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Investigating the Quality and Safety Characteristics of Skin Collagen of Bighead (*Hypophthalmichthys nobilis*), Silver Carp (*Hypophthalmichthys molitrix*), Grass Carp (*Ctenopharyngodon idella*), and Common Carp (*Cyprinus carpio*) Cultured

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Abstract

Collagen has diverse general and biomedical applications and its important role in the future of society have made it a key biopolymer for human health and well-being. Therefore, the present study was conducted with the objectives of extracting collagen from the skin of farmed carp, determining the quality characteristics of collagen, and comparing them. Collagen was extracted from the skin of carp fishes by acidic enzymatic method using 0.5 M acetic acid and pepsin in 48 hours. Collagen treatments (5 treatments) included collagen prepared from the skin of common species, grass carp, bighead, silver, and cow (control). Collagen treatments were not capable of hemolysis and did not show toxic effects on human fibroblast cells. Heavy metals (0.01-0.18 ppm) in collagen extracted from cultured carp species were within the standard range. The color (brightness) of experimental collagen (92.74-93.68) and control (92.38) showed no significant difference ($p>0.05$). Amino acids cysteine and tryptophan were not observed in collagen. Glycine and hydroxylysine amino acids (352 and 3 residues 1000g-1, respectively) had the highest and lowest amounts in collagen. Amino acids profile and collagen production efficiency (10.51-10.59%) did not show significant differences in carp fish species ($p>0.05$). Based on the results of the present study, production efficiency, safety and quality characteristics of collagen in cultured carp species did not show any significant difference ($p>0.05$), and no significant difference was observed between these characteristics and the control ($p>0.05$). Therefore, the skin of these species can be used to produce collagen and introduce it to the industry as a substitute for mammalian collagen.

Keywords: Acid hydrolysis, Cultured carp, Collagen, Pepsin enzyme, Safety

Introduction

The oceans are home to billions of plants, animals, and microorganisms, which are a huge source of natural compounds. The discovery of the ocean as an abundant source of biological compounds has a progressive effect on the research of marine biomolecules. Collagen is derived from the kola Greek word, which means glue and the French term collagen was initially described as a component of connective tissue that provides gelatin during cooking (Carvalho *et al*., 2018). Also, collagen

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is defined according to the connection with specific groups, which are defined based on the complexity and structural diversity, the presence of non-helical parts, the functions, and the ability to connect the supramolecular structures that are assigned to them. About a quarter of the total protein in mammals is collagen, which is the most abundant protein in the body and a key component of connective tissue. Type 1 collagen constitutes 90% of the collagen in the human body, and accounts for approximately 25-35% of the total protein in the fish body (Song *et al*., 2019). Marine collagen is obtained from skin, cartilage, bone, and fish scales. Collagen comprises 50-70% of cartilage and skin and 80-90% of tendons and bones without minerals. In addition, it is possible to produce it from marine vertebrate and invertebrate sources. Marine collagens have attracted much attention during the past decade, and the search for exploitation of marine collagen has increased since 2014 (Xu *et al*., 2021).

Collagen has a wide range of applications and is widely used in cosmetic and hygiene industries, ophthalmology (corneal filling), pharmaceutical, medicine (wound healing, biomedicine, preparation of medical supplies and regenerative medicine, orthopedics, surgery to make artificial skin and treatment of severe burns), prolotherapy as a strengthening of loose ligaments, blood coagulant cotton textiles, injected for the treatment of soft tissue abscess, absorbable wound dressing, (a barrier to prevent epithelial migration, helps cells to regenerate the damaged area and to build ligaments and tendons), dentistry (dental bone fillers, implants, making a permeable membrane for rebuilding the soft tissue around the periodontal gum (gum disease) and dressing pulled teeth), beauty (face mask, etc), biotechnology, tissue repair, cell migration (necessary for tissue maintenance), tissue engineering (biopolymer) and food (production of functional products). Collagen also plays important roles in cellular processes, including immune response and cell communication (Mullen *et al*., 2019; Govindharaj *et al*., 2019; Raman *et al*., 2018).

The molecular weight of collagen is 300 kilodaltons, whose length, width, and thickness are 300 nm, and the total number of amino acids in this molecule is about 3000 units. Collagen is the most abundant structural protein in animals. About 28–30 types of collagen are known, all of which are composed of three α-helices (tropocollagen) that, once secreted into the extracellular environment, can assemble into their final supermolecular organization (Cumming *et al*., 2019). Collagen molecules are naturally composed of three long helical chains of amino acid residues with non-helical ends at both ends and make different collagen structures. The basic structure of collagen consists of amino acids, mainly glycine (33%), proline, and hydroxyproline (22%). The secondary structure consists of amino acid chains that are connected in a spiral with three amino acids. They twist around each other and form a strong third structure. The basic structure of collagen is the quaternary structure related to the superhelix. The variety of collagen types is also due to the difference in the expression of genes involved in protein biosynthesis. In addition, post-translational modifications of collagens also have a significant effect on collagen diversity (Lupu *et al*., 2019; Lin *et al*., 2019). Differentiating the nature of collagen is determined by the presence of different chains, isoforms, and molecular structures of each type of collagen. At least 46 unique polypeptide chains have been found in collagen from different animals. Collagen chains are usually composed of the repeating motif Gly-X-Y, where Gly is the amino acid glycine, while X and Y are the amino acids proline and 4-hydroxyproline, respectively (Wu *et al*., 2019). This motif is distinct from other extracellular matrix components. The chains of different types of collagens differ in composition, depending on the number of repeats and the length of the fragment containing the Gly-X-Y

motif, and the amino acid residues present at the X and Y positions (Sousa *et al*., 2020).

The qualitative characteristics of collagen obtained from marine sources compared to collagen obtained from cow and pig sources include the same composition of amino acids of higher heat resistance, non-transmission of zoonotic diseases such as spongiform encephalopathy, being made of smaller peptide units compared to other collagens, easy digestion, absorption and distribution in the human body, having a large amount of amino acids lysine, glycine, proline and hydroxyproline, the essential role of lysine in the production of this protein, high biocompatibility, solubility in water, antimicrobial properties, preventing the penetration of microorganisms into wounded and damaged tissue, skin enlightenment and moisturizing, high tensile strength, high and adjustable mechanical properties, resistance to acid treatments, natural, non-allergenic, very high capacity to maintain the moisture of the skin, does not irritate the human skin, suitable for skin applications, repeatability of its effects, anti-aging and wrinkles of the skin of the face and around the eyes, non-toxicity and biodegradability (Chinh *et al*., 2019; Sukhikh *et al*., 2021). Therefore, the present study was conducted with the objectives of extracting collagen from the skin of farmed carp, determining the quality characteristics of collagen based on the hydrolysis time, and comparing them.

