Synthetic and chitosan-based polymer films were selected as the object of this study. Chitosan is a natural non-toxic oligosaccharide of animal origin capable of biological destruction. The task addressed in this work is to design a chitosan-based film with increased bacteriostatic properties for use in combined packaging material as a protective layer. The use of such films would provide for the biodegradability of packaging materials, which could make it possible to reduce the use of synthetic polymers in packaging and improve the environment.

A feature of the proposed method is that a decoction of yarrow grass is used as a chitosan solvent, which leads to the acquisition of bacteriostatic properties by the films. It has been established that the highest bacteriostatic effect is achieved in this case. The results of the research showed a significant growth retardation zone of E. coli, B. cereus, B. subtilis, P. aeruginosa, S. aureus, C. albicans, Saccharomyces and Lactobacillus strains.

The set of studies made it possible to optimize the recipe composition of films based on chitosan (%): chitosan – 2.0...2.5, glycerin – 1.0...1.5, decoction of yarrow grass – 96...97 (according to the ratio of medicinal plant raw materials:water – 1:10).

It was determined that the values of indicators of the destructive stress at the rupture of the designed films (14.0...16.0 MPa) exceed the permissible minimum, which should be 13.7 MPa for polymer films.

The designed films are not intended for independent use as packaging material but should be applied as part of combined packaging as a protective layer.

The scope of application of the current research results is the packaging of food products, namely fruit and vegetable pastes and sauces

Keywords: biopolymers, chitosan, film-forming solutions, packaging films, combined packaging, bacteriostatic properties

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DESIGN OF A NEW FILM WITH PREDEFINED PROPERTIES BASED ON CHITOSAN

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1. Introduction

The widespread use of synthetic materials in food packaging has recently caused a number of environmental problems [1]. First, the problem of disposal of plastic packaging and its uncontrolled accumulation in nature (dumps, landfills, etc.) worsen the ecological situation on Earth because it can decompose for hundreds of years. Secondly, the depletion of raw materials, in particular oil, prompts scientists to find alternative renewable raw materials for the production of packaging materials. This means that the used polymer packaging must degrade quickly enough under the influence of the surrounding environment after its use. Chemical, physical, biological, and natural factors should act synergistically and lead to polymer fermentation through the destruction of macromolecules and their transformation into low-molecular compounds [2].

In this regard, the most effective way for designing packaging films and coatings is the use of natural polymers (starch, cellulose, pectin, collagen, etc.), among which the oligosaccharide of animal origin, chitosan, has been widely studied in the last three decades [3]. The impetus for a more detailed examination was its unique physiological and ecological properties: biocompatibility, biodegradation, bactericidal, the ability to selectively bind heavy metals, etc.

Therefore, it is a relevant task to carry out studies aimed at designing biodegradable films based on chitosan.

2. Literature review and problem statement

Polymer coatings based on chitosan and its derivatives have been successfully developed in recent years abroad. Thus, in work [4], the authors improved the functional properties of films based on chitosan by mixing with other natural biopolymers, such as polysaccharides, lipids, proteins, and their derivatives. This allowed the authors to change the properties of the film according to the nature of the food products being packaged. But the designed films did not have sufficient bacteriostatic properties for long-term stor-

age of food products. Further research is needed to explore the optimal compositions of chitosan with other materials to improve its mechanical properties and overall performance as a film for packaging food products. While chitosan shows great promise as a sustainable food packaging material, its mechanical limitations need to be addressed through innovative combinations with other components to increase its market applicability.

Paper [5] investigated the migration of antioxidants in chitosan films containing plant extracts. Bio-based films added to essential oils of ginger, clary sage, or rosemary showed the highest diffusion and antioxidant activity in the simulator, highlighting their functionality and potential for use as an active packaging material for food products. The disadvantage of this advancement is the lack of data on sorption, mechanical properties, as well as toxicity of the films. Further research is needed to rank different hydroalcoholic extracts and films with essential oils for further incorporation into chitosan for food packaging, namely identification of the antimicrobial activity of new materials and their behavior in direct contact with food matrices.

Paper [6] describes the design of a composite film based on chitosan (CS) and antioxidant – banana peel extract (BPE). Different contents of BPE (4 %, 8 %, and 12 %) were added to the CS film not only as an antioxidant but also as a cross-linking agent. CS-4 % BPE composite film showed the best properties. The decrease in moisture content, water solubility, and vapor permeability of the CS-BPE composite film indicates decreased hydrophilicity. In addition, the CS-BPE composite film showed excellent antioxidant activity in various food simulants. These studies are interesting only for the processing of fresh fruits and vegetables. Films are not intended for use in combined packaging.

In [7], the effect of molecular weight on the antimicrobial activity of chitosan from *Loligo opalescens* for food packaging was investigated. The disadvantage of this work is that the antimicrobial properties of the films were evaluated only against *Escherichia coli* and *Pseudomonas putida*. Poly(lactic acid) surfaces with chitosan are able to kill most uncultured cells in food media, which is a very interesting result considering that such cells remain a public health risk. At the same time, chitosan-based surfaces reduced the number of viable, but uncultivated, and cultured biofilm cells by 73 %, 74 %, and 87 %, respectively, compared to poly(lactic acid) surfaces. Further research is needed to bring this biopolymer to an industrial level for food packaging.

In [8], the influence of the molecular weight of chitosan in the form of micro- and nanoparticles on the antibacterial activity against some pathogenic soft rot bacteria was investigated. Medium-viscosity chitosan nanoparticles show higher antibacterial activity than chitosan itself against all tested bacterial cultures. It is expected that chitosan in the form of nanoparticles could be widely used as an antimicrobial agent in food science due to their high antibacterial activity and acceptable biocompatibility. The disadvantage of the work is that the antibacterial activity was evaluated only against *Pseudomonas fluorescens*, *Erwinia carotovora* and *Escherichia coli*. Further in vivo research on micro- and nanoparticulate chitosan in the study of pathogenic bacteria is recommended.

Composite films based on chitosan with pineapple peel polysaccharides were obtained in work [9]. The application of composite films on strawberries showed that the addition of polysaccharide components can delay the rapid decay of strawberries during storage. The results of the study showed that pineapple polysaccharides have potential for applications in the field of food packaging. Mechanical properties of composite films were improved, but the authors studied antibacterial properties of composite films only against *B. subtilis*, *S. aureus* and *E. coli*. The reason for this may be the authors' lack of methodology and a laboratory base.

