Study of the Biological Properties of New Smart Packaging Materials for Use in Agricultural Industries

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Abstract. The paper studies the biological properties of film coatings created on the basis of polysaccharides (xanthan and carboxymethylcellulose) for use in various branches of the agro-industrial complex. It was found that samples of these film coatings completely decomposed in the soil in 7 days, and did in the aquatic environment in an average of 3.5 h. The effect of these film coatings on the organism of laboratory animals (rats) was studied by adding them to animal feed (experimental group). During the experiment, no deviations in behavior were noted in the experimental group of rats; no death in both the control and experimental groups of animals occurred. The studied film coating had no negative effect on the large intestine microbiocenosis and the animals’ weight. It was shown that the number of mesophilic aerobic and facultative anaerobic microorganisms in the experimental group was 3 times less than in the control one, and the number of lactic acid bacteria of the genus *Lactobacillus* was 3.3 times higher than in the control group. The results obtained indicate the biological safety of the use of the studied film coatings.

1 Introduction

Agriculture is the branch of economy aimed at providing the population with food and obtaining raw materials for industry; the security of the state also depends on the state of the agro-industrial complex. At present, agriculture is being shaped by the growing demands of consumers and expanding the possibilities of producing high-quality production based on the intellectualization, automation and robotization of processes throughout the entire cycle, from production to consumption.

Research of new "smart" packaging materials for storing food and goods is an urgent task, since this will prolong the shelf life, preserve and improve the quality of goods and products. Food films and coatings are thin layers of materials applied to plant and animal products in order to preserve their nutrients. The main functions of polymeric films are to protect products from mechanical damage, physical, chemical and biological effects [1].

The purpose of this work was to study the effect of our film coatings based on polysaccharides on the organism of laboratory animals.

2 Materials and methods

The film coating under study was prepared according to the patent for the invention «Biodegradable food film coating» (No. 2,662,008, 27.07.2018. Bull. No. 21) [5]. The composition of the film coating included xanthan (xanthan gum) (Rode Gilles, France), carboxymethyl cellulose (CMC) produced by Fluca (Switzerland), lecithin by Lecigran, Cargil, (Germany), glycerol and water.

The biodegradability of the film coatings was assessed according to GOST R 57432-2017 «Packaging. Biodegradable films. General technical conditions» [6]. Experimental studies with laboratory animals were carried out in accordance with the requirements of the Federal 01.01.1997 Law «On the Protection of Animals from Cruelty» and the provisions of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 18.03.1986).

Our experiment was carried out in the veterinary clinic of the Saratov State Agrarian University named after N.I. Vavilov on laboratory animals (rats). In the course of our research, the safety of food products protected with the film coating was assessed, considering affordable cost, versatility, acceptable optical and structural-mechanical properties, gas tightness, high resistance to microorganisms and water, and sensory acceptability [4].

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the general homeostasis of the organisms of laboratory animals. Clinically healthy laboratory animals (rats), 10 males with a live weight of 175–180 g were studied under experimental conditions.

The studied animals were kept according to generally accepted methods [7]. The rats, before setting up the experiment, were kept in quarantine for 21 days. Then, the rats were divided into 2 groups of 5 animals each: group 1 was the control one, group 2 was the experimental one. The control group animals were fed according to the generally accepted recipe of complete feed for rats in short-term experiments [8]. The diet of the experimental group of animals consisted of compound feeds with the addition of our polysaccharide film.

The microbial contamination of the large intestine contents in rats was assessed at the beginning and end of the experiment. To analyze the qualitative and quantitative microflora composition, the following differential nutrient media were used: Sabouraud, Bifidum – medium, lactobacillus, and yolk-salt agar. The contents of the colon were taken in compliance with the rules of asepsis, reaching a dilution of 10. For this, 10-fold dilutions of the material (from 1·10^{-1} to 1·10^{-10}) in sterile saline were prepared. Each dilution was plated on appropriate media, incubated for 48 h at 37 °C, then the number of grown colonies was counted and recalculated per 1 g of feces [9].

Statistical processing of the experimental results was carried out by the method described elsewhere [10]. Calculations and construction of tables were carried out using the Microsoft Office Excel 2010 included in the Microsoft Office 2010 software package.

3 Results and discussion

Previously, we have developed film coatings [11, 12], applied to food products in different ways: by spraying, brushing, and wrapping. These packaging materials had such advantageous organoleptic characteristics as transparency, uniformity, density, lack of taste and odor. Due to the above, the appearance of the product did not deteriorate. In addition, the strength of the film coatings ranged from 2.10 to 3.28 Pa, with a thickness of 0.012 to 0.088 mm and an extensibility of 10.83 to 17.12 mm, depending on the method of application.

Undoubtedly, biodegradability is a positive effect that makes it possible to classify the film coatings as “smart” packaging. According to GOST R 57432-2017 “Packaging. Biodegradable films. General technical conditions”, if film coatings decompose in the soil, then they are considered safe [6].

To confirm the biodegradability of our film coatings, experiments were carried out in natural conditions, namely, they were placed into experimental models of "soil and water".

In the course of the experiment, film coatings were made with several concentrations of polysaccharides, xanthan and CMC (%): 0.60 and 2.73 (spraying); 0.90 and 2.05 (brushing); 1.61 and 1.38 (wrapping), respectively. These concentrations were taken because we previously showed that such concentrations are optimal for the design of film coatings on food products for application in various ways [11, 12].

