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Collagen-containing products derived from the swim bladders of northern fish and their application

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Abstract. In recent years biologically active compounds are widely used in development of new polymer composite materials. In this study, collagen hydrolysate was obtained from the swim bladder of northern species of fish with alkali-salt hydrolysis followed by freeze-drying. Collagen hydrolysate has been added to rubbers based on epichlorohydrin rubber (GPCO) HYDRIN T6000 in order to improve the performance properties of the investigated rubber. The vulcanization parameter, mechanical and relaxation properties of the rubber blends containing the collagen hydrolysate have been discussed. The amino acid compositions, properties, the particle size of collagen hydrolysate have been studied. It was found that the tear resistance and compression set were significantly improved with the addition of collagen hydrolysate.

1. Introduction

The development of environmentally friendly biomaterials is a new area of technological progress of polymer composites. It is confirmed by a significant number of scientific works devoted to the preparation of new polymeric materials filled with biologically active compounds such as keratin hydrolysate, technical protein, amino acids, lignin, phospholipids which showed good performance properties. [1-6]. These fillers have a multifunctional effect and they can be considered as vulcanization accelerators, antioxidants, and also vulcanizing agents. From the literature review [7], it follows that the effectiveness of using of biologically active compounds is achieved when they are added into polar polymers, for example, into chlorine-containing polymers

One of the promising biopolymers is a collagen, which has a wide range of industrial application due to its unique properties such as non-toxicity, low antigenicity and allergenicity, biocompatibility, biodegradability. Collagen-containing raw materials are commonly treated with an alkali-salt solution which causes the protonation of collagen polypeptides and allows to obtain highly dispersed collagen hydrolysate.

One of the rubbers that containing a reactive chlorine atom in their structure is epichlorohydrin rubber (GPCO) HYDRIN T6000 (Zeon, Japan). Thus, the modification of rubber compounds with biologically active systems can be promising for obtaining new types of elastomeric materials. In this work we investigate the influence of collagen hydrolysate (GK) obtained from the swim bladder of northern species of fish on basic parameters of epichlorohydrin rubber.



2. Models and Methods

Sturgeon (*Acipenser*) swim bladders, white fish (*Carangidae*) swim bladders were obtained from a local fish processing facility. Epichlorohydrin rubber (GPCO) was used as a rubber to assess the influence of collagen hydrolysates on the main properties of vulcanizates.

2.1. Preparation of collagen hydrolysate

To remove non-collagenous proteins, swim bladders were soaked in 10 % NaOH and 10 % Na₂SO₄ at the sample/alkali-salt solution ratio of 1:3 (w/v) for 4 hours with continuous stirring. Then the samples were suspended in 0.5 M acetic acid with a sample/solution ratio 1:3 (w/v) for 24 hours under stirring. Then the samples were dialyzed against 0.1 M acetic acid for 1 day by changing the solution and then lyophilized. The swim bladders of white fish were not treated with acetic acid since they dissolved during the alkaline-salt treatment, which happens due to the nature of origin and the amino acid composition of the collagen among different fish species.

2.2. Fourier Transformation Infrared (FTIR) Spectroscopy

The nature of functional groups of collagen hydrolysate was examined by Fourier-transform infrared (FTIR) spectroscopy. The FTIR spectra were taken using Varian 7000 spectrometer in a spectral region of 4000 to 800 cm⁻¹.

2.3. Dynamic Light Scattering (DLS) and high-performance liquid chromatography

The size of particles and the zeta potential of collagen hydrolysate were measured by Dynamic Light Scattering (DLS) on the Zetasizer Nano ZS instruments (Malvern Instruments Ltd., UK).

Amino acid composition of collagen hydrolysate was determined by high performance liquid chromatography (HPLC). The hydrolysate was dissolved in 140mM Sodium Acetate trihydrate, 0.15% TEA, 0.03% EDTA, 6% CH₃CN, pH 6.1. An aliquot of 30 µL was applied to an autosampler Agilent 1260 Infinity HPLC.

2.4. Preparation of rubber blends and testing

Rubber blends were prepared in a Brabender plastograph under the following conditions: temperature 40°C; rotor speed in mixing cycle 40 min⁻¹. Collagen hydrolysate was introduced in doses of 1, 2, 3, 4, 5 phr with sulfur vulcanization system. The vulcanization characteristics of rubber compounds such as optimum cure time (t₉₀), cure rate index (CRI) were determined on MDR 3000 rheometer according to the GOST 54547-2011. The mechanical properties, determination of tear strength of the tested composites were measured according to GOST 270-93, GOST 262-93, on Autograph AGS-J machine; compression set (CS) was measured according to GOST 9.024-74 at 100°C for 72 hours.