Nanoreinforced chitosan films with antioxidant and antimicrobial properties with the addition of polyphenols were proposed in [10]. Blending of polyphenol with chitosan is reported to result in an active package with 69 % improved tensile strength, 22 % reduced vapor permeability, and 38 % increased opacity. Carrots were chosen as objects of research. The designed films showed little antimicrobial activity.

In work [11], studies of bactericidal properties and toxicity of compositions for storage of stone fruits were carried out. Compositions from extracts of medicinal plant raw materials and 2 % chitosan have been developed. The following raw materials were selected: aloe leaves, chamomile flowers, spruce bark, eucalyptus leaves, basil herbs, thyme herbs, lemon balm leaves, sage leaves, verbena herbs. All compositions were tested for fungicidal properties against *Monilinia* laxa. In all studied samples, inhibition of the growth of the causative agent of moniliosis of stone crops was recorded. All compositions showed antimicrobial activity against *Bacillus cereus* ATCC 107-02, *Escherichia coli* ATCC-25922 and *Candida albicans* ATCC-885-653. The disadvantage of the work is that the designed films are intended only for stone fruits. The reason for this is the persuasiveness of the authors in their precision approach to the research objects.

Work [12] considers the prospect of using extracts from plant raw materials for tomato storage. The study indicated the lack of research on the selection of film-forming compositions and antioxidant preparations that would take into account the effect of the specific microflora of individual fruits and vegetables. Since high solubility in water and minimal toxicity are important characteristics of the safety of substances that come into direct contact with food products, it is recommended to use water and hydro glycerin alcohol extracts of plant raw materials for the development of drug technology. Further microbiological and toxicological studies of experimental samples are necessary to establish working concentrations of extracts and to choose an effective composition.

Paper [13] investigated the storage of apricot fruits using medicinal plant extracts. To extend the shelf life, it is suggested to use a protective agent with antibacterial properties against the main fungal infections of apricot – fungi of the genus *Monilinia* (*Monilinia laxa*, *Monilinia fructicola*) and *Rhizopus stolonifer*. Water and water-alcohol-glycerin extracts of plant raw materials are recommended for use in the development of new technology – lemon balm and sage leaves, verbena grass. The disadvantage of this advancement is that the designed films are proposed to be used only for apricots. The reason for this is the persuasiveness of the authors in their precision approach to the research objects.

Taking into account current literary data, it can be stated that the level of research in this field is still low. Films from a mixture with specific components can be produced only for special applications in food technology. Most of the analyzed technological advances are intended for contact with food products such as soft drinks, confectionery, dairy products, fresh fruits, and vegetables. But many other food products are characterized by high titrated acidity, which creates an aggressive environment in the middle of the package. Packaging of such products requires other approaches that have

not yet been used. An innovative approach in this sense is to design polymer films with specified properties based on chitosan in order to use them as part of combined packaging materials.

3. The aim and objectives of the study

The aim of our work is to design a new packaging film based on chitosan and glycerin with enhanced bacteriostatic properties. This will make it possible to obtain biodegradable films and extend the shelf life of food products.

To achieve the goal, the following tasks were set:

– to investigate the rheological properties of film-forming solutions based on chitosan;

– to investigate the bacteriostatic and toxicity of films based on chitosan and to determine their rational composition;

– to investigate the possibility of stabilization of chitosan-based films;

– to study the mechanical properties of the film material.

4. The study materials and methods

Research objects:

– analog film made of polyethylene;

– research films based on chitosan.

Research hypothesis: the addition of chitosan to the composition of packaging films would help increase their bacteriostatic properties, provided they meet the requirements related to the functional and technological properties of these products.

Films were obtained under laboratory conditions from film-forming solutions prepared by mixing the components of chitosan (2...2.5 %) and glycerol (1.0...1.5 %) in solvents (water or decoction of medicinal herbs). Film formation was carried out by applying the film-forming solution on a flat horizontal glass surface, followed by complete evaporation of moisture. Evaporation of moisture was carried out under two modes: at a temperature of 20±2 °C for 1.5...3 days; at a temperature of 100±2 °C for 0.5...1 h.

Sorption properties were determined for film samples obtained in the following way:

– dried at a temperature of 20±2 °C;

– dried at a temperature of 100±2 °C;

– dried at a temperature of 100±2 °C and treated with a 20 % NaOH solution for 5, 10, 15 minutes;

– dried at a temperature of 20±2 °C and treated with a 1 % Na_2SO_4 solution for 5, 15, 30, 60, and 90 minutes;

– dried at a temperature of 20±2 °C and treated with a 1 % $ZnSO_4$ solution for 5, 15, 30, 60, and 90 minutes.

To determine the bacteriostatic properties, the following concentrations were prepared on the basis of a decoction of medicinal plant raw materials (MPR) in accordance with the requirements of the European Pharmacopoeia [14]: 10, 30, 50, 70 %. The crushed MPR was boiled in a water bath for 15 min, then removed from the water bath, cooled for no more than 10 min, and wringed.

The content of the components of essential oils and tannins in the studied decoctions was determined by the method of high-performance thin-layer chromatography [15].

Films were made on the basis of the obtained decoctions:

– based on food-grade low-molecular (water-soluble) chitosan;

– based on food-grade low-molecular-weight (water-soluble) chitosan and yarrow decoction;

– based on food-grade low-molecular-weight (water-soluble) chitosan and eucalyptus decoction.

The viscosity of chitosan solutions was determined on a Rheotest-2 rotary viscometer at 20 °C. The rheological properties of the solutions were studied under conditions of shear rates of 0.167...150 s^{-1} [16, 17].

Determination of thickness and different thicknesses. The thickness of films and combined packaging material was measured using a MK-102 micrometer.

The difference in thickness refers to the ratio of the smallest thickness of the film δ_{min} to its largest thickness δ_{max}.

