

RECOVERY OF RENEWABLE RESOURCES FROM LEATHER AND FUR INDUSTRY THROUGH DEVELOPMENT OF ADVANCED ECOLOGICAL MATERIALS

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Abstract

The aim of this research is the development of biomaterials with controlled characteristics from renewable resources generated by leather and fur industry and characterization of smart functionalities, applicable in agriculture. Keratin hydrolysates were obtained by calcium oxide hydrolysis (KerCa) in view of agriculture applications. The chemical analysis of keratin hydrolysate indicates a calcium (9.93%) and protein-rich (72.83%) composition with a long-term release of organic nitrogen which represents 12.96% of the dry substance product. The SDS-PAGE electrophoresis of KerCa highlights the characteristic bands of high molecular weight proteins (20-10 kDa) but also rich in low molecular weight peptides (6-9 kDa). Diffuse Light Scattering analysis shows the presence of particle size populations of 712 nm and of 141 nm. Experiments regarding the nutritional and biostimulation capacity on plant growth were performed with KerCa in 1:20 and 1.5:20 dilutions on lentil and wheat seeds (Tamino and Mirastar type). The germination and growth of the plants were monitored and a stimulation of 5% to 15% was recorded against the control samples. Other formulations aimed at obtaining foliar blends containing microelements, collagen and keratin hydrolysates that have been applied to wheat plants. The results indicated an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates in agriculture show that they can be the basis for obtaining new biomaterials with various ecological applications.

Key words: *renewable resources from tanning, biomaterials with controlled characteristics, smart functionalities, keratin and collagen hydrolysates, nutritional and biostimulation capacity*

1. INTRODUCTION

Keratin has recently appeared in various applications based on natural biomaterials. Besides the potential for assembly in organized structures, keratin is also constituted by bioactive elements suitable for initiating biocompatible interactions with various substrates [1].

Waste rich in keratin pollutes the environment and is released in increasing quantities as by-products from agro-industrial or leather manufacturing processes [2,3,4]. Keratin from renewable sources generated by leather and fur industry is available and easy to collect. Keratin from different origins can be specifically processed for diverse applications [5, 6].

The keratinous materials have high protein content with a composition of at least 17 amino acids that can be used in animal feed or as fertilizer for agriculture. The degradation of keratin waste can therefore provide an inexpensive source of organic nitrogen and amino acids [7].

At present, there is a particular interest in research on biodegradable materials obtained from renewable sources such as proteins, polysaccharides and lipids. The use of biodegradable materials is the most effective solution to solve environmental pollution problems caused by synthetic chemicals [8, 9].

An omnipresent biological material, keratin, has a high content of sulfur and protein. Inspired examples from the living world can provide useful solutions for designing new structural and functional materials. The keratinous materials are made of alpha-keratin (wool, hair, nails, hooves, horns, corns) and β -keratin (feathers, beaks, bird claws, reptilian claws and scales).

A comprehensive study of the biochemistry, structure and mechanical properties of keratin is of great importance for the development of new advanced ecological materials and models based on keratin [10].

Organic biomaterials obtained from keratin extracted from unprocessed by-products of the leather industry have potential for use in agriculture, bio-economy, industry and niche areas.

Keratin is a protein with specific characteristics that contains amino acids with amino ($-\text{NH}_2$) and carboxylic ($-\text{COOH}$) functional groups. It has a high content of cysteine in the amino acid sequence, with thio ($-\text{SH}$) groups, which are responsible for intra- and intermolecular sulphide (disulfide) linkages. Keratin is a biopolymer with various functional properties, applicable to the production of controlled biomaterials used in biostimulants for growth and nutrition for plants as a source of organic nitrogen and sulfur, representing an important mesonutrient. These ecological materials can contribute to the reduction of pollutant and harmful chemical compounds for insects and especially bees.

Keratin hydrolysates are used as natural fertilizers and are a source of organic nitrogen as a macronutrient. Organic amino-based fertilizers have increasingly begun to be used by agricultural producers both in the conventional production sector and in the organic production sector. Keratin and collagen hydrolysate-based fertilizers provide organic nitrogen which are immediately available to synthesize plant proteins, so amino acids become part of the plant's components with minimal energy consumption.

