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Polyfunctional Pectin Films for Food and Medical Applications

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ABSTRACT

Considering the importance in developing protective coatings to preserve the quality of food during the its shelf life and/or to use for medicinal purpose, the aim of the work was to develop novel film structures based on three pectins (Apple, Beet and Citrus) and their combination for food and medical application.

This work shows that high-purity beet pectin has preferential functional properties in comparison with apple and citrus pectins. In addition, the peculiarities of film formation are due to the physicochemical properties of pectins and substances combined with them, the mechanism of their interaction, the quantitative ratio of the components of the mixture, the optimization of which allows the preparation of films of a given structure and purpose. The developed films show interesting properties for food and medical purposes and will be tested in practical applications in food packaging and medical purpose.

Key words: pectin, film structures, film formation, polyfunctional purpose, complexing properties, antioxidant properties, antibacterial properties, food industry.

1. INTRODUCTION

One of the innovative trends in the food and medical industry is the development of new materials based on natural polymers [1]. In this context it is relevant to create fundamentally new biodegradable protective coatings based on biopolymers that can provide: effective protection of food (from microbial lesions, losses during production, storage, transportation and sale), preferential healing of wound surfaces, burns and ulcers.

Pectin is a branched heteropolysaccharide consisting of long-chain galacturonan segments and other neutral sugars such as rhamnose, arabinose, galactose, and xylose. Pectin substances are natural film [2] forming biopolymers with a wide range of beneficial effects (including sorption, antibacterial and antioxidant) on the human body [1, 3-7] and they provide interest as sources on the base of which film structures can be constructed. They form matrices with celluloses and hemicelluloses and contribute to the cell structure. Commercial pectin is commonly extracted from citrus and apple fruit and has wide application as emulsifier, gelling agent, thickener, stabilizer, and can also be used as an important ingredient in functional foods. In recent past, a new application envisaged for pectin polymers as edible films or coating. These films act as natural barrier for exchange of moisture, gases, lipids, and volatiles between food and environment, and protect fruits and vegetable from microbial contamination. The degree of esterification of pectin and other structural modifications defines the functional properties.

Pectin has been shown to strongly interact with proteins under different conditions. At pH values below their pI, they form strong complexes with soy proteins [8] sodium caseinate [9] (happy), beta-lactoglobulin [10], gelatin [11] and pea proteins [12]. The effect of low methoxyl- pectin complexes with lysozyme on the antimicrobial and physiochemical properties has also been reported [13]. A comprehensive review of the role of pectin in food processing and food packaging has been described by T. Vanitha and M. Khan [14].

It has been reported a method of obtaining food collagen film [15] for packaging meat dishes to reduce natural product loss and prevent microbiological damage using the method of heat treatment of semi-finished products based on a molding dispersion containing 2 to 6% of dry collagen, protective film-forming composition of chitosan, single tribasic organic acid, edible gelatin, water.

A composition for coating poultry carcasses [16] was developed based on an aqueous solution of a collagen-containing extract, acetic acid and glycerin.

A method has been developed to accelerate the formation of a coating on the surface of frozen fish blocks when treated

with a polymer composition of an aqueous solution of polyvinyl alcohol and modifiers (hydroxypropyl cellulose or hydroxyethyl cellulose) and then it should be removed from the surface before using fish [17].

Formulations have been developed to protect the surface of hard cheeses from microbiological spoilage based on a solution of sodium alginate, chitosan and methylcellulose, allowing them to increase their shelf life, prevent the development of mold fungi and other undesirable microflora on the surface. Essential oils of rosemary and oregano, as well as chitosan, were used as antimicrobial agents [18].

A protective composition has been developed that preserves the commodity qualities of fruits and vegetables during long-term storage, contains iron-containing polyacrylic acid, polyvinyl alcohol and water, and it is applied by immersing products in it [19].

Polymers of plant origin in the form of films are used to apply them to wounds and burn surfaces, as well as to create medical adhesives for human tissues, blood vessels and the intestines [1, 20-24]. Biopolymer compositions are effective adsorbents, especially for heavy metals.

An antiseptic pectin-containing film structure based on low-esterified beet pectin used in the treatment of purulent-inflammatory diseases and trophic ulcers was obtained [25].

