

POLYMER BIO-COMPOSITES BASED ON COLLAGEN*

by

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ABSTRACT

One of the substances suitable for preparation of biologically degradable polymer composites is collagen. It is the main component of the animal tissue of leather, bones and tendons. It belongs among the most technically important fibrous proteins. The characteristic structure of collagen, with a high degree of internal organization of molecules, is the main aspect determining the mechanical properties of this tissue. Three collagen polypeptide alpha – chains are commonly screwed into regular right-handed super-spiral. These molecules are extremely rich of glycine (26 – 28 %) and proline (over 15 %). For the intended application native collagen is relatively difficult to be processed without modification.

Collagen colloid prepared by hydrolytic decay of the conformational structure after de-amination of collagen with a shift of iso-electric point to the value < 5 was used in these experiments. Further chemical modifications was necessary, e.g. in the packaging industry, to ensure and optimize the thermal elasticity and compatibility with the tested polymers. Ethylene vinyl-acetate copolymer was used as the polymer matrix. For collagen modification, applied modifiers were based on polyhydric alcohols and amines. During the compounding process, individual compounds of the mixture were homogenized. Polymer granulates with different ratios of individual compounds in the mixture were prepared by granulation. Process-ability of the prepared granules was tested by producing foils using blowing technology. Physical and mechanical parameters of the foils were evaluated; strength, elongation, permeability to water vapor and biodegradability. The results obtained were considered mainly from the point of view of controlled biodegradation.

RESUMEN

Una de las sustancias aptas para la preparación de polímeros mixtos biodegradables es el colágeno. Es el componente principal en los tejidos animales como cuero, huesos y tendones. Pertenece entre las más importantes proteínas fibrosas para usos técnicos. La estructura característica del colágeno, con una alta ordenación interna de moléculas, es el principal aspecto determinante de las propiedades mecánicas de este tejido. Tres cadenas alfa polipeptídicas helicoidales en una super-espiral muy regular con el sentido de rotación en el de las manecillas del reloj. Estas moléculas son muy ricas en contenidos de glicina (26-28%) y prolina (superior al 15%). Para la aplicación intencionada, [polímeros biodegradables] el colágeno en su estado nativo es relativamente difícil de procesar sin modificarlo.

Una preparación coloidal por degeneración hidrolítica de la conformación estructural del colágeno por medio de desaminación, lo que causa un desplazamiento del pH del punto isoeléctrico a un valor inferior a 5 fue utilizado en estos experimentos. Modificaciones químicas adicionales fueron requeridas, v.g. en la industria de empaque [al consumidor], para asegurar y optimizar la elasticidad térmica y la compatibilidad con los [otros] polímeros siendo comprobados. Copolímeros de acetato – vinilo de etileno fue utilizado como la matriz polimérica. Para la modificación del colágeno, fueron aplicados modificantes basados en alcoholes polihídricos y aminas. Durante el proceso de agregación, los componentes individuales de la mezcla fueron homogenizados. Granulados poliméricos con diferentes proporciones de los compuestos de la mezcla se prepararon por granulación. La capacidad de los gránulos de ser procesados se comprobó usando tecnología de soplado para producir películas. Los parámetros físicos y mecánicos de las películas así producidas fueron evaluadas, incluyendo resistencia, elongación, permeabilidad al vapor de agua, y biodegradabilidad. Los resultados obtenidos se consideraron principalmente desde el punto de vista de una biodegradabilidad controlada.

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INTRODUCTION

Synthetic plastics are now widely used materials and in many areas one can hardly imagine life without them. Except the benefits based on their characteristics, they have a negative impact on the environment since waste plastics and their products are highly resistant to weathering and biological processes. Due to various forms of waste and different territorial occurrence with a varying degree of contamination, it is practically impossible to separate the waste and then recycled. Amount of produced plastics and their waste is continually growing, the capacity of dumps decreases and the cost of storage increases. Burning of the waste contributes to the creation of greenhouse gases and other toxic substances.

One of the actual problems is the implementation of an effective waste management. Systematic tasks prevent the occurrence of waste. Introduction of technologies such as minimization or recycling are preferred than passive dumping. These trends are developing based on the idea that the waste can be a valuable raw material that can be used for further production, or as a source of energy, or it may be compostable.

The development of new materials, which is currently progressing very fast, generally must be based on the following: quality of products, affordable prices and minimization of negative environmental impact throughout their life cycle. One of the possible solutions is the production of plastic matrix with such a component, which is able gradually degrade the product using the external factors.

At the development of biologically degradable polymers (BDP) and their subsequent application in a real life, the toxic harmlessness plays an important role. This is given by the composition of the product, by the process of decomposition and by the quality of resulting compost, which substances of the BDP can get into the food chain. However, the effect of different degrees of degradation of plastics on the environment (soil, water, plant root systems, terrestrial and aquatic animals) is not sufficiently investigated. Development of the products from biodegradable plastics, which meet required parameters for a given application and are biodegradable, is important, because this solves the question of raw materials and eliminates the problem with their subsequent disposal.

At present, there is already available a significant amount of commercially produced BDP, but their price and lowered competitiveness compared to conventional plastics such as PE, PP, PVC, etc. remain to be an obstacle to their widespread application. BDP are mainly used in special applications such as compostable bags for biological waste, water-soluble packaging films, also as a single use dish or shopping bags and etc. BDP can be divided by origin into three basic groups:

1. BDP based on synthetics (crude oil, natural gas, ...),
2. BDP based on renewable sources (starch, cellulose, proteins, saccharide substances, polyhydroxyalkanoates (PHA), ...),
3. BDP combined.

