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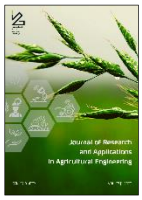

Antimicrobial Efficiency of Novel Active Packaging Based on Iron Nanoparticles Biosynthesized by Oregano Leaves Extract

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The main function of packaging materials for food is to ensure the safety and maintain appropriate, required quality. The use of active packaging, including those containing antibacterial materials or substances with antimicrobial properties, may extend the shelf life. Many of the available and used solutions are based on the application of polymer films with the addition of metal nanoparticles such as silver, gold, copper or metal oxides of magnesium, zinc and titanium. However, the aspect related to the use of iron nanoparticles is rarely found in research or publications. The aim of the research was to determine the antimicrobial effectiveness of packaging materials (parchment and foil) modified with the active layer based on an iron preparation by reducing iron salts with the use of oregano extract. Prototype packaging materials were used to carry out storage tests with the cottage cheese. The pieces of cheese were kept refrigerated for 6 weeks. During the storage tests, microbiological inoculations were performed from the surface of the packaging in contact with the product. The obtained results confirmed the antimicrobial effectiveness of the tested packages.

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1. Introduction

The packaging is a product that is made of any material, intended for storage, protection, transport, presentation of products from raw materials to processed goods.

Therefore, when considering the main functions of the packaging, we can distinguish:

- securing and protecting the product against damage, thus reducing the value in use of the product,
- promotional function – affecting the consumer at the time of making a purchase decision and influencing this decision,
- information function – the use of packaging as a carrier of information for the consumer about the properties of the product and the way of its use,
- manipulation function – facilitating the performance of activities during the storage, transport, sale or use of the product [1].

Nevertheless, classic packaging fulfilling the above basic functions is no longer sufficient nowadays, mainly due to increasing customer's expectations, but also to increase the complexity of products or the issues of sustainable development and minimizing the carbon footprint of manufactured products. For this reason, concepts such as active or intelligent packaging

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have become much more popular and the use of these packaging increases significantly each year [2].

Active packaging is one that, apart from the standard function of isolating the product from the external environment, interacts with the atmosphere surrounding the product or with the product itself, extending the storage time while maintaining product safety [3]. There is no direct migration between the absorbent used in the packaging and the product, thanks to which the product in such packaging retains its taste, color and properties. This is possible due to the interaction that occurs between the packaging and the product itself due to the absorption of gases or the release of substances capable of inhibiting the unfavorable processes taking place inside the packaging. The simplest form of active packaging is a small sachet or insert with an active substance. In the advanced technology of active packaging, the active substance is placed on the entire inner surface of the packaging. As a result, the solution is more effective, but due to the increased amount of research on the release of the active ingredient, this solution is more expensive. Depending on the function performed by this additive, active packaging can be divided into absorbing O_2, CO_2, absorbing water and steam, with antioxidant and antimicrobial properties. In packages with antimicrobial properties, the used active substances may be enzymes or bacteriocins [4], silver zeolites [5], organic acids and their derivatives [6], natural essential oils [7] or other substances with the size of nanoparticles. They allow for an effective fight against growing of microorganisms on the surface of contaminated products [8]. Among the available active packaging materials, many are based on the use of a nano-structured metal with antimicrobial properties such as gold, silver, copper [9] or metal oxides of magnesium, titanium or zinc [10]. The conducted research also verifies the effectiveness of the packaging films modified by applying bioactive substances to the surfaces. The available literature rarely provides information on active packaging with antimicrobial properties using iron nanoparticles. From a chemical point of view, they are usually iron oxides. The reported patents so far concern iron nanoparticles used as active ingredients in oxygen absorbers [11]. However, the literature reports that the effect of nano-iron in inhibiting the growth of Escherichia coli is effective. A relationship between the size and properties of nano-Fe_0 and antimicrobial activity was observed [12]. However, the use of the iron compound and its safety in relation to food raises many discussions [13–15]. Nevertheless, no detrimental effect of iron on the human body has been found, and supplements containing nano-iron are used in the treatment of anemia [16–17]. Beneficial effects are seen at doses ranging from 3–6 mg per kilogram of body weight per day, and adverse effects are seen at doses above 10–20 mg. We should also pay attention to the fact of bioavailability, which occurs in inversely proportional correlation – the smaller the particle, the greater the bioavailability of nano-iron. Nanoparticles are considered a threat to the organism when their size drops below 54 nm [18]. In Europe, nano-iron modified by netonite [19] and kaolinite [20] has been approved as food packaging material.