The bacteriostatic activity of packaging films was investigated in relation to test strains of pathogenic microorganisms *Escherichia coli, Bacillus cereus, Bacillus subtilis, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*, yeast – *Saccharomyces* and lactic acid microorganisms – *Lactobacillus*. The bacteriostatic activity of the films was determined in vitro by the method of disks placed in a nutrient medium (2 % meat-peptone agar), the microbial load of $5{\cdot}10^7$ colony-forming units (CFU) in 1 ml. 20-hour broth cultures of the above-mentioned strains were used. Only for *C. albicans*, cultures grown for 48 h on Sabouraud's liquid were taken. To study the prolongation of the bactericidal effect of chitosan films, freshly prepared samples were used. Calculations were performed after incubation of crops in a thermostat at a temperature of +36.6 °C from 1 hour to 48 hours. The antimicrobial effect of the samples of packaging films was evaluated by the presence or absence of microbial growth under and around the disks. The resulting data were analyzed according to the following parameters of the retention zone: up to 10 mm – the film does not have bactericidal properties; more than 10 mm – it does.

Studies on the toxicity of film samples were carried out in vitro by the method of disks placed on 5 % blood agar. Incubation was carried out in a thermostat at a temperature of +36.6 °C for 24 hours. The presence or absence of a zone of hemolysis (dissolution of erythrocytes) around the film disk was observed.

Determination of sorption properties. Experiments on film swelling were performed at a temperature of 20±2 °C [18].

The swelling of the films was determined by the gravimetric method and was characterized by the amount of swelling according to the following formula (1):

$$H = \frac{m_1 - m_2}{m_2}, \quad (1)$$

where m_1 is the mass of the sample after swelling, g;

m_2 is the mass of the dry sample, g.

For a more detailed study of the ability of the films to resist the influence of water, they were studied by the method of nuclear magnetic resonance (NMR) on a pulsed NMR spectrometer with an operating frequency of 16 MHz [19].

The value of T_2 (spin-spin relaxation) was found from formula (2):

$$A_e = A_0 \exp\left(-\frac{2\tau}{T_2}\right), \quad (2)$$

where A_i is the measured value of the amplitude of the sample signal for the time interval τ_i;

A_0 is the initial amplitude of the signal.

Tensile strength and elongation at break were determined on a breaking machine without pre-conditioning the film. The tests were carried out at an air temperature

of 20±2 °C and a relative humidity of 75 % on rectangular samples measuring 20×150 mm.

Since one of the most important indicators that determines the suitability of a film for use is its thickness, it was considered appropriate to choose such a viscosity of the film-forming solution that a uniform film is formed. It is known that the permissible deviation from the thickness of the film should be 20 %.

To standardize multicomponent phyto remedies, so-called marker compounds, or marker substances, are used, the presence of which is characteristic only for individual medicinal raw materials.

The ratio of MPR and solvent 1:10 was chosen as regulated by the general article on water extraction.

Given that the solubility of chitosan films is due to the presence of reactive amino groups in a protonated form, sodium, and zinc sulfates, which are capable of forming ionic bonds with protonated amino groups, were chosen as reagents for modification.

5. Results of studies on the formation of film quality based on chitosan

5. 1. Study of rheological properties of film-forming solutions

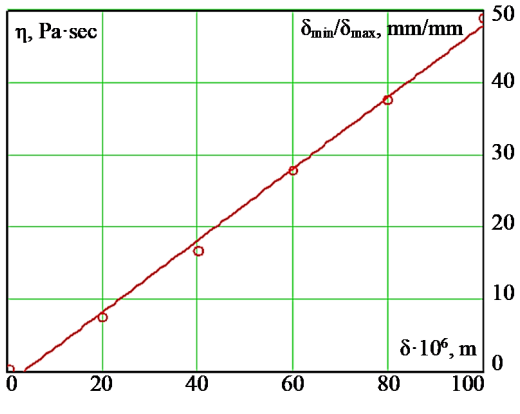
One of the most important indicators that determines the film's suitability for use is its thickness. Based on this, it was considered appropriate to choose such a viscosity of the film-forming solution that a uniform film is formed.

It is known that the permissible deviation from the film thickness should be 20 % [16]. The study of the thickness of the formed films from film-forming solutions with different concentrations of chitosan and glycerin made it possible to establish the dependence of different thickness of the films on their thickness and the viscosity of the film-forming solution. The resulting experimental data were fitted to a linear function:

$$\eta(\delta) = -0.158 + 0.05 \cdot \delta, \quad (3)$$

where η is the viscosity coefficient, Pa·s;

δ is the film thickness, μm.



The resulting dependence is shown in Fig. 1.

Fig. 1. Dependence of film different thickness on film thickness and film-forming solution viscosity

Fig. 1 shows that in order to form a uniform film, the thickness of which will not exceed the permissible 20 %, a film-forming solution with a dynamic viscosity of no more than 2 Pa·s should be chosen. At the same time, a film with a thickness of 45...47 nm is formed.

The concentration of the main components, namely, chitosan and glycerin, in the formulation of the film-forming solution was determined using a two-factor experiment. The starting factors were the volume concentration of chitosan and glycerin in the solution, and the viscosity coefficient of the obtained film-forming solution was chosen as the criterion for choosing their rational values.

The concentration of chitosan varied discretely from 1 to 3 %, and the concentration of glycerol – from 1 to 2 %. The resulting experimental values of the viscosity coefficient were fitted to the following function:

$$\eta(C_{ch}, C_{gl}) = -0.181 + 1.179 \cdot C_{ch} + 0.617 \cdot C_{gl} - 0.167 \cdot C_{ch}^2 - 0.067 \cdot C_{gl}^2 - 0.075 \cdot C_{ch} \cdot C_{gl}, \quad (4)$$

where C_{ch} is the concentration of chitosan, %;

C_{gl} – concentration of glycerol, %;

η is the viscosity coefficient, Pa·s.

The resulting surface is shown in Fig. 2.