Among biodegradable polymers made from purely synthetic materials can be classified mainly esters based on:

- copolyesters: Ecoflex (BAFS), Estar Bio (Chemical),
- polycaprolactone: CAPA (Solvay), Tone polymer (Union Carbide),
- polybutylene succinate: Bionolle (Showa Highpolymer),
- polyester amides: BAK (Bayer),
- polyester urethanes: MHP 9029 (Bayer),
- polyester copolymers: Degranil (Bayer),
- synthetic polylactide: Fortum,
- polyester: Biomax (Dupoint).

Expanding of synthetic and natural BDP production is closely related to the development of an international law and the market price relations in comparison with the prices of crude oil and natural gas.

The second and partially third group of materials deals with the problem of waste as well as with other environmental problems, since at least part of the materials in their manufacture is always biogenic – renewable origin. In addition, the primary raw material is obtained from cheap sources from agricultural commodities (starch, etc.), or it is produced as a byproduct of food production, or may be an efficient use of waste from leather production and food technology.¹

The main trends of research, development and production of biodegradable polymers in the world tend to use biogenic polymers (BP).²⁻⁴

Biogenic polymers suitable for manufacturing of plastics can be divided into the following groups:

1. BP directly extracted from biomass:
 - polysaccharides (starch and derivatives of cellulose, cotton, wood, rubber),
 - chitosan/chitin,
 - proteins (animal such as casein, collagen and vegetable such as soy, gluten),
 - fats (cross-linked triglycerides).

2. BP obtained by conventional chemical synthesis – polylactic acid (PLA),
3. BP produced by microorganisms – polyhydroxyalkanoates, bacterial cellulose, xanthan, pullulan.

At present, among the most studied and the most widely used materials in practice can be included starch and its derivatives, cellulose and its derivatives, casein, gluten and soy protein, PLA, PHA, bacterial cellulose, keratin and collagen.⁵

Collagen is a fibrous protein with the ever-repeating units: glycine, proline and hydroxyproline. This by-product of meat production finds only rare use in food production because of its low nutritional value and deficiency of essential amino acids. Industrial processing of collagen usually involves a lot of waste. The largest share of collagen waste is from manufacturing of leather, where the ratio of waste is up to 50-65 % from weight of collagen starting material.⁶⁻⁸

An important area is also the manufacture of eatable (biodegradable) bowels for packing of the meat products, which due to preserved fibrous structure of collagen have excellent mechanical properties.⁹

A small, but not insignificant amount of collagen is used in human medicine applications (bio-reconstructional tissues – vascular prosthesis, membranes, transport systems for antibiotics, steroids and other medicaments, matrix implants, hemostatic foam, burn bandages, etc.).¹⁰

Leather tannery wastes are difficult to classify as inert, because they are subject in a greater or lesser extent to biological degradation. Possibilities of effective processing and applications of tanned or non-tanned leather waste for various products were already described.^{11,12}

Collagen with low-density of crosslinking can be hydrolytically adjusted under mild reaction conditions (lower temperature, shorter time of extraction) to quality and high molecular weight gelatin. The hydrolysis of peptide bonds plays only a limited role and gelatin achieves a higher average molecular weight. More drastic hydrolysis conditions – higher temperature, longer extraction time, increased number of extractions can be applied to collagen with a higher degree of crosslinking producing gelatin of lower quality. This process increases the hydrolysis of peptide bonds, the average molecular weight of gelatin decreases and the molecular weight distribution is broadened.¹³

Controlled enzymatic hydrolysis has an undoubtedly advantage in lower energy consumption, especially when using commonly available commercial proteases of microbial origin.^{14,15} The advantage of this procedure can control

medium molecular weight of hydrolysate by choice of the reaction time of enzymatic hydrolysis.^{3,16}

In packaging technology, films or foils made of protein hydrogels have a high barrier capacity for oxygen, they prevent the oxidation of packaged materials, mainly of lipid nature.

Good workable, but extremely hydrophilic and brittle material is obtained by acidic, basic or occasionally enzymatic hydrolysis. For further applications, its chemical modification (for example with synthetic polymers) is necessary.

For thermoplastic processing of blown foils with the application in agriculture, collagen was applied as a modifier of polyvinyl alcohol. This material can be included into the third group of combined BDP, where the share of collagen hydrolysate is up to 30 % by weight and improves their physical properties and biodegradability.¹⁷

Another option to modify the properties and processability of collagen is its compounding with appropriate types of polymers. The combination of bio and synthetic polymers provides space to achieve favorable economic, commercial and environmental properties, because the biodegradable component is based on raw material of leather and food industry (e.g. collagen from leather and its hydrolysates).

The research focused not only on the selection and modification of suitable biodegradable component, research and production of biodegradable plastics and products, but also on evaluation of the effect of biodegradable products on the environment – soil, water, plant root system.¹⁸

EXPERIMENTAL

Collagen, similarly to other biopolymers, has the character of amphoteric polyelectrolyte, while the isoelectric point (IP) – determines the pH value, where the balance is between acidic and basic groups in collagen. Native fibril collagen has the equilibrium ratio COOH and NH₂ – isoelectric point – at the pH value of 7.5. IP can be moved by technologic adjustments to pH > 11, collagen changes properties as stability, reactivity, the ability of hydration, etc.

Preparation of Collagen Hydrolysate

For the preparation of biodegradable plastics, granulates and foils, collagen from secondary raw materials from leather and food industries has been modified by deamination and isoelectric point was moved to the value < 5. Collagen colloid was prepared by hydrolysis of its fibrous structure by applying of proteolytic enzymes, or lyotropic agents, salts, alkalies, acids. Parameters of prepared collagen colloids are described in Table I.

