

The Effect of Enzymatic Keratin Hydrolyzate on the Susceptibility of Cellulosic-Elastomeric Material to Biodecomposition

Y.-H Tshela Ntumba, A. Przepiórkowska, M. Prochoń

Abstract—Polymeric materials have become an integral part of every aspect of today's industry. They have wide applications, inter alia, in areas such as medicine, food industry and agriculture. In agriculture, for example, they are used for the production of pots, irrigation systems and for soil mulching. The aim of this study was the attempt to produce a biodecomposable agricultural mat, by coating cotton fabric with a blend of carboxylated styrene-butadiene latex (LBSK) containing the enzymatic hydrolyzate of keratin from cattle hair, which would serve as a material for mulching.

The production of such material allows the beneficial management of burdensome tannery waste constituted by keratin from cattle hair and at the same time, the production of agricultural mats that much faster undergo decomposition than commonly used polyethylene mats.

Keywords—Agricultural mat, biodecomposition, biodegradation, carboxylated styrene-butadiene latex, cellulosic-elastomeric material, keratin hydrolyzate, mulching, protein hydrolyzate.

I. INTRODUCTION

IN agriculture, plastics are mainly used for soil mulching, tunnels coating, direct shielding, pot production, or in irrigation systems. For mulching, plastics are used in the form of polyethylene film and non-woven polypropylene [1]. Mulching films are used to reduce the growth of weeds, reduce the loss of moisture, for the protection of plants against soil diseases and dirt. Another advantage of the utilization of these films can be the reduction of the consumption of chemicals to combat weeds, the reduction of water consumption, a faster development of crop plants, an improved quality and the achievement higher yields.

On the other hand, despite the susceptibility of polyethylene film to degradation by UV radiation, it takes about 300 years for a polyethylene film with thickness of 60 μm to degrade completely [2]. This fact has serious environmental implications. Therefore, scientists are now aiming to produce biodegradable polymeric materials which have the capacity to disintegrate under natural conditions after its period of use.

According to Majdiuk [3], biodegradable polymeric materials are polymers obtained by means of a natural process,

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by biotechnological synthesis of macromolecular compounds or products containing biodegradable fillers or modifiers (natural polymers such as polysaccharides and proteins), which have their own susceptibility to biodegradation. At the same time, the latter group of polymeric materials can also be referred to as the group of biodecomposable polymeric materials, since only the natural addition, susceptible to be assimilated by microorganisms, undergoes biodegradation, leading to a loss of consistency in the structure of the material and the deterioration of its mechanical and utilitarian properties. Such material is more susceptible to decay under the influence of mechanical and atmospheric factors.

Over recent years, researchers have been interested in the production of polymeric materials using raw materials from renewable sources. The interest in renewable resources does not only result from the propensity of these materials to biodegrade, but also from their beneficial effects on the properties of the materials obtained by their means. For example, the enhancing effect of proteins from vegetable and animal origin on the mechanical properties of polymeric materials has been demonstrated [4], [5] as well as the beneficial effect of lignocellulose and keratin on the thermogravimetric properties of those materials [6]-[8].

In order to protect the environment, attempts were made to replace synthetic polymers by natural materials which are fully susceptible to biodegradation [9]-[11], or to search for ways to improve the susceptibility of synthetic polymers to biodegradation, obtaining biodecomposable polymeric materials [5], [12], [13]. An example of obtaining such a material is discussed in this paper.

II. EXPERIMENTAL

A. Materials

- Keratin from cattle hair from Kalisz Tannery Firm (Kaliskie Zakłady Garbarskie, Poland).
- Carboxylated styrene-butadiene latex LBSK 5545, with 54% of bonded styrene, Dwory S.A., Oświęcim (Poland).
- Sulfuric acid 95%, 98,08g/mole, Chempur, Piekary Śląskie (Poland).
- Enzyme NUE 12 MP (Novo Unhairing Enzyme), Novozymes S/A (Denmark).
- Sodium hydroxide, Eurochem BGD, Tarnów (Poland).
- Gelatin powder, $M = 40000\text{--}100000$ g/mole, POCH (Poland).
- Cotton fabric with an area density of 125 g/m²,

MATIMPEX PPH (Poland).

B. Enzymatic Hydrolysis

The enzymatic hydrolysis was performed in two steps. In the first step the keratin was milled in a ball mill and treated with 0.25 M NaOH at 85°C for 2.5 hours. Then the pH was subsequently adjusted to 9, by means of concentrated sulphuric acid. The second reaction step was carried out with the addition of 12 MP NUE enzyme at 50°C for three hours. The resulting product was dried at 50°C giving keratin enzymatic hydrolyzate HE.

