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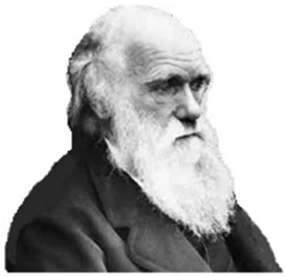

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Antibacterial activity of covered paper after storage

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ABSTRACT

The paper covered with a hydrophobic Topscreen coating was coated with the second layer. The goal of this work was to obtain the antimicrobial properties of the external coating. The samples were stored 2 months at 20 ºC. The influence of storage on the antimicrobial properties of the external coating was analyzed. The results of the study showed that paper covered with the Topscreen coating did not have an influence on the growth of *Staphylococcus aureus* cells. The second (external) layer, containing 2% polylysine as an active substance, decreased the growth of *S. aureus.* The 2-month storage of the covered paper did not influence the antimicrobial properties of coating with polylysine against *S. aureus*. It was demonstrated that the paper covered with hydrophobic coating had no influence on the growth of *E. coli* cells as well. In this case the influence of 2-month storage on the antimicrobial properties of the coating with polylysine was observed. In contrast to the results obtained for the samples that were not stored, the decrease of the growth of the bacterial cells after 24 h contact with a hydrophobic coating devoid of an active substance was noticed.

***Keywords*:** polylysine, active coatings, covered paper, *Staphylococcus aureus*

1. INTRODUCTION

Paper products are widely used in food and liquid packaging. Water resistance is one of the most important properties of paper packages [1]. The covered paper products have added value, and all improvements depend on the coatings used. The goal of the coating is to create conditions needed to a particular packaging. It is very important to choose the right coating carrier, that will act as a binder on the surface of the paper and will not diminish the existing

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properties [2]. Some of the paper products, such as corrugated package for ice-packed fish, poultry, and meat often require high water resistance [1, 3]. For example, paraffin wax emulsions and polyurethane or styrene-based copolymers are typical hydrophobic sizing agents that are applied in molten form to the surface of paper or paperboard and for improvement of the water vapor barrier property [4].

Antimicrobial packaging is an important technology to delay or inhibit the growth of pathogenic microorganisms in the food product. The active coatings are usually used for the preparation of antimicrobial packaging system by combining the advantages of coating carrier and the antimicrobial properties of the active additives It has been recognized that essential oils, chitosan, plant extracts, preservatives, acids, alcohols, nisin or nanoparticles are active against many microorganisms, such as: *E. coli*, *S. aureus*, *Bacillus cereus*, *B. atrophaeus*, *Listeria monocytogenes*, and *Candida albicans* [5-8, 11].

ε-Polylysine (PL) is one of the antimicrobials. It is a natural polypeptide recognized as safe, and the antimicrobial action of PL is attributed to its polycationic and surface nature. Polylysine is active against food pathogenic bacteria, including *L. monocytogenes*, *Escherichia coli* O157:H7, and *Salmonella typhimurium* [9, 10].

Zinoviadou *et al*. [9] used PL in whey protein films and successfully applied the films to the control spoilage flora of fresh beef. Ünalan, I.U. [10] analyzed the antimicrobial properties of edible films from whey proteins, alginate, zein, and chitosan incorporated with polylysine. Because of its antimicrobial properties, polylysine may be used as an active substance that may be added into the coating carrier to cover packaging materials.

The purpose of the study was to coat the paper covered with the hydrophobic coating. The goal was to obtain the active layer that increases the growth of *E. coli* and *S. aureus* strains before and after 2-month storage.