Materials and Methods

The waste materials needed for the preparation of collagen were obtained from the model aquatic market located in Rasht. The sample was transferred to the laboratory using Unilith-containing ice at a ratio of 2 times the weight of the skin. All extraction steps were performed at 0-4 ^{0}C. The fish skin was washed with cold water and divided into small pieces of 0.5 x 0.5 cm. For pretreatment, 0.1 m sodium hydroxide was used, which is the ratio of sample to alkali in the ratio of 1 to 10 (volume/weight). The mixture was continuously stirred for 6 hours. The alkaline solution was changed every two hours. Then, the alkali-treated skin was washed with cold water until a neutral pH. The treated skin was placed in 10% butyl alcohol, where the ratio of solid to solvent was 1:10, for 18 hours. The solvent was changed every 6 hours. The defatted skin was washed with cold water before lyophilization until a neutral pH. Collagen was extracted from the skin of cultured carp fishes by an acid-enzymatic method based on the method of Wei *et al.* (2019). To extract collagen, the lyophilized skin was placed in 0.5 M acetic acid, which has a 1:10 ratio of sample to solvent and contains pepsin (10 g per 100 g of lyophilized skin), for 48 hours. Five treatments were considered for this study. The treatments include collagen prepared from species of carp including grass carp, bighead, common and silver carp. Bovine collagen (imported) was used as a control treatment. The treatments were carried out in 3 repetitions. The quality of collagen treatments obtained through colorimetric tests using Hunter Lab (Gilbert, 2014), blood hemolysis by culture on blood agar medium (Momtahan *et al*., 2016), investigation of toxicity through culture on human fibroblasts cells (Chotphruethipong *et al*., 2022), amino acids profile using HPLC (Iranian National Standard No. 10699. 2015) and the heavy metals including arsenic, lead, cadmium, mercury, bismuth, antimony, tin, molybdenum, copper, chromium, cobalt and nickel were evaluated by acid chemical digestion method (Iranian National Standard No. 12014. 1999; AOAC, 2000). Collagen production efficiency was also determined by the hydroxyproline calculation method (Qiu *et al*., 2014). The culture media and required chemicals were obtained from Merck.

Cell culture

Human dermal fibroblast cells were cultured in T-75 flasks using Dulbecco's complete modified Eagle's medium (DMEM) containing 10% fetal bovine serum (100 units/ml), penicillin, and streptomycin at 100 µg ml^{-1} and 2 ml of L-glutamine. The cells were incubated in a humidified 5% carbon dioxide incubator (Binder Model C 170, Binder Inc, Bohemia, NY, USA) at a temperature of 37°C. Then they were cultured using a trypsin-EDTA solution of 0.25% at a concentration of 80-90%. Cell proliferation assay of collagen at concentrations of 50, 100, 200, 400, and 800 µg ml-1 were tested for cytotoxicity on human skin fibroblast cells. A culture medium without collagen was used as a control. These concentrations were tested using the MTT method. The result was reported as the percentage of cell proliferation compared to the control (Andini *et al*., 2020).

MTT method (methyl thiazolyldiphenyl-tetrazolium bromide)

After removing the supernatant from the cell culture medium, 100 µl of MTT solution was added to the cultures and kept warm for 2 hours. The cells were dissolved using 200 µl of DMSO and 100 µl of the gel was then transferred to a 96-well plate. The color expansion was determined photometrically at 540 nm. WEST-1 reagent was mixed in 5 ml of electrolyte solution and 10 µl of it was pipetted on the gels along with 90 µl of the culture medium (after removing the original culture medium). In the next step, it was incubated for 2 hours. Then the cultures were agitated for 5 minutes (100 rpm) and 100 µl of the supernatant was transferred to a 96-well plate. Photometric measurement was done at 450 nm. The initial cell density planted in collagen hydrogels on polyamide meshes was evaluated microscopically. The criterion for evaluating the best cell density was the smaller cell number and no empty cells (cell layer) between the mesh threads (ISO 10993-5).

Amino acid profile

Amino acids were measured by the electrospray ionization method. In this way, 0.5 g of the sample was acid hydrolyzed with 4 ml of hydrochloric acid solution for 24 hours at 110°C. When the sample temperature reached 24°C, it was centrifuged (Hettich Universal 320, Germany) at 4000 rpm (power gram: 3756/48) for 5 minutes. In the next step, 10 µl of the supernatant along with 1 ml of distilled water was transferred to the sample. Except for isoleucine and histidine, which included the standard leucine and 3-methylhistidine, stable isotope mixtures of each amino acid were considered as standards for calibration. After hydrolysis, calibration standards and samples were prepared by transferring 50 µl of the hydrolyzed or diluted standard to the sample. Then 50 µl of labeled stable isotope mixture as standard and 700 µl of non-centrifuged reagent were added to the sample for 5 seconds. Then, 3 µl of the prepared sample was kept at 30 ^{0}C, for amino acid analysis, and was injected into the C18 column of HPLC (- HP 1100, Germany). Mobile phases at a flow rate of 0.7 ml/min were used for separation through high-pressure liquid chromatography, and amino acids were determined at a wavelength of 254 nm (Iranian National Standard No. 10699. 2015).

Determination of yield

To determine the yield, the ratio of collagen hydroxyproline to hydroxyproline of raw skin was evaluated. The hydroxyproline content of extracted collagens was determined using a slightly modified version of the method described by Nalinanon *et al*. (2007). The samples were hydrolyzed with 6 M hydrochloric acid for 24 hours at 110° in an oven (Memmert U10, Schwabach, Germany). The hydrolyzate was clarified before filtering through Whatman No. 4 filter paper using activated carbon. To reach a pH equal to 6- 6.5, the filtered liquid was neutralized with sodium

hydroxide 10 M and 1 M. The neutralized sample (0.1 ml) was transferred to a test tube and isopropanol (0.2 ml) was added and mixed thoroughly.To the mixture 0.1 ml of an oxidant solution (a mixture of 7% (w/v) chloroamine T (w/v) and acetate/citrate buffer, pH 6, at a ratio of 1:4 (v/v)) was added and mixed thoroughly Subsequently, 1.3 ml of Ehrlich's reagent (mixture of solution A and B) was added and mixed thoroughly. The mixture was stirred and heated in a water bath (Memmert GmbH, Schwabach, Germany) at 60°C for 25 min before cooling with running water for 2-3 min. The solution was diluted to 5 ml with isopropanol. The absorbance was measured At a wavelength of 558 nm. Also, the standard curve of hydroxyproline with different concentrations from 10-60 ppm was prepared. Hydroxyproline content was expressed as mg g^{-1} of the sample (Qiu *et al*., 2014). Distilled water was used as a blank.