TABLE I
Parameters of non-crosslinked collagen colloids – Vipocol KHN and Vipocol KHN-P.

Collagen 1 – Vipocol KHN		Collagen 2 – Vipocol KHN-P – powder	
Physical and chemical parameters		Physical and chemical parameters	
Bloom at concentration 6.67% and temperature 10°C	200	Bloom at concentration 6.67% and temperature 10°C	200- 220
Particle size in Mesh, in mm	60, 0.25mm	Particle size in μm	30 - 50
Viscosity at a concentration 6.67% and temperature 60°C	2.6 mPa.s	Viscosity at a concentration 6.67% and temperature 60°C	2.8 mPa.s
pH	5.2	pH	6.5
Dry content matter	5.4%	Dry content matter	4.2%
Ash	0.4%	Ash	1.8%

Thermal resistance of collagen hydrolysate was increased by crosslinking with glutaraldehyde, formaldehyde, aluminum and dry heat. Crosslinking process increased the heat resistance of dry collagen up to temperature of 180 – 190°C, but collagen lost its elasticity and ability to behave as thermoplastic. This fact was verified by several tests. Collagen was let to dry at temperature from 120°C to 150°C and during 2 to 4 hours was incorporated only as a filler into the matrix.

Non-crosslinked collagen creates clear, strength but very brittle film and the plasticization ensured its improved compatibility with synthetic polymers.

During the BDP preparation its compounding was tested with selected polymers. Processing, physical and mechanical properties of the resulting product were regulated by a combination of suitable synthetic polymers with plasticized collagen. Prepared composites simultaneously achieved partial or complete biodegradability of the polymer system based on the mixture composition. The aim of experiments was verification of the possibility of organic polymer biocomposites preparation on mixing (compounding) extrusion line. During compounding, a homogenization of the mixture occurred at the melting temperature of the polymer matrix. The limiting factor in selection of polymer is the melting temperature of 190°C, because collagen degrades approximately at this temperature. These raw materials were specified for experimental preparation of combined polymer composite materials:

- EVAc – ethylene vinyl acetate (Evatane 1080 VN5 and Evatane 24 03) and (ESCorene FL00218 and ESCorene UL 00728) with the content of vinyl acetate (10 % and 24 %) and (18 % and 28 %),
- LDPE – low density polyethylene (Bralen FB 2 -17, Bralen

FB 08 64, Liten MB 87),

- PA 6-12 – copolymer polyamide, Microfine CoPA 6/12 and PA 12 – Rilsan AESNO P40 TL,
- Collagen 1 – Vipocol KHN – non-crosslinked, particle size 60 Mesh, 0.25mm,
- Collagen 2 – Vipocol KHN-P – non-crosslinked powder, particle size 30 - 50 μm ,
- Modifier I – based on polyhydric amines in granular form,
- Modifier II – based on polyhydric alcohols.

Monitoring of the mutual interaction between biopolymers and synthetic polymers was carried out by DSC analysis, IR spectroscopy and determination of physical and mechanical characteristics as tensile strength, relative elongation at disruption, melting index.^{13,19,20}

Foil of polyamid 12 were modified by the plasma and the high-frequency incursion of the plasma was studied to improve their surface properties.²¹

The chemical changes of polyamide 12 modified by the plasma were analyzed using the Fourier Transform Infra-Red Attenuated Total Reflection (FTIR-ATR) spectroscopy, and X-ray Photoelectron Spectroscopy (XPS). The observed changes of the surface properties of the polymer due to aging were insignificant.

RESULTS AND DISCUSSION

The experiments mainly focused on the preparation of polymer composites based on EVAc copolymers, polyolefins and polyamides in combination with collagen biopolymers.

Polymer Composites Based on EVAc Copolymers

At the beginning of experiments, we evaluated the influence of composites composition on the melt flow index (MFI). Firstly, the influence of amount of collagen, modifier and particle size of collagen was observed on the melt flow index. The results are shown in Tables II, III and IV.

TABLE II
Effect of amount of modified collagen composite.

The composition of samples EVAc with the content of 24% ethylene vinyl acetate	Melt flow index MFI [g/10 min.]
70% Evatane 24 03 + 30% (Collagen 1 – 70% + Modifier I – 30%)	9.8
60% Evatane 24 03 + 40% (Collagen 1 – 70% + Modifier I – 30%)	9.8
50% Evatane 24 03 + 50% (Collagen 1 – 70% + Modifier I – 30%)	9.8

As follows from Table II, the ratio of collagen/modifier has a fundamental influence on the properties of evaluated samples. Melt flow index remained unchanged in the same proportion of these components, despite the gradually increasing concentration of collagen – 30%, 40% and 50%.

TABLE III
Effect of amount of modifier on melt flow index of composites.

The composition of samples	MFI [g/10 min.]
Evatane 24 03	3.0
70% Evatane 24 03 + 30% (Collagen 1 – 80% + Modifier I – 20%)	2.2
70% Evatane 24 03 + 30% (Collagen 1 – 70% + Modifier I – 30%)	8.1
70% Evatane 24 03 + 30% (Collagen 1 – 60% + Modifier I – 40%)	13.5
70% Evatane 24 03 + 30% (Collagen 1 – 50% + Modifier I – 50%)	14.2

As follows from Table III, the amount of the modifier in the Collagen 1 has a significant influence on rheological properties. Higher content of the modifier (40 % or more) increased stickiness of granulate and there was a problem with the chipping of granulate.

