



28-29 September 2023,

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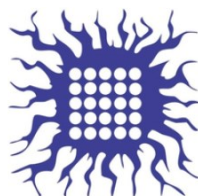
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**2<sup>nd</sup> International Conference on Chemo and Bioinformatics**

**ICCBIKG\_2023**



# BOOK OF PROCEEDINGS





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ICCBIKG 2023

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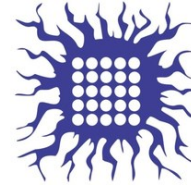
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## UV-blocking sustainable food packaging based on polyhydroxyalkanoate and bacterial pigment prodigiosin

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**Abstract:** New film materials based on bacterial biomolecules polyhydroxyalkanoate (poly(3-hydroxybutyrate-co-3-hydroxyvalerate) PHBV) and prodigiosin (PG) were produced by solvent casting as a potential food packaging material. Film precursors were obtained in a sustainable manner via microbial fermentation using waste stream-based substrates (cooking oil and second-grade canned meat, after the expiry date). The incorporation of PG into the PHBV has influenced the morphology and functionality of the obtained materials. PG acted as a nucleating agent, affecting in turn PHBV/PG film surface morphology. The films were intensively colored, transparent and blocked UV-light. An increase in PG content decreased film transparency but it did not affect UV-blocking ability. Migration experiments have shown that films possess the potential to release PG into lipophilic food simulant media where it has exhibited antioxidative action. The obtained results suggest that PHBV/PG films can be potentially used as sustainable and active food packaging materials.

**Keywords:** prodigiosin, polyhydroxyalkanoate, food packaging, solvent casting, UV-blocking

### 1. Introduction

As a major consumer of worldwide plastic production, with a product that has one of the shortest mean lifetimes (0.5 years), the packaging industry contributes significantly to the plastic waste generation. To address this, significant efforts have been put into developing biopolymer-based food packaging materials as eco-friendly alternatives to petroleum-based materials.

Polyhydroxyalkanoates (PHAs) are biodegradable thermoplastic biopolyesters produced by a wide range of bacteria as intercellular storage of carbon and energy. They are structurally heterogeneous and exhibit a variety physicochemical properties, some of which are comparable to the conventional plastics [1].

Prodigiosin (PG) is a biopigment produced as a secondary metabolite in Gram-positive and Gram-negative bacteria. The remarkable properties (*e.g.*, UV protective,

antioxidative and antimicrobial activity) made it the subject of many studies [2]. Combining these two bacterial biomolecules can lead to improved PHA-based materials which are biodegradable, sustainable and functional. The presented study proposed the PHBV-PG as a new active food packaging material with improved UV-blockage, migration and antioxidative activity that can further be successfully tuned by the concentration of prodigiosin as an active compound.

## 2. Materials and methods

### 2.1. Fabrication of PHBV/PB films

Film precursors were obtained in a sustainable manner via microbial fermentation using waste stream-based substrates. Poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV,  $M_w$   $5.4 \times 10^5$  kDa) was obtained using *Cupriavidus necator* DSM 428 and cooking oil as a carbon source [3]. Prodigiosin was produced in a bioreactor (Bio4, EDF-5.4\_1, Biotehniskais centras AS, Riga, Latvia) using *Serratia marcescens* ATCC 27117 (American Type Culture Collection, ATCC, Manassas, VA, USA) and second-grade canned meat waste (Takovo d.o.o., Serbia) as a substrate [4].

PHBV/PB films were produced using solvent casting method according to the procedure described in our previous study [5]. In brief, PHBV and PB were dissolved in  $\text{CHCl}_3$  and the obtained film solution was cast into a glass Petri dish (diameter of 6 cm). The samples were left to dry for 14 days at room temperature. In order to investigate the effect of PB content on the obtained films properties, multiple films solutions with fixed PHBV content (150.0 mg) and increasing PB content (2.5, 5.0 and 10.0 mg) were made and cast. The control was the pure PHBV film. The obtained samples were denoted as PHBV, 2.5-PHBV/PB, 5-PHBV/PB and 10-PHBV/PB, for the control and the samples with increasing PB content, respectively.

### 2.1. Characterization of PHBV/PB films

*Morphology.* PHBV/PB film surface morphology was investigated using an optical microscope using Olympus SZX10 attached to a digital imaging system.

*Transparency and UV-blockage.* Film strips ( $1 \times 4 \text{ cm}^2$ ) were placed perpendicularly to the light beam, transmission and absorption were measured in the ultraviolet and visible spectra. In addition, a qualitative assessment of the samples' contact transparency was conducted visually.