Determination of heavy metals

The acid chemical digestion determined the amounts of heavy metals arsenic, lead, cadmium, mercury, bismuth, antimony, tin, molybdenum, copper, chromium, cobalt, and nickel. To the ash (20 g) was added 50 ml of 6 M hydrochloric acid. To evaporate the acid the flask was placed in a water bath. Then 30 ml of 0.1 M nitric acid was added to this. Croze was placed in a water bath for 15 minutes. After this step, the container containing the sample was covered with aluminum foil and was placed in the environment temperature for 2 hours. Then, the contents of the flask were mixed using a glass rod. The cooled sample was filtered. The sample was transferred to a flask and filled with deionized 2 times distilled water. It was shaken to homogenize. Then, an optical atomic absorption spectrometer with a graphite furnace (Japan/ZA3700) was used to measure the light absorption of heavy metals (Wavelength of 390-410 nm) based on the calibration curve. The measuring range of the device for heavy metals is 0.15-1-30 ppm and the recycling percentage is 80-107 percent. The limit of Quantitation values is 0.02-0.01 mg kg-1 (Iranian National Standard No. 12014. 1999; AOAC, 2000).

Colorimetry (amount of color substance)

The color of the samples was determined by the Hunterlab device (color flex model USA). Color intensity was expressed using Hunter's parameters in terms of brightness (L), red-green (a), and blue-yellow (b) (Gilbert, 2014).

Blood hemolysis

A suspension was prepared from collagen in concentrations of 50, 100, 200, 400, and 800 µg ml^{-1} in distilled water. Wells were created on the blood agar culture medium. 0.5 ml of collagen solution was poured into the well. The plate was incubated at 37°C for 48 hours. The formation of a clear halo around the well-containing collagen indicates hemolysis and a positive test.

Statistic analysis

In the present study, the obtained results were analyzed using SPSS version 25 software. The results were analyzed at a significant level of 95%. One-way analysis of variance was used to compare the results of color and heavy metal tests between experimental and control treatments at a significant level of 5%. The results were expressed as mean with standard deviation.

Results and Discussion

As shown in Table 1, the amount of heavy metals in the treatments of collagen extracted from different species of carp showed no significant difference ($p>0.05$). The amount of heavy metals in the collagen of cultured carp was within the acceptable range according to the Food and Drug Administration. Although the proximity of fish breeding ponds to areas with a lot of agricultural activity or rural residential areas, the infiltration of agricultural effluents containing chemical fertilizers used in

agriculture and rice paddies, including phosphate fertilizers containing cadmium, into underground water and the entry of untreated rural sewage into the underground water, they cause an increase in the concentration of heavy metals in water and subsequently in fish.

Table 1- The results of heavy metals in collagen extracted from the skin of cultured and control carp (ppm)

Treatment Index	Bighead collagen	Common carp collagen	Grass carp collagen	Silver Carp collagen	Control	FDA limit (ppm)
Arsenic	0.07 A	0.09 A	0.21 A	0.2 A	0.06 A	3
Lead	0.02A	0.11 A	0.08 A	0.06 A	0.01 A	10
Cadmium	0.05 A	0.01 A	0.02 A	0.19 A	0.02 A	0.5
Mercury	0.12 A	0.20 A	0.05 A	0.10 A	0.05 A	1
Bismuth	0.13 A	0.02 A	0.09 A	0.02 A	0.07 A	Not determined
Antimony	0.12 A	0.02 A	0.07 A	0.04 A	0.05 A	Not determined
Tin	0.05 A	0.05 A	0.01 A	0.02 A	0.15 A	Not determined
Molybdenum	0.01 A	0.06 A	0.01 A	0.13 A	0.18 A	Not determined
Copper	0.07 A	0.02 A	0.11A	0.15 A	0.16 A	Not determined
Chrome	0.05 A	0.08 A	0.14 A	0.21 A	0.14 A	50
Cobalt	0.04 A	0.03 A	0.17 A	0.19 A	0.12 A	5
Nickel	0.09 A	0.01 A	0.02 A	0.18 A	0.13 A	0.2

Dissimilar uppercase letters in a row indicate a significant difference at the 5% level ($p<0.05$).

However, much data showed that environmental conditions are effective in the absorption and accumulation of heavy metals by fish. Heavy metals exist in surface waters in colloidal, particulate, and dissolved forms, whose behavior in natural waters depends on water chemistry such as pH, hardness, temperature, and other factors. During transport in water, these metals undergo many changes, such as dissolution, river flow, metal concentration and oxidation potential and regeneration of the river environment, sedimentation, surface absorption, and complex formation with water or soil compounds, which affect their behavior and bioavailability. In addition, the skin of farmed carp contains a lot of mucous material and prevents the binding of metals to the fish skin (Seifzadeh *et al*., 2018). Carvalho *et al.* (2018) reported the levels of mercury, lead, cadmium, and arsenic in the collagen extracted from cod fish skin were in the acceptable range. These researchers stated that the amount of lead, cadmium, mercury, and arsenic were less than 2.5 ppm, 0.25 ppm, 0.50 ppm, and 0.35 ppm, respectively. Dervan and Gulay (2023) randomly purchased 25 samples of commercial fish collagen and measured the amount of zinc, lead, arsenic, cadmium, and mercury. They did not find lead, arsenic, and mercury in the samples. The amount of cadmium in the samples was between 0.152-0.288 mg kg^{-1} and the amount of zinc was between 1.368-2673 mg kg^{-1}. The results of these researchers were higher compared to the results obtained from the measurement of heavy metals in collagen (0.01-0.18 ppm) of the present study. This difference can be due to the pollution of the primary source used for collagen production (fish skin), which may originate from the pollution of the fish habitat with sewage. In addition, cadmium is a metal that occurs naturally in the earth's crust, which may be another reason for fish contamination.

