

Antioxidant Effects of Keratin Hydrolysates

by

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Abstract

The antioxidant and antimicrobial properties of keratin hydrolysates from acid and from oxidative hydrolyses were studied. For *in vitro* studies of antioxidant properties of the keratin hydrolysates, high-molar-mass hyaluronic acid (HA) that is especially sensitive to the effect of oxidants was used as a substrate. Cu(II) ions and ascorbate-mediated degradation of hyaluronan in the absence and presence of keratin hydrolysate as antioxidant were examined using the method of rotational viscometry. Results indicate that keratin effectively inhibited HA degradation and thereby the formation of hydroxyl-, alkoxy- and peroxy-type radicals. Results of the ABTS assay show that keratin hydrolysate efficiently reduced ABTS^{•+} cation radical. The keratin hydrolysates also had antimicrobial effects on selected bacterial strains and caused decreases in formaldehyde emission from urea-formaldehyde bonded materials.

Introduction

The food and leather industries are among the largest environmental polluters and the biopolymer wastes produced in the production of meat and leather are environmental loads, mostly landfilled. This waste can be inert, biodegradable, but also dangerous.¹ Examples are low quality sheep wool from leather production and huge amounts of feather waste. About 815 000 tons of chicken feather per year are produced only in the United States,² and e.g. 77 000 tons of feather in Poland.³ Feathers are about 5 - 7% by weight of chickens.

Keratins belong to a large group of fibrous proteins, that form part of the skin layer and associated structures such as hair, nails, horn, and feathers.⁴⁻⁵ The relative molecular weight is about 50 000 kDa. The total sulfur content of keratin is in the

range from 2 to 5% on dry matter. It is assumed that keratin provides mechanical support and various protective functions in adapting vertebrates to the outside environment.⁶ Keratins can be divided into soft (epithelial) and hard (hairly).⁷ Soft keratins are often found in the lower layer of the skin, in the callus and also in the hair core. They have a lower content of sulfur amino acids – maximum 2%. Soft keratin is easier to degrade and sulfur components are weakly bonded.

Keratin insolubility is due mainly to intra-molecular disulfide bonds. A stable three-dimensional structure formed by disulfide bridges and other transverse bonds (hydrogen bridges, van der Waals forces and ionic interactions) causes keratins to be highly stable in the physiological environment. Keratins due to cross-linking with disulfide bridges are also very resistant to chemicals and enzymes.⁸⁻⁹ Therefore, to obtain soluble keratin high concentrations of acid or base solutions at high temperatures or pressures must be used.¹⁰⁻¹¹ The most-commonly used soluble keratin production technologies include hydrolysis by alkaline solutions, acids, reducing and oxidizing agents, in recent years also by enzymes. The action of oxidants on keratin causes oxidation of the disulfide bond without its cleavage or oxidative cleavage of the disulfide bond. Reductive agents (e.g. sulfides, sulfites thioglycoles) affect keratin in such a way that reductive cleavage of the disulfide bond occurs – such reaction is reversed and the disulfide bonds are re-formed by mild oxidation.

Such process results in a significant reduction of the content of certain amino-acids (i.e., methionine, lysine, tryptophan) of keratin materials.¹² This led recently to increase in usage of enzymatic hydrolysis. This process does not use very high concentrations of chemicals and in addition, the process takes place at lower temperatures and at atmospheric pressure. At present, the lower cost of enzymes may lead to cheaper process than using acidic or alkaline hydrolysis.¹³

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The keratin hydrolysates have a wide use. Thus, it is important to know the composition of keratin hydrolysates and their properties (e.g. solubility, film-forming properties, rheological properties, thermal stability). Protein hydrolysates are currently intensively used in agriculture, primarily as nitrogen fertilizers and compounds of feed ingredients. Their favorable composition makes possible to use them also as biostimulators –¹⁴ a very promising area of their application. Biostimulators are generally organic substances that stimulate plant growth to enhance their metabolism.¹⁵ Biostimulators help the healthy development of plants, strengthen their immune system and also favorably affect the yield of crops.¹⁶ The secondary effect of protein biostimulators is their action as a foliar fertilizer. Many biostimulation effects are observed at the time of application of biostimulators across the leaf.¹⁷⁻¹⁸

The prospective application of keratin hydrolysates represents the packaging industry.¹⁹⁻²² The keratin hydrolysates are suitable for preparing biodegradable films, foils, coatings or fibers. Biodegradability predestines these materials (e.g. as mulching foils) for use in agriculture.⁹

Pouring, soaking and spraying are used for preparation of films and coatings.²³ After dissolving the keratin hydrolysate, a plasticizer (e.g., glycerol) needs to be added to prevent brittleness. For better mechanical properties, films and coatings are crosslinked by physical (e.g., heat, radiation) or chemical methods by cross-linking agents (e.g. formaldehyde, starch di-aldehyde) which react with the functional groups of the protein. The biodegradability of such materials, their barrier, mechanical and other properties can be regulated by changing the crosslinking conditions.⁹ The prepared films are insoluble in the most-commonly used organic solvents.²⁴⁻²⁵

Recently there has been a strong support for keratin research because it has already led to the development of many biomaterials for use in biomedical applications based on keratin. They are based on several key properties of keratin that contribute to the overall physical, chemical and biological behavior of these biomaterials. Mainly extracted keratin proteins have the ability to self-organize and polymerize to form porous, fibrous three-dimensional matrices for cell culture. The spontaneous self-organization of keratin solutions is also interesting and has been extensively studied at the microscale as well as macroscale level.²⁶⁻²⁹

Free radicals cause degradation of a high-molar-mass glycoaminoglycan hyaluronan (HA).³⁰ The main goal of our experiments was to assess *in vitro* keratin hydrolysates as relevant (endogenous) substances with a potential to scavenge reactive oxygen species degrading HA by using rotational viscometry. The ABTS assay was used to determine radical scavenging capacity of keratin hydrolysates and thereby, their electron donor properties.

