Modern technologies for using fish wastes in the production of collagen hydrolysates and functional beverages

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Abstract. The research is devoted to the use of a cod skin hydrolysate as a component of citrus fruit beverages. It is demonstrated that the addition of a hydrolysate increases the content of protein in the beverage, slightly increases the viscosity of the beverage, and does not adversely affect its sensory characteristics. Hydrolysate is produced by enzymatic treatment of cod skin. The processing parameters allowing achieving the desired degree of destruction are determined. Physico-chemical parameters and sensory analyses of the obtained hydrolysate and its amino acid composition were analyzed. The influence of collagen hydrolysate on the physical and chemical composition of beverages, their structural and mechanical characteristics (pH and viscosity) and sensory analyses are estimated.

1. Introduction

Aquatic biological resources hold a prominent place among the food products of animal origin due to the high content protein with high amino-acid score, polyunsaturated fatty acids, vitamins, macro- and microelements. Not all types of commercial fish are used for food purposes. The reasons for this lie in historically established traditions, the unsuitability of some species for processing, the identification of new poorly studied objects of fishing, as well as the lack of sufficiently justified technological methods of processing. The processing industry faces the challenges of increasing the efficiency of raw materials use, reducing production waste, improving environmental safety and expanding the range of environmentally friendly food products [1].

According to FAO, about 40 million tons of aquatic biological resources (fish, invertebrates, and algae) per year are not used due to the lack of appropriate processing methods for food purposes. Therefore, in the near future, special emphasis will be placed upon technologies that allow maximum and environmentally-efficient use of raw materials of fish processing enterprises, including collagen-containing raw materials such as fish skin [2].

According to current regulations, the bulk of fish and fish products on sale must be skinless. As a result, at all large fish processing enterprises, fish skin is, in fact, a waste of production, subject to disposal. In reality, this in many cases means the removal of fish processing waste to the landfill. According to Roskomstat, Russia produces and processes about 4.5 million tons of fish per year. In case of deep processing of fish raw materials, the yield of collagen-containing waste (skin, scales, bones, fins) prevails, varying from 38.0 to 58.0% depending on the species composition of raw materials, i.e. from 1.7 to 2.6 million tons of waste per year, among them the fish skin accounts for
120 to 200 thousand tons per year. All this suggests the feasibility of the development of environmental technologies for processing fish waste and their subsequent use in the food industry.

Fish collagen's structure differs from that of animals; it is identical to humans [3]. That is why it is digested in the human body more efficiently. Besides, this type of collagen has absorbent and regenerating properties, so it is widely used as a component of various pharmaceutical and cosmetic products [4, 5]. Another reason for the increased interest in fish collagen is the high prevalence rate of bovine rabies, which makes it impossible to use the resulting collagen in food. Therefore, methods of complex processing of collagen-containing raw materials, including fish, are intensively developed in order to use it in the production of safe food products of directed action with preset quality parameters, including functional beverages [6]. A promising direction for the use of collagen-containing raw materials can be the development of functional beverages to restore tissues and joints in the body, improve the water-holding capacity of the skin and prevent the appearance of wrinkles by increasing the mass fraction of collagen in their composition [7].

Therefore, the aim of the research was to develop a technology of obtaining fish hydrolysate from cod skin and to define the possibilities of its application in the development of functional beverages.

2. Materials and methods

2.1. Production of cod skin hydrolysates
Collagen-containing mass obtained after cod processing and consisting of skin with scales, fins, bones and cartilage, as well as swim bladders, is used for the production of cod skin hydrolysates. The raw material was washed three times with 10% aqueous solution of citric acid with exposure, each time for 60 minutes with constant stirring and subsequent draining of the solution. The ratio of collagen-containing mass and solution is 1:1. After washing, the mass was frozen and ground on a fish cutter with 2-3 mm lattice holes.

The chopped collagen mass underwent enzymatic treatment with a complex of enzyme preparations: "food collagenosa" and "Protepsin" with a dosage of 0.1 % by weight of raw material, as well as hydro module treatment: raw materials : enzyme solution = 1: 2, treatment duration 5.5 hours, treatment temperature 35 °C with constant stirring. To inactivate the complex of enzyme preparations, the solution with hydrolysate was heated to a temperature of 70 ° C and held for 15 minutes [8].