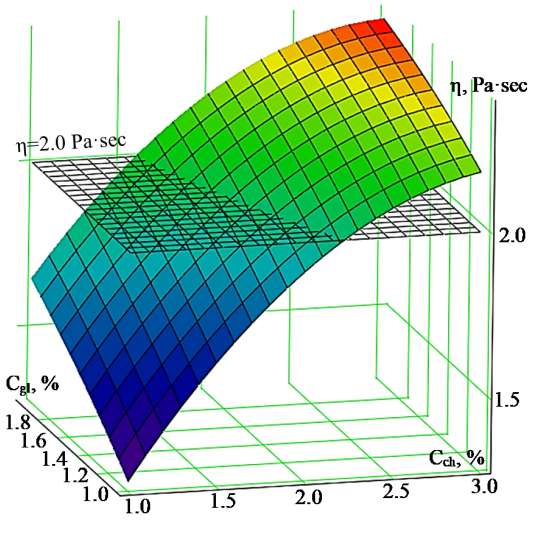


Fig. 2. Dependence of viscosity coefficient of the film-forming solution on the concentration of chitosan and glycerin in it

From the appearance of dependence of the viscosity coefficient of the film-forming solution on the concentration of chitosan and hycerin, one can see that there are ratios of the concentrations of these substances, at which the value of the coefficient is 2 Pa•s (separated in Fig. 2 by a horizontal surface). It is this value of the viscosity coefficient of the film-forming solution that is considered rational, based on the difference in thickness of the film from its thickness and the viscosity of the film-forming solution.

5. 2. Study of bacteriostatic and toxicity of films based on chitosan and optimization of their composition

Medicinal herbs were chosen from those used in medicine as anti-inflammatory and wound-healing drugs, so they have pronounced bactericidal and bacteriostatic properties: common yarrow grass and eucalyptus leaves. Data on biologically active compounds-markers contained in the selected medicinal raw materials are given in Table 1 [20].

Table 1

Dominant biologically active compounds contained in MPR

Medicinal raw materials	Substance	Content in raw materials, mg/kg	Medicinal raw materials	Substance	Content in raw materials, mg/kg
Common yarrow grass	sabinyl acetate	24.4	Eucalyptus leaves	α-pinene	39.52
	lavandulyl acetate	11.8		β-pinene	11.31
	β-caryophyllene	25.6		1,8-cineole	37.1
	1,8-cineole	12.0			
	hermacrene D	17.2		μ-cymene	4.89
	hamazulene	53.2		camphene	2.07
	viridiflorol	32.8		α-phellandrene	1.15
	camphor	13.5		other terpene hydrocarbons	2.46
	borneol	18.2			
	terpinen-4-ol	10.7		tannins	1.5
	tannins	2.8			

Analyzing Table 2, one can note that the markers of common yarrow are chamazulene, the content of which is more than 50 mg/kg. The content of sesquiterpenes (viridiflorol, β-caryophyllene, germacrene D, borneol, 1,8-cineole, terpinen-4-ol, lavandulyl acetate, camphor) exceeds 140 mg/kg of raw material. The content of tannins is 2.8 mg/kg, and the ester sabinyl acetate is 10.7 mg/kg.

The composition of eucalyptus leaves mostly includes monoterpenes (α-pinene, β-pinene, camphene, α-phellandrene), sesquiterpene 1,8-cineole, aromatic non-phenolic compound μ-cymene, and also in a small amount (up to 2.46 mg/kg) other terpene hydrocarbons. The content of tannins is 1.5 mg/kg.

For the study, decoctions of MPR of different concentrations were prepared: 10, 30, 50, 70 %.

Taking into account the fact that essential oil is well soluble in ethers, lipids and fatty oil, alcohols, but poorly soluble in water, it is extracted from MPR by maceration in alcohol. However, it is known [21] that up to 21 % of essential oil components dissolve in water. It is also known that the maximum amount of tannins is extracted with water, therefore, only decoctions are the rational form of their extraction. Their transition into water is 40...65 % [22–25]. Quantitative content of components of essential oils and tannins in prepared MPR decoctions, which were studied, are given in Table 2.

Analysis of Table 3 confirms the fact that with an increase in the concentration of MPR in the solution and a corresponding decrease in the solvent, the percentage of extraction of biologically active substances decreases. Thus, for the components of the essential oil of yarrow and eucalyptus, namely terpenoids and complex esters, the percentage of components extracted into the decoction decreases from 21 % for a 1:10 decoction to 5 % for a 7:10 decoction. Despite the fact that tannins are well soluble in water, the percentage of their extraction into the decoction also depends on its amount. Thus, the highest percentage of extraction of MPR tannins into the decoction is observed at a ratio of 1:10, namely 65 %, while at a ratio of 7:10 their extraction is 30 %.

Thus, from the considered MPR concentrations in decoctions, it was established that the optimal ratio of MPR:water, which ensures the maximum extraction of biologically active substances into the decoction, is 1:10.

In the course of the research, the bacteriostatic properties of film samples were determined on the reference strains of microorganisms, namely: *Escherichia coli* (ATCC 8739), *Bacillus cereus* (ATCC 10702), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538 – P), *Candida albicans* (ATSS 885-653), *Saccharomyces* and *Lactobacillus*.

Table 2

Biologically active compounds contained in MPR decoctions of various concentrations

Content, %	MPR:water ratio			
	1:10	3:10	5:10	7:10
Common yarrow grass				
Sabinyl acetate	0.51	1.09	1.22	0.85
Laban-dulyl-acetate	0.25	0.53	0.59	0.41
β-caryophyllene	0.54	1.15	1.28	0.89
1,8-cineole	0.25	0.54	0.60	0.42
Herma-kren D	0.36	0.77	0.86	0.60
Chamazulene	1.12	2.39	2.66	1.86
Viridis-Florol	0.69	1.48	1.64	1.14
Camphor	0.28	0.61	0.68	0.47
Borneo	0.38	0.81	0.91	0.64
Terpinen-4-ol	0.22	0.48	0.54	0.38
Tanning substances	0.18	0.42	0.56	0.59
Eucalyptus leaves				
α-pinene	0.82	1.78	1.98	1.38
β-pinene	0.24	0.51	0.57	0.40
1,8-cineole	0.78	1.67	1.86	1.30
μ-cymene	0.10	0.22	0.24	0.17
Camphene	0.04	0.09	0.10	0.07
α-phellandrene	0.02	0.05	0.06	0.04
Other terpene hydrocarbons	0.05	0.11	0.12	0.08
Tanning substances	0.10	0.22	0.30	0.32

The results of the research were evaluated visually by the degree of development of microorganisms on the surface of the experimental films and by determining the zone of delay in the development of microorganisms. The results of the study of the bacteriostatic properties of film samples are given in Table 3.

