ABSTRACT. Background: Packaging is an inseparable element of most consumer products. In addition to its primary passive protective and logistic function, it is also an excellent tool for the innovation development. One of the types of packaging innovations in the food and cosmetic industry are antimicrobial packaging. They are an example of packaging that actively protects packed products and eliminates harmful preservatives. Protection of goods against microbial spoilage extends shelf life and at the same time facilitates storage processes.

Methods: This paper aims to obtain biodegradable films based on PLA with antimicrobial properties. Four different natural antimicrobial agents were used: clove essential oil, peppermint essential oil, and two commercial powders containing nisin (Nisaplin and Novagard). The mechanical, barrier and optical properties were tested.

Results: The implementation of antimicrobial agents changed the properties of the tested bio-packaging in different rates depending on the agent. The new blends showed antimicrobial activity, however the addition of antimicrobials weakened the mechanical properties and changed the colour.

Conclusions: The biodegradable packaging materials can be used as a polymer matrix of different antimicrobial agents. They can inhibit the growth of bacteria in food or cosmetics and regarding their future use the influence on mechanical properties should be considered. Moreover, the biodegradability of biopolymers containing antimicrobial agents has barely been investigated.

Key words: antimicrobial packaging, biodegradable packaging, mechanical properties, barrier properties, innovation.

A part of this study was presented as oral presentation at the „8th International Logistics Scientific Conference WSL FORUM 2019” in Poznan (Poland), 18th-19th of November 2019.
Antimicrobial agents can be either incorporated into packaging material or covered as active coating. There are numerous types of active substances which can be used in the active packaging system: synthetic (e.g. organic acids, metals, antibiotics) or natural (e.g. natural extracts, essential oils, enzymes, bacteriocins) [Korzeniowski et al 2011; Malhotra et el. 2015].

Plant-derived essential oils (EOs) are generally accepted as great potential to be used for extending the shelf life of different foods [Talebi et all 2018]. The most cited are: rosemary, eucalyptus, oregano, thyme, cinnamon, clove and citrus fruit [Lee et al 2015, Agrimonti et al. 2019]

The effectiveness of these composites depends on product and goods combination of bio-matrix and the antimicrobial agent. Yahyaoui et al [2016] proved that the incorporation of essential oils from rosemary, myrtle and thyme in the PLA matrix can improve the mechanical properties and does not affect the colour change of the films significantly.

Therefore the aim of the study was to obtain PLA films with natural antimicrobial agents (nisin and essential oils) and investigate the effects on the optical, mechanical and antimicrobial properties of these blends. The studies are an attempt to confirm that the use of biomaterials with antimicrobial agents has a positive effect on extending the shelf life of food products by limiting contamination.

MATERIALS AND METHODS

Materials

The PLA (plylactic acid) was an Ingeo™ Biopolymer 4043D obtained from Natureworks LLC. The polymer was in the form of pellets that can be converted into film.

Antimicrobial agents used in the study were: clove essential from Etja Company, peppermint essential from PPHU KEJ
Company, Nisaplin and NovaGARD CB1-35 from Danisco Company.

The following strains of bacteria were used in this study: Clostridium perfringens, Staphylococcus Aureus, Pseudomonas aeruginosa, Yersinia enterocolitica, Salmonella enteritidis, Bacillus subtilis, Listeria innocua, Eschericha coli, Staphylococcus paratyphi and Enterococcus fecalis.

Descriptions of the samples are presented in Table 1.

| Code    | Description                  |
|---------|------------------------------|
| PLA     | Pure PLA film                |
| PLA_N   | PLA with 2% Nisaplin         |
| PLA_NG  | PLA with 2% NovaGard         |
| PLA_C   | PLA with 2% clove essential  |
| PLA_PM  | PLA with 2% peppermint essential |

**Film formation**

Films of PLA (20-40 mm) were prepared by solvent cast technique. Pure PLA films were obtained by solving 16g PLA pellets in 400 ml chloroform under magnetic stirring for 6h in the temperature of 23°C. After the films were casted, they were dried overnight (24h) at room conditions. The antimicrobial films were prepared by solving 16g of PLA pellets in chloroform under magnetic stirring for 6h with addition of 2% of antimicrobial agent.

