Conference paper

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# Problems of efficient processing and use of collagen-containing materials

**Abstract:** Collagen is an important biopolymer in numerous applications due to its special characteristics, such as biodegradability and weak antigenecity. Interest recently, has grown in fish collagen. This stems from the fact, that the use of animal collagen is unsafe due to the effects from cattle rabies disease. Furthermore, fish collagen is 96 % identical to human protein. Modern fish production is accompanied by the formation of a large number of protein-containing wastes. Depending on the degree of fish processing waste hydrolysis we obtain different products, such as feed additives or growth accelerators. Available technologies are unacceptable, because they require the consumption of large amounts of time and energy. The enzymatic method of obtaining collagen hydrolysates is the most suitable because it can be implemented under milder conditions and it prevents the destruction of amino acids, carbohydrates and other substances contained in the waste. Application of the alkaline enzymatic hydrolysis method with hydrogen peroxide pretreatment at elevated temperature provides a collagen hydrolysate, which is characterized by a high content of total nitrogen; collagen is amorphous, has fully homogeneous structure and has a balanced amino acid composition.

**Keywords:** amino acids; enzymes; POC-2014; waste.

DOI 10.1515/pac-2014-0905

## Introduction

Collagen is a general extracellular structural protein involved in the formation of connective tissues. Collagen occurs in genetically distinct forms identified as type I to type XIX. They vary considerably in amino acid composition and structure.

The main sources of industrial collagen are limited to those from pigskins and bovine hides and bones. Collagen is ductile and is used in different fields, such as leather and films, cosmetics, biomedical and pharmaceutical industries, and in food [1].

Solid tannery wastes can be used for obtaining protein hydrolysates, products of partial hydrolysis of proteins which contain the essential amino acids, trace elements, etc. Out of 1000 kg of raw hide, nearly 850 kg is generated as solid waste in leather processing. Only 150 kg of the raw material is converted into leather. Tanneries generate huge amounts of solid waste such as [2]: fleshing, 50–60; chrome shaving, chrome splits and buffing dust, 35–40; skin trimmings, 5–7; and hair, 2–5 %. Solid wastes in the leather processing constitute: beam house, 80; tanning, 19; finishing, 1 %.

**Article note:** A Special Topic article based on a presentation at the 15th International Conference on Polymers and Organic Chemistry (POC-2014), Timisoara, Romania, 10–13 June 2014.

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Collagen hydrolysates display properties that depend on the raw material source and processing method. There are two main ways of obtaining protein hydrolysates: chemical—under the influence of acids and alkalis and biological—under the actions of enzymes. The enzymatic method is the most suitable because it is implemented in softer conditions and prevents the destruction of amino acids, carbohydrates and other substances contained in the waste.

Collagen hydrolysates may be prepared through acid hydrolysis (mostly dilute $H_2SO_4$, HCl or $H_3PO_4$), alkaline hydrolysis (e.g., NaOH, KOH or $Ba(OH)_2$) [3], enzymatic hydrolysis or microbial breakdown [4].

The use of enzymes in combination with acids or alkalis and high temperatures for obtaining hydrolysates is the best method found to create collagen hydrolysates while preventing breaking down of amino acids, carbohydrates and other nutrients contained in the waste [5].

The solid wastes can be hydrolyzed and used as a useful by-product in many ways (Fig. 1).

The occurrence of bovine spongiform encephalopathy (BSE) and foot/mouth disease (FMD) along with religious constraints has resulted in an anxiety among users of collagen and collagen-derived products from land-based animals. In recent years, increasing attention has been paid to alternative collagen sources, such as fish skin, which comprise about 30 % of the total fish weight available after fish fillet preparation [6].

Modern production of fish is accompanied by the formation of a large number collagen wastes such as bones, fins, skin, scales, viscera, etc. [7] that ranged from 30 to 70 % by weight of the feedstock (Table 1).