A polymer composition from collagen and apple pectin for the treatment of infected burn wounds of IIIA grade has been reported [26]. This method increases the effectiveness of treatment by reducing the number and species diversity of the microflora and at the same time accelerating wound healing.

The objective of this study was to determine the functional properties of pectin substances such as complexing or binding ability, antibacterial activity, and antioxidant activity. These information allow to understand the direction of use of pectin substances for food and medical purposes.

2. EXPERIMENTAL (MATERIALS AND METHODS)

2.1 Materials

- Dry apple pectin (A), low degree of esterification, was obtained from “Aidigo” company (China)
- Beet pectin (B), natural, low degree of esterification, was obtained from “Danisko” company (Czech Republic)
- Citrus pectin (C), low degree of esterification, was obtained from “Danisko” company (Czech Republic)

The above three pectin’s were used as single films or to form the composite (AB, AC, CB) in the ratio 1:1.

- Clinical strains of microorganisms (*E. coli S. aureus P. mirabilis E. faecalis P. aeruginosa*) provided by the Center for Hygiene and Epidemiology of the Republic of Adygea (Russia).
- Glycerin and vegetable oil were obtained from Sigma;
- Chamomile extract, used as antioxidant, was prepared by pouring of 4.5 g of chamomile herb in 100 ml of boiling water. After 30 minutes was filtered on Whatman grade 3 filter paper.
- Chlorhexidine 0.05%, used as antiseptic, was prepared by dilution from Sigma Aldrich solution 99.5%.
- Collagen, (derived from fish raw materials) was obtained from the laboratory of the Department of Animal Products Technology of Voronezh (Russia) State University (Patent 2614273)
- Saline solution 0.9%, water was prepared by dilution with water from standard solution (15%) obtained from Sigma Aldrich.
- Food coloring: carmoisine and Patent blue (food coloring of brand KVITEN PE “Confectionery jewelry factory” (Ukraine)

2.1 Film structure preparation

To obtain a film structure, 3 g of pectin was mixed with 50 ml of water extract of chamomile (2-4%) or collagen dispersion (2%), homogenized and then left for 30 minutes at room temperature, and finally filled to 100 ml volume.

The film was formed by spreading in a Petri dishes (100 x 15 mm) and left to dry at temperature of 20-50 ° C.

2.2 Characterization of the pectin films

2.2.1 Appearance of Films

Films were evaluated visually for appearance such as smooth surface, transparency, color etc.

2.2.2 Film Forming Capacity

It is ability of film formers to form film with desired properties. Relative film forming ability was determined on the basis of rating given to each film. Rating was given out of +++++ such as poor (+), average (++), good (+++), best (++++) and excellent (+++++).

2.2.3 Film thickness

Film thickness was determined using an electron micrometer (Electronic Digital micrometer 0-25 mm) (China) with an accuracy of 0.001 mm at six different strategic locations. The average value and standard deviations were reported. The standard range for film thickness should not be less than 5 %. This is essential to assure uniformity in the thickness of the film.

2.2.4 Mass of the film

The mass of the film sample was measured by using an analytical balance LTE-150 (Gosmetr, Russia) with an accuracy of 1.0 mg. Weight variation was studied by individually weighing 6 randomly selected film strips. Average weight of films calculated. The weight of each film should not deviate significantly from average weight.

2.2.5 Residual moisture content

The residual moisture content in the film samples was determined in accordance with the method of Kchaou H. et al. [27]: 100 mg of the film was dried at a temperature of 105 ° C to constant weight in an oven (FD 115 Binder, Germany).

2.2.6 Film solubility

The solubility of the films was determined by the method of Gennadios [28], A. et al. (1998): samples of films (2 cm × 5 cm) were weighed and dissolved in 50 ml of distilled water with stirring in an ES-20/60 Shaker Incubator (Biosan) at a frequency of 250 rpm and a temperature of 25 ° C for 24 hours. After centrifugation the solutions using a Universal 320 tabletop centrifuge (HETTICH, 8000 rpm), the precipitate was dried at 105 ºC to constant weight in an oven.

2.2.7 Light transmission and transparency

The light transmission and transparency of the films in accordance with the method of Halim, A.L.A. et al [29] was determined on a spectrophotometer SF-102 (IFP, Russia) in the wavelength range from 200 to 800 nm.

