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Bactericidal Properties of Low-Density Polyethylene (LDPE) Modified with Commercial Additives Used for Food Protection in the Food Industry

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Abstract: This study investigated the influence of commercially available food preservatives: Natamax® (containing natamycin) and Nisaplin® (containing nisin) on the antimicrobial properties of LDPE film, commonly used for food packaging. Studies have shown that the addition of 3% Natamax® or, alternatively, the addition of 5% Nisaplin® provides an LDPE film with effective antimicrobial protection. The applied biocides did not significantly affect the strength and rheological properties of LDPE. However, differences in optical properties were observed. The transparency of the samples decreased slightly with the addition of 3% or 5% Natamax® (by approx. 1% and 3%, respectively). A significant change was observed in the film haze, the addition of 5% Natamax® increased this parameter by approx. 80%, while 5% Nisaplin® increased it by approx. 19%. Both Natamax® and Nisaplin® agents can be successfully used to manufacture food packaging materials with antimicrobial protection. Natamax® showed a stronger bactericidal effect, while Nisaplin® changed other properties less significantly.

Keywords: low density polyethylene (LDPE); bactericidal properties; Nisaplin®; Natamax®

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1. Introduction

Despite the intensive development of innovative technology using polymer materials in construction, medical industries and more recently in 3D printing, one of the most significant sectors using approx. 40% of this raw material is the packaging industry [1–6].

Despite the introduction of more and more biodegradable materials on the market, limited access to crude oil and the increasing prices of this raw material, the most successful materials in the production of packaging are: low-density polyethylene (LDPE), high-density polyethylene (HDPE), polyethylene terephthalate (PET) and polystyrene (PS) [7,8]. Among the above-mentioned, LDPE is used to make most film packaging [9]. Interest in conventional plastics is related to well-known methods of processing these materials [10,11]. Materials intended for packaging must have appropriate processability, good durability, barrier properties and the possibility of creating multi-layer materials or additional active properties, including biocidal and biostatic [7].

Since the onset of the SARS-CoV-2 virus pandemic in 2020, global interest in biocidal materials against viruses and other microorganisms has greatly increased [12]. The presence of pathogenic viruses, bacteria or fungi has caused increased societal anxiety. The development of innovative biocidal materials began, ranging from disinfectants, through to tools and items that ensure microbiological safety, as well as intensive work related to the development of new materials for the biomedical sector [13–15]. The search for new biocides, often based on metal nanoparticles or natural substances, has also intensified [16–19].

In the packaging industry, microbiological protection is an important factor that has the potential to extend the shelf life of the product inside the package, as well as improve the safety of the outer layer of the package. It is estimated that in combination with an appropriately modified atmosphere and barrier, it is possible to significantly extend the freshness of the packaged product, e.g., from 3 to 13 days [20,21]. The presented study focuses on bactericidal properties, which are particularly important for the packaging of meat, dairy products and bread [22,23].

Among the commercially available plastics with biocidal properties, most of them are currently modified with metal nanoparticles, the most popular of which is nanosilver [24–26]. Due to research showing that nanoparticles, after prolonged use, despite very good antimicrobial effectiveness, may have negative effects on the environment and human health, products of natural origin are becoming more and more popular [27,28]. Among natural products, we can distinguish two basic groups: plant extracts with antioxidant properties and antibiotics, bacteriocins or similar substances produced by microorganisms. Often, however, the effectiveness of natural biocides is much lower than that of nanometals, despite higher dosing of the polymer material. There are also problems regarding the high processing temperatures of polymers, which often precludes quick degradation of the natural components [29–31]. On the other hand, the use of natural substances produced by microorganisms, such as natamycin, nisin, astaxanthin, plantaricycline, lacticin, is often economically unprofitable but despite successful laboratory research results, they are not implemented in the industry [32,33]. Meanwhile, the use of products based on natural compounds such as nisin or natamycin has been practiced for many years to extend the shelf life of food products. One of the main producers of this type of product is DuPont Danisco (Stevenage, UK), which produces Nisaplin® containing nisin and Natamax® containing natamycin.

Natamycin, also known as pimaricin, is a versatile, naturally occurring antimicrobial that inhibits the growth of yeast and fungi in a wide variety of foods and beverages, as well as animal feed [34]. The compound is a tetraene polyene macrolide with a molecular weight of 665.7 Daltons. Commercial preparations of natamycin made by fermentation in a glucose-based media by selected strains of *Streptomyces* (e.g., *Streptomyces natalensis*) contain about 50% natamycin mixed with lactose. Most studies show that natamycin, despite its very strong effect on fungi, has no major effect on bacteria and viruses. This statement is often supported by fermentation efficiency studies, which are of key importance, for example, for the preservation of dairy products. There are a few studies on activity against pathogenic bacterial strains [35,36]. However, there is one study on Natamycin showing antibacterial activity [37]. The effectiveness of this biocide has been proven against pathogenic yeast strains such as *Rhodotorula mucilaginosa* and *Candida parapsilosis* [38]. As the manufacturer of Natamax® declares, the product has an antimicrobial effect in a wide range of pH values and heat treatment processes, which allows it to be used in the processing of polymeric materials.

Nisin is a polycyclic peptide made up of 24 amino acids and belongs to bacteriocins produced in the fermentation process by Gram-positive lactic acid bacteria belonging to *Lactococcus*, e.g., *Lactocococcus lactis* subsp. *lactis* [39]. Some strains of *Streptococcus* can also synthesise nisin. Nisin has been approved by the Food and Drug Administration (FDA) since 1988, while for several years it has also been used in biomedical clinical trials [40]. Nisin is widely used as a food bio-preservative, it is particularly popular in the dairy industry in the production of processed cheese. Studies have shown that nisin can prevent the development of drug-resistant Gram-positive and Gram-negative bacterial strains such as methicillin-resistant *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Clostridium difficile* or *Enterococcus bacteria* [41].