3. Results and Discussion

3.1. FTIR, DLS analyses and amino acid composition of collagen hydrolysate

The FTIR spectra of collagen hydrolysates obtained from swing bladders of both fish (sturgeon - blue line and white fish - green line) are shown in Figure 1.

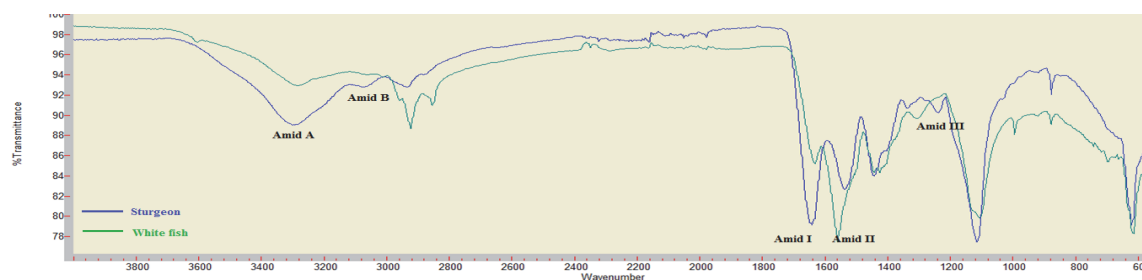


Figure 1. FTIR spectra of collagen hydrolysates.

The spectra of collagen hydrolysate obtained from sturgeon and white fish indicated amide A band (associated with N–H stretching) positions at 3293 cm^{-1} and 3281 cm^{-1} , respectively. The Amide I band which is characterized by polypeptide backbone C=O stretching vibration which was found at 1651 cm^{-1} for sturgeon collagen and at 1649 cm^{-1} for white fish collagen. The Amide II and Amide III bands were found at 1538 cm^{-1} and 1239 cm^{-1} for sturgeon collagen; at 1559 cm^{-1} and 1317 cm^{-1} for white fish collagen. The Amide II and Amide III bands represent N–H bending vibrations and C–N stretching [8].

The particle size and the zeta potential of collagen hydrolysate are important parameter that characterize good interaction of filler with rubber. It was found that the particle size of an aqueous solution of collagen hydrolysate is a polydisperse system. The particle size of collagen hydrolysate of sturgeon-varied in the range from 65 nm to 246 nm with zeta potential minus 35 mV, whereas the particle size of collagen hydrolysate of white fish are varied in the range from 95 and 146 nm with zeta potential minus 26 mV.

Amino acid composition of collagen hydrolysate obtained from swim bladders of sturgeon is shown in Table 1. According to the literature, the amino acid composition of collagen is highly specific and characterized by the presence of amino acids such as glycine, proline and alanine, and low content of sulfur-containing amino acids.

Table 1. Amino acid composition of collagen hydrolysate obtained from swim bladders of sturgeon.

Amino acid	Mol %	Amino acid	Mol %
GLY	23.23	ARG	3.33
ALA	15.25	PHE	2.59
GLU	14.30	TYR	2.46
ASP	12.06	MET	2.21
PRO	10.17	THR	1.14
LYS	3.97	TRP	1.07
LEU	3.78	VAL	0.46
SER	3.74	CYS	0.26

Glycine content of collagen hydrolysate was approximately 23% of total amino acids and there were relatively high contents of alanine, glutamic acid, asparagine, proline decreasing in that order.

According to the literature, the amino acid composition of different fish species is almost the same, although there are individual variations [7].

3.2. Vulcanization characteristics

The vulcanization parameters such as cure rate index, cure time as a function of collagen hydrolysate filling are presented in Figure 2, 3.

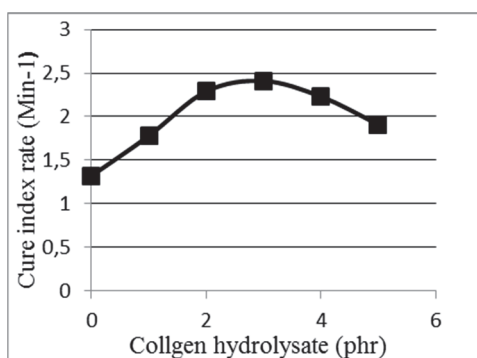


Figure 2. Cure index rate of compounds based on GPCO.

