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IMPACT OF NANOSILVER AND NANOCOPPER ON ANTIFUNGAL PROPERTIES OF PLASTIC ELEMENTS IN THE ASPECT OF THEIR USE IN AGRI-FOOD PROCESSING

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ABSTRACT

The paper presents results of microbiological research of plastic elements. Plastic elements were made of the polystyrene composition with addition of nanosilver and nanocopper with a laboratory extruder EHP 25ELine. Microbiological research was carried with regard to evaluation of antifungal impact of nanoadditives according to international standards with Cryobank (Mast Diagnostica). A positive impact of nanoadditives on antifungal properties of polystyrene elements produced by means of extrusion which is very favourable in the aspect of their use in agri-food processing.

Introduction

A dynamic progress of the plastic processing industry forces producers of plastic elements to constantly search for innovative and non-typical solutions. Additionally, constantly changing prices of primary raw materials force producers to search for cheap and resistant materials. These types of trends influence actions within plastic processing and with this background, today, we observe, inter alia, an increased effort in production of more stable compositions with favourable utility properties with addition of meal/regranulate (with its varied content and rheological properties) and composition with innovative additives including nanoadditives (Wenda et al., 2016; Malinowska-Pańczyk et al., 2010; Mroczek-Sosnowska et al., 2013; Zhang et al., 2015; Wenda et al., 2015; Jałbrzykowski et al., 2016). Nanoadditives include the following: e.g. nanosilver, nanocopper or graphene oxide. There are many scientific papers which deal with the issue of e.g. nanoparticiles of silver. However, these papers describe possibilities of application of nanoparticles of silver or the impact of nanosilver on mechanical properties and other of plastic samples or describe their general antibacterial activity (Falkiewicz-Dulik and Kowalczyk, 2016; Juraszek and Grzesiak, 2008; Zielecka et al., 2012; Gniazdowska et al., 2015). There are many stud-

ies which present results of research on the impact of nanoparticles of silver on biostability of plastic details exposed to bacteria or fungi which potentially occur in the place where elements occur (Liya et al., 2013; Rajski et al., 2016).

The paper presents results of microbiological research of plastic elements made by extrusion. Before samples were produced, granulate with a content of nanoparticles of silver and copper was prepared (by means of a compounding method). Granulate was prepared on the polystyrene basis. Due to the undertaken patent procedure, this paper does not reveal quantity participation of components of the investigated composition. It should be added that for the purpose of the research it was assumed that the produced composition will constitute a material for production of furniture elements for residential premises. Strains of fungi which may potentially occur in everyday use premises such as e.g. kitchens, storage rooms, side rooms, cellars, etc., were selected as a biologically active environment: *Penicyllium nucleatum, Aspergillus niger, Cryptococcus neoformans, Cladosporium sp.* Moreover, the research was carried out with regard to application possibilities of the developed composition as a material for production of elements of equipment for everyday use premises, such elements as: baseboards, elements of furniture etc. Such elements are of great importance in furniture finishing works but they may be also used in other premises as farm buildings, plant floors etc. At the same time, plastics with the content of nanoadditives find even greater application in the material engineering including agri-food processing.

The objective of the paper was to evaluate the impact of nanoadditives suspended in the polymer, polystyrene mass on the antibacterial properties of plastic samples produced from this mass by extrusion in the aspect of the future application in the agri-food industry.

Methods of research

Samples of the commercial polystyrene granulate (fig. 1a) and samples of the composition based on polystyrene with nanoadditive of silver and copper (nAgCu) (fig. 1b) were prepared for the research. Due to the initiated patent procedure, the recipe according to which model composition were prepared is not disclosed. The prepared samples of granulate were extruded with laboratory extruder EHP25Eline. Before the extrusion process, granulates were dried according to the recommendations for PS.

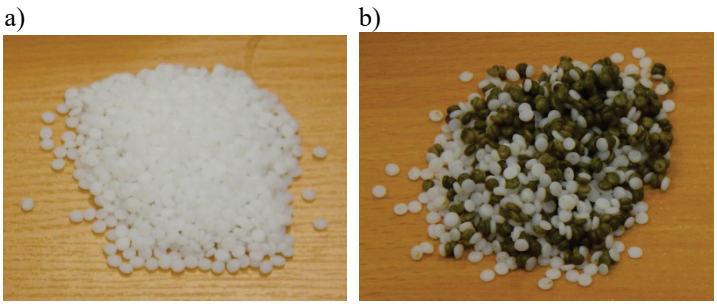

Figure 1. Samples of granulates: a) polystyrene (PS), b) polystyrene with nanoadditives of silver and copper (nAgCu)