2. EXPERIMENTAL

2. 1. Material

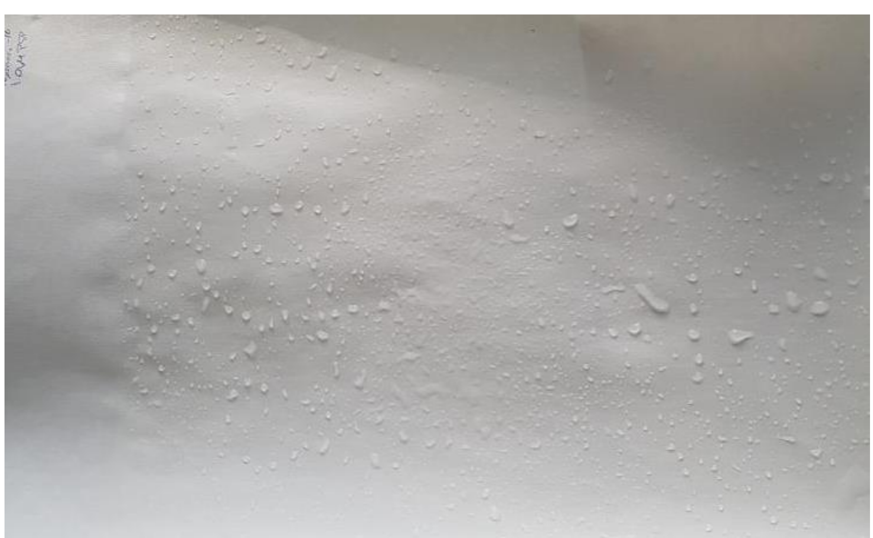

Figure 1. Paper covered with Topscreen 250 hydrophobic coating

The test microorganisms used in this study were obtained from a collection from the Leibniz Institute DSMZ (Deutsche Sammlung von Mikroorganismen und Zellkulturen). The organisms used in this study were *S. aureus* DSMZ 346, and *E. coli* DSMZ 498.

To verify the antimicrobial properties of any coatings, TSB and TSA media (Merck, Darmstadt, Germany) were used. All the media were prepared according to the Merck protocol: they were weighed according to the manufacturer's instructions, suspended in 1000 mL of distilled water, and autoclaved at 121 °C for 15 min.

Paper (120 g/m^2, Celabor, Belgium), covered with Topscreen 250 hydrophobic coating, was used as a packaging material. **Figure 1** shows the drops of water on the hydrophobic layer. Methyl Cellulose (Methocel^{TM}, Dow, Stade, Germany, was used as a coating carrier and a polylysine (Handary S.A., Belgium) was used as an active substance.

2. 2. Methods

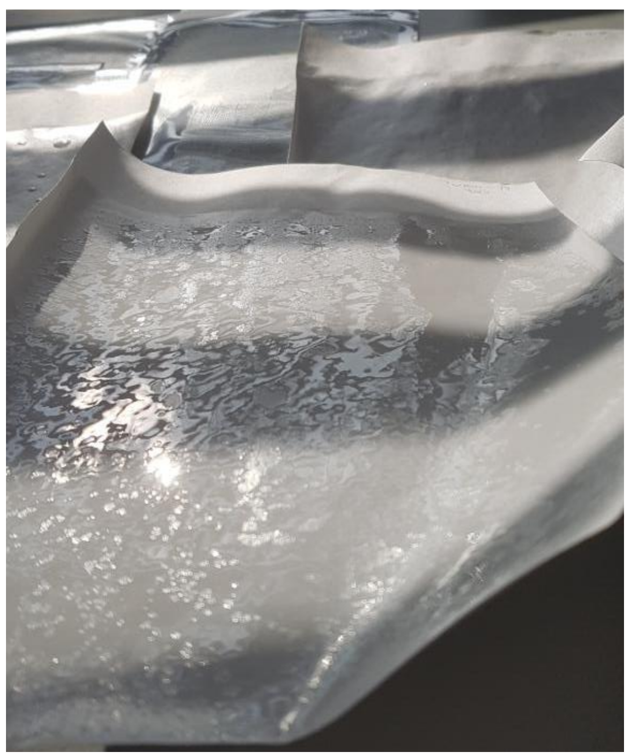

Figure 2. Paper coated with Methocel^{TM} containing polylysine (on the surface of Topscreen layer)

(1) 2 g of Methocel^{TM} was introduced into 96 mL of water. The mixture was mixed for 1 h using a magnetic stirrer (Ika) at 1500 rpm. Next, 2 g of polylysine was introduced into 98 g of mixture. The mixture was then mixed for 1 h using a magnetic stirrer (Ika) at 1500 rpm. The mixture was used to coat the paper to obtain 2% polylysine coating as a second layer (an active coating).