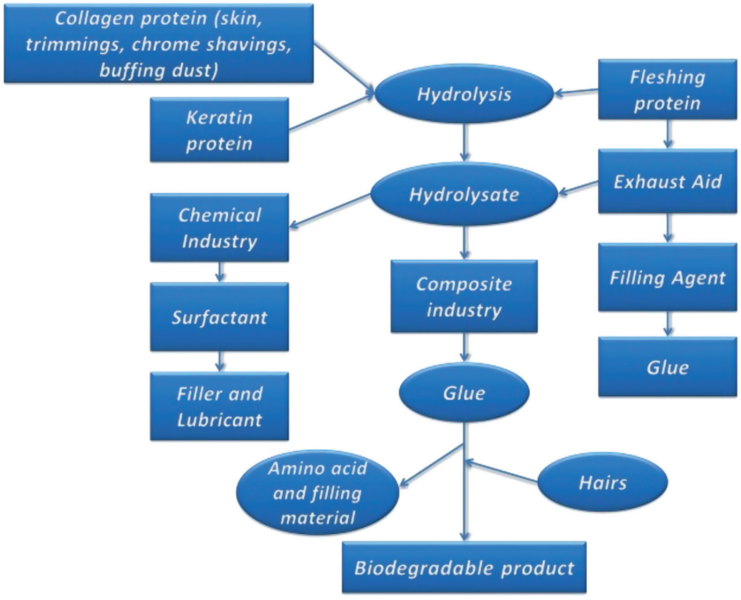


**Fig. 1** Hydrolysis for solid wastes from leather processing and their use as useful by-products.

**Table 1** The output of different fish parts after cutting.

| Part of the fish, weight % | Type of fish | | |
|---|---|---|---|
| | Scomber | Herring | Salmon |
| Muscle tissue | 61.6 | 54.5 | 54.4 |
| Leather | 3.8 | 3.5 | 4.2 |
| Head | 13.8 | 15.1 | 17.2 |
| Bones | 6.2 | 8.2 | 5.7 |
| Fish roe (milk males) | 3.8 | 7.3 | 8.1 |
| Viscera | 9.3 | 8.1 | 8.8 |
| Fins | 4.5 | 6.0 | 5.4 |
| The total mass, kg | 0.610 | 0.320 | 1.230 |

Partial use of these leads, on the one hand, to the loss of a vital protein product for use in food, feed and other purposes, and the other hand, to pollution.

Several studies have focused on the characterization of different fish collagens. Most fish collagens consist of two α-chain variants, which are normally known as $\alpha_1$ and $\alpha_2$. In addition to differences in molecular types, fish collagens have been shown to vary widely in their amino acid composition. In particular, the physical properties of the protein and the quantity of the amino acids, proline and hydroxyproline, vary significantly among fish species, and this is strictly correlated with the outside temperature of the animal's environment [8, 9].

The greatest features of fish collagen are lower denaturation temperature and viscosity than collagens of vertebrate land animals. These features distinguishing fish collagens from vertebrate land collagens are very important for food processing. This led to a recent increasing interest in fish collagens [10, 11].

The shrinkage temperatures ($T_s$), values of fish-skin collagens range from about 35 °C to 57 °C, according to the mean temperature of the environment, while mammalian collagen has a $T_s$ value of about 62 °C. By an examination of the complete amino acid analyses, fish skin gelatin hydroxyproline and mammalian gelatin are 67 and 95, respectively (residues of amino acid per 1000 total residues); the lower the amount of hydroxyproline the lower the value of $T_s$ [12]. Interchain hydrogen bonding between hydroxyl groups of hydroxyproline and backbone carbonyl groups was, on this basis, suggested as an important stabilizing feature of the collagen structure.

# Experimental procedures

## Materials

Collagen-based tanned wastes were received from the tannery "Chinbar" (Kiev) during production trials of non-chromium tanning technology [13]. The other chemicals used-sodium hydroxide p.a., hydrogen peroxide p.a., sodium carbonate p.a., hydrochloric acid p.a. were supplies by Himlaborreactiv (Kiev).

Protease Zime SB has an activity of 1500 U/g with an optimum pH 3.5–6.5, optimum temperature 38–40 °C.

## Methods

In this work alkali-enzymatic and acid-enzymatic methods were used. The level of hydrolysis was determined by Kjeldahl's method [14].

Physio-chemical properties such as moisture content, mineral substances, substances extracted by organic solvents and fatty substances were examined as per the standard procedures [15, 16].

Viscosity of collagen hydrolysate was measured with a viscometer and calculated.

*Alkali-enzymatic method of obtaining collagen hydrolysate*

This was as carried out for 6–8 h at 40 °C as follows: offal was washed with running water, crushed to the consistency of stuffing, loaded into the reactor, 50 % of water was added; 1.6 % v/w $H_2O_2$; 2 % v/w NaOH. The enzymatic hydrolysis was then carried out for 4 h, enzyme demand was 3 %. Then concentrated hydrochloric acid was adjusted to pH 4.5, heated to boiling and confined for 15–20 min. The resulting mixture was adjusted to pH 6.8 with a solution of sodium carbonate. After separation of the layers of the hydrolysate, it was evaporated to the desired concentration.

