjected to various lesions and is injured by physical or chemical means [16-17]. There have been researches in the last decades of numerous materials that could accelerate wound healing [18-20]. Collagenic hydrogels are polymeric materials with three-dimensional structure, limited cross-linking and high hydrophilicity that are applied topically. Due to these characteristics, hydrogels can absorb a high amount of water and give the wound a wet environment and at the same time have the ability to absorb the exudates produced by it [21]. Depending on the materials used, hydrogels may be natural, synthetic or semi-synthetic [22-23]. Bovine source is one of the major industrial sources of collagen, used especially in the pharmaceutical and biomedical fields [24-28]. One of the major drawbacks of bovine collagen is that almost 3% of the population is allergic to it, which is a hindrance to its use. Due to the outbreak of menacing diseases such as BSE, transmissible spongiform encephalopathy (FMD), foot-and-mouth disease (FMD), researchers have sought a safer collagen alternative. This was the marine resource, which offered multiple possibilities to harness clean raw material if it came from sea-fishing [29-31]. Although collagen extracted from fish does not form high viscosity gels, they are very convenient for certain applications, such as micro-encapsulation or obtaining light-sensitive coatings. From collagen gels, as in the case of those extracted from mammalian skin, porous films or matrices can be obtained. The present study includes original research on fish in the Black Sea. This paper is a comparative study on the physico-chemical characteristics of collagen hydrogels derived from collagen obtained from bovine skin and fish from the Black Sea. The study period was 2017-2018.

Experimental part

Materials and methods

Insoluble calf collagen fibers were obtained in the Collagen Department of the Romanian National Research & Development Institute for Textiles and Leather, from calf hide, through a special chemical enzymatic process [32]. Fish Collagen was obtained from Gray Mullet fish from the Black Sea through a bio-technological process of acid extraction [33]. Practically, two extraction variants were performed: Variant I with 0.5 M acetic acid and variant II with 0.1 M hydrochloric acid. The cross-linking agents used were tannic acid.

Chemicals

All used reagents were of analytical reagent grade and were purchased from Sigma-Aldrich, Germany. The tested microorganisms: Staphylococcus aureus (ATCC 6538P), Escherichia coli (ATCC 10536).

* email: tomescu.aneta@gmail.com , Phone: 0040722331961 All authors are considered main authors with equal contributions.

MATERIALE PLASTICE • 56 • No. 1 • 2019 http://www.revmaterialeplastice.ro 179
Collagen Biomaterials

As a support in the production of biomaterials, collagen extracted and characterized from Gray Mullet skin of the Black Sea was used and collagen from calf skin. Biomaterials have been made with hydrogels, membranes (films) and matrices. The methods of obtaining biomaterials were as follows:

- Collagenic hydrogels were obtained by mixing the adjusted gels at 0.4% and pH 7 with triethanolamine and different active substances in different concentrations from the dry substance. The hydrogels were crosslinked with 10% tannic acid over collagen and maintained at 4 °C for 24 h.
- The membranes (films) were obtained from the crosslinked hydrogels by pouring into Petri plates. Drying was carried out at room temperature, 22 °C, for 24-72 h.
- Matrices were obtained from collagen lyophilisates. The hydrogels were lyophilized using the Lacombco lyophilizer and had the same composition as the collagen hydrolyzates.

Investigation methods

Physico-chemical characteristics were obtained from the following analyzes: total nitrogen, protein, pH, dry matter content, ash content. Preliminary determination of appearance, color and determination of moisture were made. The analyzes were performed according to the Romanian Pharmacopoeia ed. X [34] and the European Pharmacopoeia [35]. The analyzed samples were harvested on collagen hydrolysates extracted from bovine skin and Gray Mullet skin obtained by acid treatment with 0.5 M acetic acid and 0.1 M HCl.

Determination of collagen content

By determining hydroxyproline, it is possible to determine the amount of collagen and thus determine its proportion of total proteins using a method described by Macovec G. named the spectrophotometric determination of hydroxyproline following Ehrlich reagent therapy [36]. To determine the percentage content of hydroxyproline, a Hellios Omega Thermo Scientific series 171008 spectrometer, suitable for use at a wavelength of 558 nm ± 2 nm. The percentage of collagen is calculated, based on the percentage of hydroxyproline, after the relationship (1) [37]:

\[ \text{Collagen content} \% = \text{HPro content} \% \times 8 \]  

(1)

Analysis by FTIR Spectroscopy

Infrared spectroscopy is sensitive to the chemical structures of molecules and is suitable for spectroscopy to determine proteins and polypeptides in different states and concentrations and is a useful tool for determining the tertiary structure of proteins and polypeptides [38]. FTIR analysis was performed with a FTIR 4200 Jasco Spectrometer.