In order to intensify the subsequent drying process, the solution with hydrolysate was condensed using an ultrafiltration unit through two-layer metal-ceramic membranes with pore sizes of 0.1-0.5 microns under the pressure of not less than 0.22 MPa. The filtrate and hydrolysate were obtained as the result of ultrafiltration. In appearance, the filtrate was a transparent liquid with a yellowish colour, whereas the hydrolysate was an opaque mass with a grayish colour.

Further, the hydrolysate was frozen and dried using vacuum freeze-drying to a 1.5-2.5% moisture content in the product [9]. The resulting dry mass was crushed in a ball mill to a powdery state. Quality parameters were estimated in the finished product.

2.2. Determination of the Content of Collagen
The content of collagen in the resultant dried samples was determined using a modification of the Neuman and Logan method and expressed in the collagen content as % of the dry matter. The method is based on the isolation of hydroxyproline in the acid hydrolysis of the product sample, carrying out a colour reaction with the products of its oxidation and measuring the intensity of the developing colour. Spectrophotometer «Specord M 40» was used to measure the colour. The obtained data on hydroxyproline were reduced to a standard form for collagen-containing products (% of total protein content), using a special index of 7.64 (for collagen).

2.3. Determination of active acidity
Active acidity was defined by a potentiometric method using Seven Go Duo pH Meter (Mettler Toledo).
2.4. Amino Acid Analysis
Amino acid content was carried out by amino acid analyzer AAA T339 (Microtechna, Praha).

2.5. Determination of moisture content
The moisture mass fraction was determined by drying at a temperature of 105 °C, to a constant weight.

2.6. Determination of protein content
Protein content was determined by the Kjeldahl method. Approximately 1 g of raw material was hydrolyzed with 15 mL concentrated sulfuric acid (H₂SO₄) containing two copper catalyst tablets in a heat block (Kjeltec 1002 system distilling Unit, Sweden) at 420 °C for 2 h. After cooling, H₂O was added to the hydrolysates before neutralization and titration. The amount of total nitrogen in the raw materials were multiplied with both the traditional conversion factor of 6,25 [10].

2.7. Determination of lipid content
The lipid content was determined using Soxhlet method of extraction.

2.8. Determination of ash content
The content of ash was determined by burning the dried fat-free suspension in a muffle furnace at t = 500-700 °C up to a constant mass.

2.9. Preparing beverages
It is known that for the synthesis of collagen in the body vitamin C is needed, which is a cofactor that ensures collagen’s production. Therefore, when developing such beverages, it is advisable to combine collagen with citrus fruits containing large amounts of vitamin C [11]. Lemon, orange and grapefruit juice are the basis of fruit beverages for this reason. Previously, the fruits were washed, scalded, peeled and pitted, crushed to a puree. After, the puree was frozen in sheets with a layer thickness (10-12) * 10⁻³ m in the freezer at a temperature of -30°C with intensive air circulation. After that, the product was exposed to vacuum freeze-drying. The primary drying temperature was equaled -30°C, the secondary drying temperature did not exceed 40 °C [12]. Freeze-dried fruits were crushed and mixed with fructose, glucose, fish collagen hydrolysate and marine salt in a «Turbula» mixer. According to the previously conducted experiments, it was determined that the most rational parameters was: mixing time - 10 minutes with the filling rate of the working chamber equal to 0.6 and the rotation speed of the bowl - 50 rpm.

The formulations are chosen in such a way that when they are restored, the osmolality of the finished beverages correspond to the isotonic drink, to ensure better digestion. This parameter is understood as the total concentration of particles (molecules, ions, colloidal particles of organic and inorganic substances) in one kilogram of solvent. The formulations of the researched beverages and the osmolality values are presented in table 1.