The aim of conducted research was to determine the influence of the nano-compound of zero valent iron introduced as an additive on the surface of foil and parchment and checking the antimicrobial properties of packaging materials prepared in this way. The received packaging was used to pack the food product and conduct storage tests to determine the impact of the packaging on the inhibition of microflora development during storage.

2. Material and methods

2.1. Synthesis and characteristic of iron-based active preparations

Methodology of obtaining active iron preparations by biosynthesis, by reduction of $FeSO_4$, $FeCl_2$ and $FeCl_3$ with the use of extract prepared from oregano leaves. The plant extract was prepared by suspending 50 g of dried oregano leaves in 800 ml of deionized water and shaking at 200 rpm for 2 hours at 80 °C. Then, the obtained extract was filtered under pressure on a Buchner funnel with the use of a soft filter. In a separate vessel, a freshly prepared solution of 0.1 M $FeSO_4 \cdot 7H_2O$; 0.1 M $FeCl_2 \cdot 4H_2O$ or 0.1 M $FeCl_3 \cdot 6H_2O$, which was added to the extract in a 1:3 volume ratio (iron salt: extract), while constantly stirring the contents of the reaction beakers on a magnetic stirrer (at a speed of 600 rpm). 0.75 M NaOH was added to the resulting solution to raise the pH to a level of 5.0–6.5 (pH was measured with indicator paper) until precipitation was obtained. The obtained suspension of active iron particles was freeze-dried to obtain a dry preparation, which was stored in a tightly closed glass container. Three samples of preparations containing iron nanoparticles, marked as OR5 (oregano/$FeSO_4$), OR6 (oregano/$FeCl_2$) and OR7 (oregano/$FeCl_3$) were synthesized. The obtained preparations were tested using the X-ray Diffraction (XRD) and X-ray Energy Dispersive Spectroscopy (EDS), thanks to which the size of active iron particles and their chemical composition were determined. The antimicrobial activity of the oregano extract and the tested samples, on YGC Agar (Yeast Extract Glucose Chloramphenicol) was inoculated a 1 ml of mixture containing the tested samples and rinses from the surface of the cheese packaging with visible signs of microbial spoilage in the ratio of 1: 1 v/v.

2.2. Iron-based active coating preparation on packaging materials

Food grade varnish (SunStar TM, SunChemical) was used to prepare the coating. Samples of iron nanoparticles with a concentration of 9% (v/v) were suspended in the varnish using an ultrasonic stirrer UP100H, Donserv (5 minutes, frequency 30 kHz) and Vortexer HS120212, HEATHROW SCIENTIFIC shakers. The slurry was prepared immediately prior to use. The tests were performed with the use of packaging materials: polyolefin foil (Ecor FPO, ECOR Product, Stęszew, Poland) and parchment (ECOR Product, Stęszew, Poland) coated with varnish (control sample) and varnish with iron nanoparticles samples (OR5, OR6, OR7). Active coatings were applied to sheets of packaging material placed on the application table, then 4 ml of the prepared suspension was poured using an automatic pipette. The suspension was spread along the packaging material by means of an application spiral rod 250 mm long and thread depth 40 µm. After the active layer was applied to the material, it was set aside to dry. In total, 6 prototypes of packaging materials were obtained (3 based on polyolefin foil and 3 based on parchment), in which the samples of white cheese were packed. In the next stage of the research, these prototypes were used to perform storage trials.

2.3. Storage tests of prototypes of active packaging materials with the use of dairy products

Storage tests were carried out with the use of curd cheeses packed in prototype packaging materials with active iron-based coatings. Curd cheese cubes weighing approximately 150 g were packed in each type of prepared packaging materials and stored for 6 weeks under 4 °C. After this time, microbiological tests were performed, including the inoculation of the inner surface of the package in contact with the cheese, performed using the imprint method using RODAC type contact plates (Plate Count Agar + Neutralizing, Liofilchem srl). Microbiological cultures (30 °C, 72 h) were performed to determine the total number of psychrophilic microorganisms.

3. Results and Discussion

3.1. Characteristics of active iron preparations obtained by biosynthesis with oregano extract

The samples of active iron preparations were analyzed by X-ray Diffraction (XRD) and X-ray Energy Dispersive Spectroscopy (EDS) and the results are shown in Tab. 1 and on Fig. 1–3.