It is possible to use pure nisin and natamycin as a component of polymers, especially as these are compounds with quite a high thermal resistance [32]. However, attempts to utilize it often indicate poor dispersion of the pure compounds in the polymer mass [42,43]. It is also economically unprofitable due to the 100% higher purchase cost of the product in its pure form compared to commercial products such as Natamax® or Nisaplin® (commercial data from September 2021). Research indicates that nisin and natamycin have anti-biofilm properties, which may be of particular importance for their use as a component of polymers [44]. There are reports of a study on the use of Nisaplin® as a component of antibacterial coatings applied with the flexographic method for meat packaging [45]. An ethylene vinyl acetate (EVA) film containing another commercial nisin-containing agent was also developed under laboratory conditions, but the study focused primarily on the release rate of nisin. There were also several reports of research on the development of biodegradable plastics with the addition of commercial products containing nisin or natamycin [46–48].

The idea behind this study was to develop a polymer material to be used as packaging material for food, which would additionally have high bactericidal activity. According to the hypothesis, the component of the polymeric material will be commercial products which are used in practice for spraying food to extend its shelf-life. This will enable, in the future, the production of packaging intended for food, in addition to other products, so that it may not be necessary to spray packaged food products with the applied biocides. Due to the use of the biocide in the entire mass of the polymer material, which therefore also ensures protection on the outer surface of the package, it is possible to use the material under development for other applications.

2. Materials and Methods

2.1. Materials

LDPE Malen E FABS 23-D022—polyethylene (Bassel Orlen Polyolefins, Płock, Poland), which is used to produce all kinds of flexible food packaging, was used for our study. It is characterised by high transparency and gloss.

The first bioactive additive was Natamax® (DuPont Danisco, Stevenage, UK) containing min. 50% of the active agent- natamycin (E235) and lactose.

The second additive used was Nisaplin® (DuPont Danisco, Stevenage, UK) containing the active agent—nisin A (E234) in the amount of min. 1000 IU/mg and sodium chloride (minimum 50%).

2.2. Material Processing

The individual active agents mentioned above were made into 10% masterbatch concentrates with polyethylene using a Bühler BTSK co-rotating twin-screw extruder (Uzwil, Switzerland) with a diameter of 20 screws and a plasticising system length of 42 L/D. Due to the limited thermal resistance of biocides, extrusion was conducted at the lowest possible temperatures in the range of 110–120 °C. LDPE was hand mixed with an appropriate amount of the masterbatch concentrates to obtain LDPE composites containing individual biocide agent concentrations of 1%, 3% and 5% in the polymer. From the obtained mixtures, flat films with a width of 100 mm and a thickness of approx. 0.04 mm were extruded using a laboratory single-screw extruder Plasti-Corder PLV 151 (Brabender, Duisburg, Germany) and cooled on a three-roll calendar. The film extrusion process was conducted at temperatures of 110–125 °C, and cooling was carried out on thermostated rollers with a temperature of 25 °C. From the obtained films, standardised test specimens were prepared with dimensions of 50 mm $\times$ 50 mm ($\pm$2 mm) according to ISO 22196 standard [49].

2.3. Bactericidal Properties

The bactericidal properties tests were carried out in accordance with ISO 22196 [49] which is an international standard compatible with JIS Z 2801:2000. The tests on the film samples were conducted with strains: *E. coli* (ATCC 8739) and *S. aureus* (ATCC 6538P). Strains were cultured according to standard guidelines for 24 h at 35 °C ($\pm$1 °C) in NA-Nutrient Agar (OXOID, Basingstoke, UK), then transplanted onto fresh media and re-cultured for 20 h.

Tests were conducted for each of the strains on three samples from each test material (containing the biocide) and on six samples of the control material (without the biocide). Half of the control samples were used to measure viable cells immediately after inoculation, and half were used to measure viable cells after 24 h of incubation. The samples did not require sterilisation prior to testing. The inoculum volume was 0.4 ml. The cover layer was a polyethylene film with dimensions of 40 mm × 40 mm (±1 mm) and a thickness of 0.06 mm. Petri dishes containing inoculated test samples (including half of the control samples) were incubated at 35 °C (±1 °C) and 90% relative humidity (RH) (±5%) for 24 h (±1 h).

For each sample tested, the number of live bacteria recovered was determined in accordance with the guidelines of ISO 22196 [49]. The antimicrobial activity was then expressed as the decimal logarithm cfu/cm^2 reduction relative to the control sample.

2.4. Mechanical, Rheological and Optical Properties

Apart from the analysis of the bactericidal properties of the produced samples, the basic film parameters were determined to evaluate the influence of the applied biocides on the mechanical, rheological and optical properties. Before testing, the samples were conditioned in accordance with ISO 291 (23 °C ± 1 °C, 50% ± 5% RH) [50].

2.4.1. Mechanical Properties under Static Stretching

The static tensile mechanical properties of the film samples were determined according to ISO 527-1 and ISO 527-2 [51,52]. The tests were conducted using TIRATest 27025 equipment (TIRA GmbH, Schalkau, Germany). Test conditions are given in Table 1.

Table 1. Test conditions for mechanical properties under static tension.