The extrusion process was carried out at a varied temperature of the extruder cylinder within 180-220ºC. In the extrusion process samples of flat bars with dimensions – thickness x width = 4 x 25 mm were used.

a) b)

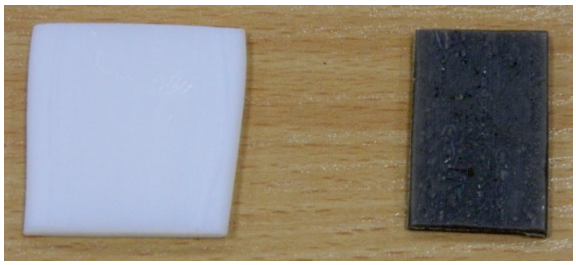

Figure 2. Image of the exemplary samples for microbiological tests: a) PS, b) PS+nAgCu

At the outlet at the extruder head, flat bars with the length of aprox. 100 mm were cut off. Then, the flat bars were placed in a dedicated form and pressed under the load of approximately 1000 kilo. As a result of pressing, flat surfaces with the thickness of approximately 2 mm with a form texture were obtained. The surfaces of samples were purposefully "marked" to facilitate interpretation of the results of microbiological research. For the purposes of the tests with the prepared elements smaller samples (fig. 2) were cut and marked as follows: polystyrene – PS, composition with nanosilver and nanocopper PS+nAgCu. After they were prepared, samples were conditioned in regular conditions for 24 hours and then microbiological tests were carried out. Microbiological tests were carried out in the Institute of Microbiology of the Medical University in Bialystok. With reference to microbiological research, firstly relevant straits of bacteria were selected. Thus, literature was reviewed which enables the selection of fungal straits. With reference to the literature review (Jain et al., 2007; Czaczyk, 2004; Bartoszewicz et al., 2007; Klotz et al., 1985) one may say that among fungal spores which commonly occur in air there are such which cause respiratory and digestive system mycosis or are responsible for alergies and asthma. Among them the most significant with regard to epidemiology in our climatic zone are fungi of *Penicylium, Aspergillus and Cladosporium type.* On the other hand, other, such as *Cryptococcus neoformans* the most often cause infections by inhalation in persons with the advanced stage of AIDS and diabetes, with transplanted organs, subjected to a long-term corticosteroid therapy. The most frequent clinical form of cryptococcosis is meningitis and encephalitis which without treating leads to death.

The main source of fungi is a microenvironment of closed rooms with high moisture, limited ventilation and weak access of light (mainly cellars, washing rooms, bathrooms or kitchens as well as farm building in rural areas). Wooden summer houses, arbours, sauna and pools also constitute a significant source of allergens of house fungi. House dust collecting in hard to access corners is a significant reservoir of allergenic fungi in rooms. It is estimated that the participation of fungi may constitute from 5 to 20% of its composition.

Taking the above into consideration, the following fungi types were selected for the research (brackets include marking of straits for the description of tests):
– Penicyllium nucleatum (Pen),
– Aspergillus niger (Asp),
– Cryptococcus neoformans (Crypt),
– Cladosporium sp. (Clad).

At the first stage of research, dynamics of biofilm formation by 4 straits of various species of fungi isolated from the environment were evaluated. Straits were stored according to the international standards with Cryobank (Mast Diagnostica). Before the experiment, straits were activated in the Saborauda liquid base and after 24-72 hours of incubation in an incubator in the temperature of 25ºC in oxygen conditions from each isolate a suspension inoculating with inoculum 10^4 CFU/ml (CFU- colony forming unit) was prepared.

Biofilm formation on the tested surfaces

Research was carried out in sterile containers where samples of plastics sterilized in 70% of ethanol were evaluated. Then, suspensions of four types of fungi with innocullum 10^4 CFU/ml of Sabourauda's broth and 50 µl 1% of solution TTC (2,3,5 - thriphenyltetrazolium chloridium SIGMA) was added correspondingly to particular containers with the tested material in order to assess the presence of red formazan which forms in the reaction of TTC reduction carried out by active methabollic bacteria. This method enables tracing the process of biofil forming by microorganisms. In the next stage of research, containers with samples were incubated in the room temperature in oxygen conditions by: 48 h, 72 h, 3 weeks.