2.2.8 Film structure

The study of the film structures was carried out on an XL-30 scanning (scanning electron microscope) with a lanthanum-hexaboride cathode.

2.2.9 Antibacterial activity

Antimicrobial activity of six pectins were investigated against *E. coli, S. aureus P. mirabilis, E. faecalis P. aeruginosa*, using agar disc diffusion technique.

The antibacterial potency of pectin was evaluated using five bacterial strains causing food poisoning diseases and /or medical interest. Two strains of Gram positive (*Staphylococcus* aureus and *Enterococcus faecalis*) and three Gram negative strains , *Escherichia* coli, *P. mirabilis* and *Pseudomonas* aeruginosa. To determine the zone of growth inhibition of microorganisms, a suspension of bacterial cells was prepared on physiological saline solution. A fresh culture of microorganisms on dense nutrient agar was used.

Drops of pectin solutions were applied to the seeded surface with a sterile pipette and were kept in a thermostat for 24 hours. Evaluation of the antibacterial activity of pectin-containing solutions was carried out by zones of growth inhibition of the studied microorganisms using a caliper as measuring device.

Turbidity

Turbidity was measured by visual comparison with standard samples of bacterial suspension kit furnished by LLC "ORMET", Ekaterinburg.

2.2.10 Antioxidant activity

The antioxidant activity of pectin membranes was evaluated by an amperometric method using quercetin as reference. The analyzer Tsevt Yauza 01.AA (Khimavtomatika, Russia) was operating at the potential of 800 mV in a flow system. The average of five replicates was taken as analytical signal. The antioxidant capacity (mg/L) was calculated by the calibration graph of quercetin (y= 0.0013x -01484) where x is the analytical signal.

2.2.11. Binding capacity

The determination of the complexing (binding) capacity of solutions (1%) of pectin substances was carried out by the titrimetric method.

Fifty ml of pectin solution was placed in a heat-resistant beaker and boiled in a water bath for 30 minutes.

The process of determination of binding capacity was carried out in 3 replicates. A working solution of lead acetate with 0.1 M in an amount of 30 ml was placed in a flask with a conical bottom of 250 ml to which 25 ml of pectin-containing solution was added. It was shaken for 30 minutes and was divided by centrifuge into fractions within 9-14 minutes unless it reached a negative reaction to lead ions with potassium bichromate. The precipitate was washed with distilled water.

The centrifugate and washings were combined and made up to the mark with distilled water in a 250 ml flask. 2 ml of buffer solution (pH 9-10), 10 ml aliquot and Eriochrome Black T (at the tip of a spatula) heated to 40 ° C were combined. The solution was titrated with 0.01 mol / dm3 solution of ethylene diamine tetra-acetate until its transition from violet to blue color.

The binding capacity was calculated as the percentage of free lead found in solution with respect the total lead added.

3. RESULTS AND DISCUSSION

3.1. The complexation ability

The degree of esterification of pectin is of overriding importance in the manifestation of complexing ability. Beet pectin, which has a natural low degree of esterification and structure, exhibits a high complexing ability in comparison with the modified low esterified pectin (apple, citrus) and it is able to show this ability even when in combination with apple and citrus pectin.

The complexing ability of various types of pectin substances and their combinations can be arranged in a row in descending order of values: AB (96.9%)> CB (90.8%)> B (88.7%)> AC (84.7%)> A (73.5%)> C (67.4%).

In order to determine the relationship between factors affecting the complexing capacity of the membrane a design of experiment (DOE) using Statistica 10 was performed. The variables were the mass fraction of pectin substances, the pH value of the medium, and the duration of the "metal (Pb2 +) - pectin" contact. In this work, Box-Benkhen design (BBD) with three factors in three levels was adopted to determine the influence of the mass fraction, pH values and the contact time, designated as independent parameters, on the complexation capacity of pectin. Variables are reported in table 1.

In the BBD design, a quadratic polynomial response equation was established and used to correlate and describe the relationship between the independent parameters and the response.

The value of the fit and the importance of the polynomial model can be recognized by the coefficient of determination, F and P value, which were investigated using ANOVA. The results of ANOVA for the six responses showed that the quadratic model p-values were less than 0.05 thus models are significant according to a 95% confidence interval.