Analysis by UV-DC Spectroscopy

Circular dichroism is an important method for analyzing the helical structure and the degree of renaturation / denaturation of samples used in biological studies. The UV-DC spectrums were obtained with ajasco J-810 spectrophotometer. The spectrum represents the mediation of four consecutive acquisitions at room temperature (25 °C).

Analysis of rheological behavior

The rheological behavior under shear stresses was determined at 23 ± 0.1 °C with a Haake VT 550 reoviscometer equipped with the medium viscosity MV1 sensor system and Rheo-Win 4 Thermo Fischer Scientific software. Comparative rheology studies have been performed on both collagen extracted from calf skin and collagen extracted from fish skin. Both samples were non-reticulated and crosslinked with 2% tannic acid. (table 1)

Rheological behavior is analyzed using equations (2) ÷ (5) shown in table 2.

Dynamic rheological measurements were performed with the Micro Fourier Transform Rheometer MFR 2100 microcontroller (GBC model), a compressor flow micro-meter equipped with a temperature control jacket connected to a Lauda E100 circulation water bath. By the Fourier analysis of the force signal, at a large number of discrete simultaneous frequencies, the main viscoelastic characteristics of the sample can be extracted: the storage G’ and loss G” module, the complex viscosity and its components. All rheological measurements were performed in triplicate (with a standard deviation of less than 15%) at 25 °C.

Determination of total antioxidant activity by chemiluminescence (ACL)

The important criteria for choosing the ACL method by photochemiluminescence includes the short time needed for the analysis and for the preparation of the sample. Components of the reagent kit for the ACL procedure: - high purity R1 - methanol; - reagent R2 - reagent buffer; - Reagent R3 - photosensitizer reagent and free radical detection reagent; - R4 reagent - the calibration standard (trolox), for quantification of lipid-soluble antioxidant substances [39]. The equipment used in the ACL method is produced by Analytik Jena AG, has made available a system that can measure the full antioxidant (ACL) capacity of a mixture of substances. The calibration curve for the standard substance trolox was built using a series of standard solutions trolox concentrations of 0.5, 1.0, 1.5, 2.0 and 3.0 nmol / sample volume. The measured

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>Collagen Type</th>
<th>Collagen Hydrogels Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1 (COL-Calf)</td>
<td>1.72% calf collagen in 0.5M acetic acid</td>
<td></td>
</tr>
<tr>
<td>Sample 2 (COL-Calf-R)</td>
<td>1.77% calf collagen, 10% tannic acid in 0.5M acetic acid</td>
<td></td>
</tr>
<tr>
<td>Sample 3 (COL-Fish)</td>
<td>0.4% fish collagen in 0.5M acetic acid</td>
<td></td>
</tr>
<tr>
<td>Sample 4 (COL-Fish-R)</td>
<td>0.6% fish collagen, 0.64% tannic acid in 0.5M acetic acid</td>
<td></td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Viscosity ( \eta ) (cP) depending on shear speed ( D ) (sec(^{-1}))</th>
<th>Shear speed ( D ) (sec(^{-1})) depending on shear stress ( \tau ) (Pa)</th>
<th>Shear stress ( \tau ) (Pa) in correlation with the selected rotation speed ( w ) (rpm)</th>
<th>shear stress ( \tau ) (Pa) depending on viscosity ( \eta ) (cP) and shear speed ( D ) (sec(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \eta = f(D) )</td>
<td>( D = f(\tau) )</td>
<td>( D = \omega * R )</td>
<td>( \tau = \eta * D )</td>
</tr>
</tbody>
</table>

\[ D = \omega * R \]  

(4)

\[ \tau = \eta * D \]  

(5)
antioxidant capacity is then quantified by comparison with a standard substance used for calibration. (trolox - vitamin E derivative). The analyzed material is based on calf collagen hydrolyzate and Gray Mullet marine fish in aqueous and alcoholic solutions of varying concentrations of ethyl alcohol.