Table 1. Formulations of beverages.

| Ingredients                        | Beverage with freeze-dried lemon | Beverage with freeze-dried orange | Beverage with freeze-dried grapefruit |
|------------------------------------|---------------------------------|----------------------------------|--------------------------------------|
| Water, %                           | 77                              | 80                               | 78                                   |
| Freeze-dried puree of lemon/orange/ | 18,9                            | 15,9                             | 17,9                                 |
| grapefruit, %                      |                                 |                                  |                                       |
| Fructose, %                        | 2,1                             | 2,1                              | 2,1                                  |
| Glucose, %                         | 1,2                             | 1,2                              | 1,2                                  |
| Fish collagen hydrolysate, %       | 0,6                             | 0,6                              | 0,6                                  |
| Marine salt, %                     | 0,2                             | 0,2                              | 0,2                                  |
Physico-chemical parameters and structural-mechanical parameters were determined in the researched beverages.

2.10. **Determination of the effective viscosity**
The effective viscosity was determined on a «Polimer RPA-1M» viscometer by converting the torque required to rotate the spindle apparatus at a constant speed when dipping the examined product at a certain depth at temperature 20 °C.

2.11. **Determination of the content of carbohydrates**
The content of carbohydrates was determined by hydrolysis with an aqueous solution of hydrochloric acid, followed by ion chromatography using electrochemical detection of the desired compounds.

2.12. **Sensory evaluation**
Sensory analyses of beverages were determined using a panel of 11 panellists. The panellists were adult healthy volunteers (aged 18 to 50) who were consumers of beverages and did not have allergies concerning the consumption of these products. The sensory evaluation included colour, smell, taste, consistency and general perception. The panellists evaluated each attribute using five-point scale to compare traditional beverages that contained no fish collagen hydrolysate. Each panellist tasted about 15-20 ml of each type of beverages. Mineral water was used to wash the mouth between tastings.

2.13. **Statistical Analysis.**
The results are presented as the values ± Standard Deviation (SD). Tukey’s test (p < 0,05) (Bower, 2009) was used to detect significant differences between treatments. P-values below 0,05 were considered significant.

3. **Results and discussion**

3.1. **Evaluation of physicochemical parameters of fish collagen hydrolysate**
Physicochemical parameters and the estimations of collagen content are important data in evaluating the potential value of fish collagen hydrolysate. Low yields of collagen extraction can be expected in industrial conditions because of the previous treatment and storage time of the raw materials. Hydrolysis would help to overcome some of the problems associated with these previous treatments, increasing the yield of a valuable product, collagen hydrolysates [1]. The results of analysis of the chemical composition of collagen hydrolysate and pH are presented in table 2.

| Parameters                                   | Values         |
|----------------------------------------------|----------------|
| Moisture content, %                          | 2,2±0,1        |
| pH                                           | 5,95±0,15      |
| Protein content, %                           | 74,4±1,2       |
| Collagen content, % of total protein content | 74,1±0,1       |
| Lipids content, %                            | 0,55±0,12      |
| Ash content, %                               | 2,2±0,2        |

These tables show that freeze-dried hydrolysate contains a significant amount of collagen-up to 74.1 % of the total protein content. It can be recommended for use as a food additive, and as a source of collagen and amino acids in foods for this reason. The solution had a white colour, it lacked the smell and taste characteristic of fish. This suggested that its addition into the composition of beverages would be promising, since it will not have a significant impact on its sensory properties.
3.2. **Analysis of amino acid composition of hydrolysate**

The amino acid composition of collagen’s hydrolysate (Table 3) is characterized by the mandatory presence of oxyproline, which is a feature of the connective tissue of fish, and the absence of tryptophan, which is an integral part of the muscle tissue of animals and fish. Glycine, Proline, and Hydroxyproline are the most important amino acids in collagen, accounting for 50% of the total amino acid content in protein. The content of Proline and Hydroxyproline is especially important for the gelling effect. Meanwhile, hydroxyproline plays a special role in stabilizing the triple helix of collagen due to its ability to bind hydrogen through the \(-\text{OH}\) group.