C. Preparation of Cellulosic-Elastomeric Material

Cattle hair keratin hydrolyzate powder was dissolved in water and the mixture was stirred for 10 minutes in a water bath at 40°C to obtain a homogeneous mixture. After this time, the latex was poured into the mixture and stirred for another 30 minutes for homogenization. The latex mixture obtained by this mean is then coated on a cotton fabric with an area density of 125 g/cm². Subsequently, the coated fabric is dried at 50°C for 30 minutes and cured for one hour at 150°C.

D. Test Apparatus

- Zetasizer nano S 90 apparatus to determine particle size.
- Zetasizer 2000 apparatus to determine Zeta potential.
- Zwick 1442 universal testing machine to determine mechanical properties.
- Zwick/Roell Shore A hardness testers, contact force 12,5 N.
- CM-3600d Konica Minolta spectrophotometer, to study changes in the colour of the obtained materials, occurring under the influence of thermo-oxidative ageing.
- Soil tests – study of biodecomposition in soil (norm PN-EN ISO 846, 2002) MEMMERT type HPP 108 climatic chamber.

III. RESULTS AND DISCUSSION

A. Characteristics of the Hydrolyzate

The average particle size of the keratin enzymatic hydrolyzate was determined by means of a Zetasizer Nano S90 apparatus, through dynamic light scattering (DLS). It was shown that about 90% of the particles had a size within a range of 110-150 nm. The nanometric size of the hydrolyzate particles indicates that it should display good dispersion in the polymeric matrix. The value of the isoelectric point of enzymatic keratin hydrolyzate is 2.6. The reduction of the band intensity in the range of 1600-1700 cm⁻¹, which probably results from broken peptide bonds, should indicate that the hydrolysis process occurred, as seen in the FTIR spectrum (Fig. 1).

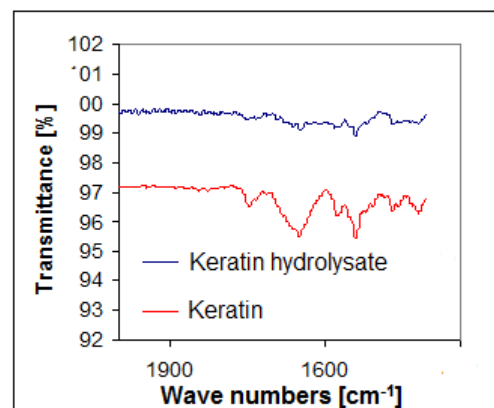


Fig. 1 FTIR spectra of cattle hair keratin and its enzymatic hydrolyzate

B. Characteristics of the Obtained Cellulosic-Elastomeric Material

Coating the fabric with latex blend involves the formation of a LBSK latex film containing enzymatic keratin hydrolyzate on the fabric. The film increases the ability of the fabric to retain water by reducing its pores and giving it flexibility, which facilitates handling.

The fabric acts as a reinforcing element, taking the strain during the utilisation of the material, and thanks to higher rigidity, it increases the shape and size-stability of the product. On account of this, cellulosic-elastomeric materials are considerably different in terms of mechanical properties from latex films which tensile strength is less than 10 MPa [14], while the tensile strength of the coated fabric is higher than 20 MPa.

The effect of enzymatic hydrolyzate of keratin from bovine hair on the degree of crosslinking α_c and the sol content Z of the cotton fabric/carboxylated acrylonitrile butadiene latex film were determined by means of the equilibrium swelling method. Mechanical properties of the coated fabric were also evaluated by means of a universal testing machine. Its hardness D in the Shore A scale was determined as well. The mechanical properties of the obtained agricultural mats are shown in Table I.

TABLE I
MECHANICAL PROPERTIES OF AGRICULTURAL MATS FILLED WITH ENZYMATIC HYDROLYZATE HE OF KERATIN FROM CATTLE HAIR

Symbol	LBSK	LBSK + HE
α_c [-]	0.34	0.37
Z [%]	8.72	14.12
TS [MPa]	21.9	23
Eb [%]	10.65	10.78
D [°Sh]	69	73

From the data presented in Table I, one can see that the addition of the enzymatic hydrolyzate of keratin from cattle hair affects the increase in the degree of crosslinking and content of sol in the swollen samples. This likely indicates that the hydrolyzate participates in the creation of additional cross-

linking with the elastomeric matrix. The increase in the degree of crosslinking is associated with the improvement of the mechanical properties, as evidenced by a 5% increase in the value of tensile strength at break compared to the material not filled with the hydrolyzate, wherein the elongation at break increases slightly. One may also notice an increase in hardness of the material filled with keratin hydrolyzate compared to the material not containing the hydrolyzate, which points to the stiffening of the material.

