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Влияние различных видов пектина на интенсивность роста штаммов *Lactococcus lactis* и *Lactiplantibacillus plantarum* и сохранение жизнеспособности микроорганизмов при инкапсулировании

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Аннотация. ***Введение.*** В статье представлено описание влияния различных видов пектина на интенсивность роста пробиотических микроорганизмов и эффективность их защиты при инкапсулировании. Использование полисахаридных матриц для микрокапсулирования рассматривается как перспективный способ повышения стабильности пробиотиков в процессе хранения и применения в составе функциональных продуктов питания**.** Целью исследования являлась оценка влияния различных типов пектина на рост штаммов *Lactococcus lactis* и *Lactiplantibacillus plantarum*, а также определение эффективности пектиновых матриц для сохранения жизнеспособности микроорганизмов при микрокапсулировании. ***Материалы и методы.*** Исследование построено на анализе интенсивности роста пробиотических культур в питательных средах с добавлением различных видов пектина, а также на оценке выживаемости микроорганизмов после инкапсулирования и в процессе хранения. Жизнеспособность клеток определяли микробиологическими методами с подсчётом колониеобразующих единиц и сравнением показателей сохранности пробиотических культур в различных пектиновых матрицах**.** ***Результаты и обсуждение.*** В ходе работы установлено, что использование пектинов в качестве матриц для микрокапсулирования способствует повышению стабильности пробиотических культур. Наиболее выраженные защитные свойства продемонстрировал цитрусовый пектин, обеспечивший наибольшую сохранность клеток исследуемых микроорганизмов в процессе хранения. Показано, что структурные особенности и степень метоксилирования пектина оказывают значительное влияние на эффективность инкапсуляции и устойчивость пробиотических клеток. ***Заключение.*** По итогам проведённого исследования установлено, что микрокапсулирование пробиотических микроорганизмов в матрицы на основе пектинов позволяет существенно повысить их стабильность и выживаемость в процессе хранения. Наиболее эффективными защитными свойствами обладали микрокапсулы на основе цитрусового пектина, обеспечившие сохранность *Lactococcus lactis* и *Lactiplantibacillus plantarum* на уровне 87,41% и 88,81% соответственно после 8 недель хранения. Полученные результаты подтверждают перспективность использования пектиновых полисахаридов для разработки биологически активных добавок и функциональных продуктов питания с пролонгированным сроком хранения и высокой биологической ценностью.

Ключевые слова: пектин, пробиотические микроорганизмы, жизнеспособность, хранение, полисахариды, стабилизация, *Lactiplantibacillus plantarum, Lactococcus lactis*

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Research article

Effect of different types of pectin on the growth rate of *Lactococcus lactis* and *Lactiplantibacillus plantarum* strains and the preservation of microbial viability during encapsulation

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Abstract. ***Introduction.*** The article describes the effect of various types of pectin on the growth rate of probiotic microorganisms and the effectiveness of their protection during encapsulation. The use of polysaccharide matrices for microcapsulation is considered as a promising way to increase the stability of probiotics during storage and use in functional foods. The aim of the study was to evaluate the effect of various types of pectin on the growth of Lactococcus lactis and Lactiplantibacillus plantarum strains, as well as to determine the effectiveness of pectin matrices for maintaining the viability of microorganisms during microcapsulation. ***Materials and methods.*** The study is based on the analysis of the growth rate of probiotic cultures in nutrient media with the addition of various types of pectin, as well as on the assessment of the survival of microorganisms after encapsulation and during storage. Cell viability was determined by microbiological methods with the calculation of colony-forming units and the comparison of the preservation parameters of probiotic cultures in various pectin matrices. ***Results and discussion***. In the course of the work, it was found that use of pectins as matrices for microcapsulation helps to increase the stability of probiotic cultures. Citrus pectin demonstrated the most pronounced protective properties, which ensured the greatest preservation of the cells of the studied microorganisms during storage. It has been shown that the structural features and the degree of pectin methoxylation have a significant effect on the encapsulation efficiency and stability of probiotic cells. ***Conclusion.*** Based on the results of the study, it was found that the microcapsulation of probiotic microorganisms into pectin-based matrices can significantly increase their stability and survival during storage. Microcapsules based on citrus pectin had the most effective protective properties, which ensured the preservation of Lactococcus lactis and Lactiplantibacillus plantarum at the level of 87.41% and 88.81%, respectively, after 8 weeks of storage. Results obtained confirm the promising use of pectin polysaccharides for the development of biologically active additives and functional food products with a prolonged shelf life and high biological value.