The proposal to test the disinfecting activity of keratin hydrolysates on selected bacteria strains was based on the chemical composition of keratin. The tested keratin samples fulfilled requirements of PhEur.7.0.5.1.3-2. Technical applications focused on testing the disinfecting activity of keratin hydrolysates on selected bacteria strains. The tested keratin samples fulfilled requirements of PhEur.7.0.5.1.3-2.³¹

Technical applications were also directed to the application of keratin hydrolysates at modification of urea-formaldehyde (UF) resin mixtures in order to reduce the release of formaldehyde from bonded wood-based panels.

Experimental

Research was focused on the biopolymer keratin due to the fact that the main characteristic of the keratin protein is the high content of thio-amino acids – methionine and cysteine.

Keratin hydrolysates were prepared using sheep wool ‘Merino’ of the following composition: nitrogen 12.15%, ash 2.53%, sulphur 2.21%, fat 7.16%. The wool was separated, washed, defatted and dried at room temperature; the hydrolysates were then prepared using either mineral acid or alkaline solution.

Hydrochloric acid was used for the acid hydrolysis, using 500 mL of distilled H₂O and 100 mL of concentrated HCl per 100 g of wool. The hydrolysis took 5 hours under reflux.

Prepared hydrolysate was filtered, partly neutralized using solid NaOH and purified using dialysis against distilled H₂O. A hydrolysate powder K-1 was obtained after drying, parameters of which were then tested.

An aqueous solution of sodium hydroxide and hydrogen peroxide was used for alkaline hydrolysis of the sheep wool. The hydrolyzing solution was prepared by adding 7 g of NaOH and 10 mL of 30% aqueous H₂O₂ into 600 mL of distilled H₂O per 100 g of wool. The hydrolysis proceeded during 5 hours at reflux.

Prepared hydrolysate was filtered, partly neutralized using concentrated HCl and purified using dialysis against distilled H₂O. A hydrolysate powder K-2 was obtained after drying, parameters of which were then tested.

Materials and Chemicals

A hyaluronan sample (sodium salt) of molar mass 970.4 kDa was purchased from Lifecore Biomedical Inc., Chaska, MN, USA. Analytical purity grade NaCl and CuCl₂·2H₂O were purchased from Slavus Ltd., Bratislava, Slovakia. L-Ascorbic acid and potassium persulfate (K₂S₂O₈ p.a. purity, max. 0.001% nitrogen) were the products of Merck KGaA, Darmstadt, Germany.

2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; purum, >99%) was from Fluka, Germany. Redistilled deionized high quality grade water, with conductivity of $<0.055 \mu\text{M}/\text{cm}$, was produced using the TKA water purification system from Water Purification Systems GmbH, Niederelbert, Germany.

Methods of Testing

X-Ray photoelectron spectroscopy (XPS) data were recorded using a Thermo Scientific K-Alpha XPS system (Thermo Fisher Scientific, UK) equipped with a micro-focused, monochromatic Al K α -X-ray source (1486.6 eV). A 400 mm micrometer X-ray beam was used at 6 mA and a potential of 12 kV. The spectra were acquired using the constant analyzer energy mode at pass energy of 200 eV.

ATR-FTIR Spectroscopy Measurements

ATR-FTIR measurements were performed using an FTIRTM NICOLET spectrometer (Thermo Scientific, USA) and a single bounce ATR accessory equipped with a Ge crystal. For each measurement, the spectral resolution was 2 cm^{-1} and 64 scans were performed.

The MALDI-TOF Spectra

Measured on an UltrafleXtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) with a 1kHz smart beam II laser. The measurements were done in reflection mode by means of the droplet technique. The matrix consisted of 3-hydroxypicolinic acid (HPA)/picolinic acid (PA)/ammonium tartrate in a ratio of 9/1/1. The matrix (1 μL) was applied on the target (ground steel) and dried at room temperature. The sample was dissolved in a mixture water/acetonitrile (1:1). The sample (1 μL) and matrix (1 μL) were mixed and added on the top of the dried matrix preparation spot and dried at room temperature.

Preparation of Stock and Working Solutions

The samples of HA, solutions of ascorbate and cupric chloride were prepared as published previously.³² For rotational viscometry and the ABTS assay we prepared stock aqueous solutions of both keratin samples (4.9 mg/mL) and diluted them twice. Concentrations of working solutions in rotational viscometry were 61.2, 30.6 and 15.3 $\mu\text{g}/\text{mL}$. Concentrations of working solutions in the ABTS assay were 5.0, 2.5 and 1.25 $\mu\text{g}/\text{mL}$. For other methods the keratin powder (100 mg) was leached for 15 min in distilled water (400 mL). The keratin extract was filtered and completed with 5 mmol/L phosphate buffer (pH 7.4) to the volume 500 mL. A series of solutions with decreasing concentration is prepared from the stock solution, preferably diluted twice compared to the previous solution.