The collagen of the common, bighead, grass carp, and silver species and control did not show toxic effects on human fibroblast cells and did not cause proliferation of fibroblast cells (Table 2). Based on the obtained results, the collagen of the common, bighead, grass carp and silver species and control species did not have the ability of hemolysis.

Table 2- Examination of the toxicity (Cell Proliferation) of collagen extracted from the skin of cultured carp species and control samples

Index	Cell Proliferation					
	Concentration (µl)					
Treatment	0	50	100	200	400	800
Common carp collagen	0.07±0.12aA	0.09±0.13aA	0.16±0.18aA	0.02±0.15aA	0.01±0.13aA	0.11±0.19aA
Grass carp collagen	0.06±0.17aA	0.02±0.11aA	0.21±0.10aA	0.01±0.14aA	0.02±0.16aA	0.09±0.23aA
Bighead collagen	0.09±0.10aA	0.05±0.20aA	0.12±0.19aA	0.03±0.17aA	0.09±0.19aA	0.08±0.24aA
Silver Carp collagen	0.05±0.22aA	0.14±0.15aA	0.23±0.17aA	0.05±0.12aA	0.01±0.08aA	0.04±0.16aA
Control	0.03±0.09aA	0.12±0.17aA	0.18±0.25aA	0.07±0.11aA	0.04±0.03aA	0.11±0.14aA

The results are presented as mean ± standard deviation.

Dissimilar uppercase letters in a row and dissimilar lowercase letters in a column indicate a significant difference at the 5% level ($p<0.05$).

Safety is one of the basic features that can be mentioned to check the quality of collagen. Since no toxic compounds are used during the various stages of carp breeding, and the compounds used to prepare collagen do not have toxic effects, therefore these features were not observed in collagen. Andini *et al.* (2020) investigated collagen prepared from the skin and scales of snakehead fish (*Channidae*) and did not observe toxicity effects. Chotphruethipong *et al.* (2022) investigated the toxic effects of salmon skin collagen on human fibroblast cells and did not determine the toxic effects. Ferreira *et al.* (2022) investigated the toxicity effects of collagen prepared from Guriguba (*Hexanematichthys parkeri*) swimming bladder on fibroblast cells and did not determine toxicity effects. Wang *et al.* (2020) evaluated the toxicity effects of collagen sponge on L929 cells of mouse fibroblast cells and observed that the sponge has no toxicity effects on the skin. Carvalho *et al.* (2018) investigated the toxic effects of collagen extracted from Codfish skin on MRC-5 lung fibroblast cells and reported the collagen to be safe for biological applications. Song *et al.* (2019) investigated the toxicity of collagen prepared from tilapia fish skin on mouse fibroblast L929 cells and human umbilical vein endothelial cells (HUVEC) and did not observe toxicity effects. The compatibility of biological materials in contact with blood is one of the most important criteria for their successful application in the body. Hemolysis refers to the release of hemoglobin from red blood cells due to their rupture. A low rate of hemolysis indicates that fewer red blood cells have been destroyed, which indicates that the biological product (collagen) is a material with favorable blood compatibility. Therefore, blood lysis is considered a new method to evaluate the toxicity of biological compounds (Momtahan *et al.*, 2016; Weber *et al.*, 2018). In the present study, collagen does not cause blood hemolysis. Therefore, Marine collagen is a safe and generally well-tolerated supplement. Wang *et al.* (2020) used a sponge prepared using tilapia fish skin collagen to investigate human wounds and found that the collagen sponge does not have hemolytic properties, and can be used as a blood coagulant. Li *et al.* (2017) showed that hemostatic effects were improved in collagen enriched with oxidized microcrystalline cellulose. Considering that no toxicity effects were observed in the present study, the results of these researchers are consistent with the results of the present study.

Table 3- Colorimetric study of collagen extracted from the skin of cultured carp species and control samples

Treatment Index	Bighead collagen	Common carp collagen	Grass carp collagen	Silver Carp collagen	Control
b (yellow bluish-)	1.86±0.93 A	1.82±0.95 A	1.85±0.42 A	1.81±0.24 A	1.47±0.73 A
a (red yellowish)	1.98±0.79 A	1.73±0.90 A	1.95±0.78 A	1.71±0.42 A	1.52±0.84A
Light	93.68±0.89A	92.76±0.98A	93.66±0.78 A	92.74±0.26A	92.38±0.71A

The results are presented as mean ± standard deviation.

Dissimilar uppercase letters in a row indicate a significant difference at the 5% level ($p<0.05$).

In terms of color, the quality of collagen can be measured. There are three values in the color scale including L (lightness), a (green-red), and b (blue-yellow), which were considered in the present study. As can be seen in Table 3, the transparency of collagen in the experimental treatments (92.74-93.68) and the control (92.38) did not show a significant difference ($p>0.05$). According to studies conducted by other researchers, there is a correlation between collagen microstructure and its transparency. Changes in collagen transparency indicate slight differences in the concentration of collagen compounds. Since the collagen was extracted from different types of carp fishes, and the concentration of amino acids detected in different collagen treatments did not differ significantly, hence the transparency did not show a significant difference in these treatments. Ramle *et al.* (2022) investigated the color characteristics of collagen prepared using acetic acid from the skin of Needlefish (*Tylosurus acus melanotus*) and reported L, a, and b as 69.77, 0.73, and 4.69 respectively. In the present study, the values of L, a, and b in carp skin collagen were determined as 92.74-93.68, 1.71-1.98, and 1.81-1.86, respectively (Table 3). The characteristics of color in the present study were evaluated better compared to other studies. The difference in the results of this study with the results of the other studies may be due to differences in the extraction method, fish species, and genetic diversity.

Table 4- Collagen production efficiency from the skin of farmed carp species and control samples%

Species	Common carp	Silver Carp	Bighead	Grass carp
Efficiency	10.59 A	10.56 A	10.57 A	10.51 A

Dissimilar letters in a row indicate the existence of a significant difference at the 5% level ($p<0.05$).