TABLE IV
Effect of the size of collagen particles on melt flow index

The composition of samples	MFI [g/10 min.]
70% Evatane 24 03 + 30% (Collagen 1 – 80% + Modifier I – 20%)	2.2
70% Evatane 24 03 + 30% (Collagen 2 – 80% + Modifier I – 20%)	3.5
70% Evatane 24 03 + 30% (Collagen 1 – 70% + Modifier I – 30%)	8.1
70% Evatane 24 03 + 30% (Collagen 2 – 70% + Modifier I – 30%)	9.8

As follows from Table IV, the size of collagen particles does not have a major impact on rheological properties of composites. Collagen No.1 KHN and No.2 KHN-P modified with the Modifier I was sufficiently compatible in mixed EVAc composite. The increasing content of the modifier agent in collagen provided a higher tensile elongation of the composite, higher melt flow index of the composite, higher compatibility with synthetic polymer and biodegradability.

Evaluation of physical and mechanical properties of foils with the application of Collagen 1 – Vipocol KHN, results are given in Table V.

The composition of foils:

- 100% Evatane 24 03 – 24% ethylene vinyl acetate,
- 50% Evatane 24 03 + 50% (Collagen 80% + Modifier I – 18% + Modifier II – 2%),
- 60% Evatane 24 03 + 40% (Collagen 80% + Modifier I – 18% + Modifier II – 2%),
- 50% Evatane 1080 VN 5 + 50% (Collagen 80% + Modifier I – 20%),
- 50% Evatane 1080 VN 5 + 50% (Collagen 80% + Modifier I – 18%+Modifier II – 2%).

For all samples of foils, there is a decrease in tensile strength compared with reference EVAc foil. Samples 2 and 3 contain a higher share of vinyl acetate component. This fact was reflected on the elasticity of bio-composite – relative elongation decreased only minimally compared to EVAc foil. Samples 4 and 5 contain a lower share of vinyl acetate and relative elongation at disruption decreased significantly.

TABLE V
Technical parameters of samples

No. of foil in direction	Tension at the yield strength [MPa]		Relative elongation at the yield strength [%]		Tensile strength at disruption [MPa]		Relative elongation at disruption [%]	
	x	s	x	s	x	s	x	s
1.	-	-	-	-	20.8	2.3	585.7	28.2
2.	6.6	0.36	4.5	0.45	8.9	1.9	495.2	29.3
3.	8.1	0.65	4.2	0.48	7.4	1.0	427.0	60.8
4.	7.1	1.30	2.6	0.81	5.4	0.92	38.5	35.2
5.	6.1	0.71	35.4	17.9	5.9	0.71	71.4	41.5

One of the objectives of the solution was to increase the permeability of combined bio and synthetic polymers against water vapor. Compounding of collagen and synthetic polymer for all samples increased permeability of water vapor (see Table VI for the results).

TABLE VI
Permeability to water vapor

Marking of samples	Permeability to water vapor [g.m ⁻² .24h ⁻¹]
Foil Evatane 24 03	6.9
Foil LDPE	1.6
2	18.4
3	20.1
4	14.7
5	17.5

To verify the parameters of composites, another series of samples were prepared based on collagen, modifier and EVAc copolymer with 18, 24 and 28 % content of vinyl acetate. The composition of selected and evaluated samples is in Table VII.

Foils were produced by blowing technology from prepared granulates. Samples were processed into foils without problems. During the production they do not tear, they have excellent flexibility and tensile elongation. The optimal amount of modifying agents – 20 % weight of collagen, weight was established by tests. Smaller amount of modifier causes

decreased collagen solubility in the polymer matrix and thus deteriorated parameters of foils, Figure 1 presents sample No. 110 – 10 % modifier to collagen. Larger content of modifier in the sample No. 109 increased stickiness, melt flow index of granulate and improved foil strength.

TABLE VII
The composition of tested samples

Sample No.	Tested samples
109	80% ESCorene FL00218 + 20% (Collagen 1 – 80% + Modifier 1 – 19.5% + Modifier II – 0.5%)
110	80% ESCorene FL00218 + 20% (Collagen 1 – 90% + Modifier 1 – 9.5% + Modifier II – 0.5%)
97	70% ESCorene FL00218 + 30% (Collagen 1 – 80% + Modifier 1 – 20%)
89	60% ESCorene FL00218 + 40% (Collagen 1 – 80% + Modifier 1 – 20%)
98	60% ESCorene FL00218 + 40% (Collagen 1 – 80% + Modifier 1 – 19.5% + Modifier II – 0.5%)
90	60% ESCorene FL00218 + 40% (Collagen 2 – 80% + Modifier 1 – 19.5% + Modifier II – 0.5%)
104	60% ESCorene UL 00728 + 40% (Collagen 1 – 80% + Modifier 1 – 19.5% + Modifier II – 0.5%)
105	60% Evatane 24 03 + 40% (Collagen 1 – 80% + Modifier I – 19.5% + Modifier II – 0.5%)
94	50% Evatane 24 03 + 50% (Collagen 1 dried at 80oC for 2.5 h – 80% + Modifier I – 19% + Modifier II – 1%)

TABLE VIII
Physical and mechanical parameters of samples.

Marking of samples	Tension at the yield strength [MPa]		Relative elongation at the yield strength [%]		Tensile strength at disruption [MPa]		Relative elongation at disruption [%]	
	x	s	x	s	X	s	x	s
in direction	x	s	x	s	X	s	x	s
89 non-dried	-	-	-	-	8.7	0.73	10.7	8.3
89 dried*	-	-	-	-	7.7	0.75	10.7	17.0
90	-	-	-	-	4.8	0.71	2.5	1.4
94	10.7	1.5	1.7	0.34	8.5	1.1	35.4	28.3
97	-	-	-	-	6.3	1.2	53.9	82.5
98	9.6	0.47	2.9	0.44	7.6	0.67	74.7	54.0
104	4.5	0.52	5.0	1.4	6.0	0.47	524.0	22.6
105	10.2	0.57	2.7	0.27	7.4	1.8	275.9	24.5
109	-	-	-	-	10.5	1.6	462.1	57.4
110	-	-	-	-	8.6	1.7	93.1	44.5

* granulate was dried 4 hours at 60°C before processing to foil.