**Mechanical properties**

**Thickness**

The thickness of the films was measured at several locations randomly around the film using a micrometre SYLVAC with 0.001mm accuracy.

**Tensile strength**

The mechanical resistance of films was performed on a Zwick Roell Z020 strength testing machine according to the PN-EN ISO 527-3 standard. Tensile strength [MPa] and elongation at break [%] were evaluated. At least five samples were tested for each film and the average value was reported.

**Puncture testing**

The test consists in determining the puncture force [N] and elongation at puncture [mm] on the Zwick Roell Z020 instrument in accordance with PN-EN 14477.

**Optical properties**

The colour measurement was made with the Focus on Color envisense NH 310. The L*a*b model was used to describe the colour of the samples tested. The L* parameter specifies the brightness (L * value = 100 means perfectly white, while 0 value – perfectly black), while a * and b * are trichromatic coordinates that specify the tone and colour saturation (Fig 2.).

Source: www.perten.com

![Fig. 2. CIE Lab colour space](source_link)

**Antimicrobial activity**

The antimicrobial effectiveness of the composite films was tested against 10 bacteria mentioned above. Suspensions of microbes were prepared, then seeded onto Petri dishes by the flooding method, consisting in applying 1ml of inoculum to the dish and pouring Agar medium.

On the solid agar the test films (dimensions of 10x10mm) were applied and incubated at 37°C. After incubation, antimicrobial activity was measured by observation of zones inhibiting the growth of microorganisms around the film samples.
RESULTS

Mechanical properties of the samples tested are shown in Table 2, 3 and 4. They are, especially tensile strength and elongation at break, very crucial parameters in exploiting different bio-based films for packaging purposes. The addition of antimicrobial agents enlarges the thickness of the PLA films (Table 2).

Table 2. Thickness

|      | Thickness [mm] |
|------|----------------|
| PLA  | 0.211±0.021    |
| PLA_N| 0.420±0.076    |
| PLA_NG| 0.490±0.072   |
| PLA_C| 0.290±0.033    |
| PLA_PM| 0.405±0.032   |

Source: own work

Table 3 represents tensile strength and elongation at break for pure PLA films and modified with antimicrobial agents. According to these data the incorporation of essential oil into PLA matrix resulted in a significant decrease in the tensile strength and elongation at break.

The same was observed by other authors [Qin et all 2017].

Table 3. Tensile strength and elongation at break

|      | Tensile strength [MPa] | Elongation at break [%] |
|------|------------------------|-------------------------|
| PLA  | 16.00±1.23             | 267±24                  |
| PLA_N| 8.07±1.65              | 18±5.0                  |
| PLA_NG| 6.19±0.92              | 3.8±0.4                 |
| PLA_C| 10.09±0.37             | 156±24                  |
| PLA_PM| 4.86±0.34             | 2.6±0.3                 |

Source: own work

The PLA sample had the lowest brightness. A positive value of the a* parameter indicates that the sample is in the yellow space, and a negative value of the b* parameter indicates that the sample is in the blue space. There is visible change in colour after the addition of antimicrobial additives. They are a little brighter and have different colours. PLA film with peppermint essential oil showed the highest brightness. In the first quarter of the colour space diagram (Fig 2.) the colour of the sample with nisin (PLA_N) was between red and yellow. The addition of essential oils turned the colour into green-yellow space (PLA_C) and dark blue and blue (PLA_PM). NovaGARD powder located the sample in the first quarter between the red and yellow colour (PLA_NG).

The results of the antimicrobial study are presented in Table 6. It shows that pure PLA film has no antimicrobial activity. The strongest antimicrobial effect was observed for the film with Nisaplin powder. Its antimicrobial activity was against all the bacteria tested - the largest against Pseudomonas aeruginosa, Bacillus subtilis (Fig. 4) and Clostridium perfringers (Fig. 3) and the smallest against Listeria innocua. Clove oil incorporated in PLA film was effective in three cases: against Bacillus subtilis (Fig 4), E coli (Fig. 5) and Pseudomonas auruginosa. For the other
bacteria, clove oil showed the bacteriostatic effect, except Clostridium perfringens that was resistant to this film.