Keywords: pectin, probiotic microorganisms, viability, storage, polysaccharides, stabilization, Lactiplantibacillus plantarum, Lactococcus lactis

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Introduction. Modern developments in the food industry are aimed at creating functional products with pronounced physiological effects and the ability to support human health. One of the most promising areas in this field is the use of probiotic microorganisms, the effectiveness of which is directly related to their viability and activity during storage and after consumption [1,2]. However, during the production of probiotic products, a significant decrease in the number of viable cells is observed due to adverse environmental factors, including fluctuations in temperature, humidity, pH, and the presence of oxygen [3, 4].

To enhance the resistance of probiotic microorganisms to stressful conditions, the use of natural polysaccharides, particularly pectins, is being actively studied. These polysaccharides possess high biocompatibility, safety, and the ability to form gel-like matrices that protect cells from destruction [5, 6]. Pectins differ in chemical structure, degree of methoxylation, and source, which determines their functional properties, including interaction with the cell surface and the formation of stable microcapsules [7, 8].

A number of studies have shown that the use of pectin as a matrix for encapsulating probiotics not only increases their resistance to the acidic environment of the stomach and storage conditions, but can also enhance cell growth activity due to a prebiotic effect [9–11]. In this regard, the study of the effect of different types of pectin on the growth rate of probiotic strains *Lactococcus lactis* and *Lactiplantibacillus plantarum*, as well as on the preservation of their viability during encapsulation, is of scientific and practical interest.

In Russia, research into the use of pectin for the encapsulation and stabilization of probiotics is actively developing [12]. Of particular interest are domestically produced pectins from beet and apple raw materials, which are characterized by low cost and high efficiency in the formation of gel structures [14, 15]. Thus, a relevant area is studying the effect of various types of pectin on the preservation of the viability of probiotic microorganisms during storage, which has practical significance for the development of new functional foods and probiotic supplements [16].

Materials and methods. Research objects. The following probiotic cultures were used as objects of study:

- Mixture of Lactococcus lactis subsp. lactis, Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. diacetylactis (sourdough for cheese). Manufacturer: Federal State Budgetary Scientific Institution "Experimental Biofactory", Russia.
- Lactobacillus plantarum (protective starter BK-Uglich-P). Manufacturer: Experimental Biofactory, Russia.

MRS broth and MRS agar were used as nutrient media. Manufacturer: Federal State Budgetary Scientific Institution “State Scientific Center for Applied Microbiology and Biotechnology”, Russia.

Encapsulation materials. Various types of pectin were used as a polymer carrier for encapsulation:

- apple pectin (highly methoxylated, HM); Manufacturer: Stoing Trading House LLC, Russia;
- citrus pectin (low methoxylated, LM); Manufacturer: Stoing Trading House LLC, Russia;
- beet pectin (low methoxylated, LM); Manufacturer: PharmMix LLC, Russia;
- tomato pectin (low methoxylated, LM); Manufacturer: PharmMix LLC, Russia;
- amidated low-esterified pectin; Manufacturer: Part of the Strength LLC, Russia.

$CaCl_2$ manufactured by Stoing, Russia, was used as a hardener . Sodium citrate manufactured by Stoing, Russia, was used to release microorganisms from the microcapsules.

Conditions for culturing probiotic microorganisms. Probiotic microorganisms were cultured in pectin solutions of varying concentrations (0.5–2.5%) at 37°C for 48 hours. Unencapsulated probiotic cultures, cultured in MRS broth under similar conditions, served as controls.