After the lapse of particular times, plates were taken out of the containers and in order to remove fungi from their surface which form a plankton suspension, they were washed three times in a sterile solution PBS with 7.2 pH. At the end of washing the plates were soaked in deionised sterile distilled water with pH=7. Then, samples were dried and transferred to microscopic observations. It should be added that finally, microscopic observations were carried out on samples which were incubated for 3 weeks.

Microscopic observations of the prepared samples were carried out with the use of the measuring microscope Olympus BX51M. The observation process consisted in the analysis of the samples surface in order to localize the bacteria straits and a biofilm and in assessment of the growth degree of a bacterial colony. A photo documentation of the analysed surfaces was made during inspection. In justified situations a computer analysis of the image was made with a program for processing and analysis of image Aphelion which is a professional tool for assessment of this type of samples, also of biological preparations. In the analysis, an algorithm was used which was developed based on own experiments and generally known recommendations (Gądek et al., 2006; Wojnar et al., 2002).

Discussion and analysis of the research results

Figure 3 presents pictures of straits cultured on a laboratory glass in conditions that are advantageous for the culture.

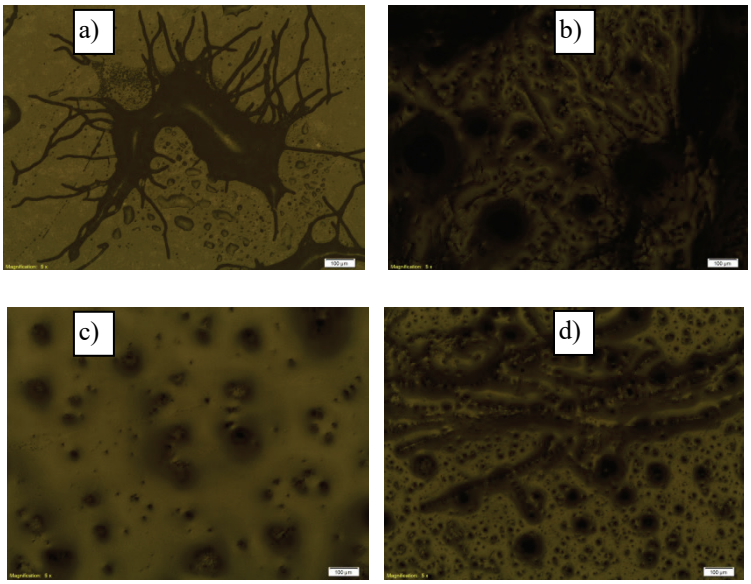

Figure 3. Microphotos of the cultured straits: a) Penicyllium nucleatum, b) Aspergillus Niger, c) Cryptococcus neoformans, d) Cladosporium sp.

The presented microphotos illustrate the image: *Penicyllium nucleatum* (Pen), *Aspergillus niger* (Asp), *Cryptococcus neoformans* (Crypt), *Cladosporium sp.* (Clad). After the structure of the culture was recognized in assimilated conditions the plastic samples were inspected. It should be mentioned that a favourable growth of the culture in case of clean PS samples and less favourable in case of PS+nAgCu samples was expected. Firstly, base samples i.e. samples of plastics kept in the same conditions as cultured straits but in clean solutions without bacteria, were observed. Figure 4 presents the image of base plastic samples. It should be added that despite the fact that samples were identically prepared in case of clean PS the surface was rougher. On the other hand, in case of PS+nAgCu big amounts of fine dark precipitation – probably of nanoadditives were observed.

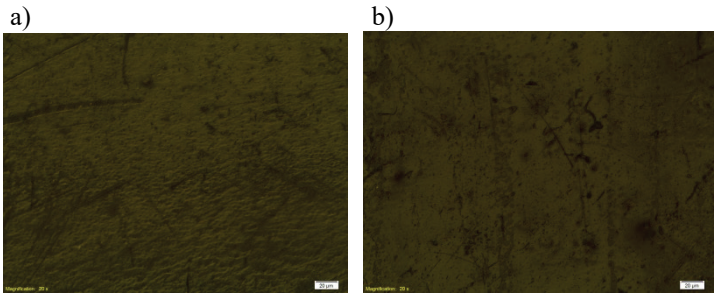

Figure 4. The image of the base plastics: a) clean PS, b) PS+nAgCu

Figure 5 illustrates exemplary results of inspection of the surface of samples PS and PS+nAgCu, where Asp bacteria were cultured. It is clear that in case of the PS sample (fig. 5a) the culture covered practically the entire surface (fig. 5c). In case of the sample PS+nAgCu the surface is clear. It may be only added that precise observations of the PS+nAgCu sample surface enabled the statement that there are places where locally groups of bacteria colonies and biofilm were reported. It may be also mentioned that the biofilm stripe seen in figure 5 probably is a result of washing the sample surface.