Table 6. Clear zones (in mm) after antimicrobial testing

|                     | PLA  | PLA_N | PLA_C | PLA_NG | PLA_PM |
|---------------------|------|-------|-------|--------|--------|
| Clostridium perfringens | 0    | 20    | 0     | 12     | 10.5   |
| Staphylococcus aureus | 0    | 18    | 11    | 10.5   | 0      |
| Pseudomonas aeruginosa | 0    | 22    | 12    | 0      | 0      |
| Yersinia enterocolitica | 0    | 12    | 10.5  | 10.5   | 0      |
| Salmonella enteritidis | 0    | 13    | 10.5  | 10.5   | 0      |
| Bacillus subtilis     | 0    | 21    | 17    | 10.5   | 10.5   |
| Listeria innocua      | 0    | 16    | 14    | 11     | 0      |
| Escherichia coli      | 0    | 16    | 10.5  | 14     | 10.5   |
| Staphylococcus paratyphi | 0    | 16    | 10.5  | 14     | 10.5   |
| Enterococcus faecalis | 0    | 16    | 10    | 12     | 10.5   |

Source: own work

Fig. 3. Antimicrobial properties of PLA (1), PLA_N (2) and PLA_C (3) against Clostridium perfringens

Fig. 4. Antimicrobial properties of PLA (1), PLA_N (2) and PLA_C (3) against Bacillus subtilis

The addition of Novagard powder showed no significant antibacterial activity. Only growth of four bacteria (Clostridium perfringens, Escherichia coli, Staphylococcus paratyphi, Enterococcus faecalis) was slightly inhibited and for the other 4 (Staphylococcus aureus, Yersinia enterocolitica, Salmonella enteritidis, Bacillus subtilis) there was the bacteriostatic effect. There was no effect for Pseudomonas aeruginosa and Listeria innocula. The film with peppermint oil had the worst results. The bacteriostatic effect was observed in the case of four bacteria: Clostridium perfringens, Bacillus subtilis, Staphylococcus paratyphi and Enterococcus faecalis.

CONCLUSIONS

This investigation showed the possibility of obtaining PLA films with natural antimicrobial substances such as bacteriocins and essential oils. Based on this research and the literature view, we can conclude that the incorporation of natural antimicrobial substances into PLA film positively influences its antimicrobial properties, however there are some differences in their activity. While pure PLA film does not show any antimicrobial activity, the highest limitation of the growth of microorganisms was for nisin. It can help maintain the high quality of products and ensure safety of consumers. The antimicrobial protection of films tested can extend food storage and protect against the adverse effects of microorganisms throughout the entire logistics chain.

Nevertheless in this study these additives weakened the mechanical properties (tensile strength, elongation at break and puncture strength) of PLA blends. It was also observed that the addition of active ingredients changed
the colour of the films, which is not always preferable.

Summarizing, the use of antimicrobial additives applied is an excellent proposition for producers looking for new ecological solutions. Moreover, it is advisable to use biodegradable materials, including PLA pellets as matrix for new active packaging. It is an innovative and excellent offer for producers who are searching for innovative ideas and who care about ecology.

For future investigations it is very important to check the biodegradability and compostability of these novel packaging systems.

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REFERENCES

Agrimonti C., White J.C., Tonetti S., Marmiroli N., 2019. Antimicrobial activity of cellulosic pads amended with emulsions of essential oils of oregano, thyme and cinnamon against microorganisms in minced beef meat, International Journal of Food Microbiology, 305, 108246, http://doi.org/10.1016/j.ijfoodmicro.2019.108246.

Ali A., Chen Y., Liu H., Yu L., Baloch Z., Khalid S., Zhu J., Chen L. 2019. Starch-based antimicrobial films functionalized by pomegranate peel, International Journal of Biological Macromolecules, 129, 1120-1126, http://doi.org/10.1016/j.ijbiomac.2019.09.068.