Tab. 1. The results of measuring of the size of iron nanoparticles by the Scherrer method

Type of preparation	Name of the sample	Crystallite size by the Scherrer method [nm]
Oregano/$FeSO_4$	OR5	no peaks for size analysis
Oregano/$FeCl_2$	OR6	10
Oregano/$FeCl_3$	OR7	50

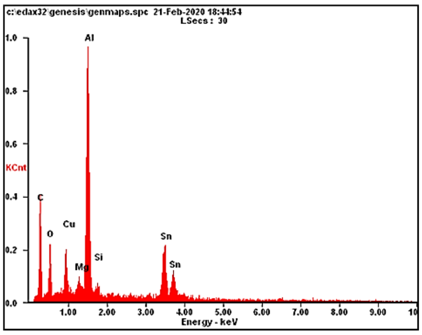


Fig. 1. The spectrum for sample OR5 for nanoparticles obtained by EDS method

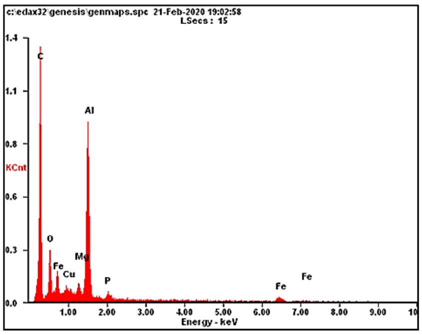


Fig. 2. The spectrum for sample OR6 for nanoparticles obtained by EDS method

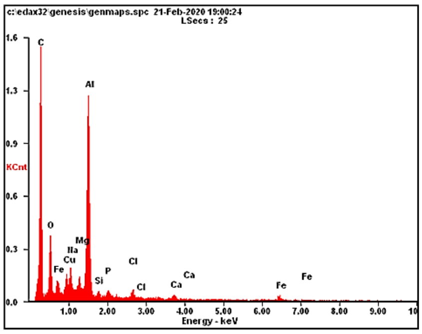


Fig. 3. The spectrum for sample OR7 for nanoparticles obtained by EDS method

The qualitative analysis of the chemical composition for all tested nanoparticles showed the presence of oxygen. On the other hand, the peak for iron is very small and it is not possible to say with 100% whether it is there. Additionally, reflections for magnesium, aluminum, silicon and tin are visible. The high intensity of the peak for C and Cu results from the preparation method, the particles were placed on a copper mesh with a carbon film.

Despite the fact, that the EDS method, not show the presence of iron or only a small amount of it was demonstrated, it was found that the obtained preparations showed high antimicrobial activity. For this purpose, prior to the application of the obtained iron preparations to the active coating, evaluation of their antimicrobial activity against the fungi present on the cheese was made. Obtained results are presented on the Fig. 4.

a) b) c) d)

Fig. 4. The antimicrobial activity of the oregano extract (a) and the tested samples: preparation OR5 (b), preparation OR6 (c), preparation OR7 (d)

As shown on Fig. 4, only in the case of preparations containing iron preparations, the growth of microorganisms was inhibited.

3.2. Storage tests of prototypes of active packaging materials with the use of dairy products

The pictures below (Fig. 5) present cottage cheese samples 42 days after packing in parchment (control sample) and in functional packaging OR5, OR6 and OR7 based on parchment.

a) b) c) d)

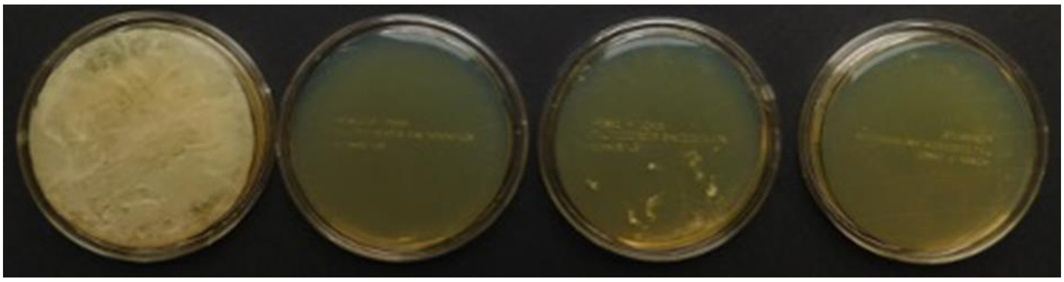
Fig. 5. Cottage cheese after 42 days of storage wrapped in parchment (a) and parchment with coating based on OR5 (b), OR6 (c), OR7 (d) iron preparations

a) b) c) d)