Determination of antimicrobial activity of biocomposites

The diffusometric method of Bauer A.W. was used with some modifications [40] with wells cut from the nonspecific nutrient agar medium. The microorganisms used for antimicrobial activity evaluation are gram-positive bacteria Staphylococcus aureus (ATCC 6538P) and gram-negative bacteria Escherichia coli (ATCC 10536). For each of the strains we prepared a standard reference solution, an Inocculum, of known concentration (3 x 10⁸/mL). We used Petri dishes in which we added 1 mL of reference solution (Inocculum) and 80 mL of nonspecific nutrient agar, sterilized and homogenized. In parallel, we also prepared 3 positive control plates to compare the results obtained. After solidifying the agar from the Petri plates we made the wells, aseptically, using a sterilized matrix of known diameter. Non-specific nutritional agar plates were filled with the known bacterial medium and we measured the inhibition halos formed by collagen hydrogels. Inhibition halos result from the activity of collagen hydrolysates on culture media by preventing the development of pathogens and their proliferation in the agar medium.

Statistical analysis

A statistical approach was designed and the experimental data were evaluated using one-way analysis of variance (ANOVA). The statistical results confirm the hypothesis that the differences between the results are either not significant (p > 0.05). The average of multiple measurements (triplicates or more) was listed in the tables together with the standard deviations.

Results and discussions

Among natural polymers, bovine collagen has been used in multiple applications. The results of the present study show the physico-chemical characteristics of collagen hydrogels from calf and Gray Mullet skin from the Black Sea. The marine collagen hydrogels were obtained by two different acid extraction processes: 0.5M acetic acid and 0.1M hydrochloric acid. Table 3 presents the results obtained for the physico-chemical characteristics of the hydrogels studied. There are higher values for calf collagen in comparison to the one obtained from fish, for ash, total nitrogen and mineral content. Comparable values for protein content and collagen content were obtained for the hydrogel made from bovine and Gray Mullet skin using the 0.5M acetic acid extraction process. The results obtained for the bovine and Gray Mullet collagen obtained using the 0.5 M acetic acid extraction process are in accord to data literature [32, 42]. The pH range is 2.5-3.5 (table 3) and is in accordance to data literature [32].

The content of collagen calculated on the basis of the percentage of hydroxyproline with equation (1) is comparable for calf collagen (92.2%) and fish collagen extracted with 0.5 M acetic acid (90.1%). Smaller values (82.3%) were obtained for fish collagen extracted with 0.1 M HCl. The highest moisture content is the hydrogel obtained from the collagen fish extracted with 0.5 M acetic acid (15.5%) compared to calf collagen (13.5%). The appearance is different: translucent gelatinous for calf collagen, white/ opaque gelatinous for fish collagen.

Analysis by FT-IR spectroscopy

The IR spectrum of the collagen hydrolyzate from both the calf skin and Gray Mullet skin has absorption bands located in the amide band, as can be seen in the FTIR spectrums obtained and presented in figure 1 (a and b). The main absorption bands of amides A, I, II and III were between 3326-1643 and 1527-1238 cm⁻¹, respectively. Amides I, II and III (1650 and 1200 cm⁻¹, respectively) are

Table 3

<table>
<thead>
<tr>
<th>Characteristics of hydrogels</th>
<th>Calf collagen</th>
<th>Fish collagen extracted with 0.5 M acetic acid</th>
<th>Fish collagen extracted with 0.1 M HCl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>translucent gelatinous</td>
<td>white gelatinous</td>
<td>white opaque</td>
</tr>
<tr>
<td>Color</td>
<td>white-yellow</td>
<td>White-yellow</td>
<td>White-yellow</td>
</tr>
<tr>
<td>Humidity [%]</td>
<td>15.5±0.1</td>
<td>15.5±0.1</td>
<td>15.0±0.2</td>
</tr>
<tr>
<td>Dry matter [%]</td>
<td>1.7±0.02</td>
<td>1.5±0.04</td>
<td>1.2±0.03</td>
</tr>
<tr>
<td>Ash 600-800 °C [%]</td>
<td>1.3±0.1</td>
<td>1.3±0.1</td>
<td>0.8±0.2</td>
</tr>
<tr>
<td>Total nitrogen mg (Kjeldal) [%]</td>
<td>16.5±0.1</td>
<td>10.9±0.2</td>
<td>8.8±0.3</td>
</tr>
<tr>
<td>Proteic substance [%]</td>
<td>96.5±0.2</td>
<td>86.5±0.1</td>
<td>78.5±0.3</td>
</tr>
<tr>
<td>pH</td>
<td>2.9±0.1</td>
<td>2.5±0.3</td>
<td>2.5±0.2</td>
</tr>
<tr>
<td>Collagen content [%]</td>
<td>92.2±0.1</td>
<td>90.1±0.2</td>
<td>82.3±0.4</td>
</tr>
</tbody>
</table>

Note: Each value is the mean ± SD of three independent measurements.
found, which is in line with data literature [43]. An absorption ratio of approximately 1, between the amide III and the 1450-1454 cm⁻¹ band, indicates that triple helical structure is intact in accordance with data literature [44]. The triple-helical structure of collagen extracted by the acidic fish skin method was confirmed from the absorption ratio between 1238 (amide III) and 1527 cm⁻¹, which is approximately 1, in accordance with literature data [45]. The same method has been used frequently in many scientific reports [43] which indicated that FTIR was a simple and practical method for assessing the triple-helical structure of collagen.