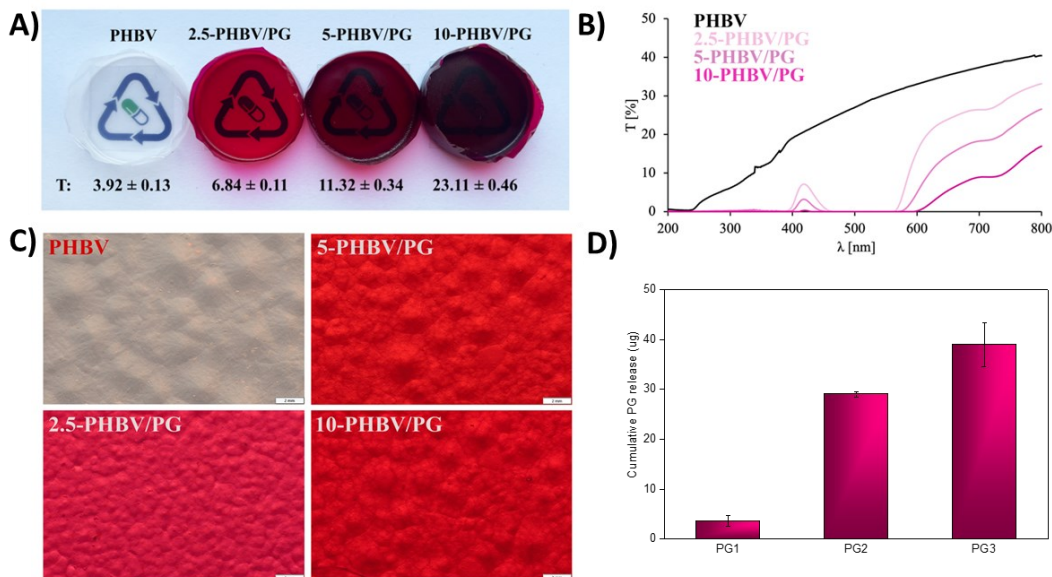
*Migration evaluation.* Migration of PB from the samples into the food was evaluated in accordance with the European Commission requirements using 10 % and 95 % aqueous ethanol solutions (v/v) as food simulant media with hydrophilic and lipophilic characteristics, respectively.

*Antioxidative activity.* Following the migration test, the food simulant media with migrated PB were analyzed for antioxidant activity against using 2,2-diphenyl-1-picrylhydrazyl (DPPH•) free radicals scavenging assay.



## 2.2. Results

Prodigiosin was successfully incorporated into PHBV biopolymer to obtain films using the solvent casting method. All samples appeared uniform, with no visible defects or phase separation, Figure 1A.



**Figure 1.** Characterization of the PHBV/PG films with increasing PG content: (A) Contact transparency and corresponding transparency value ( $T$ ,  $\text{mm}^{-1}$ ) at  $\lambda = 600$  nm; (B) Light transmission in the UV-VIS spectrum and (C) Surface morphology; (D) Migration test: cumulative prodigiosine release in lipophilic medium.

Optical properties are important characteristics of food packaging materials. Packaging material should allow visual observation of packed products, but at the same time prevent photodegradation during storage. Evaluation of light transparency and transmission indicated that the presence of PG within PHBV has significantly decreased light transmission of the obtained film materials, especially in the UV region (Figure 1A and 1B). The increase in the PG content has resulted in more intensively colored films which appeared less transparent (Figure 1A). However, all PG-containing formulations were equally efficient in UV blockage, irrespective of the PG loading.

In addition to the obvious difference in color and transparency, the film samples exhibited different surface morphology with clearly visible macroscopic spherulites (Figure 1C). The biggest spherulites were observed on the surface of the control (PHBV) sample, in contrast to the smaller and more numerous ones that appeared in PHBV/PG samples, indicating that PG acts as a nucleating agent for biopolymer crystallization. There was also a difference among the PHBV/PG samples: the lower the PG loading, the smaller and more numerous the spherulites were. PHBV has been widely recognized in the field of active packaging materials, but preferentially other active ingredients, such as eugenol, carvacrol, phenolic acid, ferulic acid, etc., have been used [6, 7, 8]. The incorporation of PG into PHBV as a polymer matrix has been reported for the first time

in our previous study [5] where the biological benefits of this system (its cytotoxicity) have been proved.

Cumulative release experiments were conducted to evaluate migration of PG from PHBV/PF films into food in potential applications as food packaging materials (Figure 1D). No PG migration was detected in the hydrophilic medium, however when placed in lipophilic medium, the release was significant, and it was directly proportional to the PG content in the films. In addition, the migrated PG exhibited antioxidant activity.

### 3. Conclusions

Bacterial biomolecules, PHA and PG, were successfully combined to produce film materials as potential sustainable and active food packaging material alternatives to conventional plastics. The incorporation of PG into the biopolymer matrix has improved the optical properties and antioxidative potential of the obtained films rendering them as suitable alternatives for packaging applications.

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