Based on the study of the bacteriostatic properties and toxicity of the investigated films, it was concluded that for further research it is more appropriate to choose a chitosan film on a decoction of yarrow.

Table 3

Diameter of the growth retardation zone of microorganisms, mm (n=5, P≥0,95)

Microorganism strain	Experimental sample									
	Control (polyethylene)	Film on water	Film on a decoction of yarrow in the ratio of MPR:water				Film on eucalyptus decoction in the ratio of MPR:water			
			1:10	3:10	5:10	7:10	1:10	3:10	5:10	7:10
E. coli (ATCC 8739)	–	11	20	18	15	14	–	–	–	–
B. cereus (ATCC 10702)	–	11	13	11	11	9	10	9	7	7
B. subtilis (ATCC 6633)	–	–	13	11	11	9	11	10	7	7
P. aeruginosa (ATCC 9027)	–	–	11	11	9	9	–	–	–	–
S. aureus (ATCC 6538-P)	–	–	16	14	12	10	11	8	6	6
C. albicans (ATCC 885-653)	–	–	19	17	14	13	12	10	8	8
Saccharomyces	–	–	18	16	14	12	13	13	10	10
Lactobacillus	–	–	24	21	19	15	15	13	10	9

5. 3. Research on the stabilization of chitosan-based films

Experimentally, it was found that variation in the concentration of chitosan in the initial solution does not significantly affect the moisture-absorbing capacity of the films. Treatment of the resulting films in an alkaline solution was used as a modification in order to transfer the films from the salt form to the basic form [26].

Samples of the films differed in the technique of preparation and processing. The results of the study of the water absorption capacity of chitosan films are shown in Fig. 3.

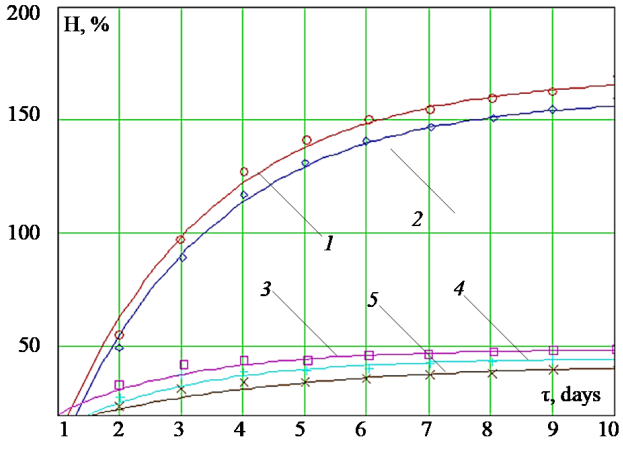


Fig. 3. Kinetic curves of swelling in a model solution of film samples: 1 — film dried at a temperature of 20±2 °C; 2 — film dried at a temperature of 100 °C; 3 — film dried at a temperature of 100 °C and treated with a 20 % NaOH solution for 5 min; 4 — film dried at a temperature of 100 °C and treated with a 20 % NaOH solution for 10 min; 5 — film dried at a temperature of 100 °C and treated with a 20 % NaOH solution for 15 min.

In this case, experimental data were fitted to a function of the following form:

$$H_i(\tau) = H_{\max i} - e^{H_{0i} - c_{wi}\tau}, \quad (5)$$

where H_0 is the initial value of the degree of water absorption, %;

$H_{\max}$ – the maximum value of the degree of water absorption, %;

c_w – approximation coefficient, %/min;

i is the sample number (1, 2, 3, 4, 5).

The values of coefficients in formula (5) are given in Table 4.

Table 4

Approximation coefficients for the swelling kinetics of film samples obtained by different techniques

Sample	Initial value of the degree of water absorption, H_0, %	Maximum value of the degree of water absorption, $H_{\max}$, %	Approximation coefficient, c_w, %/min
1	5.48	169.4	0.407
2	5.47	160.0	0.411
3	3.83	48.9	0.474
4	3.91	45.0	0.466
5	3.57	42.8	0.284

One can see in Fig. 3 that drying chitosan films at elevated temperature affects, but slightly, the moisture absorption capacity compared to films dried at room temperature (curve 2 compared to curve 1).

Exposure of chitosan films in a 20 % solution of NaOH is accompanied by the transition of chitosan from the salt form to the basic form. The transition to the main form leads to a significant increase in the packing density of chitosan molecules with a corresponding decrease in the degree of availability of chitosan links for interaction with moisture, thus reducing the degree of swelling of film samples.

The exposure time of the samples in the alkaline solution inversely affects the moisture absorption of the film (curves 3–5).

Research results show that chitosan films modified by alkali treatment, unlike untreated ones, are characterized by the absence of dissolution in water, although the swelling process takes place. Thus, after 10 days of exposure in the model solution, the samples with the size of 4×4 cm increased to the size of 6×6 cm, but later the swelling process stopped, the samples did not dissolve in the model medium.

Another technique for chemical modification of chitosan films is sulfation. Modification of the films was carried out by immersing the film samples in 1 % Na_2SO_4 and $ZnSO_4$ solutions and holding them for 5, 15, 30, 60, and 90 min. Sulfuric acid salts were chosen due to the ability of amino groups of chitosan to interact with sulfate groups. The concentration of solutions of 1 % is optimal because its increase does not affect the result while a concentration of less than 1 % does not give the desired effect.

The results of our study of dependence of the spin-spin relaxation of water protons on the modification technique are shown in Fig. 4.