2. MATERIALS AND METHODS

2.1. Materials

The recovered crude wool (LB) (Fig.1) was degreased (LD) (Fig. 2) in a float with 1% Na_2CO_3 , 1% NH_3 (solution of 25% concentration), 1% detergent by stirring for 12 hours at 60°C in a FAVE equipment (Fig. 3). The alkaline hydrolysis of the degreased wool was done in water at 80°C , in a float of 1: 20, with 10% CaO , by stirring for 12 hours and 8 hours statically.



Fig. 1. Recovered crude wool (LB)



Fig. 2. Degreased wool (LD)



Fig. 3. FAVE equipment

2.2. Methods

The wool recovered from the waste resulting from the processing of natural sheepskins was degreased and hydrolyzed by the alkaline method in the presence of calcium oxide in order to obtain keratin hydrolysates with high degree of cleavage of macromolecular chains. The method for keratin solubilisation is presented in Figure 4.

The keratin hydrolysates obtained by alkaline hydrolysis in the presence of calcium oxide is rich in organic nitrogen and minerals which can serve as growth biostimulant for cultivated plants in poor soils.

Raw wool was chemically characterized by determining the dry matter, ash, extractable substances and total nitrogen content, the data obtained being listed in Table 1.

Table 1. Chemical characterization of the raw wool (LB)

No.	Characteristics UM , %	Method Standard	Raw wool, LB
1	Dry matter	SR EN ISO 4684:2006	86.67
2	Ash*	SR EN ISO 4047:2002	12.83
3	Total nitrogen*	SR EN ISO 5397:1996	12.83
4	Extractable substances*	SR EN ISO 4048:2002	5.33

*values reported on a dry matter basis

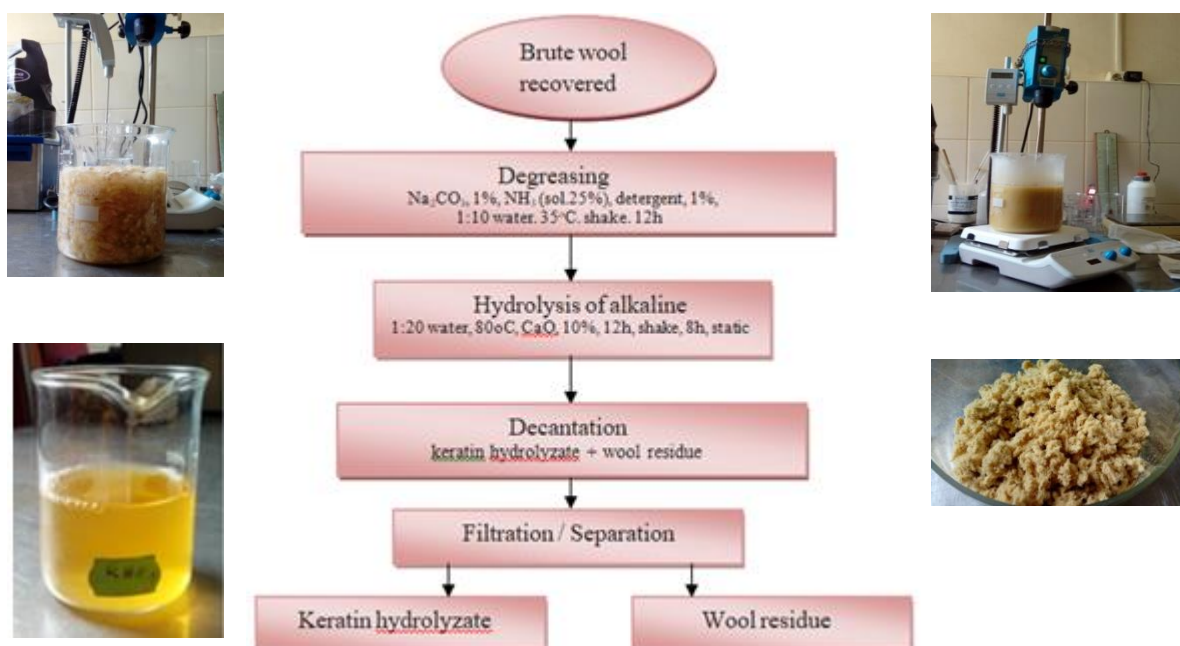


Figure 4. Method for keratin solubilisation by alkaline hydrolysis.

From analyses obtained for crude wool (LB) and degreased wool (LD), different values are noticed for ash and extractable substances (Table 2). Values of degreased wool (LD) are lower than those of the raw wool sample, which highlight the removal of impurities (lignin, soil, lanoline etc.) during the washing process (Table 2).