As shown in Table 4, collagen yield did not show any significant difference between experimental treatments ($p>0.05$). Hydroxyproline is unique to collagen as an amino acid, and its amount is not high in other proteins. It was considered to check collagen efficiency (Silva *et al.*, 2014). Ahmed *et al.* (2019) reported that collagen production efficiency from big eye tuna skin extracted with 0.5 M acetic acid at 4 C° for 48 hours and a pepsin concentration of 0.2 g per gram of skin was 16.7%. They also expressed 13.5% collagen production efficiency from tuna skin extracted with 0.5M acetic acid at 4°C for 72 hours. Tan *et al.* (2018) found the production efficiency of collagen from Catfish skin extracted with hydrochloric acid at 0.118 -23.6 IU g-1 pepsin concentration and 4°C to be 59.03%, and the yield of collagen production from Catfish skin extracted with hydrochloric acid at 4°C and 60 hours was found to be 5.00 -

42.36%. Junianto *et al.* (2018) found the extraction efficiency of collagen from Nilem fish skin extracted with 0.5, 0.7, and 0.9 M acetic acid in concentrations of 0.5, 1, and 1.5% pepsin and under 4°C 5.25-6.18%. Zhang *et al.* (2019) determined the production efficiency of collagen from golden pompano skin extracted with 0.5 M acetic acid at 4 °C and within 48 hours 21.81%. Arumugam *et al.* (2018) expressed the production efficiency of collagen from sole fish skin using 0.5 M acetic acid at 25°C and 32 hours 19.9%. Oslan *et al.* (2022) reported the yield of acid-soluble collagen from the skin of Purple-Spotted Bigeye Snapper to be 5.79% and pepsin-soluble collagen to be 6.65%. In the present study, 48 hours were spent for collagen extraction, and the collagen extraction efficiency (10.51-10.59%) did not show any significant difference between different species of carp. Researchers have expressed the difference in the obtained results under the influence of various parameters such as temperature, stirring, time, solvent-to-solid ratio, and solvent concentration, which affect collagen extraction efficiency (Menezes *et al.*, 2020).

Table 5- The profile of collagen amino acids extracted from the skin of farmed carp species (Residues 1000g^{-1})

Samples Amino acids	Bighead collagen	Common carp collagen	Silver Carp collagen	Bighead collagen	Grass carp collagen
		Non–essential amino acids			
Aspartic acid	45A	43B	43B	43B	43B
Tryptophan	20A	0B	0B	0B	0B
Serine	33A	28B	28B	29B	29B
Glutamic acid	75A	74A	74A	74A	74A
Glycine	330B	352A	352A	352A	352A
Cysteine	1.24A	0B	0B	0B	0B
Arginine	50B	52A	52A	52A	52A
Proline	121B	125A	125A	125A	125A
Alanine	119B	129A	129A	129A	129A
Tyrosine	3A	4A	4A	4A	4A
		Essential amino acids			
Valine	21A	20A	20A	20A	20A
Isoleucine	11A	9B	9B	9B	9B
Histidine	5B	9A	9A	9A	9A
Lysine	26B	32A	32A	32A	32A
Leucine	23B	27A	27A	27A	27A
Methionine	6B	12A	12A	12A	12A
Phenylalanine	3B	7A	7A	7A	7A
Threonine	18B	20A	20A	20A	20A
Hydroxyproline	94A	78B	78B	78B	78B
Hydroxylysine	7A	3B	3B	3B	3B
Imino acids	215A	193B	193B	193B	193B

Dissimilar uppercase letters in a row indicate the existence of a significant difference at the 5% level ($p<0.05$).

As can be seen in Table 5, the amino acids in the collagen extracted from the skin of farmed carp did not show any significant difference with each other ($p>0.05$). Tryptophan and cysteine were not observed in carp collagen. Amino acids glycine, alanine, proline, and hydroxyproline had the highest amounts. Hydroxylysine had the lowest amount. In general, collagen and proteins absorb a lot of ultraviolet rays due to peptide connections and side chains. Protein absorbs light at a wavelength of 280 nm in the best way. Also, amino acids such as histidine, tryptophan, phenylalanine, and tyrosine have absorption bands between 250 and 288 nm. While the collagen extracted from the skin has a lower absorption wavelength because the amino acids

glycine, proline, and hydroxyproline form the triple helix of collagen, the maximum absorption of which occurs at a wavelength of 230 nm (Rosmawati *et al.*, 2018). In the collagen samples of the present study, the maximum absorption peak was observed between 230 and 240 nm, which was influenced by the simultaneous use of acetic acid and pepsin in the extraction of collagen from fish skin. In addition, absorption values between 200 and 220 nm were attributed to structural materials such as ester or carboxylic compounds. Fish collagen varies widely in amino acid composition. According to studies conducted by other researchers, the living environment has a strong effect on the composition of amino acids in collagen extracted from fish species (García-Sifuentes *et al.*, 2016). However, since the species under study were cultivated species of the same fish that were raised in similar water environments, therefore, the composition of collagen amino acids extracted from their skin did not show any difference. Data on the amino acid composition of pure collagen showed that collagen contains tryptophan, which is a rare amino acid in collagen extracted from carp fish scales. Since the skin of the fish contains scales, This amino acid along with cysteine should have been determined in the collagen amino acid profile, but because the pre-treatment stage and successive washes caused the scales to be separated from the skin, therefore these amino acids were not included in the collagen amino acid profile in the present study (Gauza Włodarczyk *et al.*, 2017). Chinh *et al.* (2019) showed the amino acid sequence in the collagen obtained by acid method from freshwater carp scales as threonine (21), proline (110), glutamic acid (77), arginine (50), serine (38), alanine (117), glycine (306), aspartic acid (49), cysteine (32), histidine (6), lysine (26), leucine (24), phenylalanine (15), valine (19), identified isoleucine (12), methionine (12), tyrosine (21) and hydroxyproline (89) residues $1000g^{-1}$ in the collagen of carp scales. In the present study, cysteine was not observed, aspartic acid (43), glutamic acid (74), tyrosine (4), isoleucine (9), threonine (20), and hydroxyproline (78 residues per $1000g^{-1}$ were observed in smaller amounts. Hydroxylysine (3) and leucine (27) were also determined. Serine (28) and methionine (12) residues $1000g^{-1}$ were reported in the same amount in different treatments. Glycine (352), arginine (52), proline (125), alanine (129), valine (20), histidine (9), and lysine (32) residues $1000g^{-1}$ were observed in more quantity. Moses *et al.* (2018) analyzed the amino acid composition of tilapia collagen and showed that glutamic acid (11.96–15.14 g $100g^{-1}$) and aspartic acid (7.69-9.427.69 g $100g^{-1}$) are the most amino acids of collagen. In the present study, glycine and alanine accounted for the highest amounts of amino acids with 352 and 129 residues $1000g^{-1}$, respectively. Carvalho *et al.* (2018) reported the amino acid sequence in collagen extracted from cod fish including alanine (91.48), arginine (30.45), aspartic acid (38.82), cysteine (1.28), glutamic acid (56.08), glycine (266.12), histidine (5/01), hydroxylysine (6.65), hydroxyproline (39.60), isoleucine (5.61), leucine (16.51), lysine (19.62), methionine (15.04), valine (12.02), tyrosine (2.25), threonine (16.89), serine (53.87), proline (62.69), phenylalanine (12.70), and n-isobutyl glycine (13.75) residues $1000g^{-1}$. In the results of this study compared to other studies isobutyl glycine and cysteine were observed in collagen. The amount of amino acids alanine, aspartic acid, glycine, hydroxyproline, isoleucine, leucine, lysine, valine, threonine, and proline were higher in collagen. However, the amount of amino acids arsenine, glutamic acid, histidine, hydroxylysine, methionine, tyrosine, serine, and phenylalanine was lower. The difference in the amino acid profile of the present study compared to the results of other studies can be might be due to the difference in the primary source of collagen production, species, tissue, and genetic diversity. Chuaychan *et al.* (2016)