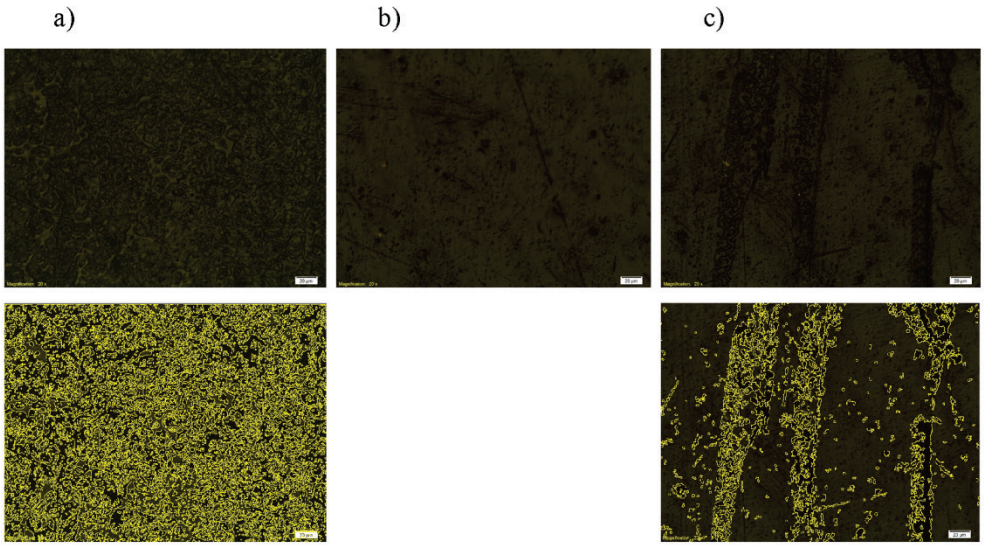

Figure 5. Image of the samples surface after microbiological tests in the Asp environment: a) PS, b) and c) PS+Ag

It would mean that during the culture was held, bacteria grouped on the surface of PS+nAgCu; however, as a result of washing, they were almost completely removed therefrom. Only thin, not numerous strips were left. This fact proves that bacteria were not inclined to fix to the surface PS+nAgCu, which may prove antibacterial activity of nanoadditives. To carry out a quantity assessment of this situation, calculations with Aphelion program were carried out. Results of this analysis (fig. 6) show clearly that in case of the sample PS+nAgCu, the amount of bacteria groups permanently fixed to the surface (395 on the surface of approx. 1 mm^2) is half lower than in case of the sample PS (741, on the surface of approx. 1 mm^2). It may be stated clearly and without additional tests that PS+nAgCu material is a less favourable base for development and growth of the bacteria film in comparison to clear PS. The situation for all the remaining investigated samples is analogous.

Figure 7 presents the selected results of observation of the surface where Clad bacteria were cultured. It may be stated that in this case, the situation is the same as in case of Asp bacteria. The surface of the PS sample is practically entirely covered with the biofilm layer

with participation of Clad bacteria (fig. 7a). In case of PS+nAgCu sample, only single areas may be found where local bacteria groups (fig. 7c) may be seen.

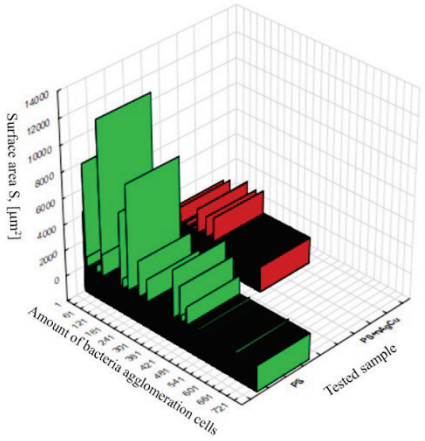

Figure 6. Results of the computer image analysis with the use of Aphelion program – sample PS+nAgCu in Aps environment