Analysis by UV-DC spectroscopy
UV circular dichroism (UV-DC) measures the differential absorption of circularly polarized light. Amide links of proteins found in highly ordered regions - helixes α and folded structures α - have specific optical activity due to orientation (fig.2). To avoid saturation of the detector, the sample concentration should be small enough (<0.125 mg/mL). From figure 2 (a and b) the following can be highlighted: close wavelength values were obtained (198.5 nm for fish collagen and 198 nm for bovine collagen); the values obtained for the maximum wavelengths were also close (221.5 nm for fish collagen and 222 nm for bovine collagen); the values for the zero ellipticity point for fish collagen (215.5 nm) and for the zero ellipticity point for calf collagen (214 nm) are comparable; similar values were obtained for Rpn parameter value 0.10 for fish collagen and Rpn value 0.12 for bovine collagen.

Rheological behavior
Improvement of mechanical properties can be achieved through crosslinking. The disadvantage of chemical crosslinking is reducing the biocompatibility of hydrogels and these become cytotoxic [46]. Among the beneficial effects of reticulation, it can be mentioned: improvement of mechanical properties, increase of proteolytic resistance and solvents [48], reduction of antigenicity [49]. In the case of tannic acid crosslinking, only pH acidic hydrogels were prepared because AT rapidly and relatively tightly binds to collagen only under acidic conditions, at pH values ranging from 2 to 4. Above the pH value of 7, its binding is slow and incomparably weaker. The researched collagen hydrogels are presented in table 1. Rheological parameters and obtaining the reograms and flow curves were made with the set of equations (2) + (5) of table 2.

Characterization by dynamic rheological behavior for calf collagen
Rheological measurements in a stationary system affect the structure of the dispersed systems, due to the shear forces to which they are subjected, while the ones in the dynamic regime do not affect the systems so that the rheological parameters determined maintain their actual values. Apparent viscosities of different concentrations of calf collagen were determined in successive dilutions of 1, 0.5, 0.25% shown in figure 3. It can be concluded that the apparent viscosity increases with increasing collagen concentration. The 1% collagen solution exhibits a rheological profile characteristic of elastic behavior with G’ (storage modulus) much greater than G” (the loss of viscosity) and almost independent of frequency. For crosslinked collagen, the mixture becomes characteristic of a viscoelastic fluid, but for high concentrations of collagen, (fig.3.B).

This can be explained by a concentration of tannic acid, which is sufficient to ensure a good combination of tannic acid in chains, leading to a transient network.
over 10 rad / s having predominantly gel nature. Figure 5.a shows the dependence of the storage and loss modules on the angular frequency for the collagen sample of the calf at 25°C. The sample has a gel point at approx. 3.8 rad / s, is predominantly viscous under the gel point and predominantly gel over the gelification point. Figure 5.b shows the dependence of storage and loss-of-angles for sample 1 for fish at 25°C. The sample has a predominantly viscous character for \( \omega < 65 \) rad / s and is at the gel point for \( \omega > 65 \) rad / s.

**Determination of total antioxidant activity by chemiluminescence (ACL)**

From the examination of curves obtained using the Photochem instrument with ACL kit (Analytic Jena AG) which are shown in figure 6 shows that all samples with collagen hydrolysates show antioxidant activity reported as trolox units equivalents (mmol / sample volume). Analyzing the calf collagen and collagen fish hydrogels mixed with water, 40% ethyl alcohol and 50% ethyl alcohol we concluded that in all mixtures made of collagen fish shows values higher than total collagen for total antioxidant capacity expressed as trolox units equivalents (mmol/sample volume), as shown in figure 7.

For example, in the case of mixtures with water, the calf collagen has an ACL of 0.368 nmol trolox/sample volume, and the fish collagen has an ACL of 0.456 nmol trolox/sample volume.