Rotational Viscometry

The dynamic viscosity of the reaction mixture (8 mL; 0.15 M aqueous NaCl) containing HA (20 mg), ascorbate (100 μM) plus Cu(II) ions (1 μM) in the absence and presence of keratin in concentration 61.2, 30.6 and 15.3 $\mu\text{g}/\text{mL}$ was monitored by a

Brookfield LVDV-II+PRO digital rotational viscometer (Brookfield Engineering Labs., Inc., Middleboro, MA, U.S.A.) at $25.0 \pm 0.1^\circ\text{C}$ and at a shear rate of 237.6 s^{-1} for 5 h in a reservoir-spindle couple made of Teflon. Viscosity values were collected in 3-min intervals.

ABTS Assay

Radical-scavenging activity was measured according to a modified ABTS method. To prepare ABTS⁺ radical cation, an aqueous solution of K₂S₂O₈ (3.3 mg of K₂S₂O₈ in 5 mL of distilled H₂O) was added to ABTS (17.2 mg), and the resulting solution was stored 14 h in the dark. Finally, the dark-green ABTS⁺ radical cation solution (1 mL) was diluted with distilled H₂O (60 mL) and used in the ABTS assay. The investigated samples comprised 2 mL of the diluted ABTS⁺ solution with addition of 50 mL of the keratin stock solution (4.9, 2.45 and 1.2 mg/mL). UV/VIS spectra were recorded in time interval 2 - 30 min using a UV-VIS 1800 spectrophotometer (Shimadzu, Germany).

Antimicrobial Conservation Efficiency

Used microorganisms:

Escherichia coli CCM 3954, Staphylococcus aureus CCM 4516, Pseudomonas aeruginosa CCM 1961, Candida albicans CCM 8215, Aspergillus brasiliensis CCM 8222.

Tested sample, keratin powder was tested to specification PhEur.7.0. Item 5.1.3-2 Criterion A – Accredited test.

Formaldehyde Emissions

Test method JIS A 1460 (2001). Building boards. Determination of formaldehyde emission. Desiccator method.

The volume of desiccator: 9-11 dm³

Loading coefficient: 1800 cm²

Temperature: $20 \pm 0.5^\circ\text{C}$

Test duration: 24 h

The analytical method: acetylacetone method with spectrophotometric evaluation.

Preparation of birch (*Betula*) veneer panels: Five layers plywood were prepared to determine the formaldehyde emission under the following conditions: pressure of 1.8 MPa, temperature of 105°C , pressing time 6 min. Samples were kept at the temperature of $20 \pm 2^\circ\text{C}$ and relative humidity of $65 \pm 5^\circ\text{C}$ for 7 days.

Results and Discussion

The experimental work has focused on the investigation of modifications of natural polymer keratin and its hydrolytic treatment directed mainly to cleavage of disulfide bond to thiol groups S–H, resp. O–SO₃ bond and influence of these changes on antioxidant properties, antimicrobial conservation efficiency and possibility to reduce formaldehyde emissions.³³

Two types of samples of low-molecular dried keratin hydrolysates K-1 and K-2 were prepared and parameterized from sheep wool under laboratory conditions (Table I and Table II).

Table I
Parameters of keratin K-1.

Chemical composition of keratin K-1 hydrolysate

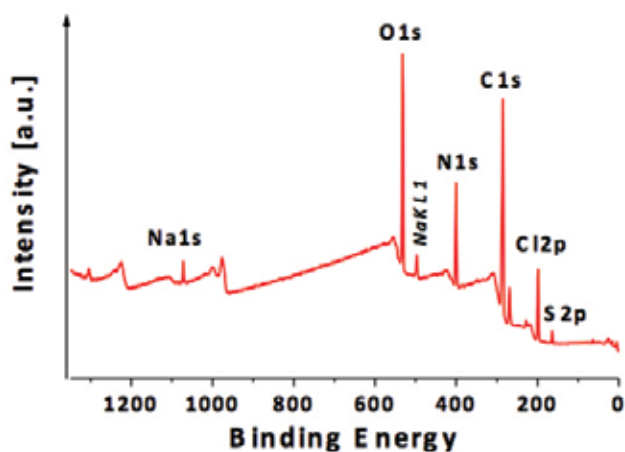
C 1s	O 1s	N 1s	S 2p		Cl 2p	Na 1s
58.9%	18.0%	15.2%	1.2%		5.7%	1.0%
			sulfide/ C-S	SO ₃		
			88.4%	11.7%		

Table II
Parameters of keratin K-2.

Chemical composition of keratin K-2 hydrolysate

C 1s	Na 1s	N 1s	S 2p		O 1s
62.3%	4.4%	12.0%	1.6%		19.6 %
			sulfide/ C-S	SO ₃	
			40.3 %	59.7%	

