Purification of model solutions from copper($^{2+}$) ions with aqueous extracts from hydrolysates of protein-containing keratin

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Abstract. A hydrolysate based on keratin-containing raw materials (sheep wool) was obtained by alkaline hydrolysis. The optimal conditions of the hydrolysis process have been established. IR spectroscopic studies have shown that the assumed structure of the samples corresponds to the content of amino acids with a peptide bond in them. The experiments have determined that the greatest degree of removal of copper ions from model solutions, with a concentration of these ions of 1 mol/l, is observed when an aqueous extract from sheep wool keratin hydrolysate is added. It was found that the greatest decrease in the concentration of Cu^{2+} ions was observed when the extract was added to the 4 cm^3 model solution and the removal of ions was 82%. The formation of amino acids with copper ions by internal complex compounds was revealed, in which the Cu(II) atom is associated with oxygen and nitrogen atoms of amino groups. In this case, chelate structures consisting of five-membered cycles arise.

1 Introduction

Currently, a new innovative direction in the field of environmental protection is intensively developing on a global scale – the use of waste from the processing of agricultural raw materials to remove various pollutants from aquatic environments, including heavy metal ions (ITM) [1-4].

The main advantage of these reagents is that these materials have an extensive raw material base, are cheaper and simpler in terms of production and disposal methods in comparison with industrially used reagents. Heavy metals, whose ions are not biodegradable and accumulate in the reservoir, currently occupy one of the priority positions among the dangerous factors in the general pollution of the environment by pollutants. Wastewater from electroplating workshops, mining, ferrous and non-ferrous metallurgy, mechanical engineering and other industries serve as sources of water pollution with heavy metal compounds [5-6].

One of the most toxic elements widely used in industrial production and consumption is honey and its compounds.

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It is known that wool fibers can be converted into soluble protein using denaturing agents-acids, alkalis, etc. In comparison with the acid hydrolysis of keratins, alkaline hydrolysis is usually preferred. The protein hydrolysates obtained during hydrolysis consist of amino acid residues [7-9].

Wool consists of 90% of keratins of fibrillar structure, which has an α-spiral shape. It consists of 20 to 23 amino acids. The chemical structure of wool, purified from various contaminants, is a biopolymer – keratin, which belongs to the group of protein substances. The elemental composition of keratin is, %: C–50.3-52.5; H–6.4-7.3; N–16.2-17.7; O–15.0-20.7; S–0.7-5.0 [10-11].

The simplest formula corresponding to the elementary composition of wool contains 39 carbon atoms and is the same as the formula of keratin ($C_{39}H_{65}N_{11}SO_{13}$), since wool corresponds to keratin in its chemical properties [12].

2 Materials and methods

The presence of functional groups in the amino acids that make up the keratin of wool causes high sorption characteristics of the latter in relation to metal ions.

The purpose of this work was to determine the optimal conditions for the hydrolysis reaction of keratin-containing cheese (sheep wool), providing the production of aqueous extracts containing protein compounds for the removal of heavy metal ions from aqueous media.

The process of hydrolysis of sheep wool was carried out in the following order: first, the wool was cleaned from dirt, oil and other contaminants. In this case, the wool is mechanically loosened and subjected to a chemical hydrolysis process using water, heating and reagents. To do this, we took 50 g of wool and placed it in a chemical heat-resistant flask, and then 200 ml of 2% sodium hydroxide solution and 600 ml of distilled water were added to it, heated to a temperature of 90-95 °C, hydrolyzed for 8-10 hours. After the dark cinnamon-colored solution was filtered, the filtrate was used for further investigation.

Isolation of free amino acids. Precipitation of proteins and peptides of the aqueous extract of the samples was carried out in centrifuge cups. To do this, 1 ml (exact volume) was added to 1 ml of the test sample 20% THUK. After 10 minutes, the precipitate was separated by centrifugation at 8000 rpm for 15 minutes. After separating 0.1 ml of the filler liquid, it was freeze-dried. The hydrolysate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid.

To determine the composition and structure of the hydrolysis reaction products, pre-dried keratin hydrolysate samples were examined on a SHIMADZU Fourier infrared spectrometer in the range of 4000-400 cm^{-1} at room temperature.

The spectrophotometric method of studying the Cu^{2+} model solution and the effect of the extractant on the removal of the same ions from aqueous solutions was carried out in a SF-46 spectrometer with software.

3 Results and discussion

Phenylthiocarbamyl derivatives (FTC) of amino acids were obtained by reaction with phenylthioisocyanate using the Steven A., Cohen Daviel method. The identification of amino acid derivatives was carried out by HPLC. HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector. column 75x4.6 mm Discovery HS C18. Solution A: 0.14M CH_3COONa + 0.05% TEA pH 6.4, B: CH_3CN. The flow rate is 1.2 ml/min, absorption

is 269 nm. Gradient %V/min: 1-6%/0-2.5min; 6-30%/2.51-40min; 30-60%/40.1-45min; 60-0%/50.1-55min (Figure 1) [6].