Parameter	Value
Temperature (°C)	23 ± 1
Humidity, RH (%)	50 ± 5
Test speed (mm/min.)	50
Length of the measurement section, L_0 (mm)	50
Sample width (mm)	10

2.4.2. Mass Melt Flow Rate, MFR

The melt flow rate for the film samples was determined according to ISO 1133 [53]. The tests were performed using a capillary plastometer LMI 4003 (Dynisco, Franklin, MA, USA). The measurements were performed at a temperature of 190 °C with a load of 2.16 kg.

2.4.3. Light Transmittance

The analysis of changes in the light transmittance of the samples after the addition of biocides was carried out in accordance with ISO 13468-1 using a test stand equipped with a Hazemeter M 57 photometric device (Diffusion Systems, London, UK) equipped with a light source in the form of a 12 V tungsten lamp [54].

2.4.4. Haze Tests

Tests were carried out in accordance with ASTM D 1003-11 [55] using the same test stand as for light transmittance (Section 2.4.3). The haze value is determined by passing light through a sample, then entering a transmittance value divided by 100 into the measuring system, and then directing the light into a light trap. The optical system then measures the value of the scattered light.

2.5. Statistical Analysis

Figures are presented as mean ± standard deviation. For microbiological activity tests, the obtained results were analysed using the Past 321 program (Softpedia, Past 321, Bucharest, Romania, 2018). Significant differences were determined based on one-way analysis of variance (ANOVA) using Tukey's and Mann–Whitney's test for $p < 0.05$.

3. Results

3.1. Films

As a result of the extrusion, samples of LDPE films containing 1%, 3% and 5% Natamax® or Nisaplin® were obtained. The control sample was LDPE film containing no biocide of the same thickness as the other films (Figure 1). The addition of biocides did not significantly affect the colour of the samples. Moreover, no significant differences were observed in the samples in the scanning electron microscope (SEM) images. Only at 5% concentration of biocides were single agglomerates were noticed (Figure 1).

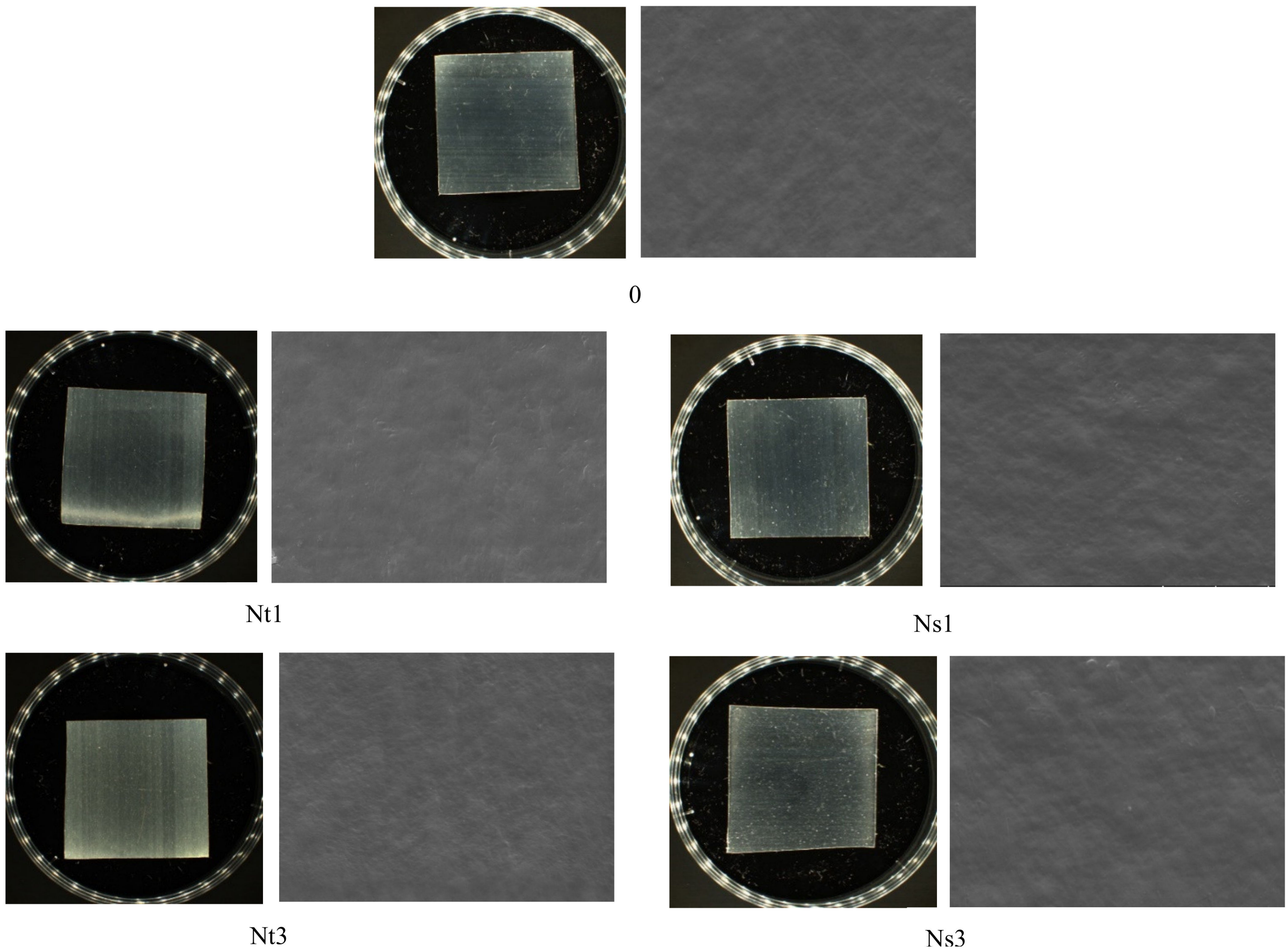
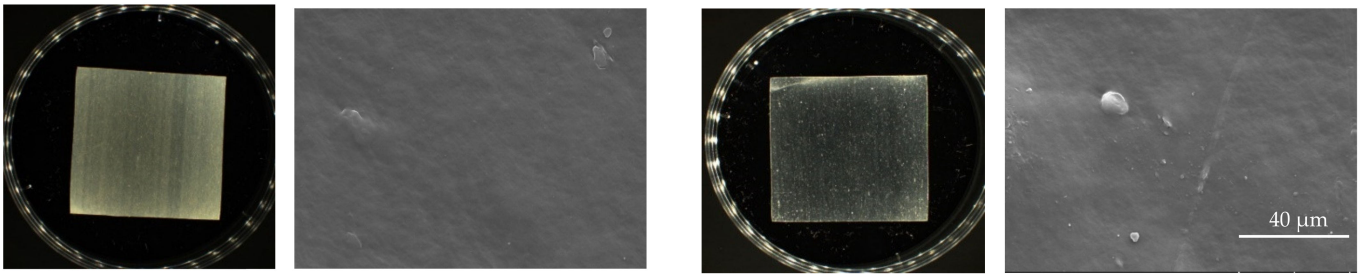