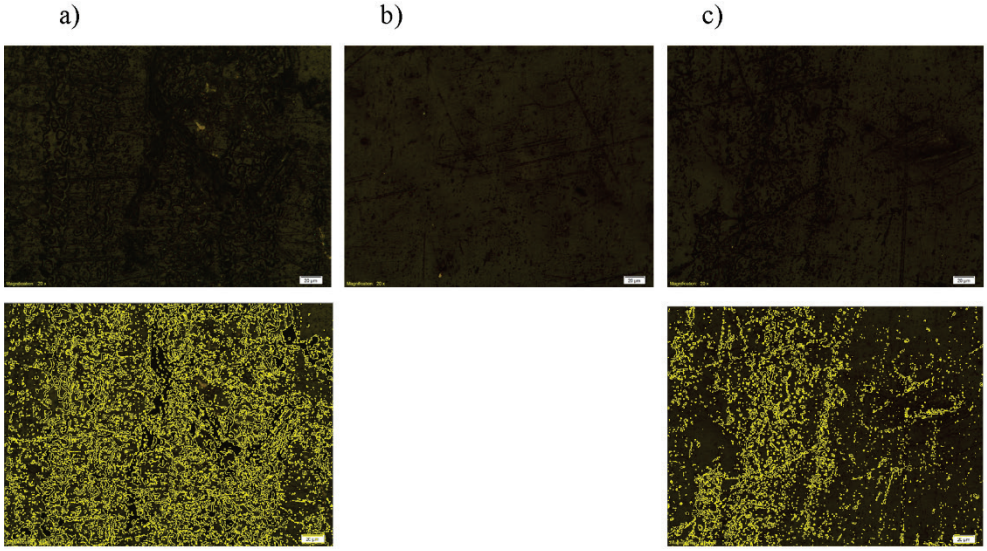

Figure 7. Image of the samples surface after microbiological tests in the Clad environment: a) PS, b) and c) PS+Ag

In this case, as before, one may say that there are clear differences in the intensity and growth of the bacteria film cultured on the surface of PS i PS+nAgCu. The PS base is a good place for the growth of the analysed culture in comparison to the base PS+Ag.

Figure 8 contains micropictures of the analysed samples after microbiological tests in the Crypt environment. And in this case the same tendencies were reported as in the previous research. We may only add that on the PS sample surface in case of Crypt bacteria, generally considerably less bacteria colonies were reported than in case of Asp and Clad bacteria. Simultaneously, as previously, a higher growth of bacteria colonies was reported than for PS base in comparison to PS+nAgCu base.

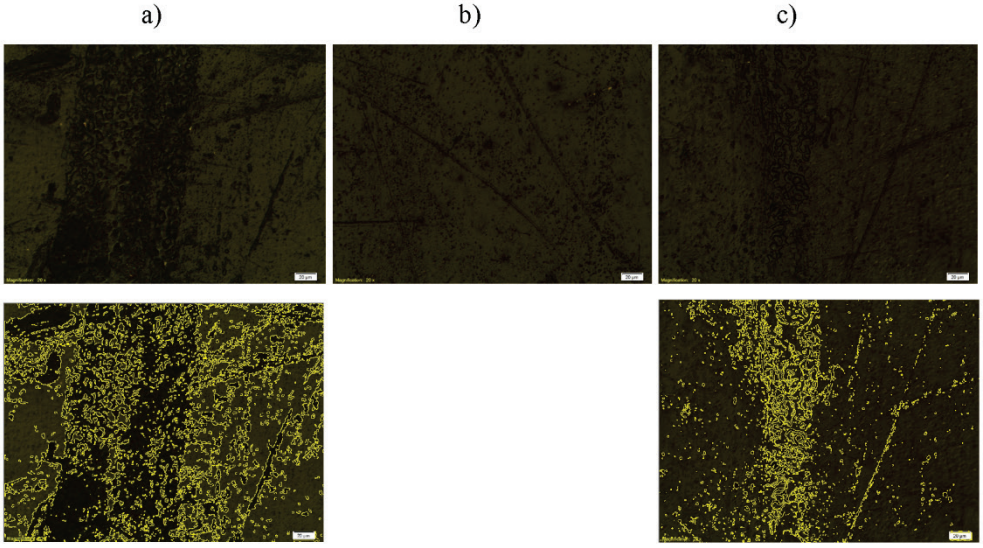

Figure 8. Image of the samples surface after microbiological tests in the Crypt environment: a) PS, b) and c) PS+Ag

As the last ones, figure 9 presents the selected results of observations of the sample surface after microbiological tests in the Pen environment. Also in this case, the same trends as in previous samples were reported. Presence of the bacterial film on the entire sample surface with single discontinuity was reported. While, in case of sample PS+nAgCu a considerably lower number of bacteria colonies, more specifically its single, local groups, were determined.