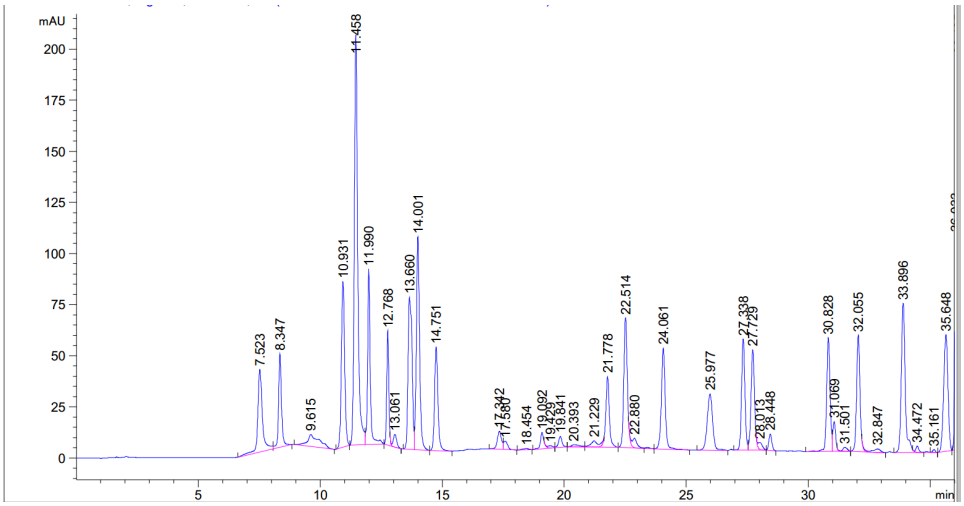

Fig. 1. Chromatogram of creatine hydrolysate.

Accordingly, a model solution containing Si^{2+} 1mol/dm^3 ions, prepared by dissolving the corresponding sample of penthydrous copper sulfate (50 g) in 1 dm^3 of distilled water, had the following parameters: pH–3.74, density–1.4001 mg/cm^3, color–blue. The course of the experiment was as follows: in five flat-bottomed flasks containing 100 cm^3 of a pre-prepared model solution, one or another extract was added in a volume from 1 to 10 cm^3. The addition of the latter led to the formation of a dispersed brown phase due to the formation of amino acid complexes with copper ions. The dispersed brown phase was removed by filtration, dried and weighed, and the filtrate was analyzed for changes in physico-chemical parameters. According to the data obtained, dependences were constructed demonstrating the effectiveness of using the extract in the purification of model waters from Si^{2+} ions.

The obtained results of high-performance liquid chromatography (HPLC) of keratin hydrolysate are shown in Table 1. As can be seen from the table, the amino acid composition consists of the following amino acids: aspartic and glutamic acid, serine, glycine, cysteine, threonine, arginine, alanine, proline, tyrosine, valine, methionine, histidine, isoleucine, leucine, phenylalanine, lysine.

Table 1. Amino acid content in sheep's wool.

The name of the Amino Acids	**Hydrolysis of amino acids**	**The name of the Amino Acids**	**Hydrolysis of amino acids**
	Concentration mg/g		**Concentration mg/g**
Aspartic acid	7.012374	Proline	8.663317
Glutamic acid	14.47531	Tyrosine	6.526913
Serin	4.700157	Valin	9.513951
Glycine	7.4561	Methionine	0.523553
Asparagine	0.00	Histidine	10.66514

Glutamine	0.00	Isoleucine	4.678963
Cysteine	2.267844	Leucine	12.9017
Threonine	7.278442	Tryptophan	0.00
Arginine	20.76491	Phenylalanine	3.809746
Alanin	3.663457	Lysine	3.244828

To determine the structure of keratin hydrolysates, the IR spectra of samples of hydrolysates synthesized at a temperature of 100 °C were studied. Fluctuations at 1580 and 865 cm^{-1} correspond to the presence of –NH groups, 1453 and 1405 cm^{-1} groups of -CH_2. Fluctuations in the 3367-3371 cm^{-1} region are also observed in the IR spectra, which indicates the presence of -NH_2 groups; absorption bands in the 2963 cm^{-1} region belong to –CH bonds. The CONH peptide group gives absorption bands in the range of 3600-1400 cm^{-1} of the IR spectrum, characterizing the bonds –OH, -NH, -CH, C=O. IR spectra of keratin hydrolysates showed the presence of amide and sulfide groups. The region of amide groups corresponds to amide (1435 cm^{-1}); the region of sulfoxide groups corresponds to cysteine 1080 cm^{-1}. Absorption bands in the region of 2960-2930 cm^{-1} are symmetric and asymmetric valence vibrations of CH_3–CH) groups. The absorption bands at 1450-1400 cm^{-1} are attributed to asymmetric deformation vibrations of the CH_3 groups. Absorption bands in the region below 1400 cm^{-1} are believed to be associated with vibrations in which the entire polypeptide skeleton of the molecule participates [7-9].