Fig. 6. Cottage cheese after 42 days of storage wrapped in polyolefin foil (a) and foil with coating based on OR5 (b), OR6 (c), OR7 (d) iron preparations

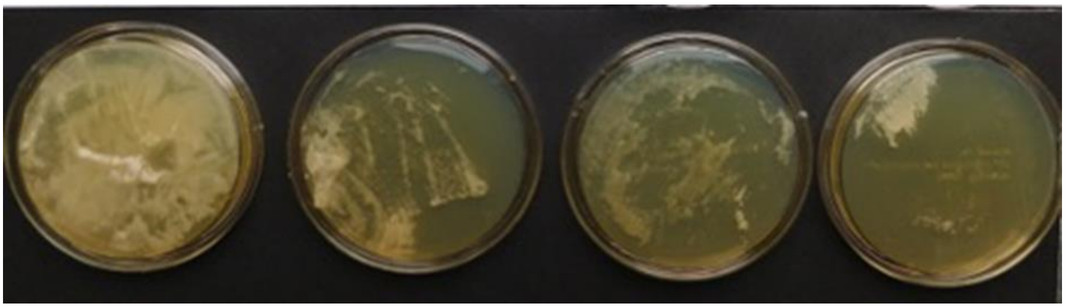
The Fig. 6 show cheese curd samples 42 days after packing in polyolefin foil (control sample) and functional packaging based on this foil.

The microbiological tests performed to determine the total number of psychrophilic microorganisms showed that all packages containing active iron preparations based on parchment inhibited the growth of microorganisms on the surface of the packed product (Fig. 7). Only when the layer containing the OR6 preparation is used, a slight presence of the cultured microorganisms can be noticed.



a) b) c) d)

Fig. 7. Microorganisms grown on the contact plates; the inoculations were performed from the surface of the control package (parchment) (a) and from the surface of functional packages OR5 (b), OR6 (c) and OR7 (d) after 42 days of storage of the samples



a) b) c) d)

Fig. 8. Microorganisms grown on the contact plates; the inoculations were performed from the surface of the control package (polyolefin foil) (a) and from the surface of functional packages OR5 (b), OR6 (c) and OR7 (d) after 42 days of storage of the samples

In the case of the polyolefin foil, it was more difficult to obtain tight packaging of the product and the coating peeled off partially from the surface of the film, which was probably the reason for the lower inhibition of microbial growth in this type of packaging. However, compared to the control, also in this case, a significant reduction in the number of microorganisms was observed on the surface of the package in contact with the product (Fig. 8). The smallest number of microorganisms was present on the surface of the OR7 package.

Due to the increasing popularity of "bio" products on the market, which are produced and produced without the use of preservatives, these products require protection against deterioration using active packaging materials [21]. The greater susceptibility of this type of products to spoilage necessitates the use of new solutions to protect and extend the shelf life of food, to avoid the risk of consumer health [22]. Therefore, more and more research is directed precisely in this field. The inhibition of microbial growth on the food surface was observed using the ZVI/PLA coating applied to the polyolefin film. These films with the addition of 3% ZVI (w/w) inhibited the growth of microorganisms, and the storage tests were carried out in the same period [23]. A similar effect was also obtained with the use of chitosan-PVA-TiO_2 films and chitosan / carboxymethyl cellulose/ZnO nanoparticles films. The conducted research confirmed that both packaging materials made of parchment and foil with the addition of iron nanoparticles obtained with the use of oregano extract effectively prevent contamination of the inside and the surface of the cheese during the entire six-week storage of the product. Active packaging inhibited the growth of microorganisms on the surface of the product.

4. Conclusions

Based on the obtained research results, it can be concluded that the use of functional packaging based on iron nanoparticles obtained by reducing iron salts with the use of oregano extract, effectively inhibits the growth of microorganisms on the surface of the curd cheese in contact with the packaging. Cheese wrapped in prepared prototype packaging materials was stored for 6 weeks in refrigeration conditions and after this time, a significant inhibition of the growth of microorganisms on the surface of the product was detected. There was observed that the packaging based on parchment, practically complete inhibited the growth of microorganisms. The foil-based packaging significantly reduced the growth of microorganisms. The extended shelf life of the cheeses packed in the developed packaging gives a promising prognosis for their use as active food packaging.

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