Table 1: The experimental design levels of the Box-Behnken (BBD) method and the independent variables

	Factors	Units	Factor levels		
			max	medium	min
			+1	0	-1
Amount of protein	X_1	%	1,5	1	0,5
pH of the medium	X_2	pH	4	3	2
Time of contact metal-pectin	X_3	min	60	40	20

The second-degree polynomial functions that modeled the complexation ability process are shown in the following equations:

$A = +67.29 + 1.97\ Mass + 2.21pH + 2.79\ time + 0.57\ Mass \times pH - 0.54\ Mass \times time + 0.65\ pH \times time + 1.42\ Mass^2 - 1.75\ pH^2 + 0.22\ time^2$

$B = +61.96 + 1.86\ Mass + 2.05\ pH + 1.89\ time + 0.76\ Mass \times pH - 1.19\ Mass \times time + 0.56\ pH \times time + 1$

$C = +84.14 + 0.94 Mass + 1.21\ pH + 1.80\ time + 0.45\ Mass \times pH - 0.21\ Mass \times time + 0.41\ pH \times time + 1.25\ Mass^2 - 0.29\ pH^2 - 0.12\ time^2$

$AB = +78.72 + 1.34\ Mass + 2.01pH + 1.92\ time + 0.73\ Mass \times pH - 0.41 Mass \times time + 0.49\ B \times C + 1.49\ Mass^2 - 1.26 \times pH^2 + 1.16 \times time^2$

$AC = +90.13 + 1.63\ Mass + 1.81\ pH + 1.92\ time + 0.65\ Mass \times pH - 0.24\ Mass \times time + 0.46000\ pH \times time + 2.21\ \times Mass^2 - 0.49\ pH^2 + 0.18 \times time^2$

$CB = +78.33 + 1.31\ Mass + 2.02\ pH + 2.56\ time + 0.77\ Mass \times pH - 0.51\ Mass \times time + 0.53\ pH * time + 1.95\ Mass^2 - 1.03\ pH^2 + 0.29\ time^2$

These equations show that each output is provided by a combination of second order polynomials. The latter are formed, in turn, by combination of input variables.

3.2. The antibacterial activity

The antibacterial activity of pectin solutions (1%) of apple, citrus, beet, and their combinations was tested in relation to the clinical strains of the microorganisms Escherichia coli, Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa. Results are reported in Tables 2.

Table 2: The results of the study of the antibacterial activity of pectin substances and their combinations at 5 and 10 units of turbidity

Pectin	Clinical strains of microorganisms Microorganism growth retardation, mm							
	E. coli		*P. mirabilis*		*E. faecalis*		*P. aeruginosa*	
Turbidity units	5	10	5	10	5	10	5	10
A	10	4	10	5	0	0	0	0
C	5	0	5	3	5	0	20	5
B	10	5	5	4	15	5	10	0
AC	10	0	10	7	0	0	0	0
AB	20	10	1	8	0	0	5	0
CB	15	0	5	7	0	0	12	3

The data in Tables 2 show that all pectin and their combinations have antibacterial activity against the microorganisms studied, with the exception of Staphylococcus aureus (all pectin) and Enterococcus faecalis (all combinations of pectin), regardless of the concentration of microorganisms.

However, an increase in concentration of microorganisms (from 5 to 10 units of turbidity), a decrease in antibacterial activity was observed, with the exception of beet pectin with respect to the microorganism Enteraococcus faecalis;

A synergic and negative effect was observed for all combined pectins (AB, AC, AD) membranes to the following microorganisms: *Escherichia coli* (5 turbidity units); *Proteus mirabilis* (10 units of turbidity); Enterococcus *faecalis* (5 and 10 units of turbidity); Pseudomonas *aeruginosa* (5 units of turbidity).

3.3. Antioxidant activity

The antioxidant activity of different types of pectin substances and their combinations is reported in table 3.

Table 3: Antioxidant activity of the investigated water pectin solutions (1.5%)

Pectin solutions	Antioxidant activity, mg/cm^3
A	14,98
C	1,22
B	21,59
AC	2,32
AB	17,43
CB	17,08

All the pectin under study shows antioxidant capacity (AA) whereas beet pectin in combination with apple and citrus (AB and CB) shows an increase of their their antioxidant activity. Apple pectin in combination with citrus also increases this indicator, but to a lesser extent than beet.