Paper covered with a hydrophobic Topscreen layer was coated with Methocel™ containing polylysine (**Figure 2**). The paper was covered using Unicoater 409 (Erichsen, Hemer, Germany) at 25 °C with a roller at a diameter of 25 µm. The coatings were dried for 10 min at a temperature of 50 °C.

The samples were stored 2 months at 20 ºC. The paper samples, covered with a hydrophobic Topscreen layer, devoid of second layer (control samples) and paper covered with a hydrophobic Topscreen layer and with the antimicrobial coating (as an external layer) were cut into square shapes (3 cm × 3 cm). The antimicrobial properties of samples were carried out according to ASTM E 2180-01 standard before and after 2 months of storage [ASTM Standard test method for determining the activity of incorporated antimicrobial agent(s) in polymeric or hydrophobic materials, E 2180-01].

3. RESULTS

The results of the study showed that the paper covered with hydrophobic Topscreen coating did not have an influence on the growth of *S. aureus* cells. The second (external) layer, containing 2% polylysine as an active substance, decreased the growth of *S. aureus*. It was observed that the decrease of the number of bacterial cells was higher than 4 log (**Figure 3**). The 2 months storage of the covered paper did not influence the antimicrobial properties of coating with polylysine.

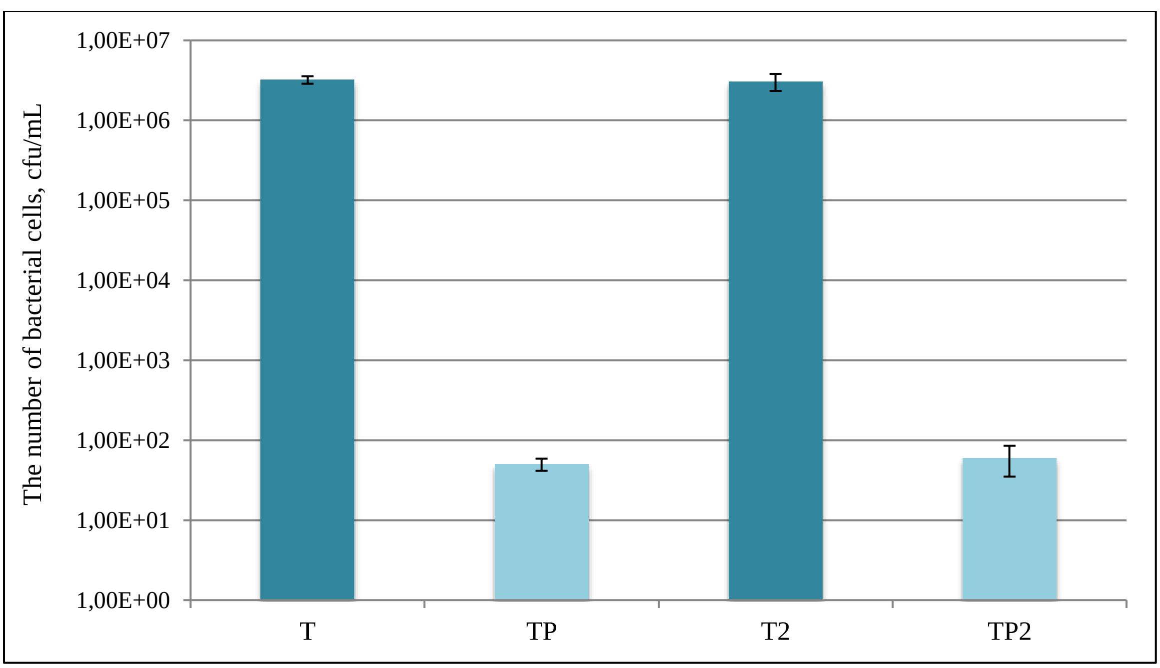

Fig. 3. The activity of the coating with the polylysine against *S. aureus* before and after 2 months of storage