Table 2. The value for obtaining a calibration graph of a $CuSO_4$ solution at various concentrations (CF-46).

Point number	**Volume for the prepared solution, V ml, (1 mol/l $CuSO_4$)**	**Obtained concentrations, C_m (mol/l)**	**Optical density, D (λ =620 nm)**	**Molar absorption coefficient, ε**
1	2.5	0.05	0.12	11.7 10^{-2}
2	5.0	0.10	0.14	6.8 10^{-2}
3	10.0	0.20	0.30	7.3 10^{-2}
4	12.5	0.25	0.32	6.2 10^{-2}
5	20.0	0.40	0.50	6.1 10^{-2}
6	25.0	0.50	0.59	5.8 10^{-2}
7	30.0	0.60	0.70	5.7 10^{-2}
8	37.5	0.75	0.80	5.2 10^{-2}
9	40.0	0.80	0.85	5.19 10^{-2}

To determine the concentration of copper sulfate in aqueous solutions, a calibration graph was drawn up depending on the concentration at wavelength λ = 620 nm (Figure 2).

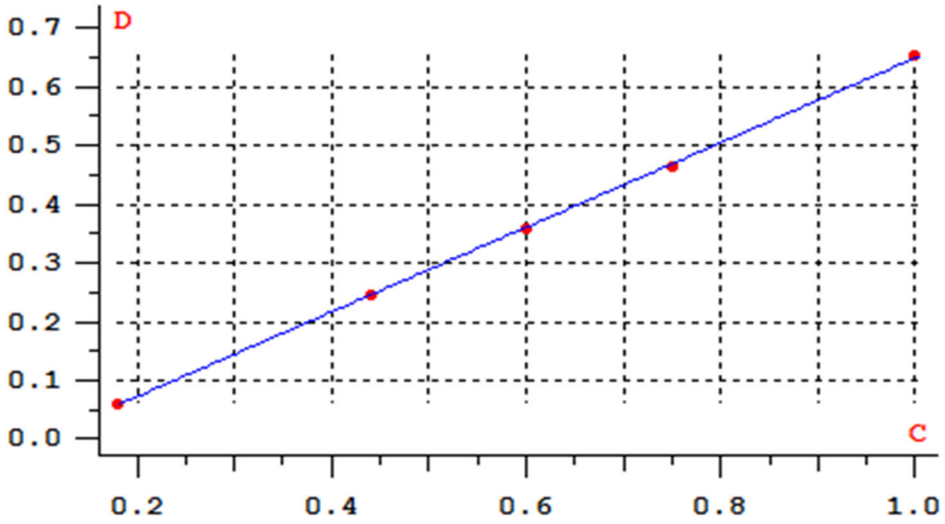

Fig. 2. Calibration graph of an aqueous standard solution of $CuSO_4$ at various concentrations.

The extractant consisting of a mixture of amino acids forms complexes with copper($^{2+}$) ions, they are internal complex salts; in them, the Cu(II) atom is associated not only with oxygen atoms, but also with nitrogen atoms of amino groups. The bond between the copper and nitrogen atom is carried out by additional valences (due to an unsupported pair of nitrogen electrons of the amino group and free d-orbitals of copper). In this case, annular structures consisting of five-membered cycles arise. The copper atom in such intracomplex compounds has a coordination number of 4, does not have an ionic character, and such complexes are characterized by stability, and, as a rule, insoluble in aqueous media. Tabular data and graphs of the dependence of the residual ion content in filtrates are shown in Table 3 and Figure 3-4.