For blends with 40% ethyl alcohol, the calf collagen records an ACL of 0.280 nmol trolox/sample volume, and the fish collagen records an ACL of 0.323 nmol trolox/sample volume, and for blends with 50% ethyl alcohol, the calf collagen records an ACL of 0.097 nmol trolox / sample volume and fish collagen records ACL of 0.205 nmol trolox/sample volume. The collagen hydrolyzate in aqueous solution has antioxidant activity in the analyzed samples. ACL (total antioxidant capacity) decreases with increasing sample dilution. Figure 7 shows that the aqueous collagen hydrolysates exhibit the highest values for ACL. (Fish collagen 0.456 nmol trolox/sample volume and calf collagen with 0.368 nmol trolox units equivalents/sample volume).
Table 4

<table>
<thead>
<tr>
<th>Bacterial Strains</th>
<th>Staphylococcus aureus (mm)</th>
<th>Escherichia coli (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calf collagen gel 1%</td>
<td>4.5±0.3</td>
<td>4.0±0.2</td>
</tr>
<tr>
<td>Calf collagen gel 2.5%</td>
<td>6.0±0.4</td>
<td>5.8±0.1</td>
</tr>
<tr>
<td>Fish collagen gel 1%</td>
<td>5.0±0.3</td>
<td>4.8±0.2</td>
</tr>
<tr>
<td>Fish collagen gel 2.5%</td>
<td>6.5±0.2</td>
<td>6.0±0.5</td>
</tr>
</tbody>
</table>

Note: Each value is the mean ± SD of three independent measurements.

Collagen biomaterials

Collagen hydrogels obtained from calf collagen (fig. 8a) are different in appearance from those obtained from Gray Mullet fish collagen (fig. 9a).

To obtain the collagen membranes, the crosslinked hydrogels with tannic acid were poured into Petri dishes, after a prior agitation to remove the air, the free-drying method was applied. Collagen calf membranes (fig. 8b) can be compared to those in fish collagen (fig. 9b).

Antimicrobial activity

The in vitro antibacterial potential of collagen hydrogels against both gram-positive and gram-negative bacteria is summarized in table 4.

Calf collagen presents zones of inhibition between 4.5-6 mm of diameter, for *Staphylococcus aureus*, higher than *Escherichia coli* with zones of inhibition between 4.0-5.0 mm of diameter. *Gray Mullet* fish collagen also has inhibition zones between 5.0-6.5 mm diameter for *Staphylococcus aureus*, larger than *Escherichia coli* with zones of inhibition between 4.8-6.0 mm in diameter. Fish collagen exhibits better antimicrobial activity compared to calf collagen.

Conclusions

The paper presents physico-chemical characteristics of collagen hydrogels derived from bovine skin compared to collagen extracted from *Gray Mullet* skin from the Black Sea. Collagen hydrogels from marine fish were obtained by two different acid extraction processes: 0.5 M acetic acid and 0.1 M hydrochloric acid. From the comparison of physico-chemical characteristics we can draw the following conclusions:

- Fish collagen extracted from Gray Mullet fish using the acetic acid 0.5 M method shows superior values to that obtained with the 0.1 M HCl acid process for all the characteristics analysed and is comparable to the results obtained for calf collagen. The highest collagen content value obtained is from calf collagen (92.2%) comparable to that from fish collagen (90.1%) obtained using the 0.5 M acetic acid process.

- From analysis by FT-IR spectroscopy, the triple helical collagen structure for both fish and calf collagen was confirmed in accordance with literature data [43 ÷ 45].

- From analysis by UV-DC spectroscopy, the results show that during the extraction the triple helix structure of the collagen wasn’t affected and both the collagen hydrogels obtained from fish and calf can be utilized for biomaterials.

- From the rheological behaviour we concluded that all hydrogels are found to have pseudoplastic behaviour, but viscosities are much higher at a given concentration for
those using 0.5 M acetic acid extracts from marine fish, becoming predominantly elastic. The collagen rheograms in fish are similar to those of the calf, the curves being descending and depending on the shear forces.

From the ACL study (total antioxidant capacity) presented by the hydrogels from calf and marine fish in aqueous and alcoholic solutions it is found that all samples showed antioxidant activity. It is also found that collagen hydrolyzate exhibits higher antioxidant activity in 40% ethyl alcohol solutions over the same collagen concentrations in 50% ethyl alcohol solutions.

-Study on those strains showed a more intense activity on *Staphylococcus aureus* than on *Escherichia coli*, which proves the effectiveness of these biomaterials for the intended destinations, namely skin infections.

From the collagen hydrolyzers, biomaterials with multiple topical applications can be made in the form of hydrogels, membranes and collagen matrices, both from calf collagen and fish collagen. Biomaterials made from *Gray Mullet* fish in the Black Sea have notable advantages due to the antioxidant and antimicrobial properties they possess.

**References**