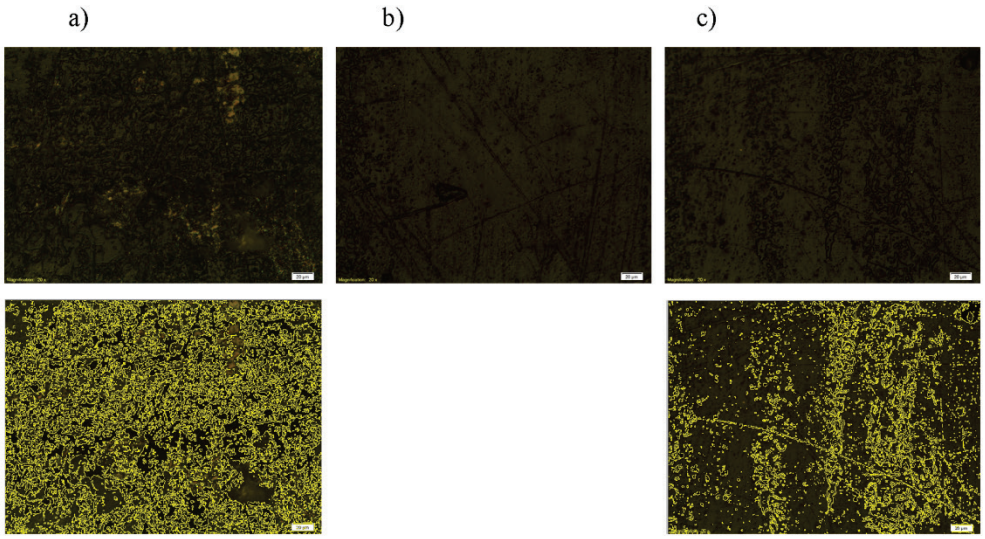

Figure 9. Image of the samples surface after microbiological tests in the Pen environment: a) PS, b) and c) PS+Ag

Conclusions

Based on the investigations which were carried out and their analysis, the following conclusions were expressed:

1. Presence of bacterial colonies both in samples with PS and samples with PS+nAgCu were reported.
2. On the samples with clean PS it was observed that practically the entire surface of the sample was covered with a layer of a stable biofilm with a strong bacteria growth. It may result from the fact that the PS surface is a good (favourable) base for growing bacterial colonies.
3. On the surface of samples PS+nAgCu presence of bacterial colonies was also reported. However, it should be added that these colonies were placed only locally in single spots. It means that the surface PS+nAgCu is a less favourable base for the growth of sown bacterial colonies in comparison to the base of clean PS.
4. In the context of the issues concerning a complex destination of the material (furniture elements for residential premises), it may be said that a considerably unfavourable impact of the PS+nAgCu composition was reported (in comparison to clean PS) on the behaviour of 4 investigated fungi colonies. The tests confirm antibacterial activity of this composition.
5. Plastics with the content of such additives may be successfully used in various fields of economy. Generally, a raising interest in this type of composition in the material engineering has been reported. They may be also successfully applied in the agri-food processing as structural materials in dairy plants, food industry, agricultural farms etc.

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WPŁYW NANOSREBRA I NANOMIEDZI NA WŁAŚCIWOŚCI ANTYGRZYBICZE DETALI Z TWORZYW SZTUCZNYCH W ASPEKCIE ICH ZASTOSOWANIA W PRZETWÓRSTWIE ROLNO-SPOŻYWCZYM

Streszczenie. W pracy przedstawiono wyniki badań mikrobiologicznych detali z tworzyw sztucznych. Detale tworzywowe wykonano, jako kompozycję polistyrenu (PS) z zawartością dodatków nanosrebra i nanomiedzi, za pomocą laboratoryjnej wytłaczarki EHP 25ELine. Badania mikrobiologiczne prowadzono w kierunku oceny antygrzybiczych oddziaływań nanododatków według międzynarodowych standardów stosując Cryobank (Mast Diagnostica). Stwierdzono korzystny wpływ nanododatków na antygrzybicze właściwości detali z polistyrenu wytwarzanych metodą wytłaczania, co jest bardzo korzystne w aspekcie ich zastosowania w przetwórstwie rolno-spożywczym.

Słowa kluczowe: nanododatki, srebro, miedź, tworzywa sztuczne, oddziaływania antygrzybicze