K-1



K-1

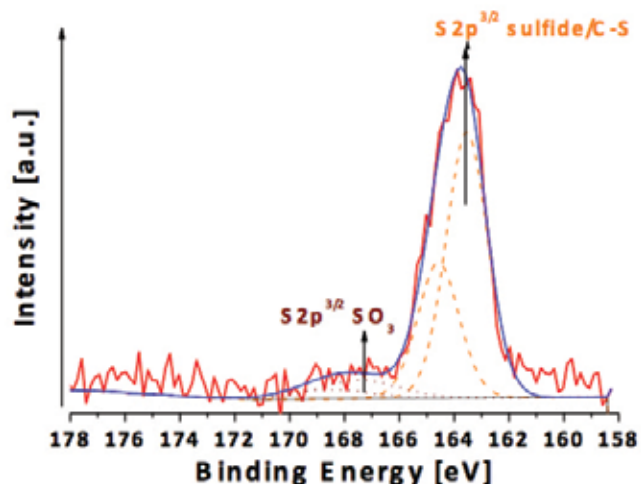
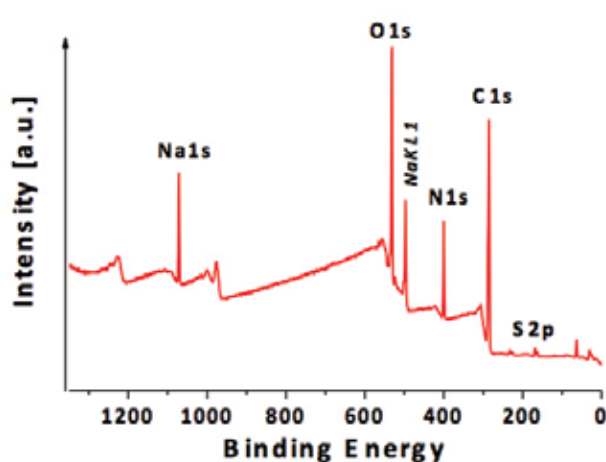


Figure 1. X-Ray photoelectron spectroscopy spectra analysis of keratin K-1 hydrolysate.

K-2



K-2

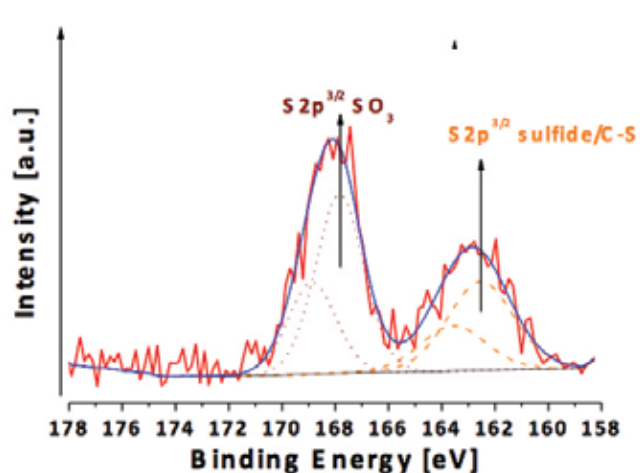


Figure 2. X-Ray Photoelectron Spectroscopy spectra analysis of keratin K-2 hydrolysates.

(N1s signal at ~ 400 eV), sodium (Na1s signal at ~ 1072 eV), chlorine (Cl2p signal at ~ 198 eV) and sulfur as C-S (S2p signal at ~ 163 eV) and SO₃ (S2p signal at ~ 168 eV) (Figure 1 and Figure 2).

Changes in the spectra of keratin hydrolysates are mainly expressed in the content of chlorine and sulfur, in proportion between the reduced form of C-S (S2p signal at ~163 eV) and SO₃ (S2p signal at ~ 168 eV) – oxidized form. Keratin K-1 has a higher content of reductive sulfur bonds.

Infrared FTIR-ATR Spectroscopy

Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) was used for investigations of the surface chemical composition. Samples of both keratins K-1 and K-2 were evaluated by IR spectral analysis of which results are presented in Figure 3.

ATR-FTIR spectra of both keratin samples are similar. There are observable main peaks typical for protein-type materials. Main differences between IR spectra of keratin samples are observed in the area of valence vibrations, where a broad, non-resolved band with local maxima at 3146 cm⁻¹, 3049 cm⁻¹, 2971 cm⁻¹, is present for sample K1. These can be assigned to -OH, -NH₂ and -CH functional groups. On the other hand, sample K-2 has this main broad band more resolved, with approximately the same local maxima. Additionally, an apparent peak at 2624 cm⁻¹ is present for both samples K-1 and K-2 (as a part of the broad peak at 3800 – 2200 cm⁻¹), that could be assigned to S-H bond.

Peak characteristics for the amide structure are present in the area of deformation vibrations, peaks with the maxima at 1660 cm⁻¹ correspond to amide I range, peaks assigned to the range of amide II are at maxima at 1400 – 1500 cm⁻¹ and peaks with the maximum at 1224 cm⁻¹ correspond to amide III range.

Peaks representing the amide structure are more resolved for sample K-2 (blue) compared to sample K-1 (red).

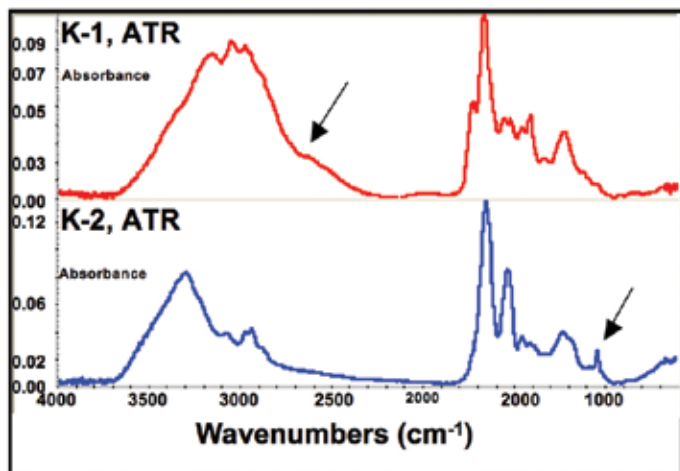


Figure 3. FT-IR spectra analysis of keratins K-1 and K-2 hydrolysates.