C. Thermo-Oxidative Ageing

It is widely known from the literature [15], [16] that proteins and modifications thereof have antioxidant properties, what is also shown by the example of carboxylated styrene butadiene latex filled with the acidic and enzymatic hydrolyzate of keratin from cattle hair [14]. The effect of enzymatic hydrolyzate of keratin from cattle hair on the accelerated thermo-oxidative ageing of cellulosic-elastomeric materials was assessed. The thermo-oxidative ageing factor S was determined. This factor is the measure of the changes that occurred in the material as a result of circulating air at the temperature of 70 °C for 7 days. The closer the factor is to unity, the stronger the resistance to accelerated thermo-oxidative ageing.

$$S = (TSb \cdot Eb) \text{ after ageing} / (TSb \cdot Eb) \text{ before ageing}$$

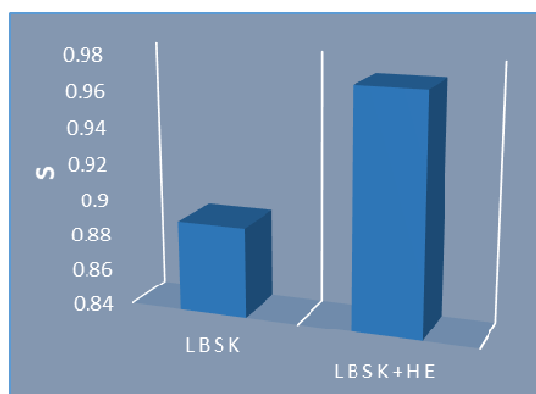


Fig. 2 Resistance of cellulosic-elastomeric material to accelerated thermo-oxidative ageing

Fig. 2 shows that the addition of the enzymatic keratin hydrolyzate clearly improves resistance to accelerated thermo-oxidative ageing. This happens because keratin is a protein with a high content of cysteine. As a result of keratin hydrolysis, free -SH groups are formed. They are prone to form stabilizing -SO* radicals even under slightly oxidative conditions, thereby keratin hydrolyzate gains antioxidant properties and acts as an inhibitor of thermo-oxidative ageing processes.

Changes in the colour of the samples after thermo-oxidative ageing were determined by means of a CM-3600d model spectrophotometer. The colour of the samples after ageing were compared the colour of samples before ageing. The data shown in Fig. 3 are values of dE^*ab coefficient, which

informs us about the difference in colours between an aged and a one before ageing.

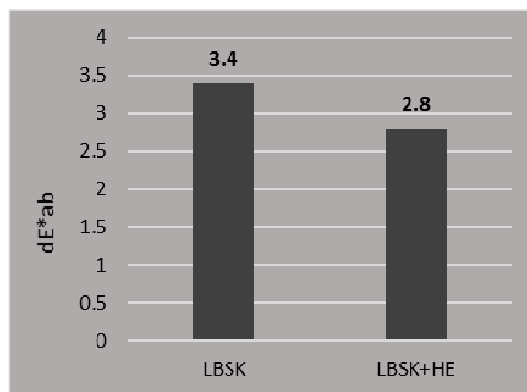


Fig. 3 Effect of the enzymatic hydrolyzate of keratin on the colour shift of LBSK latex-coated fabrics subjected to accelerated thermo-oxidative ageing

Fig. 3 indicates a smaller change in colour in the case of the material containing keratin hydrolyzate because of the lower value of the dE^*ab coefficient as compared to the control material not containing the hydrolyzate. This is advantageous due to the fact that the material containing keratin hydrolyzate maintains its colour to a greater extent, therefore fulfilling its function of restricting the access of light to the surface of soil for a longer period of time.

D. Biodecomposition of Agricultural Mats

The effect of enzymatic keratin hydrolyzate on the susceptibility of the agricultural mat, obtained by coating a 125 g/cm² cotton fabric with carboxylated styrene-butadiene latex, to undergo biodegradation has also been assessed.

A very important parameter in the process of biodecomposition, as well as biodegradation, is the presence of water. Water is the main medium for supplying the material with microorganisms but it also washes out the hydrolyzate from the material, which may be an additional source of protein for the cultivated plants. For this reason the equilibrium swelling test of the samples in water Q_w was also carried out. The results are shown in Fig. 4.