Figure 1. *Cont.*



Nt5 Ns5

Figure 1. Sample images (real photos and photos taken with a scanning electron microscope-SEM, Hitachi SU 8010, Tokyo, Japan). Abbreviations: 0- pure LDPE; Nt 1,3,5 (samples with 1%, 3% and 5% Natamax®); Ns 1,3,5 (samples with 1%, 3% and 5% Nisaplin®).

3.2. Antibacterial Properties

According to the methodology, the antibacterial properties of polymer materials are determined by measuring the reduction in antibacterial activity (R), i.e., based on the difference in the logarithm of the number of living cells found on the material containing the biocide and the control material after inoculation with bacteria and incubation. An antimicrobial product is assumed to have antimicrobial efficacy when $R \geq 2.0$. The number of viable bacterial cells after sample incubation is presented in Table 2. The results met the validation conditions according to ISO 22196 [49].

Table 2. Number of viable bacterial cells after incubation CFU/cm^2 (mean ± standard deviation in brackets). Statistically significant differences in the columns are marked with different letters for $p < 0.05$. Abbreviations: as in Figure 1.

Sample	*E. coli*	*S. aureus*
0	1.2×10^4 (1600) e	1.1×10^4 (1700) f
Nt1	7.0×10^2 (90) c	2.5×10^3 (280) d
Nt3	4.0×10^1 (10) a	3.0 (1) a
Nt5	3.0×10^1 (5) a	6.0 (2) b
Ns1	1.0×10^3 (200) d	5.6×10^3 (300) e
Ns3	3.3×10^2 (60) c	8.7×10^2 (70) c
Ns5	6.0×10^1 (8) b	4 (2) ab

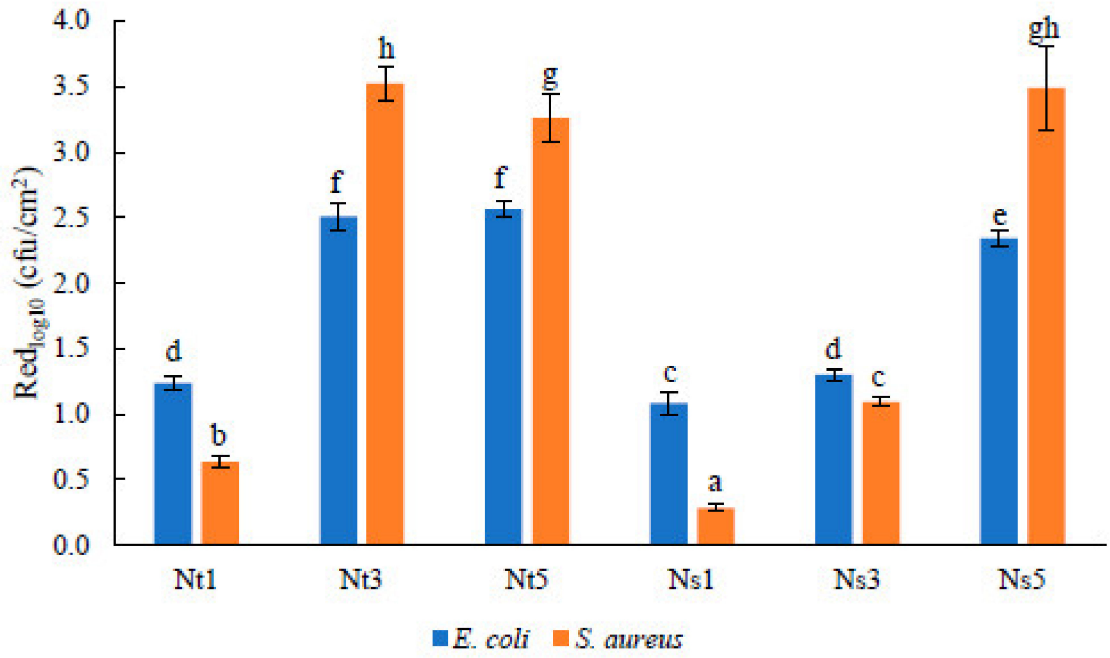
In the presence of samples containing biocides, a significantly lower number of recovered microorganisms were observed in each case.