Figure 1. Subjective evaluation of qualitative parameters of EVAc foils with modified collagen samples No. 109 and 110.

Prepared foils were tested and evaluated on shear strength and permeability to water vapor, results are given in Tables VIII and IX.

TAs follow from these results, the amount of modified collagen and vinyl acetate in copolymer EVAc has not a significant influence on values of the strength at disruption. However, it possesses a large effect on the relative elongation at disruption. Samples with high content of vinyl acetate No. 104 and 105 reached high values of elongation also at 40 and 50 % content of collagen. With increasing content of vinyl acetate and modified collagen, water vapor permeability also increases.

Samples No. 109 and 110 of EVAc foils with collagen scanned by electron microscope are presented in Figure 2.

TABLE IX
Permeability to water vapor.

Marking of samples	x [g.m ⁻² .24h ⁻¹]
Foil collagen 89	14.1
Foil collagen 97	12.1
Foil collagen 98	16.1
Foil collagen 104	26.6
Foil collagen 105	18.9
Foil Escorene FL 00218	7.5
Foil Escorene UL 00728	16

The images prepared by electron microscopy show that the sample No. 110 is not enough homogeneous. Collagen is not equally dispersed into the EVAc matrix. In contrast, collagen is more homogeneous in EVAc matrix sample no. 109 at 20 % modification. Based on obtained results from testing, the optimal amount of modifying agents was determined.

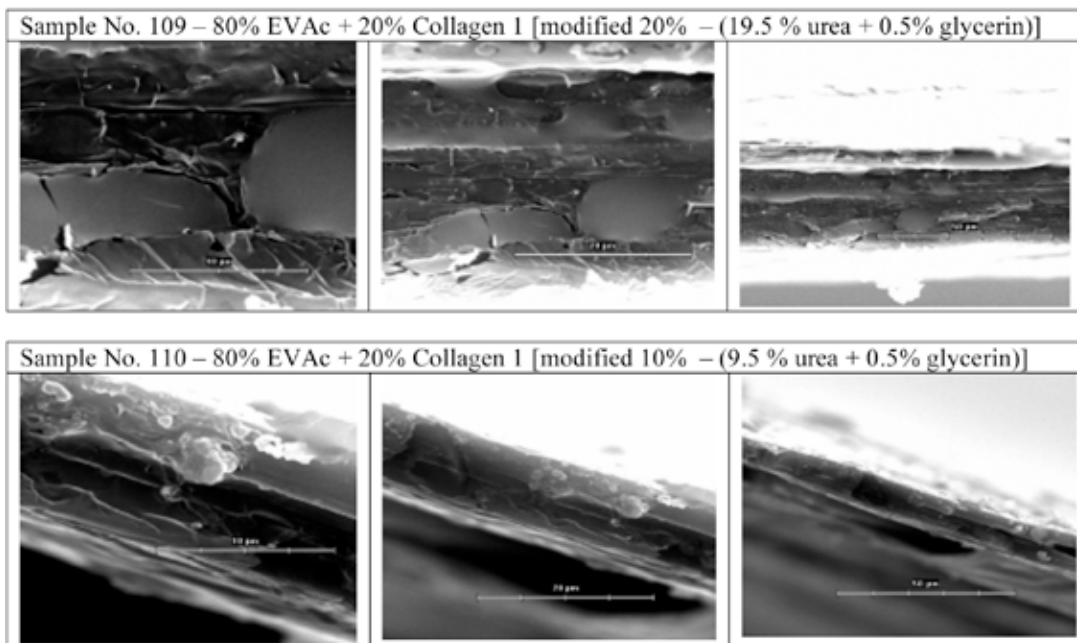


Figure 2. Comparison of qualitative parameters of EVAc foils No. 109 and 110 with modified collagen.



Figure 3. Aging and biodegradation of granulate and foils.

In further tests, biodegradability of polymer bio-composites was investigated. Prepared foils based on EVAc No. 90, 94, 97, 89, 98, 104, 105 were placed in laboratory conditions into the soil and the influence of soil bacteria on degradation of material was investigated. Samples of foils were after 24 h and 60 days picked from the soil. Images of foils are presented in Figure 3.

As the figures illustrate, biodegradability of foils is mostly influenced by the content of vinyl acetate in the polymer matrix, as well as by the content and type of collagen. In samples No. 104 and 105, the content of vinyl acetate is 28 and 24 % and content of collagen with the modifier is 40 %. Despite these two samples after 60 days are only partially degraded, sample No. 98 with the same composition and 18 % content of vinyl acetate in polymer matrix was significantly degraded. The strength of foil was decreased by 71 % after 45 days. The reason for this decrease can be that the higher content of vinyl acetate is significantly reinforcing functional groups of individual components in mixtures and thus creates stronger bonds at samples No. 104 and 105. At the collagen content with modifier of 50 %, there is also a significant degree of degradation in comparison with the same sample with lower content of collagen.

Stability of foils (109, 104, 98) was evaluated after immersion to water for 24 hours, as was indicated at experiments, that some samples are soluble in water, Figure 4.



Figure 4. Samples after 24 hour immersion in water.

Samples were analyzed by FT-IR after immersion in soil and water, IR spectral analysis and comparison of samples is presented in Figures 5 and 6.