The IR spectra of sample K-1 shows a peak at 1722 cm⁻¹ that is present most probably due to hydrolysis of the -COOH group (C=O).

A small number of weak bands is present for both samples K-1 and K-2 in the range of 1000 – 1130 cm⁻¹ that could be assigned to various sulfuric oxides formed during keratin hydrolysis.

It can be stated that main strips typical for protein-type materials (inter-band corresponding to the OH, CH, C=O, NH, C-O-C bonds are observable in all the spectra. These spectra differ mainly in the field of deformation vibrations.

The MALDI-TOF Spectra

Measured on an Ultrafle Xtreme MALDI-TOF/TOF mass spectrometer (Bruker Daltonics, Germany) with a 1kHz smart beam II laser. Samples of keratins K-1 and K-2 were evaluated by MALDI-TOF analysis and the spectra are presented in Figures 4 and 5.

Changes in samples of keratin hydrolysates are mainly in molecular weights. The keratin K-2 sample is less hydrolyzed and has higher molecular weights in the range (550 – 4000) Da.

Rotational Viscometry

As shown in Figure 6, oxidative degradation of HA was monitored by using Cu(II) ions (1.0 μM) and ascorbate (100 μM), the so-called Weissberger oxidative system (WBOS) in the

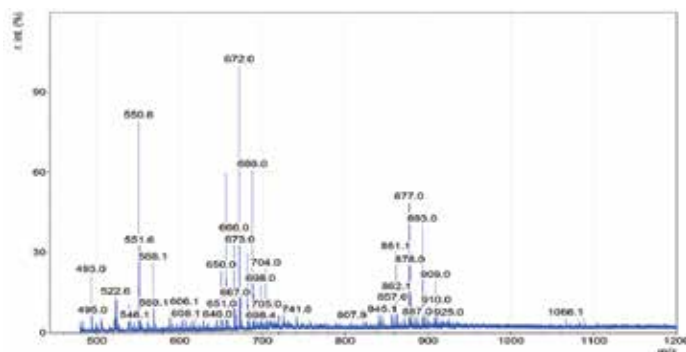


Figure 4. MALDI-TOF analysis of keratin K-1 hydrolysates.

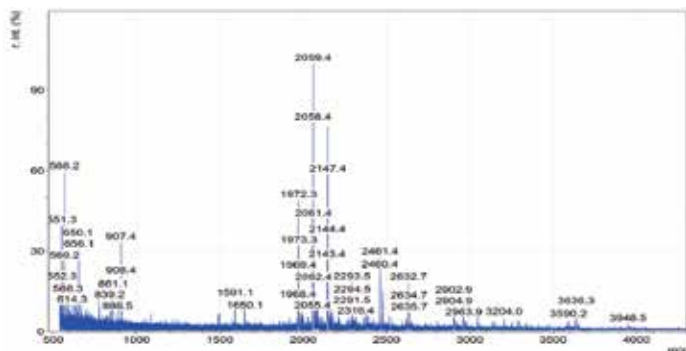


Figure 5. MALDI-TOF analysis of keratin K-2 hydrolysates.

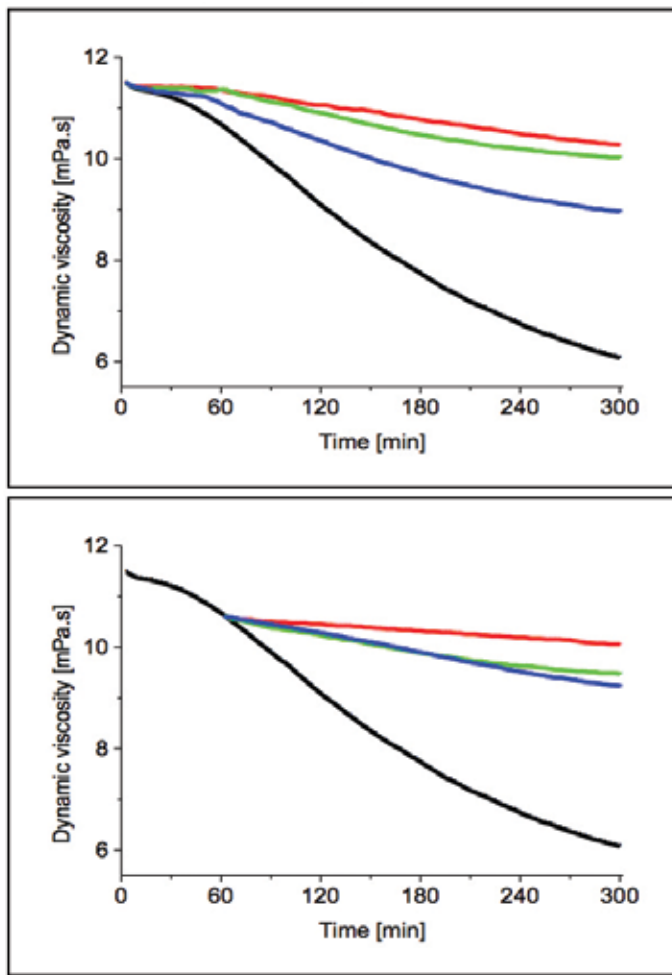


Figure 6. Time dependent changes in dynamic viscosity of the HA solution exposed to oxidative degradation by WBOS (black) and in the presence of keratin K-1 at concentrations 61.2 (red), 30.6 (green) and 15.3 (blue) $\mu\text{g/mL}$. Keratin was added to the HA mixture before HA degradation (top panel) or 1 h later (bottom panel). (Ed Note: Colors top to bottom at 300 min are red, green, blue and black.)

absence and presence of keratin K-1 (a keratin hydrolysate prepared by acid hydrolysis) at concentrations 61.2; 30.6 and 15.3 $\mu\text{g/mL}$. First, HA was subjected to WBOS, whereas dynamic viscosity of the HA solution decreased by 5.4 mPa.s. within 5 h (black curve). Addition of keratin K-1 resulted in a less rapid $\cdot\text{OH}$ radical-mediated HA degradation reaching the decrease in dynamic viscosity of the HA solutions in a range from 1.2 to 2.5 mPa.s. (red, green and blue curves).