The lowest number of *E. coli* bacteria (cfu/cm^2) was recorded for samples modified with 3% and 5% Natamax®. After incubation of the bacteria in the presence of the samples containing Nisaplin®, a lower number of *E. coli* (cfu/cm^2) was also observed than in the control samples. For samples containing 1% Natamax®, a similar number of bacterial cells was observed as for samples with 3% Nisaplin® (Table 2).

The applied biocides reduced the number of *S. aureus* to a greater extent than that of *E. coli*. The lowest number of *S. aureus* bacteria was observed after incubation with samples containing 3% Natamax® and 5% Natamax® and Nisaplin® (Table 2).

Subsequently, based on the number of bacteria (Table 2), R was calculated for each variant. The results are shown in Figure 2.

(a)



(b)

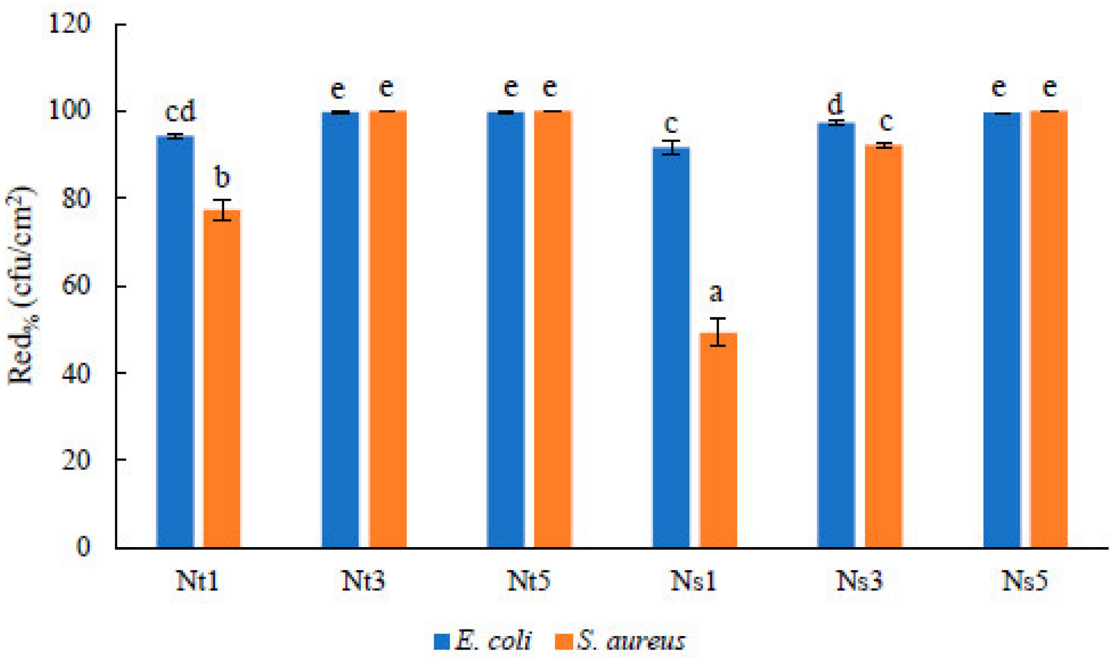


Figure 2. Antimicrobial activity (R) values of samples containing biocides relative to control samples (without biocide): (**a**) reduction in the decimal logarithm cfu/cm^2 (Red$_{log10}$), (**b**) percentage reduction cfu/cm^2; all R values are statistically significant in relation to control samples. Statistically significant differences between the variants in the graph are marked with different letters, for $p < 0.05$. Abbreviations: as in Figure 1.

The higher the antimicrobial activity, the higher the R index. As the concentration of each biocide increased, an increase in antimicrobial activity was observed (Figure 2).

Satisfactory antimicrobial activity ($R \geq 2.0$) is customarily taken from the Japanese equivalent of the standard used (JIS Z 2801). For the *E. coli* strain, a satisfactory value was observed for the samples with 3% and 5% Natamax® (R = 2.5 and R = 2.6, respectively) and 5% Nisaplin® (R = 2.3). For 3% and 5% Natamax®, the values did not differ significantly from each other (Figure 2a) [56].

Similarly, to *E. coli*, satisfactory antimicrobial activity against *S. aureus* ($R \geq 2.0$) was observed for samples with 3% and 5% Natamax® (R = 3.5 and R = 3.3, respectively) and 5% Nisaplin® (R = 3.5), with no significant difference between the 5% Nisaplin® samples and the Natamax® samples (Figure 2a).

Despite the lack of sufficient antimicrobial activity for the remaining variants, it was calculated that for most samples containing the biocide, the number of both bacterial strains (*E. coli* and *S. aureus*) was reduced by over 90% compared to the number of bacteria in control samples without the biocide. Lower values were obtained only for samples containing 1% of each biocide (approx. 77% for Natamax® and approx. 49% for Nisaplin®) (Figure 2b).

3.3. Mechanical, Rheological and Optical Properties

Subsequently, the mechanical, rheological and optical properties of the developed materials were measured to see if the addition of a biocide led to significant changes in the selected film parameters (Table 3).

Table 3. Comparison of selected LDPE film parameters with and without biocides (mean ± standard deviation in brackets). Statistically significant differences ($p < 0.05$) compared to the control (0) are marked with asterisks. Abbreviations: as in Figure 1.