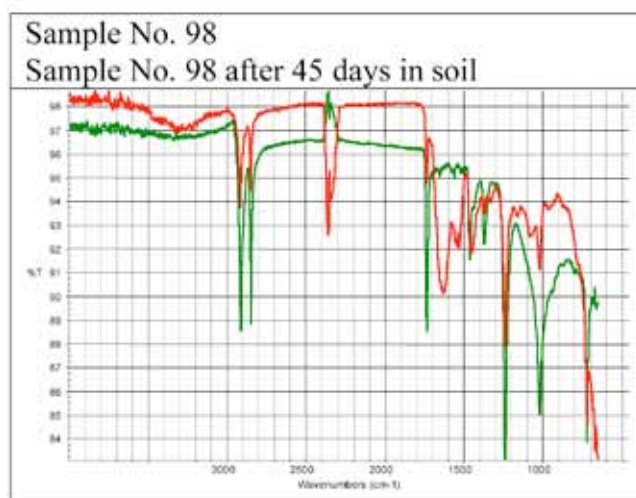


Figure 5. FT-IR spectra.

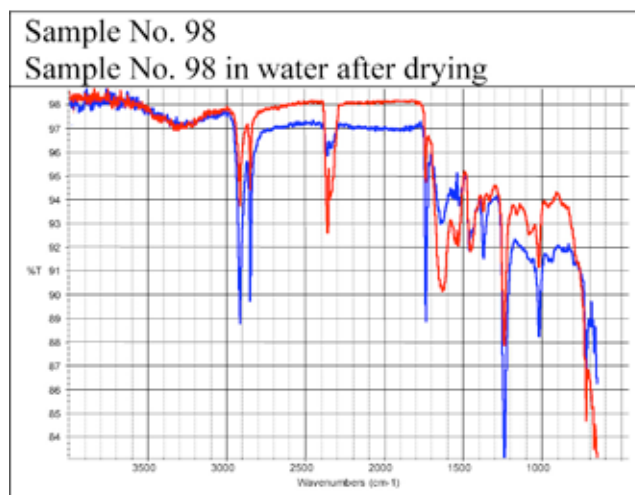


Figure 6. FT-IR spectra.

As indicated from the IR spectra for the sample No. 98, collagen was degraded from the foil under action of the soil. No peaks typical for nitrogen compounds are at wavelengths from 1550 to 1650 cm^{-1} , that would correspond to the presence of collagen in the material.

Tests of basic eco-toxicological analysis of bio-composite granulate – sample No. 98 were carried out on seeds of higher plants *Sinapis alba L.* (according to Slovak technical standard STN 83 8303: 1999: Testing of dangerous properties of wastes. Eco-toxicity. Acute toxicity tests on aquatic organisms and growth inhibition tests of algae and higher cultivated plants).

Ash was determined gravimetrically by annealing at 550°C. Nitrogen in the samples was determined by dry combustion method (immediate dynamic combustion of the sample in a stream of super pure oxygen) after subsequent oxidation-reduction reactions of gaseous products and their thermo-conductive detection in FLASH EA 1112 Thermo Finnigen.

Results of eco-toxicological test given in Table X were counted according to following relation:

$$IC_n = \frac{D(k) - D(t)_{n-1}}{D(k)} \cdot 100 \quad (\%)$$

where:

IC_n is inhibition of the root growth (%) for given dilution n ,

$D(k)$ is average length of the root in control (mm),

$D(t)$ is average length of the root in tested concentration (mm),

n is dilution.

Sample No. 98 EVAc composite with collagen composite was eco-toxicologically tested on seeds of higher plants *Sinapis alba L.*:

- water extract was prepared as follows: weighed ratio 5g of sample/50 ml demineralized water, 6 hours mixing and following sedimentation for 18 hours,
- test was carried out in water extract after filtration without dilution,
- inhibition influence of extract on growth of root *Sinapis alba L.* was not proved,
- opposite effect was proved – stimulation of the root growth.

TABLE X
Results of basic eco-toxicological analysis of bio-composite granulate – sample No. 98.

Ash [%]	Nitrogen* [%]	Average length of root in control	Average length of root in collagen extract	ICn [%]
0.0424	2.87, 3.13, 2.98, 3.12, 3.22, 3.17	33.7 mm	37.3 mm, 38.1 mm	+ 12 %

Polymer Composites Based on Polyolefin

Using polyolefin polymer matrix, there was a non-homogeneous incorporation of collagen into the polymer matrix. The mutual compatibility was very low and there was

even a degradation of the material already during the preparation of composite granules. Compatibility was not improved even by coupling agent. Experiments confirmed that polyolefin polymer composites can be prepared with use of dried collagen only, without modifiers. Considering obtained results after compounding, composites based only on polyolefin and collagen has not been tested.

To improve the parameters of polyolefin composites, there was also tested preparation of composite with different ratio of EVAc to LDPE. This procedure enabled to prepare granulate, but in further processing this granulate exhibited worse properties than pure granulate based on EVAc sample No. 41. Properties of composites were improved when LDPE was added to the granulate EVAc + collagen, sample No. 37, only after compounding. Parameters of samples No. 37 and 41 were compared with the sample No. 43, which was prepared on the basis of EVAc copolymer and modified collagen 1.

Composition and testing of prepared samples:

37.50% (Evatane 24 03 + 50% (Collagen 1 – 80% + Modifier I – 18% + Modifier II – 2%) – 90% + Bralen FB 64 – 10% - added after compounding,

41.60% (Evatane 24 03 + 40% – Bralen FB 08 64 – 40%) + 40% (Collagen 1 – 80% + Modifier I – 18% + Modifier II – 2%)

43.50% Evatane 1080 VN 5 – 50% + 50% (Collagen 1 – 80% + Modifier I – 18% + Modifier II – 2%)

Subjective evaluation of qualitative parameters of foils sample No. 37 EVAc with modified collagen, PE was added only after compounding and sample No. 43 foil prepared only by compounding of EVAc with collagen. Prepared foils were transparent with homogenous structure, Figure 7.