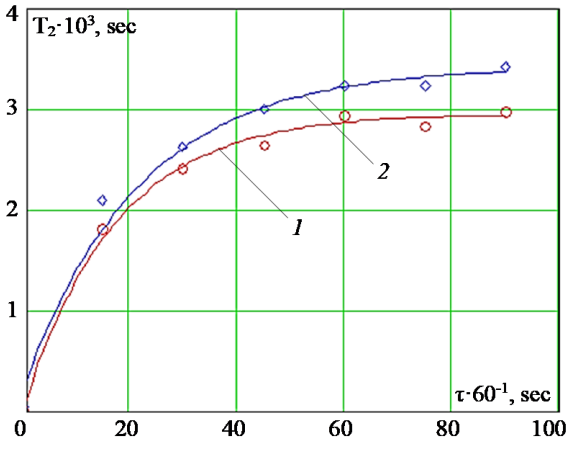


Fig. 4. Dependence of spin-spin relaxation of water protons on the modification technique with 1 % Na_2SO_4 solution and 1 % $ZnSO_4$ solution

The experimental data on the NMR study of dependence of the spin-spin relaxation of water protons on the modification technique of film samples were fitted to a function of the following form:

$$f_i(\tau)=a_i-e^{b_i-c_i\tau}, \quad (6)$$

where a_i, b_i, c_i are approximation coefficients.

For the dependences shown in Fig. 4, the values of approximation coefficients are given in Table 5.

Table 5

Value of approximation coefficients in the dependence of the spin-spin relaxation of water protons in the film samples in Fig. 4

Sample	$a_{i0}\cdot10^3$, s	$b_i\cdot10^3$, s	$c_i\cdot10^3$, %/s
1	1.09	2.954	58
2	1.17	3.415	46

It should be noted that in physical terms, the coefficient a_{i0} refers to the maximum spin-spin relaxation time T_2 of water protons in the investigated films, the coefficient b_i to the initial T_2 value, and the coefficient c_i to the rate of T_2 change depending on the duration of modification of the film samples.

From Fig. 4 one can see that the maximum T_2 value, and, accordingly, the maximum water absorption for the film sample treated with Na_2SO_4 has a smaller value. Based on this, further studies were conducted for films treated with Na_2SO_4 since reduced water absorption for such products as packaging films is a more acceptable functional and technological property.

Fig. 5 shows the results of measuring the spin-spin relaxation of samples of chitosan films modified with a 1 % Na_2SO_4 solution and kept in a model solution.

The experimental data were fitted to a function of form (6). Approximation coefficients for these experimental data are given in Table 6.

From Fig. 5 one can see that with an increase in the duration of exposure of the films in the model solution, the spin-spin relaxation time increases, which may be evidence of water sorption by chitosan films.

Table 6

Value of approximation coefficients in the dependence of the spin-spin relaxation of water protons in the film samples in Fig. 5

Sample	$a_{i0}\cdot10^3$, s	$b_i\cdot10^3$, s	$c_i\cdot10^3$, %/s
1	2.624	0.621	73
2	3.024	0.627	58
3	3.256	0.926	56
4	3.624	0.944	43
5	3.720	1.010	54

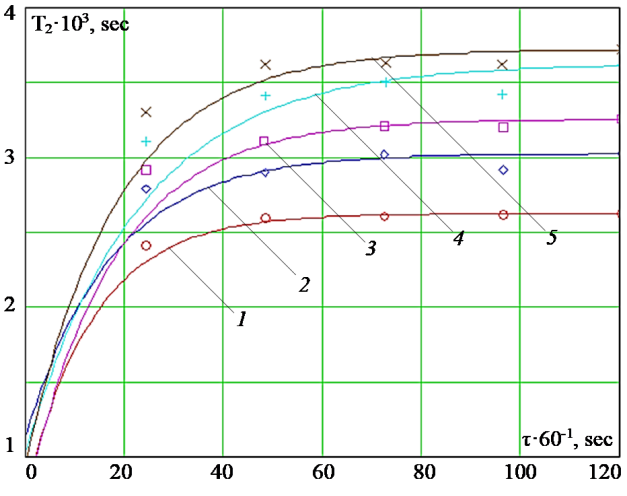


Fig. 5. Influence of the length of exposure τ in the model solution of films modified by Na_2SO_4 on the state of water in them for different durations of modification, min.: 1 – 60; 2 – 30; 3 – 15; 4 – 10; 5 – 5

The spin-spin relaxation time increases rapidly during the first 24 hours of exposure of the modified films in the model environment. At this time, water sorption by chitosan films occurs, that is, an increase in the number of water molecules, which lengthens the time of spin-spin relaxation. Then the swelling process slows down and stops (the spin-spin relaxation time becomes constant).

5. 4. Studying the mechanical properties of the film material

The strength characteristics of the films were studied at a stretching speed of 100 mm/min. The results of the research are given in Table 7.

Table 7

Results of investigating the mechanical properties of film packaging materials

Indicator	Control (polyethylene)	Unmodified chitosan film	Modified chitosan film
Tensile strength, MPa	21.0±0.9	16.0±0.7	14.0±0.6
Relative elongation at break, %	210.0±20	15.0±1.0	7.0±0.3

One can see from Table 7 that the breaking stress in modified films is 14.0 MPa, and in unmodified films – 16.0 MPa. Thus, our data indicate that after the modification of the films, the destructive stress at rupture decreases. Thus, in the case of modified films, the destructive stress at break is

reduced by 53.3 %. Despite the low values of the destructive stress of the designed films compared to polyethylene, their values exceed the permissible minimum, which should be at least 13.7 MPa for packaging films.

As a result of the research, it was noted that the relative elongation of unmodified chitosan films is significantly greater than that of modified ones and is 15.0 and 7.0 MPa, respectively. Thus, the modification of the films reduces the indicators of destructive stress and strength at stretching. This means that the film becomes stronger but less elastic.

6. Discussion of results related to the formation of quality of a new film based on chitosan

The study of the different thicknesses of the films obtained from film-forming solutions with different concentrations of chitosan and glycerin made it possible to establish the dependence of different thicknesses of the films on their thickness and the viscosity of the film-forming solution (Fig. 1).

At the same time, it was established that in order to form a uniform film, the index of thickness variation of which will not exceed the permissible 20 %, a film-forming solution with a dynamic viscosity of no more than 2 Pa·s should be chosen. The film, which is obtained under the condition of the viscosity coefficient of the film-forming solution of 2 Pa·s, has a thickness of 45...47 nm, which meets the technical requirements for packaging films.

It should be noted that the formation of the viscosity of the film-forming solution is largely determined by such components as chitosan and glycerin. The conducted two-factor experiment, where the initial factors were the volume concentration of chitosan and glycerin in the solution, provides an opportunity to determine their rational values. In this case, the viscosity coefficient was chosen as the selection criterion (Fig. 2).