	σ (MPa)	ε (%)	MFR (g/10 min.)	T (%)	H (%)
0	11.9 (0.05)	596.7 (2.08)	2.1 (0.06)	91.5 (0.10)	40.7 (0.20)
Nt1	11.8 (0.05)	588.0 (2.00) *	2.2 (0.06)	91.2 (0.06)	51.3 (0.15) *
Nt3	11.8 (0.06)	572.0 (2.00) *	2.2 (0)	90.4 (0.06) *	66.2 (0.10) *
Nt5	11.7 (0.07)	564.0 (2.00) *	2.3 (0.1)	88.4 (0.20) *	73.2 (0.59) *
Ns1	11.8 (0.04)	596.7 (2.52)	2.1 (0)	91.8 (0.35)	41.0 (0.10)
Ns3	11.8 (0.04)	582.8 (1.53) *	2.2 (0.06)	91.1 (0.87)	46.0 (1.10) *
Ns5	11.8 (0.04)	579.3 (3.06) *	2.3 (0.06)	91.4 (0.15)	48.3 (0.61) *

Abbreviations: σ (MPa)—tensile strength; ε (%)—elongation at break; T (%)—transparency; H (%)—haze.

Among the mechanical properties, the values of tensile strength (σ, MPa) and elongation at break (ε, %) were analysed. The addition of biocides did not significantly affect the σ values (Table 3). The addition of Natamax® and >1% Nisaplin® caused a slight decrease in the ε value (Table 3, Figure 3). After adding Natamax®, ε decreased in proportion to the biocide content: 1% of the biocide caused a decrease in this parameter by approx. 1%, 3% of the biocide caused a decrease of approx. 4% and 5% of the biocide caused a decrease of approx. 5%. After adding Nisaplin®, ε decreased, respectively: for 3% biocide a decrease of approx. 2% and for 5% biocide a decrease of approx. 3% (Figure 3).

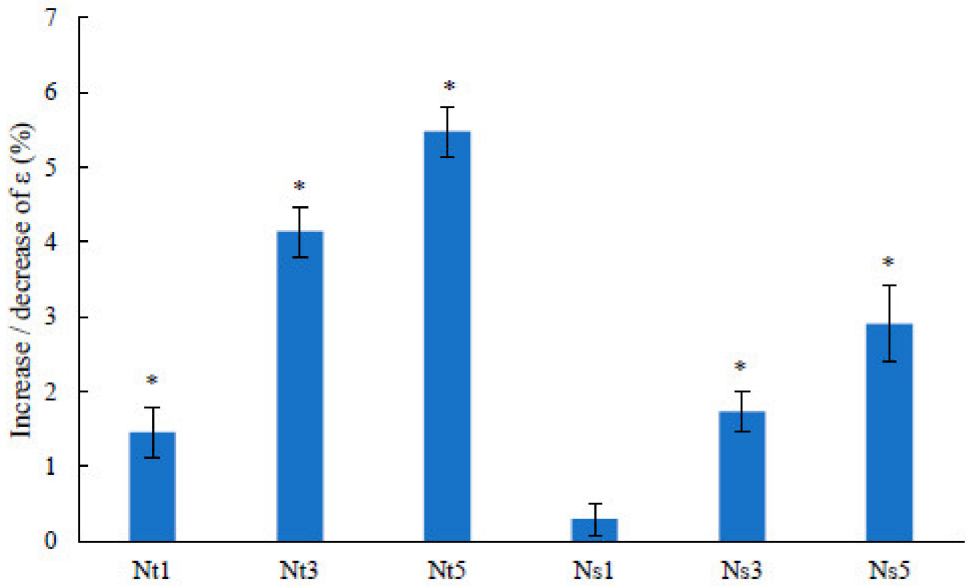


Figure 3. Elongation at break (percentage differences compared to samples without biocide). Statistically significant differences ($p < 0.05$) compared to the control (0) are marked with asterisks. Abbreviations: as in Table 1.

There was no significant influence of the biocides content on the rheological properties of the LDPE film. Changes in MFR values were within the measurement tolerance of the study (Table 3).

As described above, after extrusion of the film, discolouration of the film was visually noticed upon the addition of biocides (Figure 1). In connection with the above, the changes in optical properties of the samples were verified using the transparency (T, %) and haze (H, %) analyses (Table 3). The analysis of the T value showed a slight decrease in the parameter after the addition of both biocides. However, a significant difference was only noted after adding 3% or 5% Natamax® (approx. 1% and 3%, respectively) (Table 3, Figure 4a).

(a)