*Ion-exchange liquid-column chromatography method*

To conduct qualitative and quantitative analyses of amino acid composition of the collagen-containing material of the resulting hydrolyzate, ion-exchange liquid-column chromatography with the 339 M automatic analyzer (Microtechna, Czech Republic) was employed.

**The aim of this work** is to develop a method to use collagen-containing wastes and to determine a rational way of using the obtaining collagen-based materials.

## Results and discussion

In the research work alkaline-enzymatic methods have been used for obtaining the hydrolysates.

The chemical composition of chrome-free tanned leather industry wastes are characterized by rather high hide substance and can be used to obtain hydrolysates (Table 2). The high mineral content is probably associated with the presence in leather shavings such as mineral slats like chloride and sodium carbonate as a result of their use in acid-salt treatment and pH adjustment during processing of phosphonium compounds.

On the main stage, physico-chemical properties of collagen hydrolysate obtained by alkaline enzymatic method with an additional treatment of hydrogen peroxide at elevated temperature were determined. The results of determinations are given in Table 3. The degree of hydrolysis was determined by total nitrogen content in the final product.

The application of the alkaline enzymatic hydrolysis method in conjunction with the processing of waste by hydrogen peroxide at elevated temperature provides a collagen hydrolysate, which is characterized by a high content of total nitrogen, collagen is amorphous and has a fully homogeneous structure [17]. Obtained hydrolysate balanced amino acid composition.

Waste mackerel (offal) obtained after butchering of the fish was also studied. Typically, these wastes are not reused and disposed at landfills. Wastes were preserved using NaCl (100 % v/w salt). The chemical composition of the fish waste is presented in Table 4.

After the alkali-enzymatic hydrolyses the level of hydrolysis was monitored by total nitrogen content of the product, it was 12.2 g/L. The disadvantage of the obtained product was a dark brown color and an unpleasant “fishy” smell.

**Table 2** Physico-chemical properties of chrome-free tanned wastes.

| Index | Values |
|---|---|
| Content in leather wastes, % | |
| Hide substance | 62.1 |
| Moisture content | 22.0 |
| Mineral substances | 8.4 |
| Substances extractable by organic solvents | 0.8 |
| Total soluble substance | 12.6 |

**Table 3** Physico-chemical properties of collagen hydrolysate.

| Index | Values |
|---|---|
| Content in the collagen hydrolyzate, g/L | |
| Mineral substances | $15.0 \pm 0.5$ |
| Dry matter | 14.23 |
| Total nitrogen content | $23.6 \pm 0.5$ |
| Phosphorus, g/L | 0.5 |
| Viscosity | |
| Specific viscosity | 0.9 |
| Relative viscosity | 1.9 |
| Kinematic viscosity, $mm^2/s^2$ | 2.22 |
| Density, $kg/m^3$ | 1.0415 |
| pH | 6.5 |

**Table 4** Physico-chemical properties of the salted fish waste.

| Content in the waste, % | |
|---|---|
| Moisture | 43.5 |
| Mineral substances | 38.6 |
| Fatty substances | 5.9 |
| Total nitrogen content | 7.9 |

To intensify the process of hydrolysis, further treatment with hydrogen peroxide was used. The effectiveness of this treatment was confirmed in previous studies using waste from the leather industry [18]. Unfortunately, in the case of fish waste, such treatment was not effective. Although the total nitrogen content in the final product was 15.4 g/L. The unpleasant smell of the product was still present as a result of the formation of peroxides via partial oxidation of the fats.

For the acid-enzymatic hydrolysis, solutions with different concentrations of acetic acid and enzyme were used. Consumption of enzyme was 1.3 %. The degree of hydrolysis was determined by the total nitrogen content in the final product (Table 5).

Decreasing the alkali washing time leads to an increase in the mineral content in the final product. The increasing of the acid-enzymatic hydrolysis reaction speed has positive effect on the quality of the final product, indeed the content of total nitrogen increases. Alkali washing was applied for saponification and elimination of fats and washing out non-protein components.

Fish collagens show a wider variation in composition. Their hydroxyproline and, to a lesser extent, proline contents are lower than those of mammalian collagens (Fig. 2). This is compensated for by other hydroxyamino acids such as serine and threonine.