The level of antioxidant activity can be arranged in descending order: B (21,59) > AB (17,43) > CB (17,08) > A (14,98) > AC (2,32) > C (1,22).

It should be noted that, in contrast to the antioxidant activity, the complexing ability of combinations of pectin with beet is higher than that of pectin without this combination.

The combination A and C shows a very low antioxidant capacity even if A alone has a good antioxidant capacity. This effect is not observed in the combination CB.

3.4. Characteristics of films

Previous study (Patent N°2342955) showed that the optimal conditions for the formation of pectin film structure are: the ratio of components, the homogeneity of the film-forming composition and the drying temperature. Mass and thickness of the films depends on the amount of solution taken for the film formation. However, when adding a dye, the film is formed, but it could not have freely removed from the mold, and when adding vaseline oil, the film is not formed. Each pectin and combinations were submitted to the same process of preparation including all the foreseen supplements (table 3). However, we found that the best film structures were obtained for the sample n. 1 (pectin, water), n. 3 (pectin, infusion of chamomile, vegetable oil), and n. 7 (pectin, collagen, water, glycerin). The sample n.1 was considered as reference.

Table 4: Characteristics of the process of film formation of the obtained film structures

Sample number	Composition for film formation	Film forming capacity	Appearance
1	Pectin, water	+++++	Transparent, smooth, good appearance
2	+ chlorhexidine	+++	Transparent slight rough
3	+ chamomile infusion and vegetable oils	++++++	Transparent, smooth, good appearance
4	+ collagen	------	Does not form film
5	+ saline	++++	Semitransparent, smooth
6	+ camomile infusion	+++	Smooth, trasparent
7	+ collagen and glycerin	+++++	Transparent, smooth, good appearance

Some organoleptic and physicochemical parameters of these film structures are reported in table 5.

Table 5: Physical and chemical indicators of pectin-containing film structures with the best structural characteristics and enhanced composition

Film Samples (Apple)	Thickness mm	Weight g	Residual moisture, %	Water solubility %	Transparency	Apparence	Color	Odor	Consistency
1	0,040	70,6	7,3	99,85 %;	8,95	Dense, uniform	white	No odor	Uniform
3	0,048	58,8	6,9	99,89	8,93	Dense, uniform	cream	No odor	Uniform with inclusion
7	0,242	69,2	6,6	99,79	2,2	Dense, uniform	Light cream	No odor	Uniform with inclusion

Data of Tables 5 show that the organoleptic characteristics of the structures obtained, as a rule, have similar indicators: homogeneity, no damage, and no odor. The average film weight is 66.2 g, and solubility in all samples is over 99%.

The different residual moisture content of the film may be due to additional ingredients in the construction of the film (collagen, glycerin, vegetable oil) and their water-holding ability, but not pectin, since apple pectin with the same water holding power (water-holding capacity) was used in all samples.

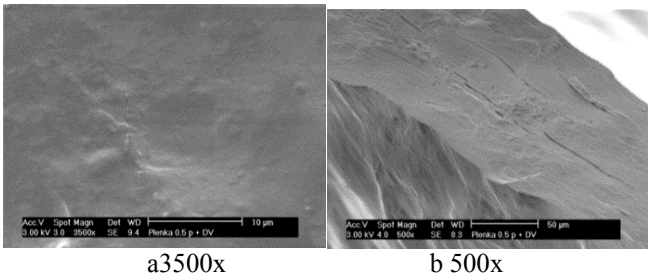
It is shown that the transparency of the films in samples 1, 3 is the same, whereas the smooth fragments in sample 7 have the highest index of the compared options, and the fragment with inclusions has a very low transparency (2.2). This confirms the importance of dissolution of the components in the film-forming mixture.

All films were dried at different temperatures. Obviously, the drying rate of the film formation depends on the temperature. At a temperature of 20 °C, full drying of the film occurs within 48 hours, but increasing temperature up to 60-70 ° C time can be reduced to about 3 hours. For more viscous solution drying time is short and when the solution is homogeneous, the film structure is transparent and brilliant like in processed samples No. 1, 3, and 7 of table 5.