Table 2. Chemical characterization of the degreased wool (LD)

No.	Characteristics UM , %	Method Standard	Degreased wool, LD
1	Dry matter	SR EN ISO 4684:2006	87.46
2	Ash*	SR EN ISO 4047:2002	2.48
3	Extractable substances*	SR EN ISO 4048:2002	0.34

*Values reported on a dry matter basis

Characterization of keratin hydrolysate for applications in agriculture

The keratin hydrolysate obtained by alkaline hydrolysis was characterized by physical-chemical analysis, SDS-PAGE electrophoresis, Diffuse Light Scattering (DLS), ATR -FTIR, contact angle and surface tension.

Physico-chemical analysis of keratin hydrolysate

The content in dry matter, ash, total nitrogen, protein, pH, amine nitrogen, calcium oxide (Table 3) highlights the functionality of the keratin hydrolysate obtained in the presence of calcium oxide as follows: ash content (12.50%) and calcium oxide (9.18%) show that keratin hydrolysate is rich in mineral substances; total nitrogen (14.06%) and protein (79.02%) represent a source of nutrients with slow release properties for the development of plant crops.

Table 3. Physical and chemical characteristics of the keratin hydrolysate

No.	Characteristics, UM	Standard Method	Values KerCa
1	Dry matter, %	SR EN ISO 4684:2006	5.12
2	Ash*, %	SR EN ISO 4047:2002	12.50
3	Total nitrogen*, %	SR ISO 5397:1996	14.06
4	Protein*, %	SR ISO 5397:1996	79.02
5	pH, units of pH	STAS 8619/3:1990	11.27
6	Ammonia nitrogen**, %	Metoda ICPI	3.21
7	Calcium oxide*, %	Metoda ICPI	9.18

*values reported on a dry matter basis, **value reported on protein basis

Analysis of keratin hydrolysate by SDS-PAGE electrophoresis.

The obtained keratin hydrolysates were characterized by determining the molecular weight distribution using SDS-PAGE electrophoresis. One of the most important factors in obtaining bioactive peptides with functional properties is their molecular weight. The analyses results showed that keratin hydrolysates are polydispersions of free polypeptides, oligopeptides and amino acids.

The analyses were carried out according to Laemmli [11] method using Coomassie Blue Stain R250 for protein visualisation. The analysis results showed the presence of predominantly low molecular weight keratin hydrolysate for 6-3 kDa and other fractions of 6-17 kDa (Fig.5). According to the literature data [12], these bands correspond to high sulfur keratin (20-10 kDa) and to keratin with free amino acids, mainly glycine / tyrosine (6-9 kDa).

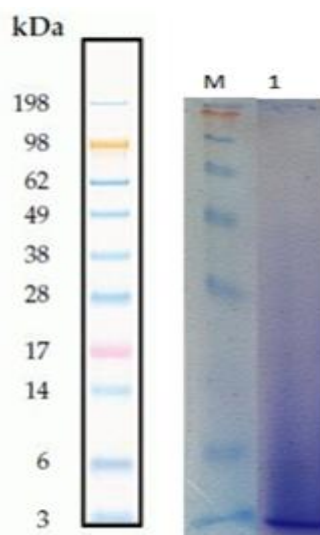


Figure 5. SDS-PAGE electrophoresis for KerCa keratin hydrolysate

Determination of particle size by DLS technique

The keratin hydrolysate was analyzed using Zetasizer Nano ZS from Malvern. Measurements were made to determine particle size and Zeta potential, and the results obtained are shown in Table 4.

Table 4. Particle sizes and Zeta potential

No.	Keratin hydrolysate	Size, nm			Pdl	Zeta potential, mV
		Main populations	Average			
1	KerCa	141	712	296	0.521	-7.75

From Table 4 and Figures 6 and 7 we can see the presence of two major populations, at 141 nm and 712 nm, an average of 296 nm, the polydispersity of 0.521 and the Zeta potential of - 7.75 mV which means a high tendency of agglomeration.