After cultivation, 5 ml of pectin solution samples were treated with a 2% sodium citrate solution and then centrifuged at 12°C for 5 minutes. The centrifugation procedure was repeated three times until a pure probiotic sediment was obtained. The method for isolating microorganisms from the pectin matrix was consistent with published data [13].

Encapsulation of probiotic microorganisms. Probiotic encapsulation was achieved using ionic gelation. Pectin solutions containing probiotic cultures were injected with an insulin syringe into a curing agent solution – a 2% $CaCl_2$ solution. The resulting microcapsules were soaked in saline and stored at 5°C.

The viability of probiotics in microcapsules was analyzed after 12 hours, 24 hours, 1 week, 4 weeks and 8 weeks of storage.

Release of microorganisms from microcapsules. The release of probiotic microorganisms from pectin microcapsules was carried out by treating the capsules with a 2% sodium citrate solution, after which a quantitative analysis of viable cells was carried out.

Determination of the concentration of probiotic microorganisms. The concentration of probiotics was determined using a densitometer, as well as by counting colony-forming units (CFU/g) on MRS-agar nutrient medium.

For this purpose, serial tenfold dilutions of the test samples were performed, after which 1 ml of the suspension was added to sterile Petri dishes containing melted and cooled MRS agar to 37°C. Incubation was carried out at 37°C for 48 hours, after which the number of colonies grown was counted, taking into account the dilution, and the viable cell concentration (CFU/g) was calculated.

The results are presented as M ± m, where *M* is the mean value and *m* is the standard error of the mean.

Evaluation of probiotic growth efficiency. To assess the growth efficiency of probiotic microorganisms, the generally accepted relative growth index was used [14], calculated using the formula:

$$E = \frac{N1}{N0} * 100\%, \quad (1)$$

where N_0 is the concentration of probiotic microorganisms in 1 g before the start of cultivation;

N_1 is the concentration of probiotic microorganisms in 1 g after the end of cultivation.

Calculation of losses and encapsulation efficiency. Losses of probiotic microorganisms during storage were calculated using the formula:

$$P = 100*(1 - \frac{A1}{A}) \% \quad (2)$$

where P is the loss (%), A is the initial value of CFU/ g, A_1 – Final CFU/g value after completion of the storage process.

The following formula was used to calculate the encapsulation efficiency:

$$EE = 100\% - P \quad (3)$$

in accordance with the methodological approaches used in studies on microencapsulation of probiotics [15] .

Statistical data processing. All experimental studies were performed in triplicate. Results are presented as the arithmetic mean with the standard error of the mean. Statistical processing of the experimental data was performed using *Statistica 10* and *Microsoft Excel software packages* , employing analysis of variance methods. The statistical significance of differences in the "efficacy" indicator, expressed as a percentage, was assessed using the χ^2 (chi-square) test. Differences between the compared groups were considered significant at a significance level of $p < 0.05$.

Results and discussion. Probiotic microorganisms were cultured simultaneously on MRS nutrient medium and in pectin solutions; the initial concentration of probiotic microorganisms was $1*10^8$ CFU/g. Table 1 presents the results of a study of the growth rate of probiotic microorganisms during cultivation on various types of pectin. The assessment was carried out using densitometry data, which allowed us to determine the dynamics of biomass accumulation during fermentation. Upon completion of fermentation, tenfold dilutions of each replicate were cultured to determine the viability and activity of the probiotic cultures. The samples were incubated in a thermostat at 37°C for 48 h, after which the number of colony-forming units (CFU) per 1 g of sample was counted. For each variant, the arithmetic mean CFU value was calculated, reflecting the growth rate and activity of the probiotic microorganisms. Based on the obtained data, an integrated assessment of the growth intensity of cultures on various types of pectins was made and the growth efficiency of microorganisms was calculated relative to the initial indicators; the results are presented in Table 1.