The results of this research demonstrated that the paper covered with hydrophobic Topscreen coating had no influence on the growth of *E. coli* cells. A more than 2 log decrease of the number of bacterial cells after 24 h contact with Methocel^{TM} layer, containing polylysine, was noted. As emphasised below (**Figure 4**), the influence of 2-month storage on the antimicrobial properties of the coating with polylysine was observed. In contrast to the results obtained for the samples that were not stored, the decrease of the growth of the bacterial cells after 24-h contact with a hydrophobic coating devoid of an active substance was observed. It means that the 2-month storage influenced the antimicrobial activity of the coatings, but slightly.

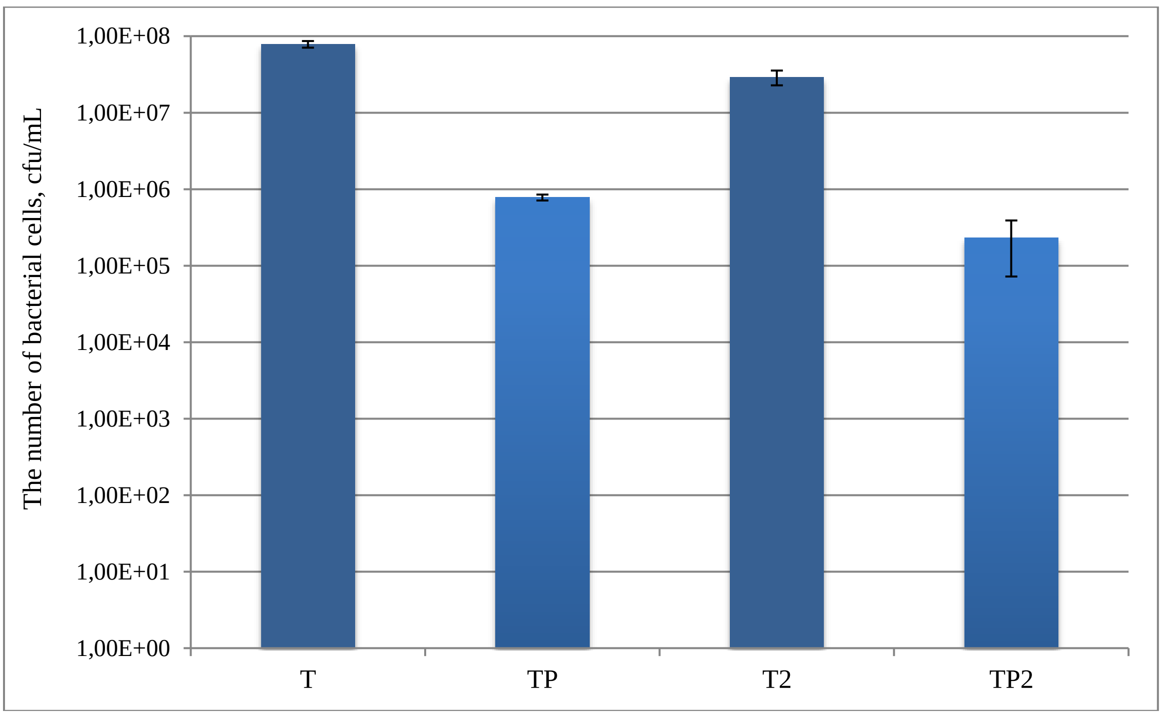

Fig. 4. The activity of the coating with the polylysine against *E. coli* before and after 2 months of storage

4. CONCLUSIONS

A. Methocel^{TM} coatings, containing polylysine, were active against *S. aureus* and *E. coli* strains.
B. 2 months of storage influenced the antimicrobial activity of the coatings against *E. coli*.

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