The results of the two-factor experiment indicate different effects of chitosan concentration and glycerol concentration on the viscosity of the film-forming solution. This is evidenced by the different slope of the secants to the OC_{ch} axis and to the OC_{gl} axis, respectively, in the $O\eta\times OC_{ch}$ plane and in the $O\eta\times OC_{gl}$ plane, respectively. The results indicate that the concentration of chitosan has a greater effect on the viscosity of the film-forming solution than the concentration of glycerol.

Obviously, this is explained by the fact that the rheological behavior of chitosan is characterized by a pseudoplastic flow of chitosan solutions, in which the drop (decrease) in viscosity occurs already at low shear stresses. It can be assumed that this is caused by the destruction of associates of chitosan macromolecules with different lengths of acetyl blocks, which differ in stability. As for dilute solutions, the stable behavior of chitosan solutions is achieved as a result of the destruction of intramolecular hydrogen bonds, while at the same time the viscosity of the system increases due to the strengthening of interchain contacts in the polymer. Research has also shown that chitosan solutions are unstable, their viscosity decreases sharply during the first day, therefore it is recommended to prepare films immediately after preparing chitosan solutions. The results of these studies coincide with literature data [27].

As, based on studies of the film thickness variation depending on its thickness and the viscosity of the film-forming solution, a value of the viscosity coefficient of 2 Pa·s is considered rational, then the rational concentrations of chitosan and glycerin were chosen precisely from the range for which the viscosity coefficient was not less than this is the value. At the same time, an assumption was accepted since the mechanism of interaction between chitosan and glycerin is complex and difficult to predict from the point of view of the rheological properties of the solution they form, rational concentrations of these substances were chosen within the studied ranges.

Thus, rational concentrations of chitosan and glycerin, which form a solution with a dynamic viscosity of 2 Pa·s, were chosen: chitosan – 2.0...2.5 %, glycerin – 1.0...1.5 %.

One of the fundamental issues that need to be solved when designing protective coatings from high-molecular compounds is to provide them with additional antimicrobial properties since all polymers, especially those of natural origin, have insufficient resistance to the action of microorganisms.

The biological activity of the films can be explained by the presence of a positively charged amino group in the chitosan molecule, which is a polycation and has the property of binding to the surface of negatively charged cells. At the same time, there is a loss of the protective mucopolysaccharide layer of the cell wall, disruption of cell division and synthesis of the cell wall.

Studies of the biological activity of films based on chitosan (Tables 3, 4) showed that the most pronounced bacteriostatic effect was found in films on a decoction of yarrow (a significant retention zone was noted on all strains of microorganisms). The greatest antibacterial effect was manifested against strains of *E. Coli*, *S. aureus, C. albicans, Saccharomyces* and *Lactobacillus* (size of zones 20, 16, 19, 18, and 24 mm, respectively). To a lesser extent, the antibacterial effect was revealed against the strains of *B. sereus*, *B. subtilis* (zone size 13 mm) and *P. aeruginosa* (zone size 11 mm). A film sample on a decoction of eucalyptus has worse bactericidal properties and detects such microorganisms as *B. cereus*, *B. subtilis*, *St. aureus*, *C. Albicans*, *Saccharomyces* and *Lactobacillus*. The increased bacteriostatic film on the decoction of yarrow grass is explained by the higher content of tannin and terpene compounds in the decoction, in particular sesquiterpenes (chamazulene, viridiflorol, β-caryophyllene, germacrene D, borneol, 1,8-cineol, terpinen-4-ol, lavenderol acetate, camphor). In the decoction of eucalyptus leaves, monoterpenes (α-pinene, β-pinene, camphene, α-phellandrene) predominate. As is known, monoterpenes are quite volatile substances, unlike sesquiterpenes, which are less susceptible to oxidative processes and less volatile than monoterpenes. Thus, it can be assumed that a part of monoterpenes evaporates during the preparation of films on a decoction of eucalyptus, which reduces its bacteriostatic activity. In addition, tannins have significant bacterial activity against microorganisms. The mechanism of their action involves the folding of the protein on the surface of the microorganism, the formation of a stable insoluble membrane around it, which leads to the termination of its vital activity. Or the solution of tannins slowly penetrates through the shell of microorganisms into the protoplasm and nucleus, concentrating in them, paralyzes vital activity and causes their death as a result. The content of tannins in a decoction of yarrow grass is almost twice as high as in a decoction of eucalyptus leaves, which affects the bacteriostatic effect of the finished film [28].

It was established that with an increase in the concentration of MPR in the decoction on the basis of which the films were made (ratio 3:10, 5:10, 7:10), their bacteriostatic activity decreases. This is due to the high concentration and interaction of the components of the decoction, in particular tannins and terpenoids, which inhibits (and sometimes completely prevents) the diffusion of the components into the nutrient medium.

A comparative analysis of films made on MPR decoctions of different concentrations made it possible to establish that the rational ratio of MPR:water during the preparation of the decoction is 1:10, which makes it possible to maximally extract substances that have a bacteriostatic effect.

During the evaluation of the toxicity results, it was found that there was no lysis on the nutrient medium around the control film and test samples. Therefore, none of the studied samples showed toxic properties.

Our set of studies has made it possible to establish a rational recipe composition of films based on chitosan (%): chitosan – 2.0...2.5, glycerin – 1.0...1.5, decoction of yarrow grass – 96...97 (for the MPR:water ratio – 1:10).

Chitosan films have high vapor permeability and sorption capacity [29], which leads to unlimited swelling followed by dissolution in a moist environment. This is due to the presence of hydroxyl groups (–OH) in the chitosan molecule, which easily attract water molecules. Since many food products are characterized by a high moisture content (70...80 %), it is important that the biodegradable packaging material retains its properties throughout the entire period of use. In this regard, the designed films need to be modified to obtain the necessary characteristics.

Analyzing the data in Fig. 4, it should be concluded that the alkali-treated films have a water-absorbing capacity almost 5 times lower than untreated samples. Therefore, the lower degree of moisture absorption of chitosan films in the basic form compared to the salt form is an indisputable advantage because it provides the possibility of a longer stay of the film in contact with the food product without destruction. Thus, by changing the conditions for the preparation of the films, or using alkali modification, it is possible to obtain polymer coatings with significantly different rates of moisture absorption.