Table 1. Evaluation of the growth rate of probiotic microorganisms

Nutrient medium	Strain	Pectin concentration, %	Average CFU/g value according to densitometry indicators	Average CFU/g value based on 10th dilution	E, %
MRS – broth	*L. lactis*	0	(4.51±14)*10 ^{8}	(4.8±15)*10 ^{8}	451
	L. planturum		(8.13±0.24)*10 ^{8}	(8.1±0.24)*10 ^{8}	813
Apple pectin (HM)	*L. lactis*	0.5	(2.3±0.07)*10 ^{8}	(2.2±0.06)*10 ^{8}	230
		1	(3.83±0.11)*10 ^{8}	(3.9±0.13)*10 ^{8}	383
		1.5	(4.44±0.13)*10 ^{8}	(4.25±0.12)*10 ^{8}	444
		2.0	(5.38±0.16)*10 ^{8}	(5.25±0.15)*10 ^{8}	538
		2.5	(5.23±0.16)*10 ^{8}	(5.15±0.14)*10 ^{8}	523
	L. planturum	0.5	(3.6±0.11)*10 ^{8}	(3.9±0.12)*10 ^{8}	360
		1	(5.17±0.16)*10 ^{8}	(5.1±0.15)*10 ^{8}	517
		1.5	(6.22±0.19)*10 ^{8}	(6.15±0.18)*10 ^{8}	622
		2.0	(7.73±0.23)*10 ^{8}	(7.7±0.22)*10 ^{8}	773
		2.5	(7.68±0.23)*10 ^{8}	(7.7±0.21)*10 ^{8}	768
Citrus pectin (HM)	*L. lactis*	0.5	(2.62±0.08)*10 ^{8}	(2.7±0.09)*10 ^{8}	262
		1	(4.08±0.12)*10 ^{8}	(4.0±0.11)*10 ^{8}	408
		1.5	(5.48±0.16)*10 ^{8}	(5.25±0.15)*10 ^{8}	548
		2.0	(6.02±0.18)*10 ^{8}	(6.0±0.17)*10 ^{8}	602
		2.5	(5.98±0.18)*10 ^{8}	(6.0±0.17)*10 ^{8}	598
	L. planturum	0.5	(3.31±0.1)*10 ^{8}	(3.35±0.11)*10 ^{8}	331
		1	(6.24±0.19)*10 ^{8}	(6.15±0.17)*10 ^{8}	624
		1.5	(7.68±0.23)*10 ^{8}	(7.75±0.24)*10 ^{8}	768
		2.0	(9.11±0.27)*10 ^{8}	(9.1±0.25)*10 ^{8}	911
		2.5	(9.0±0.27)*10 ^{8}	(9.0±0.26)*10 ^{8}	900
Beet pectin (LM)	*L. lactis*	0.5	(2.83±0.08)*10 ^{8}	(2.9 ± 0.09)*10 ^{8}	283
		1	(3.41±0.1)*10 ^{8}	(3.3±0.11)*10 ^{8}	341
		1.5	(3.95±0.12)*10 ^{8}	(4.0±0.12)*10 ^{8}	395
		2.0	(4.38±0.13)*10 ^{8}	(4.25±0.13)*10 ^{8}	438
		2.5	(4.35±0.13)*10 ^{8}	(4.3±0.13)*10 ^{8}	435
	L. planturum	0.5	(3±0.09)*10 ^{8}	(3.0±0.09)*10 ^{8}	300