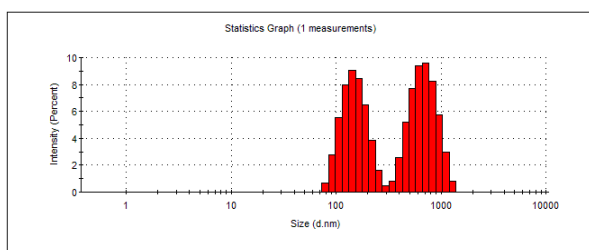


Figure 6. Size distribution histogram of keratin hydrolysate particles

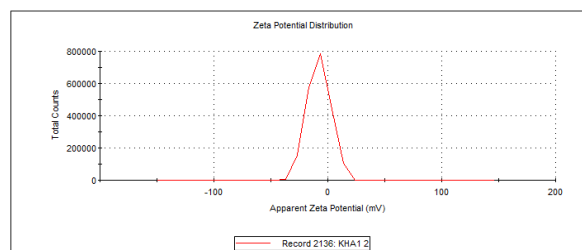


Figure 7. Zeta potential for keratin hydrolysate

Characterization by ATR -FTIR

For the ATR -FTIR spectra a FT-IR 4200 JASCO spectrometer was used at 16 scans on the 4000-400cm⁻¹ scale, with a scanning speed of 2.2KHz and a resolution of 4cm⁻¹, single beam, with an ATR accessory with diamond sensor.

The infrared absorption spectrum of keratin hydrolysate shows characteristic absorption bands attributed to peptides (-CONH), known as amide I, amide II, amide III and sulfur compounds (Fig.8).

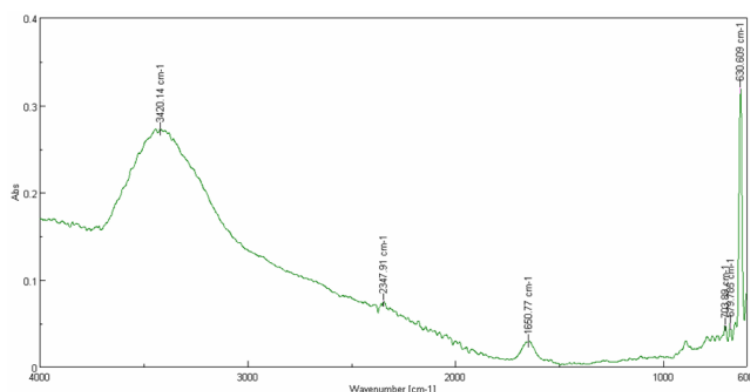


Figure 8. The ATR-FTIR spectrum of the keratin hydrolysate

The 3400 cm^{-1} primary amide band characteristic of $\nu_{\text{N-H}}$ stretching vibration, the 3440 cm^{-1} secondary amide band also specifies $\nu_{\text{N-H}}$ stretching vibration and the 1650 cm^{-1} tertiary amide band width specific for $\nu_{\text{C=O}}$, these being present in the obtained keratin hydrolysate spectra. The $600\text{-}700\text{ cm}^{-1}$ band is specific for sulfur compounds and $\nu_{\text{C-S}}$ stretching vibration and the $800\text{-}900\text{ cm}^{-1}$ band for sulfur compounds and $\delta_{\text{S-H}}$ saturation vibrations [13].

Determination of contact angle and surface tension

A VCA Optima type device was used to measure the contact angle. The keratin-glass hydrolyzed contact angle was determined by establishing a 5-foot capture protocol per minute for one minute. In this case, the value of the contact angle provides information about the wetting capacity of the liquid. Thus, the lower the angle of contact (Table 5), the more easily the sample can be absorbed when used in plant foliar dispersions.

Table 5. Contact angle and surface tension values for KerCa

Keratin hydrolysate	Contact angle, °	Surface tension, mJ/m ²
KerCa	9.25	40.77

Surface tension is the general property of liquids to take geometric shape with the smallest area in the absence of external forces. This is due to the action of the cohesion forces between the fluid molecules.

The value of surface tension of keratin hydrolysates confirms the results from the contact angle, namely the presence of low intermolecular forces, which lead to a large capacity of wetting the samples.

A low surface tension facilitates the dissolution of any specific activity compounds used to obtain new products with applications in agriculture.

Characterization of wool residue

The residual wool resulting after alkaline hydrolysis was milled (Fig. 9) and chemically characterized by the determination of dry matter, ash, and total nitrogen (Table 6). The high content of ash suggested that this material can be used for soil acidity correction.