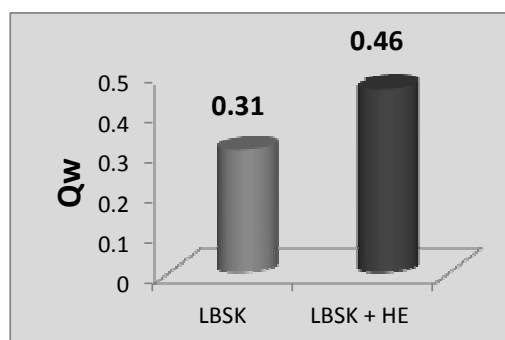


Fig. 4 Effect of enzymatic hydrolyzate on the equilibrium swelling Q_w of coated fabrics in water

From Fig. 4 it follows that the presence of enzymatic hydrolyzate of keratin in the material increases its ability to swell in water, which also improves the biodegradability.

The test of the biodecomposition process of the cellulosic-elastomeric material consisted in placing standard dumbbell-shaped samples into universal soil in a climatic chamber and incubating them for 30 days at 30°C at a relative humidity of 80%. Conditions were chosen to reflect as much as possible the conditions in the natural environment. After that period of time, the tensile strength and appearance of the samples were assessed. The results of the mechanical properties before and after biodecomposition are provided in Fig. 5.

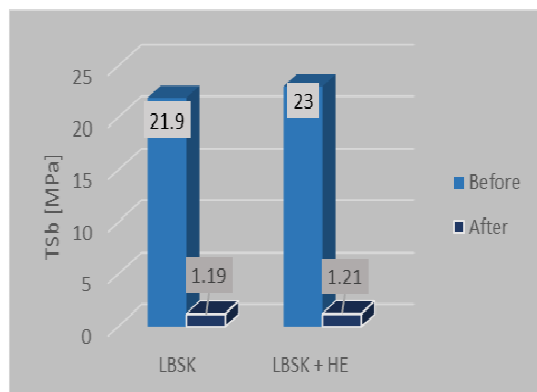


Fig. 5 Effect of the enzymatic hydrolyzate of keratin on the susceptibility of coated fabrics to biodecomposition

Fig. 5 shows that the mechanical properties of the coated cotton fabric significantly deteriorate after the biodecomposition process: tensile strength values of samples after biodecomposition are much smaller than the values before biodecomposition.

As expected, the presence of keratin hydrolyzate resulted in a faster decomposition of the sample compared to the sample not containing the hydrolyzate. The relative percentage change in tensile strength samples is somewhat higher for the sample containing the enzymatic hydrolysed keratin. Fig. 6 shows pictures of fabrics coated with LBSK latex before and after biodecomposition in universal soil under a microscope.

From Fig. 6 one can gather that pores in the material with enzymatic hydrolyzate are bigger and the colour of the material is darker after biodecomposition. That may imply a more advanced biodecomposition process than in the material without enzymatic keratin hydrolyzate from cattle hair. Such material could be used for mulching in agriculture.

Mulching is meant; inter alia, to reduce the evaporation of water from the soil surface, maintaining its proper humidity [17]. For this purpose, a dark material is applied. This condition is fulfilled in the case of agricultural mats containing enzymatic keratin hydrolyzate, which provides it with a brown colour. The shade of the colour depends on the thickness of the coated layer.

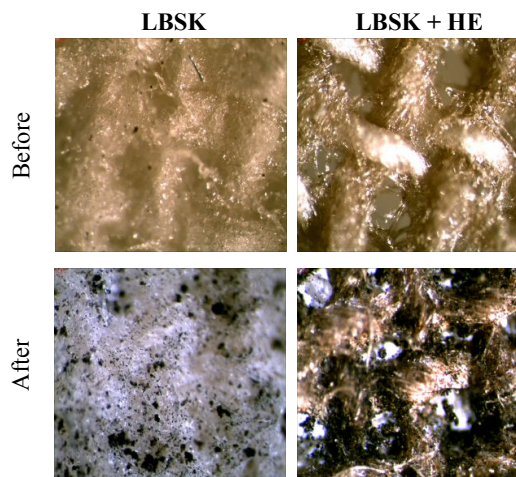


Fig. 6 Microscope pictures of fabrics coated with LBSK latex before and after biodecomposition in universal soil

IV. CONCLUSION

The enzymatic keratin hydrolyzate is characterized by nanometric particle sizes, which facilitates its introduction into the elastomer matrix, granting the final product better mechanical properties through the increase of the degree of crosslinking. Additionally, its antioxidant properties cause the resulting agricultural mat to be resistant to accelerated thermo-oxidative ageing. This is advantageous because the material fulfils its role of restricting the access of light to the soil surface and maintaining appropriate humidity. The presence of the hydrolyzate also increases the absorption of water by the material, which facilitates and accelerates its biodecomposition compared to the material that contains no hydrolyzate. The susceptibility of the tested materials to biodecompose is evidenced by the substantial decrease in tensile strength of the mat after 30-day incubation in universal soil, which is above 94%.