Aswathy J., Heera K.V., Sumi T.S., Meritta J., Shiji M., Praveen G., Indu C. Nair, Radhakrishnan E.K., 2019. Starch-PVA composite films with zinc-oxide nanoparticles and phytochemicals as intelligent pH sensing wraps for food packaging application, International Journal of Biological Macromolecules, 136, 395-403, http://doi.org/10.1016/j.ijbiomac.2019.06.018.

Boyaci D., Iorio G., Sozbilen G. S., Alkan D., Trabattoni S., Pucillo F., Farris S., Yemenicioğlu A., 2019. Development of flexible antimicrobial zein coatings with essential oils for the inhibition of critical pathogens on the surface of whole fruits: Test of coatings on inoculated melons, Food Packaging and Shelf Life, 20, 100316, http://doi.org/10.1016/j.fpsl.2019.100316.

Heydari-Majd M., Ghanbarzadeh B., Shahidi-Noghabi M., Najafi M.A., Hosseini M. 2019. A new active nanocomposite film based on PLA/ZnO nanoparticle/essential oils for the preservation of refrigerated Otolithes ruber fillets, Food Packaging and Shelf Life, 19, 94-103 http://doi.org/10.1016/j.fpsl.2018.12.002.

Fonseca L., dos Santos Cruxen C.E., Bruni G.P., Fiorentini A.M., da Rosa Zavareze E., Lim L., Dias A. 2019. Development of antimicrobial and antioxidant electrospun soluble potato starch nanofibers loaded with carvacrol, International Journal of Biological Macromolecules, 139, 1182-1190, http://doi.org/10.1016/j.ijbiomac.2019.08.096.

Kashiri M., López-Carballo G., Hernández-Muñoz P., Gavara R., 2019. Antimicrobial packaging based on a LAE containing zein coating to control foodborne pathogens in chicken soup, International Journal of Food Microbiology, 306, 108272, http://doi.org/10.1016/j.ijfoodmicro.2019.108272.

Lee, H., Kim, K., Choi, K.-H., Yoon, Y., 2015. Quantitative microbial risk assessment for Staphylococcus aureus in natural and processed cheese in Korea. J. Dairy Sci. 98, 5931–5945. http://doi.org/10.3168/jds.2015-9611.

Lan W., Zhang R., Ahmed S., Qin W., Liu Y., 2019. Effects of various antimicrobial polyvinyl alcohol/tea polyphenol composite films on the shelf life of packaged strawberries, LWT, 113, 2019, 108297, http://doi.org/10.1016/j.lwt.2019.108297.
Czaja-Jagielska N., Praiss A., Walenciak M., Zmysłona D., Sankowska N., 2020. Biodegradable packaging based on PLA with antimicrobial properties. LogForum 16 (2), 279-286. http://doi.org/10.17270/J.LOG.2020.391

Liu B., Xu H., Zhao H., Liu W., Zhao L., Li Y. 2017. Preparation and characterization of intelligent starch/PVA films for simultaneous colorimetric indication and antimicrobial activity for food packaging applications, Carbohydrate Polymers, 157, 842-849. http://doi.org/10.1016/j.carbpol.2016.10.067

Malhotra B, Keshwani A and Kharkwal H., 2015. Antimicrobial food packaging: potential and pitfalls. Front. Microbiol. 6:611. http://doi.org/10.3389/fmicb.2015.00611

Qin Y., Li W., Liu D., Yuan M., Li L. 2017. Development of active packaging film made from poly (lactic acid) incorporated essential oil, Progress in Organic Coatings 103, 76–82. http://doi.org/10.1016/j.porgcoat.2016.10.017

Talebi F., Misaghi A., Khankari A., Kamkar A., Gandomi H., Rezaeigolestani M. 2018. Incorporation of spice essential oils into poly-lactic acid film matrix with the aim of extending microbiological and sensorial shelf life of ground beef, LWT, 96, 482-490. http://doi.org/10.1016/j.lwt.2018.05.067

Wang K., Lim P.N., Tong S.Y., Thian E.S. 2019. Development of grapefruit seed extract-loaded poly(ε-caprolactone)/chitosan films for antimicrobial food packaging. Food Packaging and Shelf Life, 22, 2019,100396. http://doi.org/10.1016/j.fpsl.2019.100396