Table 6. Physical and chemical characterization of the resultant wool residue

No.	Characteristics UM , %	Method Standard	Wool residue
1	Dry matter	SR EN ISO 4684:2006	85.84
2	Ash*	SR EN ISO 4047:2002	14.95
3	Total nitrogen*	SR ISO 5397:1996	undetected

*values reported on a dry matter basis



Figure 9. Milled wool residue

3. RESULTS

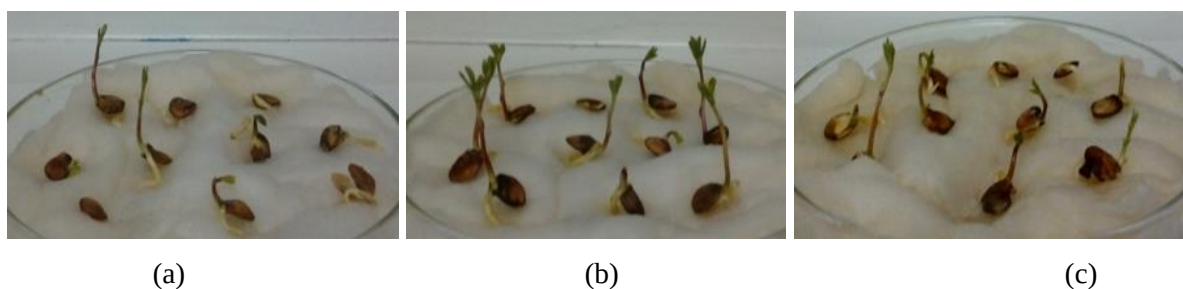
Testing and evaluating the functionality of keratin hydrolysate with applications in agriculture

The functionality of keratin hydrolysate has been tested and evaluated by use in treatments applied to various types of seeds.

The keratin hydrolysate obtained by alkaline hydrolysis in the presence of calcium oxide has been used in various concentrations to treat lentils and wheat seeds. The survey was done during 5-6 days on seed germination and 19 days on plant growth.

Testing and evaluating the functionality of keratin hydrolysate on lentil seeds

The lentils were germinated in two dilutions of 1:20 and 1.5: 20 of keratin hydrolysate samples and water. Seeds from the control samples were germinated only with water. Evaluation of the effect of keratin hydrolysate on lentil plants was followed for 19 days and indicated an increase in lentil plants length by 10% in the case of samples treated with 1:20 KerCa and by 5% in the case of samples treated with 1.5: 20 KerCa versus the control sample (Fig.10).



(a)

(b)

(c)

Figure 10. Lentil seeds germination after 5 days

(a) control, treated (b) with 1:20 KerCa, (c) with 1.5:20 KerCa

Testing and evaluating the functionality of keratin hydrolysate with applications in wheat seed farming

Two kinds of wheat seeds have been treated with keratin hydrolysate: Tamino and Mirastar.

Keratin hydrolysate was used in the same dilutions in water of 1:20 and 1.5: 20 as compared to the control sample treated with water. The results of the treatments indicated a growth of wheat plant length by 15% when using dilution of 1: 20 and by 10% when using the dilution of 1.5: 20 as compared to the control.

The biostimulatory effect of keratin hydrolysate was also highlighted in an extended experiment on a larger number of wheat grains by treatment with a 2.5% solution. Mirastar and Tamino wheat seeds were treated with KerCa by 10 minutes wetting. The seeds were grown on cotton and in the soil.

After 5 days the number of germinated seeds was 10% higher for KerCa-treated seeds than for the control samples germinated in both medium, cotton wool and ground.

The germination rate of Mirastar wheat seeds treated with keratin hydrolysate was determined and a higher germination rate after five days of experiment was noticed (Fig.11).

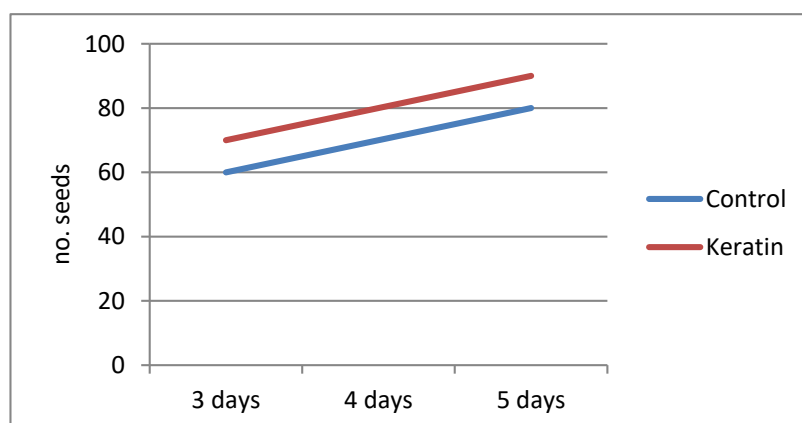


Figure 11. Germination rate for Mirastar wheat type

In the KerCa-treated Tamino type seeds, a higher germination rate is observed after the first three days as compared to the control seeds (Fig.12).