reported that collagen extracted using acid and pepsin from spotted golden goatfish scales had a high glycine content (196 and 198 (residues $1000g^{-1}$, respectively). In the present study, collagen had a high amount of glycine (352 residues $1000g^{-1}$). The difference in the results of the present study with the aforementioned study was due to the use of a combination of acid and pepsin for collagen extraction.

Conclusion

The safety features and other quality features such as transparency and aggregation were not significantly different between carp species and were within the acceptable range. Also, the yield of collagen extraction did not show any significant difference between different species of carp. A time of 48 hours is suggested for extracting collagen from cultured carp and replacing it with mammalian collagen.

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References

1. Ahmed, R., Haq, M., & Chun, B.S. (2019). Characterization of marine derived collagen extracted from the by-products of bigeye tuna (*Thunnus obesus*). *International Journal Biological Macromoleculs, 135(1),* 668–676. https://doi.org/10.1016/j.ijbiomac.2019.05.213
2. Andini, A., Prayekti, E., Wulandari, D.D., & Nidianti, E. (2020). Cytotoxicity assay using brine shrimp lethality test on collagen chitosan wound dressing sterilized by ultraviolet Light. *Indonesian Journal of Medical Laboratory Science and Technology, 2(1),* 21-26. https://doi.org/10.33086/ijmlst.v2i1.1467
3. Association of Official Analytical Chemists (AOAC). (2000). Fish and Marine Products Treatment and Preparation of Sample. *AOAC Standard No. 937.07*. Washington, DC: U.S. Patent and Trademark Office.
4. Arumugam, G.K.S., Sharma, D., Balakrishnan, R.M., Babu, J., & Ettiyappan, P. (2018). Extraction, optimization, and characterization of collagen from sole fish skin. *Sustainable Chemistry and Pharmacy, 9(2018),* 19–26. https://idr.nitk.ac.in/jspui/handle/123456789/11243
5. Blanco, M., Vázquez, J.A., Pérez-Martín, R.I., & Sotelo, C.G. (2017). Hydrolysates of fish skin collagen: an opportunity for valorizing fish industry byproducts. *Marine Drugs, 15(5),* E131. https://doi.org/10.3390/md15050131
6. Carvalho, A.M., Marques, A.P., Silva, T.H., & Reis, R.L. (2018). Evaluation of the potential of collagen from Codfish skin as a biomaterial for biomedical applications. *Marine Drugs, 16(12),* 495. https://doi.org/10.3390/md16120495
7. Cao, J., Duan, Q., Liu, X., Shen, X., & Li, C. (2019). Extraction and physicochemical characterization of pepsin soluble collagens from golden Pompano (*Trachinotus blochii*) skin and bone. *Journal Aquatic Food Product Technology, 28(3),* 837–847. https://doi.org/10.1080/10498850.2019.1652216
8. Chinh, N.T., Manh, V.Q., Trung, V.Q., Lam, T.D., Huynh, M.D., Tung, N.Q., Trinh, N.D., & Hoang, T. (2019). Characterization of collagen derived from tropical freshwater Carp fish scale Wastes and its amino acid sequence. *Natural Product Communications, 14(7),* 1–12. https://doi.org/10.1177/1934578X19866288
9. Chotphruethipong, L., Hutamekalin, P., Nilsuwan, K., Sukketsiri, W., Aluko, R.E., Abdul, N.R., & Benjakul, S. (2022). Combined effects of defatted hydrolyzed collagen from salmon skin and