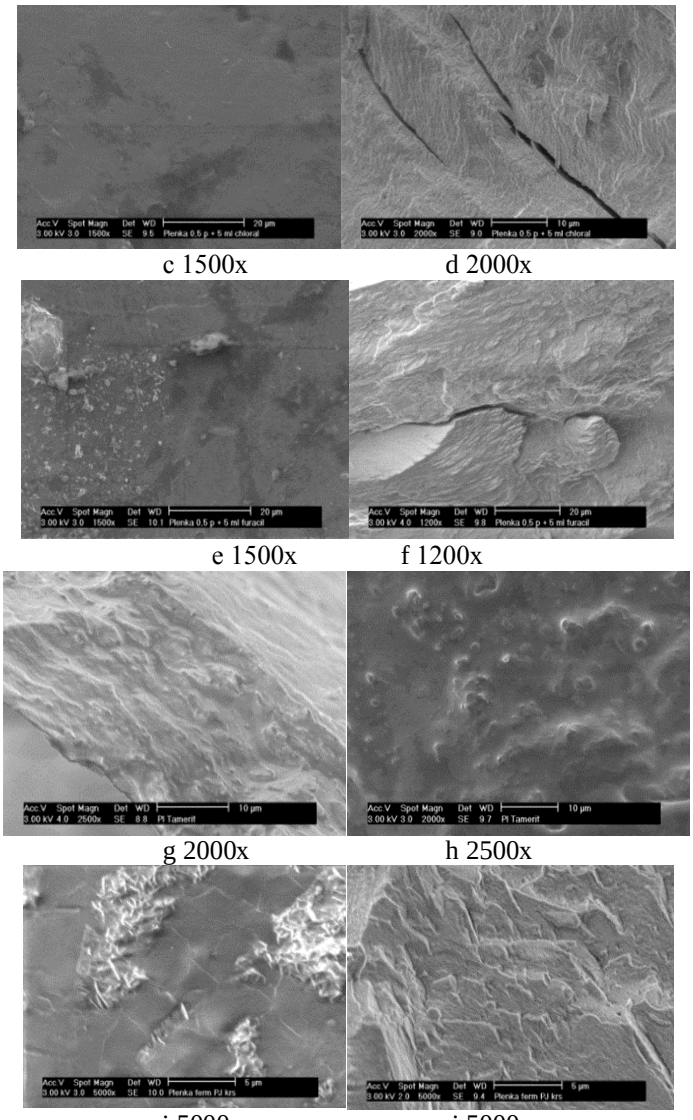
In order to study the influence of the drying temperature on the drying rate of the films, solutions of pectin of the same concentration were applied to the surface of the Petri capsule and dried at a temperature of 40, 50 and 60°C.

It was found that the films from beet pectin at 40 and 60 °C turned out to be brittle, easily broken, and thin but a temperature of 50 °C it remains intact and dense. Same behavior was shown by the apple pectin film.

A comparative analysis shows that there are similar and distinctive morphological features of the structures of the studied objects. The images characterize the topology of the film surface. Films obtained from pectin with distilled water are almost smooth. The microstructure of the film cut shows that there is a specific “pattern” due to the mechanism of interaction of the substances combined with pectin.



a3500x b 500x



c 1500x d 2000x

e 1500x f 1200x

g 2000x h 2500x

i 5000x j 5000x

Figure 3: The microstructure of the pectin films and their slices (accelerating voltage of 3 kV): with distilled water (a, b), chlorhexidine - antiseptic (c, d), furatsilin - antiseptic (e, f), tamerite - immunomodulator (g, h), multienzyme preparation of cattle (i, j);

The membrane characteristics show their potential application in the food industry in shelf life application and in medicine as wound healing or antioxidant protection. The films appear flexible and without being brittle.

Formulation prepared using apple pectin and propylene glycol as plasticizer had good appearance and high folding endurance

4. CONCLUSIONS

In this study, the capability of three pectin and their combination to form film structures to be used in the food and medical sectors was demonstrated. The properties of the membranes produced offer interesting prospects for the use of safe protecting coatings of food to increase the shelf life or in medicine as wound protection. Furthermore, the membrane are inexpensive easy to prepare and present satisfactory antibacterial and antioxidant properties. Work is in progress for their testing in medical situation in collaboration with the Medical Institute of Maikop State Technological University.

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