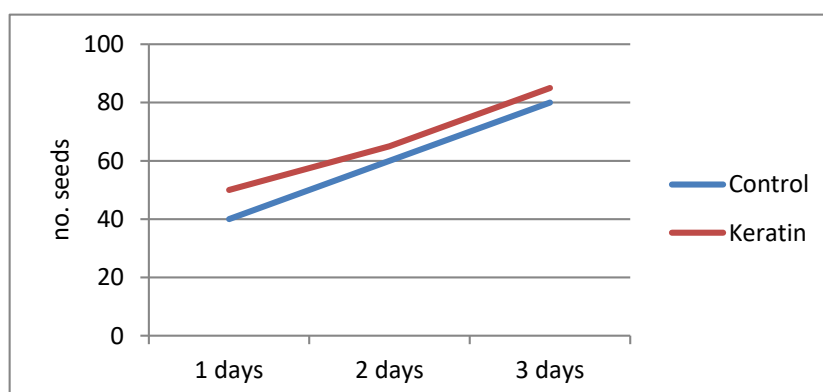


Figure 12. Germination rate for Tamino wheat type

Measurement of plant length of the Mirastar wheat type, germinated in cotton wool, shows an accelerated growth effect in the presence of KerCa treatment (Fig.13).

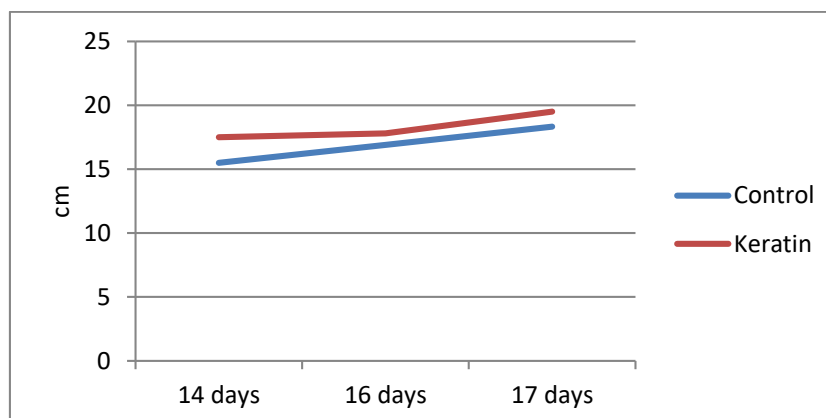


Figure 13. Average plant length for Mirastar wheat type as compared to the control

Measurement of plant length in the Tamino wheat type, increased on cotton wool, shows an accelerated growth effect in the presence of KerCa treatment in the early days (Fig.14).

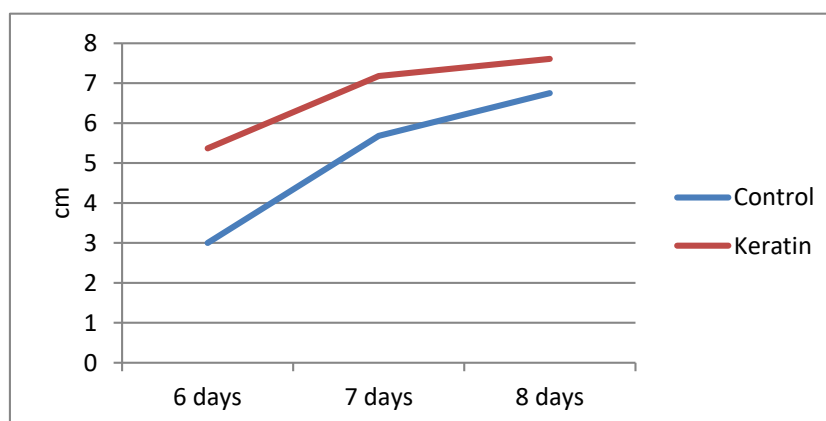


Figure 14. Average plant length for Tamino wheat type

It can be concluded that keratin hydrolysate has biostimulating effects for the germination of lentil seeds in the case of concentrations of 1:20 and 1.5: 20. Experiments on wheat seeds (Tamino and Mirastar) indicated a biostimulatory effect in the first 5 days of germination in the presence of keratin hydrolysate.