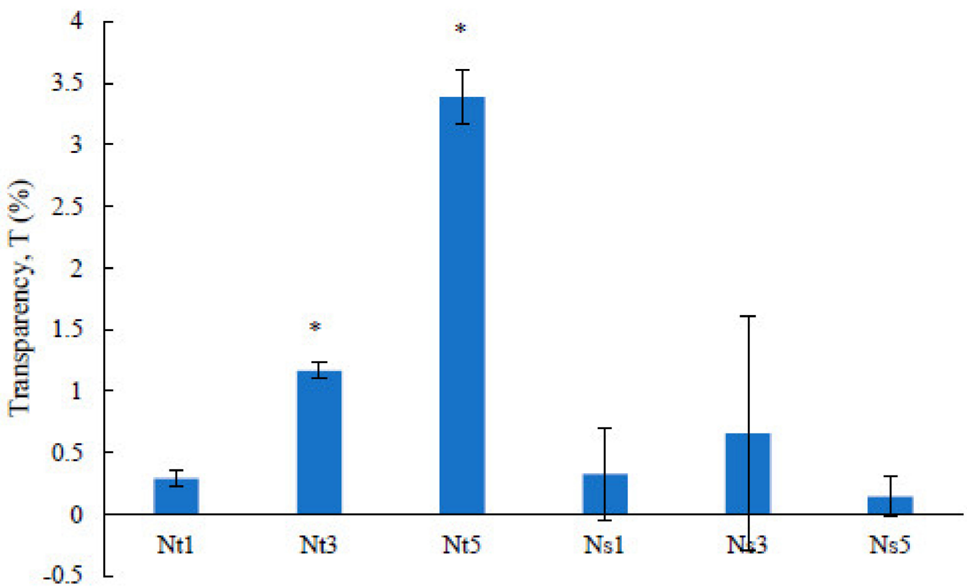


Figure 4. *Cont.*

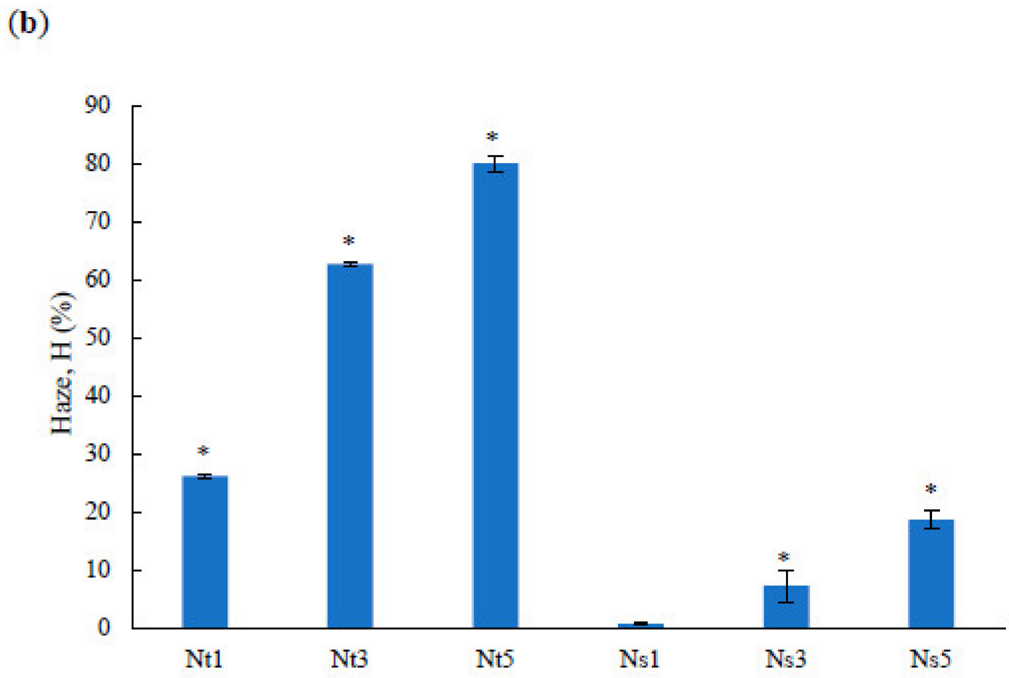


Figure 4. Optical properties (percentage differences compared to samples without biocide): (**a**) transparency; (**b**) haze. Statistically significant differences ($p < 0.05$) compared to the control (0) are marked with asterisks. Abbreviations: as in Figure 1.

The biocides used, especially Natamax®, had a much greater impact on the value of H. Natamax® caused a large increase in H from approx. 26% with 1% biocide content to approx. 80% with 5% biocide content. On the other hand, in the presence of Nisaplin®, the differences were much smaller and existed only with >1% biocide content: by approx. 7% with 3% biocide content and by approx. 19% with 5% biocide content (Table 3, Figure 4b).

4. Discussion

As part of the study, LDPE films containing biocides commercially used for food preservation were developed. The effect of additives Natamax® (containing natamycin) and Nisaplin® (containing nisin) on the antimicrobial properties of the material was analysed and whether these properties were sufficient for the material to be used as food packaging with biocidal properties. Additionally, selected properties of the developed materials such as strength, rheological and optical properties were determined to verify whether the addition of a biocide would deteriorate the functional properties of LDPE films used for packaging.

After using each of the biocides as a component of the LDPE film, very high antimicrobial properties against *E. coli* and *S. aureus* bacteria strains were found.

It was particularly surprising to find bactericidal properties for the Natamax® modified samples. This agent contains a minimum of 50% natamycin, which, until now was considered only as a biocide directed against fungi and yeasts [44]. The effect of natamycin on bacteria has so far been studied mainly against fermentation bacteria. In our research, we used pathogenic strains (*E. coli* and *S. aureus*) and the biocides used reduced the number of *S. aureus* to a greater extent than that of *E. coli*. The differentiation of the effectiveness of biocides on individual strains of microorganisms with different metabolic mechanisms was described, among others, by Orlo et al. [57]. While developing the antimicrobial effectiveness of natural compounds, they separately analysed the effectiveness against food spoilage bacteria (*Shewanella putrefaciens*, *Brochothrix thermosphacta*, *Lactobacillus plantarum*) and food-borne pathogenic bacteria (*E. coli*, *S. aureus*, *P. aeruginosa*). Among all examined, the greatest activity was found against the pathogenic strain *S. aureus*.

The highest effectiveness of Natamax® was found at 3% and 5% content in the LDPE film. After incubation with samples containing 1% Natamax®, a similar number of bacterial cells was observed as for the samples with 3% Nisaplin®. In addition, the results showed that for most samples containing the biocides used, the number of both bacterial strains (*E. coli* and *S. aureus*) were reduced by over 90% compared to the number of bacteria in control samples without biocide. Lower values were obtained only for samples containing 1% of each biocide (approx. 77% for Natamax® and approx. 49% for Nisaplin®). Such a high antibacterial effectiveness of the applied biocides could be indirectly influenced by a change in the structure of the film, which could result in stronger adhesion of bacterial cells to the film. In addition, it is believed that the high extrusion temperature of the samples activated the biocide release from the polymer mass.