vitamin C on proliferation and migration of human fibroblast cell. *Fishes, 7(5)*, 265. https://doi.org/10.3390/ fishes7050265
10. Chuaychan, S. (2016). *Production and characterization of collagen, gelatin, and gelatin hydrolysate powder from scales of spotted golden Goatfish*. Feculty of Food Science and Technology, Prince of Songkla University, Songkhla, Thailand.
11. Cumming, M.H., Hall, B., & Hofman, K. (2019). Isolation and characterisation of major and minor collagens from Hyaline Cartilage of Hoki (*Macruronus novaezelandiae*). *Marine Drugs, 17(4)*, 223. https://doi.org/10.3390/md17040223
12. Devran, D., & Gulay, O.Y. (2023). Investigation of possible heavy metals and antibiotic residues in commercial collagen. *Mehmet Akif Ersoy Üniversitesi Veteriner Fakültesi Dergisi, 8(1)*, 31–36. https://doi.org/10.24880/maeuvfd.1218939
13. Junianto, J., Iskandar, I., Rizal, A., & Damayanti, W. (2018). The influence of concentration of acetic acid and pepsin enzyme in Nilem fish skin collagen extractionto the amount of rendement produced. *World News Natural Science, 21(2018)*, 164–170.
14. Ferreira, A.C., Bomfim, M.R.Q., & da Costa Sobrinho, C.H.d.B. (2022). Characterization, antimicrobial and cytotoxic activity of polymer blends based on chitosan and fish collagen. *AMB Express, 12(1)*, 102. https://doi.org/10.1186/s13568-022-01433-7
15. García-Sifuentes, C.O., Lugo-Sánchez, M.E., Scheuren-Acevedo, S., Martínez-Porchas, M., & Peralta-Martínez, V. (2016). Amino acid profile of collagen fractions extracted from by-products of Ophistonemalibertate and Scomberjaponicus, CyTA. *Journal of Food, 14(2)*, 296-301. https://doi.org/10.1080/19476337.2015.1101711
16. Gauza-Włodarczyk, M., Kubisz, L., & Włodarczyk, D. (2017). Amino acid composition in determination of collagen origin and assessment of physical factors effects. *International Journal Biology Macromolecule, 104(PtA)*, 987–991. https://doi.org/10.1016/j.ijbiomac.2017.07.013
17. Gilbert, S.W. (2014). *Applying the hedonic method*. National Institute of Standards and Technology Technical Note 1811. https://doi.org/10.6028/nist.tn.1811
18. Iranian National Standard No. 12014. (1999). Heavy metal measurement of cosmetic products. Iranian Standard and Industrial Research Institute.
19. Iranian National Standard No. 10699. (2015). Livestock, poultry, and aquatic feed - measuring the amount of amino acids. Iranian Standard and Industrial Research Institute.
20. Gómez-Limia, L., Carballo, J., Rodríguez-González, M., & Martínez, S. (2022). Proximate composition and amino acid profle of European eel skin: infuence of body weight. *European Food Research and Technology, 248*(1053–1063), 1437–1446. https://doi.org/10.1007/s00217-022-03978-0
21. Govindharaj, M., Roopavath, U.K., & Rath, S.N. (2019). Valorization of discarded Marine Eel fish skin for collagen extraction as a 3D printable blue biomaterial for tissue engineering. *Journal of Cleaner Production, 230(2)*, 412–419. https://doi.org/10.1016/j.jclepro.2019.05.082
22. ISO 10993-6. (2016). *Biological evaluation of medical devices*. Part 6: tests for local effects after implantation.
23. Li, H., Cheng, W., & Liu, K. (2017). Reinforced collagen with oxidized microcrystalline cellulose shows improved hemostatic effects. *Carbohydr Polym, 165(2)*, 30–38. https://doi.org/10.1016/j.carbpol.2017.02.023
24. Lin, X., Chen, Y., Jin, H., Zhao, Q., Liu, C., Li, R., Yu, F., Chen, Y., Huang, F., Yang, Z., Ding, G., & Tang, Y. (2019). Collagen extracted from bigeye Tuna skin by isoelectric precipitation: physicochemical properties, proliferation, and migration activities. *Marine Drugs*, 17(5), 261. https://doi.org/10.3390/md17050261
25. Menezes, M.D.L.L.R., Ribeiro, H.L., Flávia de Oliveira, M., & de Andrade Feitosa, J.P. (2020). Optimization of the collagen extraction from Nile tilapia skin (*Oreochromis niloticus*) and its

hydrogel with hyaluronic acid. *Colloids and Surfaces B: Biointerfaces, 189(2),* 110852. https://doi.org/10.1016/j.colsurfb.2020.110852

26. Momtahan, N., Panahi, T., Poornejad, N., Stewart, M.G., Vance, B.R., Struk, J.A., Castleton, A.A., Roeder, B.L., Sukavaneshvar, S., & Cook, A.D. (2016). Using hemolysis as a novel method for assessment of cytotoxicity and blood compatibility of decellularized heart tissues. *ASAIO Journal, 62(3*), 340-8. https://doi.org/10.1097/MAT.0000000000000373
27. Moses, S., Agbaji, E.B., Ajibola, V.O., & Gimba, C.E. (2018). Amino acid composition and proximate analysis in Tilapia (*Oreochromis mossambicus*) Fish from dams and rivers in Zamfara state, Nigeria. *Journal Applied Science Environment Management, 22(6),* 8899–905. https://doi.org/10.4314/jasem.v22i6.10
28. Mullen, A., Bayon, Y., Pandit, A., & Raghunath, M. (2019). The collagen suprafamiliy: From biosynthesis to advanced biomaterial development. *Advanced Materials, 31(1),* 6–11. https://doi.org/10.1002/adma.201801651
29. Nalinanon, S., Benjakul, S., Visessanguan, W., & Kishimura, H. (2007). Use of pepsin for collagen extraction from the skin of bigeye snapper (*Priacanthus tayenus*). *Food Chemistry, 104(2),* 593-601. https://doi.org/10.1016/j.foodchem.2006.12.035.
30. Oslan, S.N.H., Shapawi, R., Mokhtar, R.A.M., Noordin,W.N.M., & Huda, N. (2022). Characterization of acid and pepsin-soluble collagen extracted from the skin of purple-Spotted Bigeye Snapper. *Gels, 8(10),* 665. https://doi.org/10.3390/gels8100665
31. Qiu, B., Wei, F., Sun, X., Wang, X., Duan, B., Shi, C., Zhang, J., Zhang, J., Qiu, W., & Mu, W. (2014). Measurement of hydroxyproline in collagen with three different methods. *Molecular Medicine Reports, 10(2),* 1157–1163. https://doi.org/10.3892/mmr.2014.2267
32. Raman, M., & Gopakumar, K. (2018). Fish collagen and its applications in food and pharmaceutical industry: A review. *EC Nutrition, 13(2018),* 752–767. https://api.semanticscholar.org/CorpusID:54176016
33. Rosmawati, Abustam, E., Tawali, A., & Said, M.I. (2018). Amino acid and collagen content of Snakehead (*Channa striata*) fish skin and bone. *Scientific Research Journal, 6(1),* https://api.semanticscholar.org/CorpusID:212409859
34. Ramle, S.Z., Oslan, S.N.H., Shapawi, R., Mokhtar, R.A.M., Noordin, W.N.M., & Huda, N. (2022). Biochemical characteristics of acid-soluble collagen from food processing by-products of Needlefish Skin (*Tylosurus acus melanotus*). *Appliued Science, 12(24*), 12695. https://doi.org/10.3390/app122412695
35. Seifzadeh, M., Golashahi, E., & Safiyari, Sh. (2018). Study the concentrations of lead and cadmium in farmed rainbow trout in Talesh of Guilan. *Animal Science Research Journal, 28(2),* 65–79. https://sid.ir/paper/365590/en
36. Song, W.K., Liu, D., Sun, L.L., Li, B.F., & Hou, H. (2019). Physicochemical and biocompatibility properties of type I collagen from the skin of Nile Tilapia (*Oreochromis niloticus*) for biomedical applications. *Marine Drugs, 17(3),* 137. https://doi.org/10.3390/md17030137
37. Sorushanova, A., Delgado, L., Zhuning, W., Shologu, N., Kshirsagar, A., Raghunath, R., Sousa, R.O., Alves, A. L., Carvalho, D.N., Martins, E., Oliveira, C., Silva, T.H., & Reis, R.L. (2020). Acid and enzymatic extraction of collagen from Atlantic cod (*Gadus morhua*) swim bladders envisaging health-related applications. *Journal of Biomaterials Science, Polymer Edition, 31(3),* 20–37. https://doi.org/10.1080/09205063.2019.1669313
38. Sukhikh, S., Noskova, S., Ivanova, S., Ulrikh, E., Izgaryshev, A., Larichev, T., Kozlova, O., Prosekov, A., & Babich, O. (2021). Comparative analysis of collagen-containing waste biodegradation, amino acid, peptide and carbohydrate composition of hydrolysis products. *Applied Science, 11(23),* 11511. https://doi.org/10.3390/app112311511