		1	(4.21±0.12*10 ^{8}	(4.15±0.12*10 ^{8}	421
		1.5	(4.82±0.14)*10 ^{8}	(4.9±0.15)*10 ^{8}	482
		2.0	(5.33±0.16)*10 ^{8}	(5.2±0.14)*10 ^{8}	533
		2.5	(5.28±0.16)*10 ^{8}	(5.25±0.15)*10 ^{8}	528
Tomato pectin (LM)	*L. lactis*	0.5	(1.6±0.05)*10 ^{8}	(1.75±0.06)*10 ^{8}	160
		1	(2.31±0.07)*10 ^{8}	(2.2±0.07)*10 ^{8}	231
		1.5	(2.78±0.08)*10 ^{8}	(2.8±0.09)*10 ^{8}	278
		2.0	(3.13±0.09)*10 ^{8}	(3.05±0.09)*10 ^{8}	313
		2.5	(3.07±0.09)*10 ^{8}	(3.1±0.09)*10 ^{8}	307
	L. planturum	0.5	(2.1±0.06)*10 ^{8}	(2.05±0.06)*10 ^{8}	210
		1	(2.48±0.07)*10 ^{8}	(2.35±0.07)*10 ^{8}	248
		1.5	(3.25±0.1)*10 ^{8}	(3.2±0.1)*10 ^{8}	325
		2.0	(3.71±0.11)*10 ^{8}	(3.8±0.12)*10 ^{8}	371
		2.5	(3.73±0.11)*10 ^{8}	(3.8±0.12)*10 ^{8}	373
Amidated pectin	*L. lactis*	0.5	(1.82±0.05)*10 ^{8}	(1.85±0.05)*10 ^{8}	182
		1	(2.47±0.07)*10 ^{8}	(2.35±0.07)*10 ^{8}	247
		1.5	(2.84±0.09)*10 ^{8}	(2.85±0.09)*10 ^{8}	284
		2.0	(3.29±0.1)*10 ^{8}	(3.25±0.1)*10 ^{8}	329
		2.5	(3.27±0.1)*10 ^{8}	(3.2±0.1)*10 ^{8}	327
	L. planturum	0.5	(2.18±0.07)*10 ^{8}	(2.15±0.07)*10 ^{8}	218
		1	(2.68±0.08)*10 ^{8}	(2.8 ± 0.09) *10 ^{8}	268
		1.5	(3.33±0.1)*10 ^{8}	(3.25±0.1)*10 ^{8}	333
		2.0	(3.91±0.12)*10 ^{8}	(3.95±0.13)*10 ^{8}	391
		2.5	(3.87±0.12)*10 ^{8}	(3.95±0.12)*10 ^{8}	387
Reliability of differences	*L. lactis*	0.5	$p < 0.001$	$p < 0.001$	$p < 0.001$
		1	$p < 0.001$	$p < 0.001$	$p < 0.001$
		1.5	$p < 0.001$	$p < 0.001$	$p < 0.001$
		2.0	$p < 0.001$	$p < 0.001$	$p < 0.001$
		2.5	$p < 0.001$	$p < 0.001$	$p < 0.001$
	L. planturum	0.5	$p < 0.001$	$p < 0.001$	$p < 0.001$
		1	$p < 0.001$	$p < 0.001$	$p < 0.001$
		1.5	$p < 0.001$	$p < 0.001$	$p < 0.001$
		2.0	$p < 0.001$	$p < 0.001$	$p < 0.001$
		2.5	$p < 0.001$	$p < 0.001$	$p < 0.001$