As a result of hydrolytic decomposition of fish collagen, the number of basic amino acids increased due to breaking down of the peptide bonds, arginine content increased to 9.59 %. Hydrolysate content of the essential amino acid histidine in fish collagen is 1.37 %. Very important amino acids for the nutrition of young animals are: isoleucine and leucine (1.72 and 5.04 %), methionine (2.03 %), threonine (3.92 %), and phenylalanine (2.84 %), the latter is higher in fish collagen hydrolysate than in hydrolysate from cattle skins.

The presence of potentially reactive amino groups, gives us the ability to change the properties of hydrolysates [19]. Potentially, the most reactive capable groups of proteins are those containing the amino acids serine (whose primary group is the –OH group), hydroxyproline (secondary –OH), threonine (secondary -OH), tyrosine (phenolic –OH), aspartic and glutamic acids containing the group –COOH and lysine and arginine containing alkaly groups [20]. Hydrolysates can be chemically modified [21] by the application of crosslinking reagents (in particular aldehydes, starch, enzymes).

Thus, the amino acid composition of the obtained hydrolysate is balanced. It can be used as an organic fertilizer and as growth promoter in animals food, and after further modifications as a component of composite materials and biopolymers.

**Table 5** Characteristics of the methods and products of hydrolysis from fish waste.

| Variant | Washing with alkali | Carrying hydrolysis | | | | Content, g/L | | |
|---|---|---|---|---|---|---|---|---|
| | | Alkali | Enzyme | Acid | $H_2O_2$ | Nitrogen | Dry residue | Ash |
| 1 | – | + | + | – | – | 12.2 | 218.9 | 124.4 |
| 2 | – | + | + | – | + | 15.4 | 215.3 | 120.2 |
| 3[a] | + | – | + | + | – | 11.2 | 15.2 | 4.9 |
| 4[b] | + | – | + | + | – | 14.3 | 22.3 | 9.0 |

[a]Alkali washing duration 24 h. Alkali-enzymatic hydrolysis duration 4 h, at 40 °C.

[b]Alkali-enzymatic hydrolysis duration 4 h, at 40 °C and 8 h. Room temperature (left overnight). Alkali washing duration decreased to 1.5 h. Alkali was dosed in 3–4 receptions after 30 min.

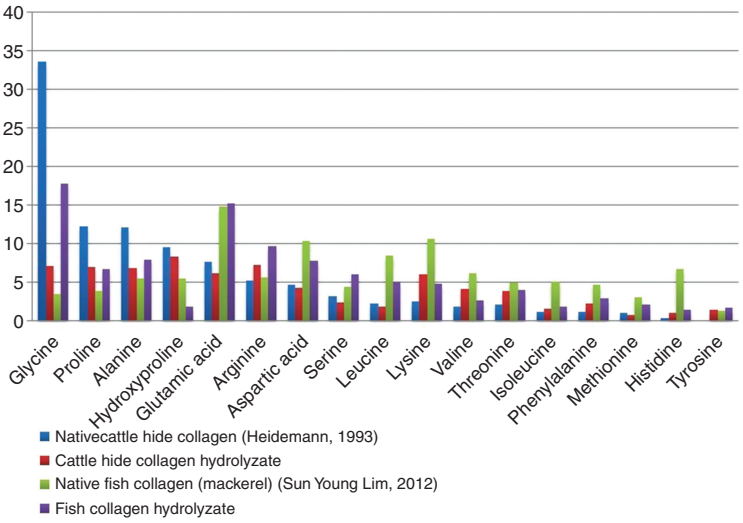


**Fig. 2** Amino acid composition of fish and cattle hide hydrolysate (%).

## Conclusion

A method was developed for the disposal of collagen-containing mackerel (*Scomber scombrus*) waste to produce collagen-based biomaterials for various purposes. The method involves the acid-enzymatic hydrolysis of the waste in a solution of acetic acid and enzyme, enzyme demand was 1.3 % in conjunction with the previous washing carried out with alkali to remove soluble proteins.

Reducing the duration of alkali washing leads to an increase in mineral content in the final product. The increasing of the acid-enzymatic hydrolysis has positive effects on the quality of the final product and the content of total nitrogen increases.

The amino acid composition of the resulting hydrolysate is balanced. It can be used to produce organic fertilizers and growth promoters in animals food, because it contains essential amino acids (isoleucine, leucine, methionine, threonine and phenylalanine). After further modifications, which are determined by the presence of functional groups of amino acids, hydrolysate can be used as a component of composite materials and biopolymers.

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