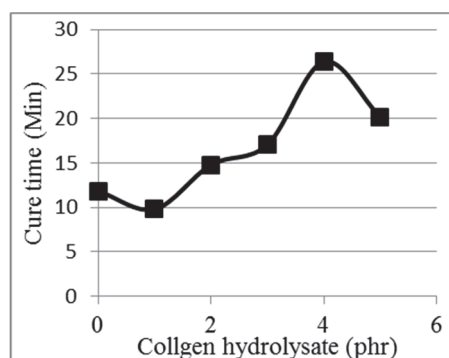


Figure 3. Cure time of compounds based on GPCO.

The data showed that the increase of collagen hydrolysate content in blends resulted to the increase of the cure rate index till the filler reached 3 phr after that the slight decrease in cure rate index was observed (Figure 2).

On the other hand, the cure time decreased and then increased with increasing of collagen hydrolysate content in the blends (Figure 3).

According to the literature, the fillers based on proteins containing amino groups are natural accelerators of rubber vulcanization [7].

3.3. Mechanical properties

The studies of mechanical properties of blends filled with collagen hydrolysate showed that the modifier did not caused a significant effect on the investigated properties. The data obtained was within the error in accordance to the GOST for this type of measurement.

At the same time, the addition of the collagen hydrolysate promotes an increase in the resistance to tear of the elastomer composites (Figure 4).

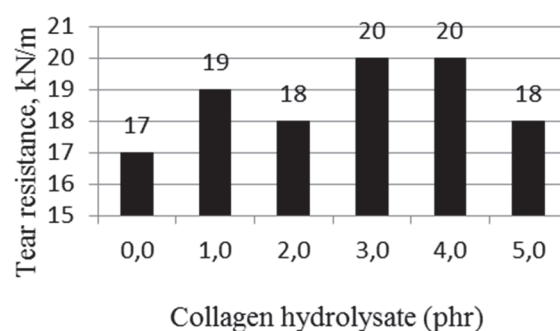


Figure 4. Tear resistance of compounds based on GPCO.

The maximum value of this parameter is achieved at 3 and 4 phr addition of collagen hydrolysate and reached to 20 kN/m, while for the comparison specimen it reached to 17 kN/m, which is 18% higher than that for unfilled GPCO. The collagen hydrolysate can greatly enhance the tear resistance of GPCO.

3.4. Compression set

Figure 5 illustrates the compression set of collagen hydrolysate filled GPCO blends. Compression set is an important operating property for sealing rubbers, which characterizes the relaxation properties and high performance elastomers after aging. It characterizes the ability to recover after the load is removed after thermal aging. Low values of this parameter characterize high recoverability of the material.

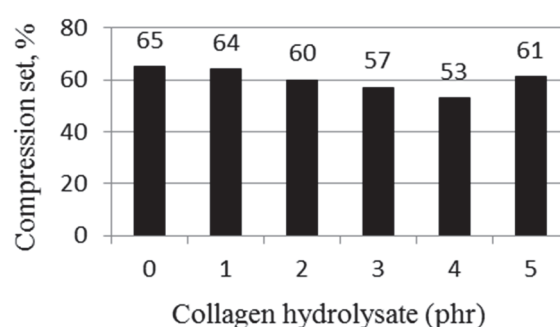


Figure 5. Compression set of compounds based on GPCO.

The use of collagen hydrolysate as modifying additive of elastomer composites based on GPCO caused a reduction of the level of compression set accumulation.

The lowest value of the compression set was achieved at 4 phr and reached 53%, whereas for the comparison specimens it reached to 65%, which was 19% lower than for unfilled rubber. Reduction in the level of compression set may occur because collagen hydrolysate can contribute to the occurrence of relaxation processes due to an increase of the mobility of macromolecule segments of the elastomer matrix.

4. Conclusion

Thus, a new environmentally friendly material based on collagen from the new source was obtained. The material can be used in various industries due to its unique properties such as rich amino acid composition and the nanometer particle size of collagen hydrolysate. Furthermore, the results showed that after alkali-salt treatment, the nature of collagen was preserved.

One of the possible areas of applications of collagen hydrolysate is a modification of rubber compounds. Collagen hydrolysate obtained from the swim bladder, has a multifunctional effect and can be considered as a vulcanization accelerator. Moreover, it can participate in the processes of crosslinking between the elastomer matrix, which was confirmed by the reduction in the level of compression set.

It should be noted that the collagen hydrolysate obtained from waste fish raw materials is environmentally friendly biomaterials and it will contribute to the development of resource-saving technologies in the processing of rubber.

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