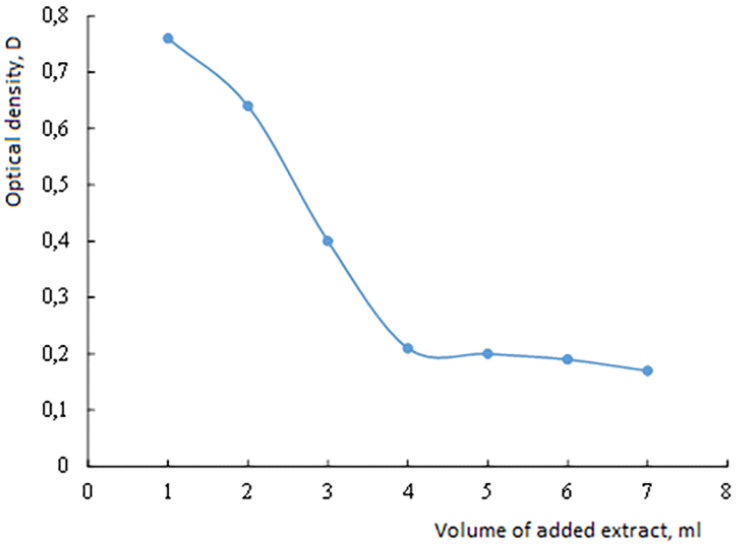

Fig. 3. Dependence of the residual concentration of Cu(II) ions in filtrates on the volume of added extracts.

As follows from the graphs shown (Figure 3), the greatest decrease in the concentration of Cu^{2+} ions is observed when 4 cm^3 of the extract is added to the model solution. The lowest residual concentration of Cu(II) ions is observed in the case of 10 cm^3 addition of a slightly acidic extract to the model drain, the highest–when adding ESH. This circumstance is explained by the fact that in this pH range (pH 3.7), copper complexes with amino acids have the lowest solubility value and precipitate [10]. As can be seen from the graph (Figure 4).

With the addition of an extractant, the molar repayment coefficient increases and the copper concentration decreases. This proves the extraction of copper ions from an aqueous solution.

Table 3. The data obtained by spectrophotometric analysis of the effect of keratin extracts on 1 mol/l of $CuSO_4$ solution (Volume=10ml).

Number points	Extract volume, V_{ml}	Optical density, D	Concentration, C_m(mol/l)	Molar extinction coefficient, ε
1	1	0.76	0.66	$5.63 \cdot 10^{-2}$
2	2	0.64	0.56	$5.58 \cdot 10^{-2}$
3	3	0.40	0.35	$5.58 \cdot 10^{-2}$
4	4	0.21	0.18	$5.7 \cdot 10^{-2}$
5	5	0.20	0.16	$6.1 \cdot 10^{-2}$
6	6	0.19	0.15	$6.19 \cdot 10^{-2}$
7	7	0.17	0.13	$6.39 \cdot 10^{-2}$

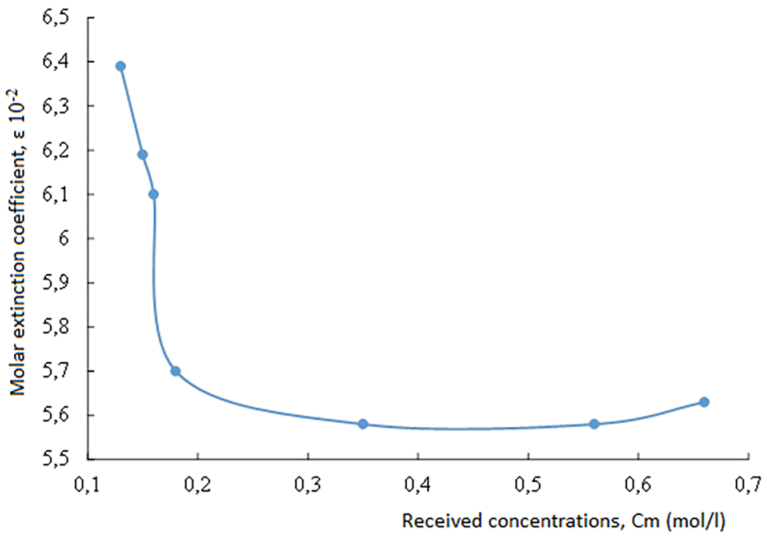

Fig. 4. Dependence of the molar repayment coefficient on the concentration of copper ions (2+).

The calculations determined that the degree of removal of Cu(II) ions, taking into account dilution of the model solution with extracts, was 82.0%.

4 Conclusion

The Thus, the amino acid composition of keratin hydrolysates from sheep wool was established in the results of the conducted studies, and the optimal hydrolysis condition was also established.

IR spectroscopic studies have shown that the assumed structure of the samples corresponds to the content of amino acids with a peptide bond in them.

The experiments have determined that the greatest degree of removal of copper ions from model solutions, with a concentration of these ions of 1 mol/l, is observed when an aqueous extract from sheep wool keratin hydrolysate is added.

It was found that the greatest decrease in the concentration of Cu^{2+} ions was observed when the extract was added to the 4 cm^3 model solution and the removal of ions was 82%.

The formation of amino acids with copper ions by internal complex compounds was revealed, in which the Cu(II) atom is associated with oxygen and nitrogen atoms of amino groups. In this case, chelate structures consisting of five-membered cycles arise.

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