Table 3. Amino acid composition of the freeze-dried fish collagen hydrolysate.

| Amino acid composition | g/100 g of protein |
|------------------------|-------------------|
| Lysine                 | 5.66              |
| Histidine              | 1.61              |
| Arginine               | 6.08              |
| Aspartic acid          | 7.29              |
| Threonine              | 2.53              |
| Serine                 | 4.23              |
| Gultamic acid          | 12.22             |
| Proline                | 10.34             |
| Tryptophan             | -                 |
| Cysteine               | 0.10              |
| Glycine                | 21.20             |
| Alanine                | 6.59              |
| Valine                 | 2.94              |
| Methionine             | 0.03              |
| Isoleucine             | 2.57              |
| Leucine                | 4.64              |
| Tyrosine               | 0.03              |
| Phenylalanine          | 2.51              |
| Hydroxyproline         | 7.51              |
| Hydroxylysine          | 1.95              |

3.3. **Analysis of quality parameters of beverages**

Table 4 shows that collagen content and protein content in investigated beverages was averaged 0.1% and 0.4%, respectively. The content of carbohydrates ranged from 7.4% in beverage with freeze-dried lemon to 15.9% in beverage with freeze-dried grapefruit, which is associated with a different content of carbohydrates in the raw fruit. The values of active acidity of beverages were closer to the neutral medium. The effective viscosity of the obtained products was slightly higher than the viscosity of water (1.03 kPa \(\cdot\) s).

Table 4. Chemical composition, active acidity and effective viscosity of the beverages.

| Parameters                        | Beverage with freeze-dried lemon | Beverage with freeze-dried orange | Beverage with freeze-dried grapefruit |
|-----------------------------------|---------------------------------|----------------------------------|--------------------------------------|
| Protein content, %                | 0.43±0.02                       | 0.4±0.03                         | 0.41±0.02                            |
| Collagen content, % of total protein content | 0.090±0.002                  | 0.096±0.002                      | 0.103±0.001                         |
3.4. Sensory evaluation of prepared beverages

The sensorial properties of fruit beverages containing fish hydrolysates proved to be comparable to control samples produced without hydrolysate. The addition of hydrolysate did not affect the colour of the product. Also, what is very important, the beverages had a rich fruit taste without specific fish taste and smell. The panellists noted that the addition of fish collagen hydrolysate had virtually no effect on the consistency of the beverages.

4. Conclusion

A rational technology for obtaining collagen-containing hydrolysate from cod processing waste has been developed, including enzyme treatment of the crushed mass, increasing the concentration of the hydrolysate solution by ultra filtration, freezing, vacuum freeze-drying and grinding the freeze-dried product to a powder.

It is demonstrated that the proposed technology of collagen isolation allows obtaining a high-quality hydrolysate with a content of collagen equal to 74.1 % of the total protein content. Sensory evaluation of the hydrolysate indicated that the odour characteristic of fish raw materials was absent. Therefore, it can be recommended for use as an additional source of protein in a wide range of products without deterioration of sensory parameters.

A line of beverages based on citrus fruits is offered. It was determined that the addition of collagen-containing hydrolysate in an amount of 0.6% slightly affects the viscosity of beverages, while increasing the content of protein, increases its availability and does not have a negative impact on the sensory characteristics of beverages.

References

[1] Blanco M, Vázquez J A, Pérez-Martín R I and Sotelo C G 2017 Mar Drugs 15 131
[2] Karayannakidis P D and Zotos A 2016 Fish Processing By-Products as a Potential Source of Gelatin: A Review J. of Aqua. Food Product Tech 25 65-92
[3] Sanchez A, Blanco M, Correa B, Perez-Martín R I and Sotelo C G 2018 Mar Drugs 16 144
[4] Koyama Y 2016 J Nutr Food Sci 6 504
[5] Moskowitz R 2000 Seminars in Arthritis and Rheumatism 30 87–99
[6] Hashim P, Mohd Ridzwan M S, Bakar J and Mat Hashim D 2015 Collagen in food and beverage industries Int. Food Res. J. 22 008
[7] Abuine R, Rathnayake A U and Byun H-G 2019 Biological activity of peptides purified from fish skin hydrolysates Fish. and Aquatic Sci. 22 14
[8] Zarubin N Yu, Bredihina O V, Semenov G V and Krasnova I S 2016 J. Vest. AGTU: fish. ind. 3 138-44
[9] Semenov G V and Krasnova I S 2018 Freeze-drying food (Moscow: Deli plus) p 292
[10] Kjeldahl J 1883 Fresenius’ J. Anal. Chem. 22 366-82
[11] Bilek S Y and Bayram S K 2015 J of Func. Food 14 562-9
[12] Semenov G V, Tikhomirov A A and Krasnova I S 2016 Int. J. of Appl. Eng. Res. 11 8056-61