The KerCa product made by hydrolysis in the presence of calcium oxide has specific properties for agricultural applications. Physical and chemical analyses show that the hydrolysate is rich in minerals, nitrogen and protein substances needed in the plant growth process.

Other formulations aimed at obtaining foliar blends containing 1:1:0.5 microelements, collagen and keratin hydrolysates that have been applied to wheat plants in a dilution of 0.3:100. The results indicated an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates in agriculture show that they can be the basis for obtaining new biomaterials with various ecological applications.

4. DISCUSSION

Alkaline hydrolysis with CaO is a reliable method for keratin hydrolysate preparation with low molecular weight of 6-20 kDa and with average particle size of 296 nm. These characteristics showed biostimulant properties for lentil and wheat seeds and plants due to the polydisperse composition of keratin hydrolysate composed by fraction with free sulphur content and with free amino acids. The chemical composition of KerCa product revealed a content of total nitrogen 14.06% and protein 79.02% and represents a source of nutrients with slow release properties for the development of plant crops.

The infrared absorption spectrum of keratin hydrolysate shows characteristic absorption bands attributed to primary amide at 3400 cm^{-1} , secondary amide band at 3440 cm^{-1} and tertiary amide band at 1650 cm^{-1} . The $600\text{--}700\text{ cm}^{-1}$ band and the $800\text{--}900\text{ cm}^{-1}$ band are specific for sulfur compounds, characteristic for keratin.

The low surface tension of prepared keratin hydrolysate facilitates the dissolution of any specific activity compounds used to obtain new products with applications in agriculture. The keratin hydrolysate obtained by alkaline hydrolysis in the presence of calcium oxide has been used in two concentrations dilutions in water of 1:20 and 1.5: 20 to treat lentils and wheat seeds. The survey was done during 5-6 days for seed germination and 19 days for plant growth. Evaluation of the effect of keratin hydrolysate on lentils plants was followed for 19 days and indicated an increase in lentil plants length by 10% in the case of samples treated with 1:20 KerCa and by 5% in the case of samples treated with 1.5: 20 KerCa versus the control sample. Two kinds of wheat seeds have been treated with keratin hydrolysate: Tamino and Mirastar. The results of the treatments indicated a growth of wheat plants by 15% when using dilution of 1: 20 and by 10% when using the dilution of 1.5: 20 as compared to the control. The biostimulatory effect of keratin hydrolysate was also highlighted in an extended experiment on a larger number of wheat seeds germinated on cotton and in the soil by treatment with a 2.5% solution. After 5 days the number of germinated seeds was 10% higher for KerCa-treated seeds than for the control samples grown on cotton wool and ground.

Other formulations aimed at obtaining foliar blends containing 1:1:0.5 microelements, collagen and keratin hydrolysates have been applied to wheat plants in dilution of 0.3:100. The results indicated an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates in agriculture show that they can be the basis for obtaining new biomaterials with various ecological applications.

5. CONCLUSIONS

It can be concluded that the keratin hydrolysate has biostimulating effects for the germination of lentil seeds at the concentration of 1:20 and 1.5: 20. Experiments on wheat seeds indicated a biostimulatory effect in the first 5 days of germination in the presence of keratin hydrolysate.

The KerCa product made by hydrolysis in the presence of calcium oxide has specific properties for agricultural applications. Physical and chemical analyses show that the obtained keratin hydrolysate is rich in minerals, nitrogen and protein substances required in the plant growth process. The KerCa keratin hydrolysate was used in wheat (Tamino and Mirastar) and lentil seeds treatment and showed improved germination rate and plant growth stimulation.

The experimental on obtaining foliar blends containing microelements, collagen and keratin hydrolysates that have been applied to wheat plants showed an increase in plant length by 20% as compared to the water-treated control samples, which demonstrates a stimulating growth effect. The good results obtained in applications of keratin and collagen hydrolysates in agriculture show that they can be the basis for obtaining new biomaterials with various ecological applications.

ACKNOWLEDGMENTS

The present work was supported by Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number 55PTE_ BIOFOL_CER and by Ministry for Research and Innovation, project number 16 Nucleu / 2018, PN 18 23 01 04_SMART_PIEL.

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