The results showed that 3% Natamax® content, or alternatively 5% Nisaplin® content, provides an LDPE film with effective antimicrobial protection.

Other researchers have also attempted to include natural biocides mainly in biodegradable plastics. Since conventional plastics still dominate the market as raw materials for the production of food packaging, it was considered necessary to apply natamycin and nisin to LDPE. On the other hand, Settier-Ramírez et al. [21] developed active packaging containing other natural components, including phytic acid, which extended the shelf-life of chilled confectionery cream from 3 to 13 days. The material was found to be highly active against pathogenic Gram-negative bacteria (*E. coli*, *Salmonella enterica* and *Pseudomonas fluorescens*) and against Gram-positive bacteria (*Listeria monocytogenes*).

Although the studies with natamycin concerned mainly fungi, several studies were undertaken showing the high antibacterial activity of nisin [43,58]. Research on the antimicrobial effectiveness of cellulose nanocrystals containing nisin against selected lactic acid bacteria causing spoilage of meat was carried out, among others, by Gedarawatte et al. [59]. They confirmed the possibility of using the developed polymer material as a reinforcing agent in active food packaging. In the studies of Gedarawatte et al. [59], a nisin content of 2–2.5 mg/mL resulted in the inactivation of microorganisms. In our study, the highest effectiveness was obtained at a concentration of 3% or 5% of the agent, which, according to the manufacturer's declaration, contains about 50% of the active substance.

However, also in the case of nisin, the results of various researchers are not unequivocal. Not all studies confirm the high effectiveness of this biocide. Alirezalu et al. [60] developed an antimicrobial polyamide and alginate casing containing nisin nanoparticles to inactivate bacteria in a nitrogen-free Frankfurt sausage [60]. The results showed a very weak inhibitory effect against *E. coli*, *S. aureus*, fungi and yeasts and the total number of viable microorganisms after 45 days at 4 °C.

Such divergent data on the effectiveness of the use of a selected biocide in the antimicrobial protection of a polymeric material may be caused by bad dispersion in the polymer mass. There are reports on the agglomeration ability of compounds with biocidal activity [61]. The method of processing the polymer material is very important to ensure proper dispersion. In our case, special attention was paid to mixing and the residence time of the material in the extruder, which is known to be of key importance for maintaining the appropriate durability of the processed material [62].

The applied biocides, apart from ensuring adequate antimicrobial protection, did not significantly affect the tensile strength, and the effect on elongation at break was relatively small (maximum decrease of approx. 3% in the case of 5% Nisaplin®). Additionally, the rheological properties did not change significantly. Similarly, Holcapkova et al. [63] found that the content of nisin powder (0.15% ww) had no major effect on the mechanical properties when compared to pure polylactide (PLA).

However, in this respect, the opinion of scientists is not clear, because in other studies, Jin and Liu [64] found that the addition of nisin significantly reduced the mechanical properties of the polymer material. Similarly, Czaja-Jagielska et al. [65], after the use of Nisaplin®, found a significant effect of this agent on the weakening of the mechanical properties of

PLA film, not excluding the developed material from being used as a packaging material for food and cosmetics.

Similarly, Wang et al. [43], despite the high antimicrobial activity of the natamycin agar film, observed a reduction in the tensile strength of the film (from 17.61 MPa to 14.94 MPa), as well as a reduction in transparency, an increase in haze and a change in colour.

Additionally, in the presented study, after extrusion of the film, some changes in the colour of the film were visually noticeable after adding biocides, but the transparency analysis only showed a slight decrease in this parameter after the use of both biocides. The only significant difference in the values of this parameter was found after adding 3% or 5% Natamax® (decrease by approx. 1% and 3%, respectively). Much greater differences were observed in the haze values, where Natamax® had a more significant influence on this parameter as well (a decrease of up to about 80% with 5% biocide content), whereas Nisaplin® with the same content decreased the haze by about 19%.

Summarising the conducted study, both Natamax® and Nisaplin® agents can be successfully used to manufacture food packaging materials with antimicrobial protection. Natamax® showed a stronger bactericidal effect, while Nisaplin® changed other properties less significantly. Subsequently, it seems necessary to study the long-term properties of the developed materials, as well as the impact on extending the shelf life of the packaged products. As stated, among others, by Davidson and Harrison [35], the results of laboratory tests need to be confirmed in real conditions, where in each environment the processes may be different and unpredictable. Maintaining good sanitation of food contact materials is an important factor in controlling the risk of microbial contamination of food [66]. It is likely that in the future, use of the developed materials will provide sufficient protection to packaged food without the need to spray it, as is current practice.

The obtained results confirming high antibacterial activity suggest that the films can be used not only as a packaging material for food, but also for other types of applications. More so, because no considerable influence was found, of the components used on the strength properties of the polymer material. However, in the work undertaken, a slight change in the haze colour caused by the proposed additives should be considered. However, this is typical of many natural components [67].

5. Conclusions

The results showed that the addition of natural biocides in the form of commercial additives Natamax® and Nisaplin® during the extrusion process provides LDPE films with antimicrobial protection against *E. coli* and *S. aureus*. High efficiency was found for samples containing 3% Natamax®, while a similar effect was obtained after the use of 5% Nisaplin®. It is possible to use the developed films as a packaging material, with an antimicrobial effect, intended for food packaging, e.g., for meats. In the case of Natamax® film, the most important change in the film is the reduction in transparency and a significant increase in haze, which makes it impossible to use this additive in highly transparent packaging. Importantly, no significant influence of the biocides used on the mechanical properties of the film was found.

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