Based on the obtained results it can be concluded, that composite based on EVAc polymer matrix with collagen and modifier, sample no. 43, is more homogeneous than foils amended by PE, which was added after compounding, sample No. 37.

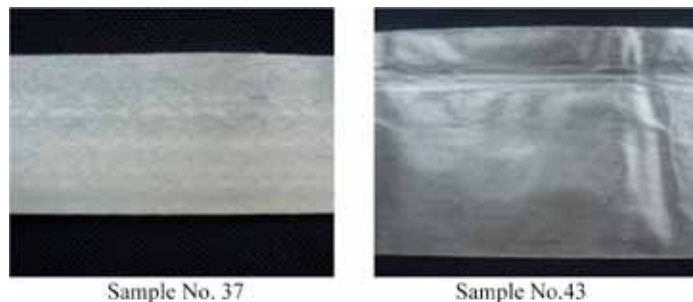


Figure 7. Subjective evaluation of sample No. 37 EVAc and sample No.43

The infrared spectral analysis (FT-IR) was used to determine the mutual interaction of individual components of the composite to find out the cause of material degradation at using LDPE matrix. The IR spectra of individual components of prepared samples of foils No. 37 and 43 were compared and they are presented in Figures 8 and 9.

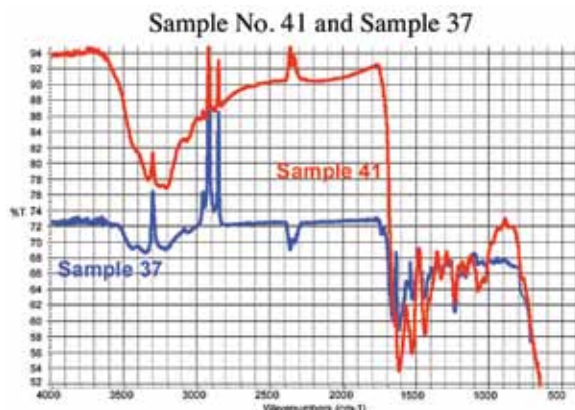


Figure 8. FT-IR spectra.

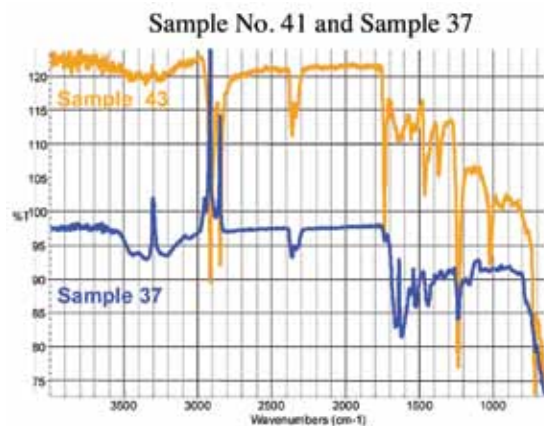


Figure 9. FT-IR spectra.

Spectral analysis confirmed different course of spectra at tested samples. For samples No. 37 and No. 41 the peaks of 1640 cm^{-1} are corresponding to the C=O group. In comparison with the sample No. 43, peaks of 1240 cm^{-1} corresponding to the C–O functional groups are significantly weaker for samples No. 37 and 41. These bonds are probably disrupted, what is explained by decrease of elastic vinyl acetate component in the composite No. 41. In the area of $3500\text{--}3150\text{ cm}^{-1}$, two new bands appear, that are not present in the sample No. 43. These correspond to the N–H functional groups. They can be from a modifier I or the ammonia NH_3^+ cation, which could be created at collagen degradation, as supported by the

smell of ammonia in the preparation of samples No. 41 and No. 37 containing LDPE. For samples No. 37 and No. 41, the peak of 1380 cm^{-1} is also absent. This can correspond to the CH_3 functional groups.

Polymer Composites Based on Polyamides – (PA)

Samples of polymer composites with different type of PA and with modified collagen 1 were experimentally prepared. PA12 and copolymer PA6/12 were taken due to lower melting temperature ($140\text{--}180^\circ\text{C}$), considering that temperature of collagen hydrolysate decomposition is 268°C and dried collagen hydrolysate is 273°C (results of the DSC analysis). In the case of PA6, the lowest possible temperature of processing was 220°C . At this temperature, degradation of collagen started and prepared polymer granulate had dark color. In the case of application of urea as modifier at compounding, foaming of extruded strings and degradation of material appeared. Therefore, further experiments were aimed on PA12 and copolymer PA 6/12, which have lower temperature of melting. Considering the sensitivity of polyamides to humidity, collagen was dried before compounding at 150°C for 4 h. The preparation of foils based on copolymer PA6/12 was tested by extrusion on laboratory Brabender with following cooling on rollers. Strength properties of foils reached values from 9.9 to 24.4 MPa, relative elongation at disruption was from 4.9 to 193.7 %.

The images prepared by electron microscopy show that the sample No. 111 (string) is not enough homogeneous. Collagen is not equally dispersed into PA matrix. Dispersion of collagen was improved by the preparation of film molding. Sample no. 112 – 50 % modification of collagen provided improved homogenization of the collagen matrix.

Based on the obtained results from testing of the foils preparation, the optimal amount of modifying agents was determined, which is – up to 50 % weight by weight of collagen.