Pieces of the film coatings with a diameter of 10 cm were buried in the soil under natural conditions to a depth of 3 cm (Fig. 1). Throughout the experiment, at intervals of 24 h, a visual inspection of each film coatings in the soil was carried out. The film coatings, being in the soil, became cloudy after 24 h, after 48 h the integrity of the film changed, namely, small pieces were formed, and after 72 h no noticeable changes occurred. Subsequently, from 96 to 120 h, a constant decrease in the size of the film samples was observed (Fig. 2). After 144 h, the film coatings were halved.

The complete dissolution of the film coating in the soil occurred after 168 h. It means that the film coating decomposes in natural environmental conditions in 7 days (Fig. 3). The decomposition rate of the film coatings is influenced by a number of indicators, such as: the thickness of the film coating, its composition, climatic conditions of the environment, etc. In this regard, film coatings of the same diameter were buried in another type of soil (peat soil model) for 7 days. Average daily temperature was +18±3 °C. A change in structure was noted on the second day; partial decomposition with pronounced inclusions was observed on the fourth day; and there was complete decomposition on the seventh day.
As a result, it was shown that changes in soil composition, seasonality, and average daily temperature did not significantly affect the biodegradability of our film coatings.

To confirm biodegradability in an aqueous medium, film samples 10 cm in diameter were immersed into water (Fig. 4). Complete dissolution was noted within 3–4 h (Fig. 5).

Table 1. Feeding diet for rats in short-term experiments, %

| Ingredient          | Control group | Experimental group |
|---------------------|---------------|-------------------|
| Barley              | 40.0          | 40.0              |
| Wheat               | 42.6          | 42.6              |
| Fodder yeast        | 6             | 6                 |
| Meat and bone meal  | 3             | 3                 |
| Bone meal           | 1.2           | 1.2               |
| Herbal flour        | 4             | 4                 |
| Chalk               | 1.7           | 1.7               |
| Table salt          | 0.5           | 0.5               |
| Premix              | 1             | 1                 |
| Film coating solution | –           | 3–5              |

On the 30th day of the experiment, the experimental animals were euthanized. When observing the experimental animals after feeding, no deviations in behavior from the control group were noted.

Every day, throughout the entire experiment, the laboratory animals were weighed, and they were clinically examined; no changes in the general state of their body and no deviations in behavior were observed, no impairments to motor activity or appetite were found. Throughout the experiment, no external signs of intoxication were observed in the rats of all the groups. The animals were active, their reaction to external stimuli was not disturbed, and the body temperature of the rats remained within the physiological values, according to their age characteristics. During the experiment, no death was observed in both the control and experimental groups of animals (Fig. 6).

As can be seen from Figure 2, complete dissolution of the film coating occurred on average within 210 min (3.5 h).

As a result of the experiment, it was revealed that the samples of our film coatings completely decomposed in the soil within 7 days, and did in the aqueous environment in an average of 3.5 h. In addition, it was noted that the film coatings with the lowest concentration of xanthan underwent the fastest degradation.

In the course of further studies, the safety of the film coatings was assessed on laboratory animals (rats).

The control group of animals was fed according to the generally accepted recipe of complete feed for rats in short-term experiments [8]. The film coating prepared according to the patent «Biodegradable food film coating» [5] was added into the diet of the experimental group of animals. For this, the film coating was sprayed onto the finished dry mixture and dried for 55–70 min.

The feeding diet for rats in short-term experiments is presented in Table 1 [8].
In the course of microbiological studies of the intestinal contents of the control and experimental groups of animals, the number of mesophilic aerobic and facultative anaerobic microorganisms, as well as lactobacilli (*Lactobacillus*) and fungal cells were estimated. The qualitative and quantitative composition of the large intestine microflora of the laboratory animals plays an important role in the occurrence or development of disorders of their digestive canal. Table 2 presents the results of our study of the species and quantitative composition of the colon microflora of the laboratory animals. Based on the data in Table 2, it can be seen that the QMAFAnM in the experimental group was 3 times less than in the control one. In turn, the number of lactic acid bacteria (*Lactobacillus*) in the experimental group was 3.3 times higher than in the control one.

**Table 2.** Microbiocenosis of the large intestine contents of laboratory animals

| Samples            | QMAFAnM, CFU/g | Lactobacillus, CFU/g | Yeasts and molds, CFU/g |
|--------------------|----------------|---------------------|------------------------|
| Control group      | 30·10⁵         | 3·10⁵               | –                      |
| Experimental group | 10·10⁶         | 10·10⁵              | –                      |

Thus, we can conclude about the positive effect of the film coating on the internal microflora state of the large intestine of animals.

**4 Conclusion**

The studied film coating is tasteless, odorless and colorless, easy to chew, it is used as a thin film on food products and performs barrier functions, allowing maintaining the organoleptic characteristics of products during their shelf life. The film coatings are biodegradable, and decompose without residue in the soil and aqueous environment in 7 days and 3.5 h, respectively. Complete decomposition took place in natural conditions, which speaks of their environmental safety. Our film coatings had no negative effect on the rat organism, which indicates the biological safety of the use of our film coatings for laboratory animals.

Thus, the results of our studies allow us to say that the developed film coatings based on polysaccharides are environmentally and biologically safe.

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