Yahyaoui M., Gordobil O., Díaz R. H., Abderrabba M., Labidi J., 2016. Development of novel antimicrobial films based on poly(lactic acid) and essential oils, Reactive and Functional Polymers, 109, 1-8. http://doi.org/10.1016/j.reactfunctpolym.2016.09.001

Zhao Y., Teixeira J.S., Saldaña M. D.A., Gänzle M. G., 2019. Antimicrobial activity of bioactive starch packaging films against Listeria monocytogenes and reconstituted meat microbiota on ham, International Journal of Food Microbiology, 305, 108253. http://doi.org/10.1016/j.ijfoodmicro.2019.108253

Zheng K., Xiao S., Li W., Wang W., Chen H., Yang F., Qin C. 2019. Chitosan-acorn starch-eugenol edible film: Physico-chemical, barrier, antimicrobial, antioxidant and structural properties, International Journal of Biological Macromolecules, 135, 344-352. http://doi.org/10.1016/j.ijbiomac.2019.05.151.

BIODEGRADOWALNE OPAKOWANIA NA BAZIE PLA O WŁAŚCIWOŚCIACH PRZECIWDRONBOUSTROJOWYCH

STRESZCZENIE. Wstęp: Opakowanie jest nieodłącznym elementem większości produktów dostępnych na rynku. Oprócz pierwotnej, pasywnej funkcji ochronnej i logistycznej jest także doskonałym narzędziem do rozwoju innowacji. W poszczególnych ogniwach łańcucha dostaw role opakowań w sprawnym przemieszczaniu towarów od producenta do odbioru odgrywa dużą rolę. Odpowiednio zaprojektowane i wykonane opakowania nie tylko w istotny sposób wpływać na obniżenie kosztów, ale także zapewnić jakość i bezpieczeństwo całego łańcucha. Z punktu widzenia ekologicznego coraz częściej wykorzystuje się opakowania na bazie surowców odnawialnych, np. na bazie PLA.

Podstawowym zadaniem opakowań jest zabezpieczenie produktu przed niekorzystnymi zmianami jakie są zgodne z zakresem przechowywania. Jednym z rodzajów innowacji opakowań jest opakowanie przeciwdrobnoustrojowe - sposób aktywnej ochrony zapakowanego produktu przed psuciem mikrobiologicznym, co ma na celu otwarcie przestrzeni do spożywczej, a jednocześnie utrzymać procesy przechowywania.

Metody: Celem badania jest uzupełnienie biodegradowalnych folii na bazie PLA o właściwościach przeciwdrobnoustrojowych. Zastosowano cztery różne naturalne środki przeciwdrobnoustrojowe: olejek goździkowy, olejek miętowy i dwa proszki zawierające nizynę (Nisaplin i Novagard). Przetestowano właściwości mechaniczne, barierowe, antymikrobiologiczne i optyczne.
Wyniki: Dodatek środków przeciwdrobnoustrojowych zmienił właściwości badanych prób w różnym stopniu. Badane folie wykazały aktywność przeciwdrobnoustrojową, jednak dodanie środków przeciwdrobnoustrojowych osłabiło właściwości mechaniczne i zmieniło ich kolor.

Wnioski: Przytoczone badania potwierdziły, że PLA można stosować jako matrycę polimeryczną dla różnych środków przeciwdrobnoustrojowych. Mogą hamować rozwój bakterii w żywności lub kosmetykach, a przy ich przyszłym zastosowaniu należy wziąć pod uwagę wpływ na właściwości mechaniczne. Testowane folie mogą korzystnie oddziaływać na zapakowane produkty w całym systemie logistycznym, wydłużając ich termin przydatności do spożycia, a jednocześnie ich biodegradowalność sprawia, że są opakowaniami przyjaznymi środowisku.

Słowa kluczowe: opakowania przeciwdrobnoustrojowe, opakowanie aktywne, opakowanie biodegradowalne, właściwości mechaniczne, właściwości barierowe

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