A similar protective effect of keratin K-1 was observed when added to the hyaluronan solution during oxidative degradation, where alkoxy-/peroxy- radicals rather than $\cdot\text{OH}$ radicals are produced (bottom panel).

Next, keratin K-2 at concentration 30.6 $\mu\text{g/mL}$ was assessed as a substance to prevent HA degradation (Figure 7). The decreases

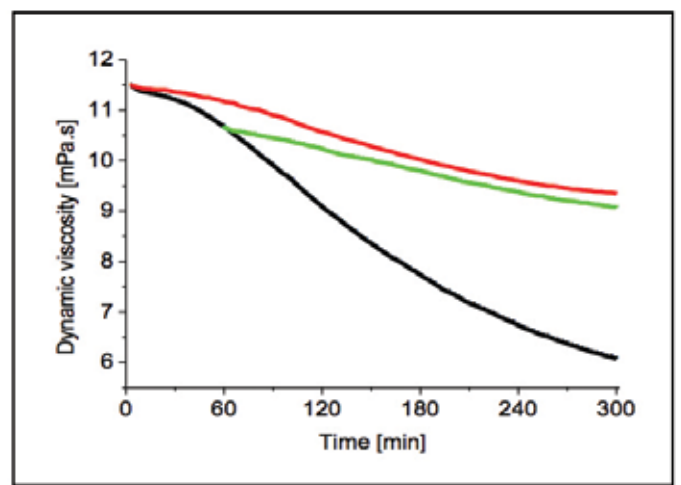


Figure 7. Time dependent changes in dynamic viscosity of the HA solution exposed to oxidative degradation by WBOS (black curve) and in the presence of keratin K-2 at concentration 30.6 $\mu\text{g/mL}$. Keratin K-2 was added to the HA mixture before HA degradation (red curve) and 1 h later (green curve).

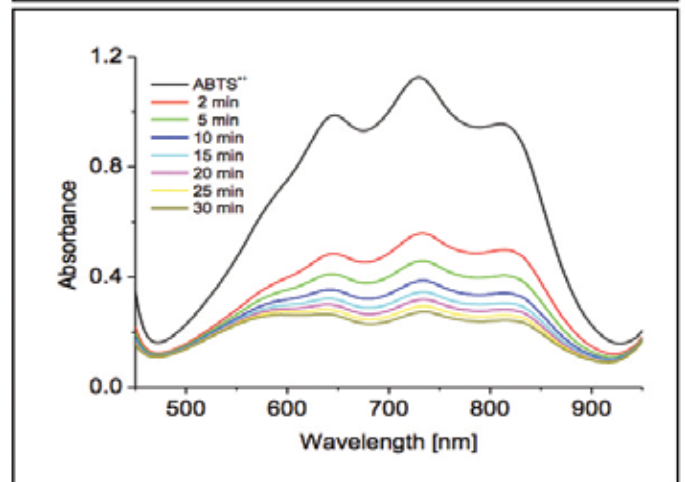
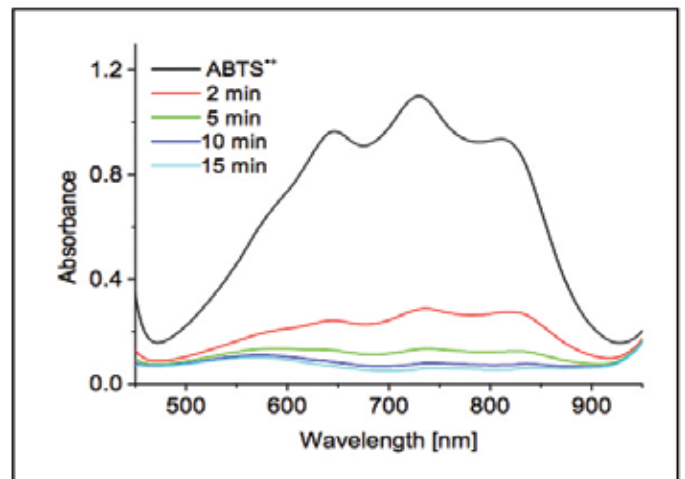


Figure 8. Scavenging of the ABTS^+ cation radical by keratin K-1 (top panel) and keratin K-2 (bottom panel) at concentration 120 $\mu\text{g/mL}$.

in dynamic viscosity of the HA solution were 2.13 and 1.6 mPa.s. within 5 h when adding keratin K-2 before HA degradation begins (red curve) or 1 h later (green curve), respectively.

ABTS Assay

As shown in Figure 8, top panel keratin K-1 at concentration 120 $\mu\text{g}/\text{mL}$ effectively scavenged the ABTS^+ cation radical within 15 min. In contrast, results of a measurement of keratin K-2 (bottom panel) within 30 min showed a continuous and less rapid scavenging of the ABTS^+ cation radical.