Thermal modification, which is carried out by heating the film for a certain time at a temperature above 100 °C, creates conditions for a certain stabilization of the properties of the film, but such modification leads to the loss of the material's flexible properties and the appearance of brittleness.

Another method of modification is the treatment of chitosan films with chemicals, which leads to the formation of a water-insoluble polyelectrolyte complex and surface modification, which is accompanied by the loss of solubility of the films in water [30]. Sulfation was used in the work.

One can see from Fig. 5 that the spin-spin relaxation time increases with the duration of exposure of film samples in sulfate solutions. A shorter spin-spin relaxation time is characteristic of the films modified with the Na_2SO_4 solution, which indicates the blocking of a larger number of reactive amino groups than in the case of the $ZnSO_4$ solution. At the stage of modification of films lasting 5...15 min, the time of spin-spin relaxation increases rapidly, then the duration of holding in solutions does not affect the relaxation parameters.

Based on the research results shown in Fig. 6, it was determined that it is advisable to use a Na_2SO_4 solution with a modification time of 30...60 min, which gives the lowest spin-spin relaxation time. This time is enough to block the free amino groups of chitosan, which cause water dissolution. In addition, the results show that the modification allows blocking free amino groups of chitosan and stabilizing the film, that is, reducing its sorption properties.

Thus, comparing modified chitosan films with unmodified ones that immediately dissolve in water, one should note the positive effect that makes it possible to obtain polymer coatings with given properties, in particular, insolubility in water.

Polymeric materials must have appropriate mechanical strength. Therefore, in the work, the operational characteristics were investigated, namely, the mechanical strength of new chitosan films was determined in order to determine the possibility of their use as part of a combined package.

Our data (Table 5) indicate that under the action of the modifier (1 % Na_2SO_4 solution) bonds are formed between amino and sulfate groups, which act as a stress concentrator that disrupts the intermolecular interaction of the polymer, contributing to a decrease in strength and elasticity of the obtained films.

Since chitosan-based films are not intended for independent use as a packaging material, our results in terms of physical and mechanical indicators are quite sufficient for the formation of a protective layer as part of a combined package.

A peculiarity of our research is that the solvent of chitosan (water-soluble) was not water but a decoction of yarrow grass, which contains many tannins and terpene compounds. The best ratio of chitosan:decoction, which leads to the maximum extraction of these substances, is 1:10.

In addition, unlike [7, 9, 11], we studied the bacteriostatic properties of the films on the standard of microorganism strains. The following strains were identified: *Escherichia coli* (ATCC 8739), *Bacillus cereus* (ATCC 10702), *Bacillus subtilis* (ATCC 6633), *Pseudomonas aeruginosa* (ATCC 9027), *Staphylococcus aureus* (ATCC 6538-P), *Candida albicans* (ATCC 885-653), *Saccharomyces* and *Lactobacillus*. This makes it possible to use the designed films for a wider range of food products and for their longer storage without preservatives.

The limitations of the work are that in order to obtain a film with rational indicators (bacteriostaticity, mechanical characteristics), it is necessary to strictly observe the ratio of the components of the chitosan solution.

As a drawback of the work, one should note that the modification of hydrochloric acid, although it stabilizes the water-absorbing capacity of the films, leads to a decrease in their strength (by 12.5 %) and elastic (by 53.3 %) characteristics. This makes it impossible to use them subsequently in an independent form but only as part of the combined packaging material. To provide water-repellent properties, it is necessary to look for other possibilities.

Thus, the designed chitosan film could be used as part of a combined packaging material for a wide range of food products with the possibility of long-term storage. The further development of our research will involve designing a combined packaging material using a new chitosan film. In addition, further research could aim at finding opportunities to increase the strength and elastic characteristics of chitosan films in order to use them as an independent packaging material.

7. Conclusions

1. Our studies of different thicknesses of films obtained from film-forming solutions with different concentrations of chitosan and glycerin have established that to form a uniform film, the index of different thicknesses of which will not exceed the permissible 20 %, one should choose a film-forming solution with a dynamic viscosity of no more than 2 Pa·s. Using a two-factor experiment, in which the initial factors were the volume concentration of chitosan and glycerin in the solution, it is possible to determine the rational concentrations of chitosan and glycerin, which form a solution with a dynamic viscosity of 2 Pa·s. These values are equal to chitosan – 2.0...2.5 %, glycerin – 1.0...1.5 %. The film, which is obtained with a viscosity coefficient of the film-forming solution of 2 Pa·s, has a thickness of 45...47 nm, which meets the technical requirements for packaging films.

2. It has been shown that the preparation of films from chitosan based on MPR decoctions leads to an increase in their antibacterial efficiency. It was established that the film material based on a decoction of yarrow grass has the highest bacteriostatic properties against test strains of pathogenic microorganisms, fungi, yeasts, and lactic acid microorganisms. Our set of studies have made it possible to obtain a rational recipe composition of films based on chitosan and decoction of yarrow, which has high bacteriostatic properties (%): chitosan – 2.0...2.5, glycerin – 1.0...1.5, decoction of yarrow grass – 96 ...97 (for the MPR:water ratio – 1:10).

3. The designed chitosan films were modified by various methods. It has been proven that the modification with NaOH solution makes it possible to reduce the moisture absorption capacity of the films but does not eliminate it completely. Modification with salts of sulfuric acid ($ZnSO_4$ and Na_2SO_4) makes it possible to stabilize the moisture-absorbing capacity of the films but gives them brittleness and fragility, which makes it possible to subsequently use them only as part of the combined packaging material.

4. It was established that the destructive stress of modified films is 14.0 MPa, which exceeds the permissible minimum, which should be at least 13.7 MPa for packaging films. The film could be used as part of the combined packaging material.

Conflicts of interest

The authors declare that they have no conflicts of interest in relation to the current study, including financial, personal, authorship, or any other, that could affect the study, as well as the results reported in this paper.

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Data availability

All data are available, either in numerical or graphical form, in the main text of the manuscript.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the current work.

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