REFERENCES

- [1] D. Briassoulis, "Mechanical behaviour of biodegradable agricultural films under real field conditions. *Polymer Degradation and Stability*, 2007, 92, 1115 – 1132.
- [2] Kyrikou I. and Briassoulis D. Biodegradation of Agricultural Plastic Films – A critical review. *J. of Polymers and the Environment*, 2007, 15 (2), 125-150.
- [3] Majdiuk E. Materiały tworzywowe przyjazne dla środowiska. *Przetwórstwo Tworzyw*, 2002, 4 (90), 105-114.
- [4] Jong L. Reinforcement Effect of Biomass Carbon and Protein in Elastic Biocomposites. *Polymer Composites*, 2013, 34 (5), 697–706.
- [5] Prochoń M., Przepiórkowska A., Tshela Ntumba Y.-H. The Effect of Waste Fodder Potato Proteins on the Mechanical Properties of Carboxylated Acrylonitrile-Butadiene Rubber. *ISRN Polymer Science*, 2012, (2012), 5 pages
- [6] Monteiro S. N., Calado V., Rodriguez R. J. S., Margem F. M. Thermogravimetric behavior of natural fibers reinforced polymer composites—An overview. *Materials Science & Engineering A*, 2012, 557, 17–28.
- [7] Prochoń M., Janowska G., Przepiórkowska A., Kucharska-Jastrzabek A. Thermal properties and combustibility of elastomer–protein composites. *J. of Thermal Analysis and Calorimetry*, 2012, 109 (3), 1563-1570
- [8] Prochoń M. and Przepiórkowska A. Innovative Application of Biopolymer Keratin as a Filler of Synthetic Acrylonitrile-Butadiene Rubber NBR. *Journal of Chemistry*, 2013, (2013), 8 pages

- [9] Schettini E., Sartore L., Barbaglio M., Vox G. Hydrolyzed Protein Based Materials for Biodegradable Spray Mulching Coatings. *Acta horticulturae* 01/2012, 952, 359-366.
- [10] Immirzi B., Santagata G., Vox G., Schettini E. Preparation, characterisation and field-testing of a biodegradable sodium alginate-based spray mulch. *Biosystems Engineering*, 2009, 102 (4), 461–472
- [11] Kasirajan S. and Ngouajio M. Polyethylene and biodegradable mulches for agricultural applications: a review. *Agron. Sustain. Dev.*, 2012, 32, 501–529
- [12] Tshela Ntumba Y.-H., Przepiórkowska A., Prochoń M.. Próby wykorzystania odpadowej sierści bydłowej do uzyskania biorozkładalnych materiałów elastomerowych. *Przetwórstwo Tworzyw*, 2011, 5 (143), 405 - 408.
- [13] Shubhra Q.T.H., Alam A.K.M.M., Khan M. A. , Saha M., Saha D., Gafur M.A. Study on the mechanical properties, environmental effect, degradation characteristics and ionizing radiation effect on silk reinforced polypropylene/natural rubber composites. *Composites, Part A*, 2010, 41, 1587–1596
- [14] Tshela Ntumba Y.-H., Prochoń M., Przepiórkowska A. Porównanie wpływu rodzaju hydrolizatu keratyny na właściwości mechaniczne karboksylowanego lateksu butadienowo-styrenowego. *Przetwórstwo tworzyw*, 2012, 6 (150), 687 – 691, 2012
- [15] Balakrishnan B., Prasad B., Rai A.K. In vitro antioxidant and antibacterial properties of hydrolysed proteins of delimed tannery fleshings: comparison of acid hydrolysis and fermentation methods. *Biodegradation*, 2011, 22, 287-295
- [16] Guo H., Kouzuma Y., Yonekura M. Structures and properties of antioxidative peptides derived from royal jelly protein. *Food Chemistry*, 2009, 113, 238-245
- [17] Kaniszewski S., Dyśko J., Babik J. 2009. Wpływ nawadniania i fertygacji kroplowej azotem na plonowanie warzyw korzeniowych. *Infrastruktura i Ekologia Terenów Wiejskich*. Nr 2009/ 03