Figure 9 illustrates the results of scavenging the ABTS^+ cation radical using keratin K-1 or K-2 at lower concentration 60 $\mu\text{g}/\text{mL}$. As evident, keratin K-1 (top panel) was again more effective in reduction of ABTS^+ cation radical than did keratin K-2 (bottom panel).

As depicted in Figure 10, both keratins at the lowest concentration 30 $\mu\text{g}/\text{mL}$ reduced the least efficiently the ABTS^+ cation radical. Keratin K-2 scavenged again the radical more slowly compared to keratin K-1.

A lower efficiency of scavenging the ABTS^+ cation radical by using keratin K-2 compared to keratin K-1 was observed also at lower concentrations 60 and 30 $\mu\text{g}/\text{mL}$.

The ABTS assay was used to determine IC_{50} values of the keratin hydrolysates. The reached results showed antioxidant properties of both keratin hydrolysates. The IC_{50} value of keratin K-1 is comparable with the one of vitamin C (Table III).

Table III
 IC_{50} values of keratin samples K-1 and K-2 and the reference vitamin C.

ABTS assay	Samples		Reference
	K-1	K-2	Vitamin C
IC_{50} (mg/mL)	0.023	0.035	0.02

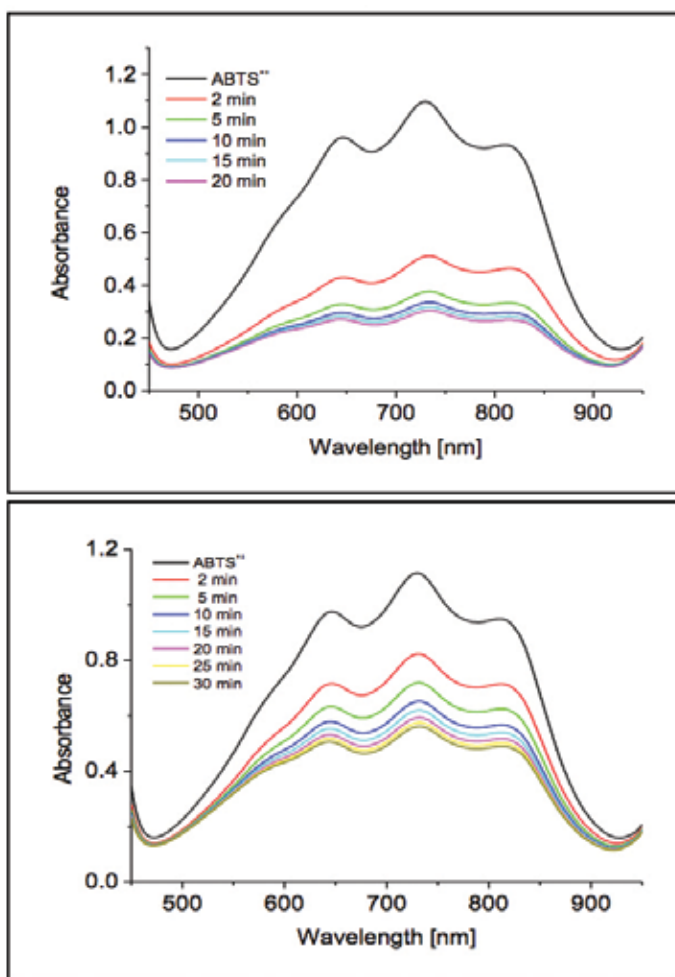


Figure 9. Scavenging of the ABTS^+ cation radical by keratin K-1 (top panel) and keratin K-2 (bottom panel) at concentration 60 $\mu\text{g}/\text{mL}$.

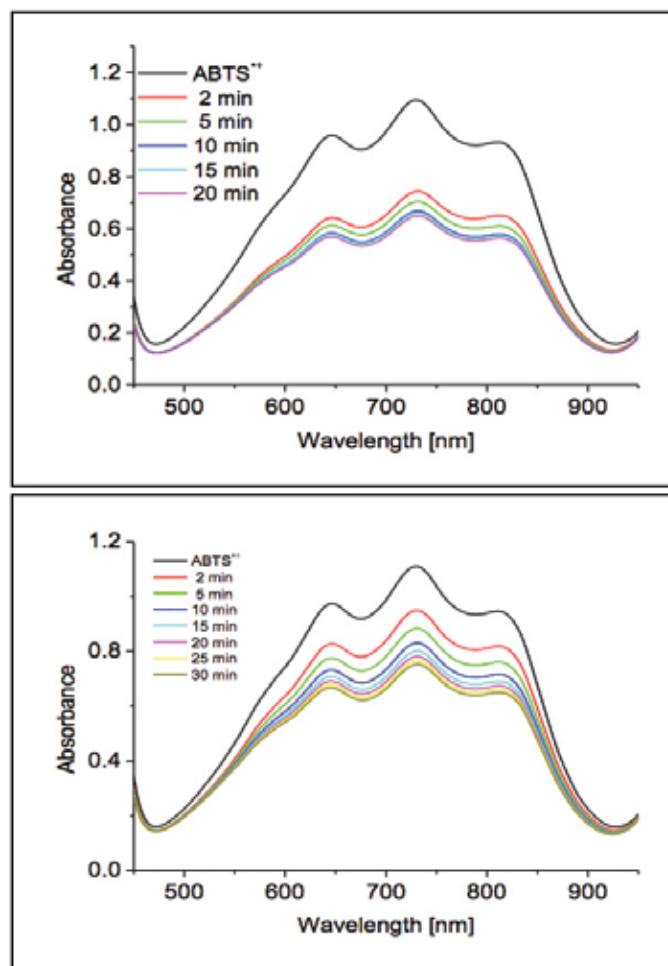


Figure 10. Scavenging of the ABTS^+ cation radical by keratin K-1 (top panel) and keratin K-2 (bottom panel) at concentration 30 $\mu\text{g}/\text{mL}$.