39. Tan, Y., & Chang, S.K.C. (2018). Isolation and characterization of collagen extracted from channel catfish (*Ictalurus punctatus*) skin. *Food Chemistry, 242(1)*, 147–155. https://doi.org/10.1016/j.foodchem.2017.09.013
40. Xu, N., Peng, X.L, Li, H.R., Liu, J.X., Cheng, J.S.Y., Qi, X,Y., Ye, S.J, Gong, H.L, Zhao, X.H., Yu, J., Xu, G., & Wei, D.X. (2021). Marine-derived collagen as biomaterials for human health. *Frontires Nutrition, 8(3)*, 702108. https://doi.org/10.3389/fnut.2021.702108
41. Zhang, W., Zheng, J., Tian, X., Tang, Y., Ding, G., Yang, Z., & Jin, H. (2019). Pepsin-soluble collagen from the skin of Lophius litulo: A preliminary study evaluating physicochemical, antioxidant, and wound healing properties. *Marine Drugs, 17(12)*, 708. https://doi.org/10.3390/md17120708
42. Wang, T., Yang, L., Wang, G., Han, L., Chen, K., Liu, P., Xu, S., Li, D., Xie, Z., Mo, X., Wang, L., Liang, H., Liu, X., Zhang, S., & Gao, Y. (2021). Biocompatibility, hemostatic properties, and wound healing evaluation of tilapia skin collagen sponges. *Journal of Bioactive and Compatible Polymers, 36(1)*, 44–58. https://doi.org/10.1177/0883911520981705
43. Weber, M., Steinle, H., Golombek, S., Hann, L., Schlensak, C., Wendel, H. P., & Avci-Adali, M. (2018). Blood-Contacting biomaterials: in vitro evaluation of the Hemocompatibility. *Frontires Bioenginering Biotechnology, 6(3)*, 99. https://doi.org/10.3389/fbioe.2018.00099
44. Wei, P., Zheng, H., Shi, Z., Li, D., & Xiang, Y. (2019). Isolation and characterization of acid-soluble collagen and pepsin-soluble collagen from the skin of hybrid sturgeon. *Journal of Wuhan University of Technology-Mater Science Edition, 34(2019)*, 950–959. https://doi.org/10.1007/s11595-019-2143-6
45. Wu, J., Kong, L., Zhang, J., & Chen,W. (2019). Extraction and properties of acid-soluble collagen and pepsin-soluble collagen from silver carp (*Hypophthalmichthys molitrix*) scales: Prerequisite information for fishery processing waste reuse. *Polish Journal Environmental Studies, 28(4)*, 2923–2930. https://doi.org/10.15244/pjoes/93742

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بررسی ویژگی‌های کیفی و ایمنی کلاژن پوست گونه‌های سرگنده (*Hypophthalmichthys nobilis*)، معمولی (*Cyprinus carpio*)، علف‌خوار (*Ctenopharyngodon idella*) و نقره‌ای (*Hypophthalmichthys molitrix*) کپور ماهیان پرورشی

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چکیده

تنوع زیاد کاربردهای کلاژن و نقش مهم آن در آینده جامعه، سبب شد که به پلیمر زیستی کلیدی برای سلامت و رفاه انسان تبدیل شود. بنابراین مطالعه حاضر با هدف استخراج کلاژن از پوست ماهی کپور پرورشی، تعیین خصوصیات کیفی کلاژن و مقایسه آنها انجام شد. کلاژن از پوست ماهی کپور به روش آنزیمی اسیدی با استفاده از اسید استیک ۰/۵مولار و پپسین طی مدت زمان ۴۸ ساعت استخراج شد. تیمارهای کلاژن (۵ تیمار) شامل کلاژن تهیه شده از پوست گونه‌های معمولی، علف‌خوار، سر گنده و نقره‌ای کپور ماهیان و گاو (شاهد) هستند. تیمارهای کلاژن قادر به همولیز خون نبودند و اثرات سمی روی سلول‌های فیبروبلاست انسانی نشان ندادند. فلزات سنگین (۰/۱۸–۰/۰۱ ppm) در کلاژن استخراج شده از گونه‌های کپور پرورشی در محدوده استاندارد قرار داشتند. رنگ (روشنایی) کلاژن آزمایشی (۹۳/۶۸–۹۲/۷۴) و شاهد (۹۲/۳۸) تفاوت معنی‌داری نشان ندادند ($P> ۰/۰۵$). اسیدهای آمینه سیستئین و تریپتوفان در کلاژن مشاهده نشد. اسیدهای آمینه گلیسین و هیدروکسی لیزین (به‌ترتیب ۳۵۲ و ۳ residues $1000g^{-1}$) بیشترین و کمترین میزان کلاژن را داشتند. پروفایل اسیدهای آمینه و راندمان تولید کلاژن (۱۰/۵۹–۱۰/۵۱ درصد) تفاوت معنی‌داری را در گونه‌های ماهی کپور نشان ندادند ($P> ۰/۰۵$). بر اساس نتایج مطالعه حاضر، بین راندمان تولید، ایمنی و ویژگی‌های کیفی کلاژن در گونه‌های کپور پرورشی تفاوت معنی‌داری مشاهده نشد ($P> ۰/۰۵$). این ویژگی‌ها بین کلاژن آزمایشی و شاهد تفاوت معنی‌داری نشان ندادند ($P> ۰/۰۵$). از این رو پوست این گونه‌ها را می‌توان برای تولید کلاژن به کار گرفت و جایگزین کلاژن پستانداران کرد.

واژه‌های کلیدی: آنزیم پپسین، ایمنی، کپور ماهیان پرورشی، کلاژن، هیدرولیز اسیدی

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