CONCLUSIONS

Based on the experimental, laboratory and semi-production tests, the following conclusions can be stated:

1. Ethylene vinyl acetate copolymers with different content of vinyl acetate were specified for the preparation of combined polymer bio-composites. Polymer composites containing up to 60 % weight of collagen hydrolysate Vipocol KHN and KHN-P has been prepared with required tensile properties of prepared foils,
2. Polymer bio-composites with controlled biodegradation, as well as with defined physical and mechanical properties can be prepared by a mutual combination of individual components of mixtures and by a different content of vinyl acetate in the copolymer.

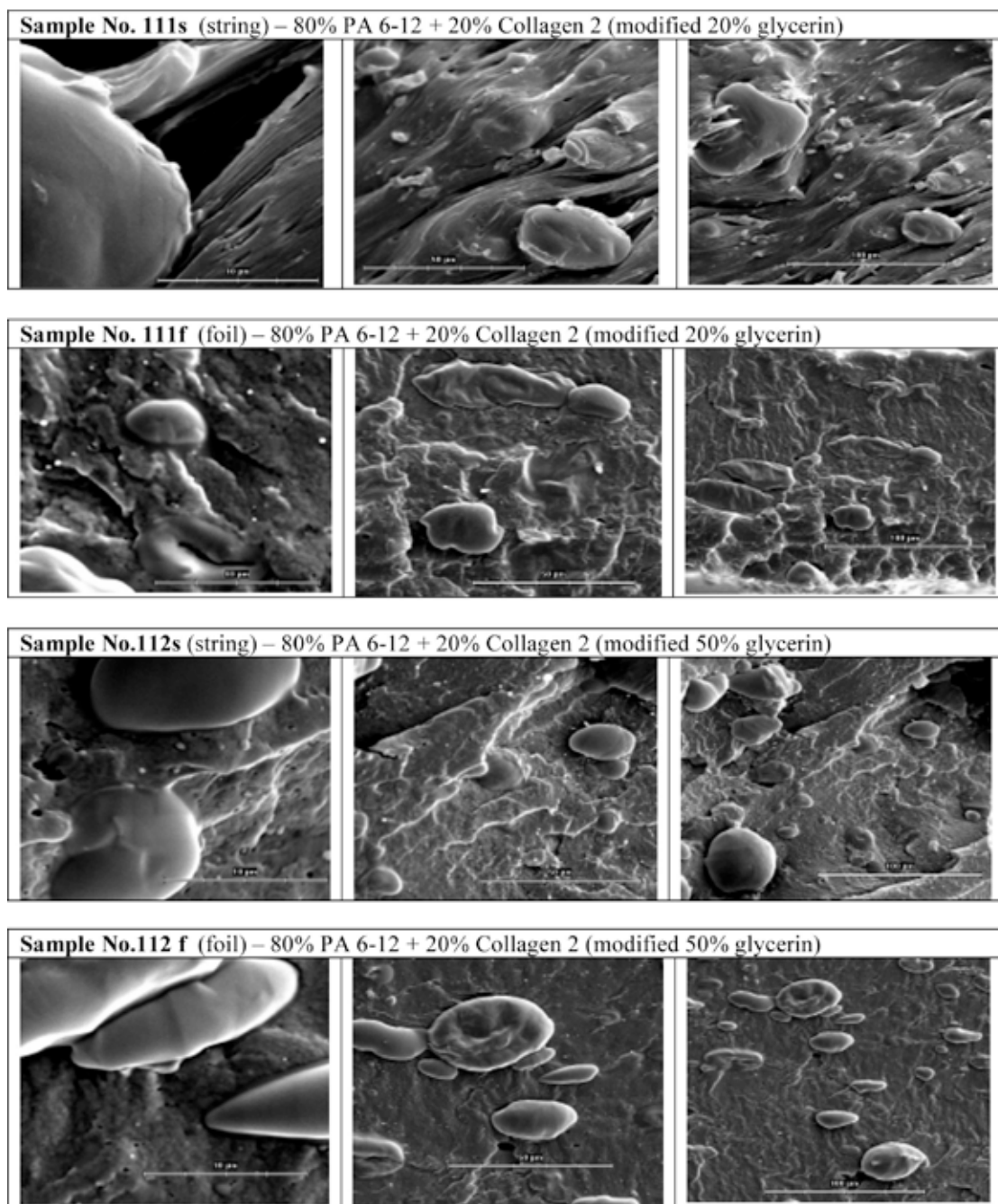


Figure 10. Comparison of qualitative parameters of PA 6/12 foils No. 111 and 112 with modified collagen

TABLE XI
Technical parameters of prepared foils

Marking of samples	Tension at the yield strength at disruption [MPa]	Relative elongation at disruption [%]	Permeability to water vapor [g.m ⁻² .24h ⁻¹]
Foil EVAkol 18 20	10.5	462.1	10.2
Foil EVAkol 18 40	7.6	74.7	16.1
Foil EVAkol 28 40	6.0	524.0	26.6

For realization in the praxis, there were proposed following polymer composites and foils:

EVAkol 18 20, EVAkol 18 40 and EVAkol 28 40 – polymer bio-composites based on EVAc copolymer and modified collagen hydrolysate.

Foils EVAkol 18 20, EVAkol 18 40 and EVAkol 28 40 – foils produced by technology of blowing from polymer bio-composites.

Technical parameters of prepared foils are given in Table XI.

Based on the results of the acute toxicity test on aquatic organisms and growth inhibition test on algae and higher plants can be concluded that aqueous extract of collagen is inert since the tests were negative. An inhibitory effect of the extract on the growth of *Sinapis alba L.* root was not proven. The opposite effect was shown, which means the stimulation of root growth by about 12%.

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