Antimicrobial Conservation Efficiency

Assessment of disinfectant activity of keratin hydrolysates – keratins K-1 and K-2 (Table IV and Table V).

Tested sample powder of keratin **conforms** in tested parameters to specification PhEur.7.0. Item 5.1.3-2 Criterion A.

Tested sample powder of keratin **conforms** in tested parameters to specification PhEur.7.0. Item 5.1.3-2 Criterion A.

Natural hydrolyzed sulfur-containing keratin biopolymers provided antimicrobial effect on selected bacterial strains, which allowed reduction of even exclude supplementary dosing of preservatives.

Table IV
Antimicrobial conservation efficiency of keratin K-1.

Used microorganism	Log reduction					Test method	TS
	0 day	2 days	7 days	14 days	28 days		
<i>Escherichia coli</i> , CCM 3954	6	4	NR	NR	NR	PhEur. item 5.1.3	A
<i>Staphylococcus aureus</i> CCM 4516	6	2	NR	NR	NR	PhEur. item 5.1.3	A
<i>Pseudomonas aeruginosa</i> , CCM 1961	6	3	NR	NR	NR	PhEur. item 5.1.3	A
<i>Candida albicans</i> CCM 8215	5	1	NR	NR	NR	PhEur. item 5.1.3	A
<i>Aspergillus brasiliensis</i> CCM 8222	5	2	NR	NR	NR	PhEur. item 5.1.3	A

Purity test: conforms

Neutralization efficiency: conforms

NR – microorganism no recovery

TS – Test type

A – Accredited test

Table V
Antimicrobial conservation efficiency of keratin K-2.

Used microorganism	Log reduction					Test method	TS
	0 day	2 days	7 days	14 days	28 days		
<i>Escherichia coli</i> , CCM 3954	6	3	4	4	NR	PhEur. item 5.1.3	A
<i>Staphylococcus aureus</i> CCM 4516	6	2	3	NR	NR	PhEur. item 5.1.3	A
<i>Pseudomonas aeruginosa</i> , CCM 1961	6	2	3	4	4	PhEur. item 5.1.3	A
<i>Candida albicans</i> CCM 8215	5	1	3	3	3	PhEur. item 5.1.3	A
<i>Aspergillus brasiliensis</i> CCM 8222	5	2	3	3	3	PhEur. item 5.1.3	A

Purity test: conforms

neutralization efficiency: conforms

NR – microorganism no recovery

TS – Test type

A – Accredited test

Table VI
Test results – formaldehyde emission by the desiccator method.

Sample	Extinction	Emission [mg/l]
0 – UF resin + 20% filler + 5% hardener – standard	0.0533	0.587
1 – UF resin + 20% filler + 5% hardener + 1% K-1	0.0501	0.551
2 – UF resin + 20% filler + 5% hardener + 2% K-1	0.0357	0.410
3 – UF resin + 20% filler + 5% hardener + 5% K-1	0.0348	0.368
4 – UF resin + 20% filler + 5% hardener + 1% K-2	0.0488	0.534
5 – UF resin + 20% filler + 5% hardener + 2% K-2	0.0487	0.533
6 – UF resin + 20% filler + 5% hardener + 5% K-2	0.0477	0.521

Reducing Formaldehyde Emissions

Keratin hydrolysates K-1 and K-2 were tested to determine their influence on release of formaldehyde from wood based panel – plywood bonded with UF resins. Measured values of extinctions of tested samples confirmed the lowering of formaldehyde emissions for each concentration of keratin hydrolysate in comparison with the reference sample. The most significant decreasing of formaldehyde up to 37% was obtained for 5% dosage of keratin K-1. Samples of keratin K-2 lowered formaldehyde emission only about 2.5% in comparison with the reference sample (Table VI).

Determined results confirmed that both types of keratin additives lower formaldehyde emissions from UF bonded plywood.³³

Previous research³⁴ tested modification of UF resin with keratin through copolymerization reaction. The results show that the addition of keratin in amount 5% is the best choice. By optimizing the synthesis process, the low toxicity modified urea-formaldehyde resin was prepared.

Conclusions

A growing interest in the biopolymer keratin, due to its biocompatibility, biodegradability, mechanistic hardness and natural occurrence, has led to development of materials for biomedical applications.

The experimental work has focused on the investigation of modifications of natural polymer keratin and its hydrolytic

treatment directed mainly to cleavage of disulfide bond to thiol groups S–H, resp. O–SO₃ bond.

It was discovered and confirmed that the technological process used for the preparation of the hydrolysates greatly effects the final composition (distribution of molecular weights of the hydrolysates), which in turn contributes to the overall physical, chemical and biological behavior of these biomaterials

Keratin hydrolysate K-1, prepared using acidic hydrolysis, had a higher content of reducing sulfur groups that ensured the antioxidant effect comparable to that of vitamin C. Moreover, the disinfectant effect could allow for lowering or complete removal of preservative additives e.g. in cosmetic products. Lastly, the effect on the decrease of formaldehyde emissions from glued plywood panels could allow for their reclassification into a class with the lowest formaldehyde emission amount.

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