Note: A one-way analysis of variance was performed on the CFU/g values to assess the effect of pectin type and concentration on the growth of probiotic microorganisms. The significance of differences in the "Efficacy, %" column was assessed using the χ^2 (chi-square) test to compare the distributions of efficacy values between the study groups. Differences were considered statistically significant at $p \leq 0.05$.

Comparison of the final growth rates of Lactococcus strains lactis, Lactobacillus planturum cultivated on different types of pectins is shown in Figure 1.

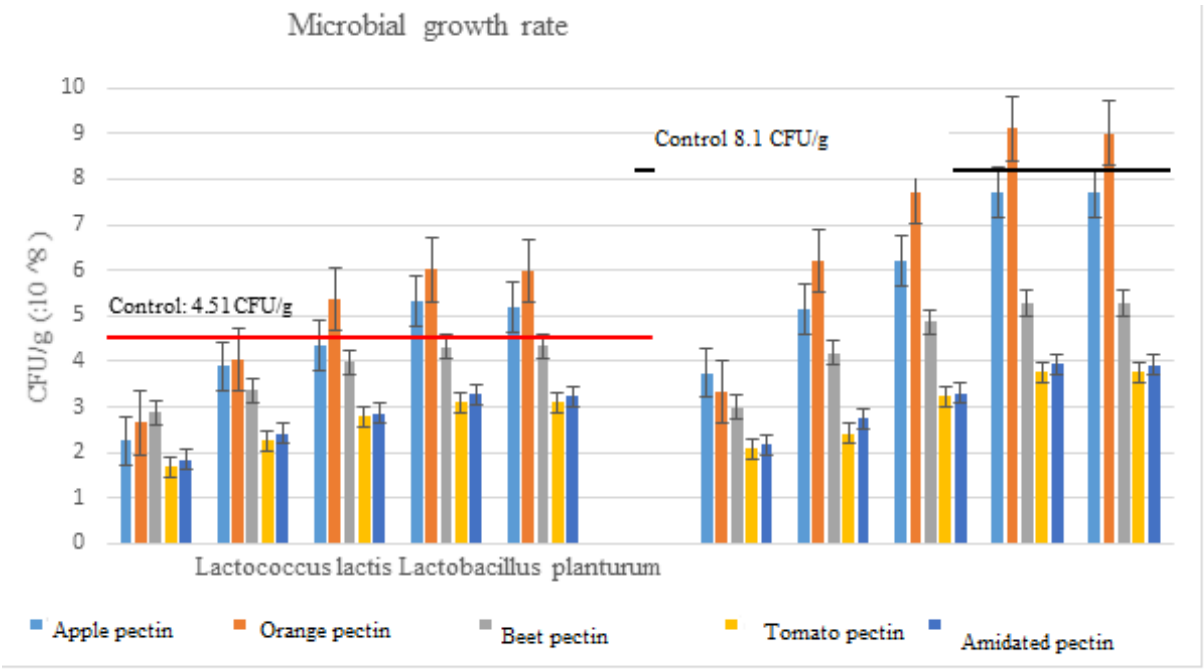


Figure 1. Dependence of the growth intensity of probiotics (*Lactococcus lactis; Lactobacillus plantarum*), cultivated on different types of pectins

The graph shows the dependence of the growth rate of probiotic microorganisms. The highest growth rate of microorganisms of the strain Lactococcus lactis is observed in solutions of apple, orange and beet pectins at concentrations from 1.5 to 2.5%. The highest growth rate of microorganisms of the strain Lactobacillus plantarum was observed in 2–2.5% pectin solutions. When comparing the results obtained with the control, the Lactococcus strain showed the highest growth efficiency. lactis was detected in samples cultured in a 2% solution of citrus pectin - 602% and in a 2% solution of apple pectin – 538%, the Lactobacillus strain planturum – on a 2% citrus pectin solution – 911%. No significant differences were found between the growth rates of probiotic strains cultured on a 2% pectin solution and on a 2.5% pectin solution.

During the comparison of the results of this experiment with the control group, it was established that the growth intensity of probiotic microorganisms cultivated on tomato and amidated pectins is low, on beet pectin it is average (for the L. lactis strain it is approximately 100% lower, and for the Lb. planturum strain it is 270% lower than the control group indicators), on citrus and apple pectins it is high (for the L. lactis strain it is approximately 100% higher for samples cultivated on a 2% citrus pectin solution and 50% higher for samples cultivated on a 2% apple pectin solution, and for the Lb. planturum strain it is 100% higher for samples cultivated on a 2% citrus pectin solution and 40% lower for samples cultivated on a 2% apple pectin solution relative to the control group indicators).

For the next experiment, the following types of pectins were selected: citrus 2%, apple 2% and beet 2%.

During the experiment, microcapsules were obtained, after which the growth rate of the encapsulated probiotic cultures was assessed, and the rate of viable cell loss during the microencapsulation process was calculated. The experimental results are presented in Table 2.

Table 2. Encapsulation efficiency of 2% pectin solutions of Lactococcus lactis and Lactobacillus plantarum strains

Composition of the capsule	Encapsulation efficiency, %
Apple pectin 2%, L. lactis	87.24
Apple pectin 2%, L. planturum	91.04
Citrus pectin 2%, L. lactis	90.85
Citrus pectin 2%, L. planturum	91.22
Beet pectin 2%, L. lactis	89.35
Beet pectin 2%, L. planturum	90.7

The data presented in Table 2 demonstrate that during the encapsulation process, some probiotic microorganisms are washed from the surface of the microcapsules, resulting in a loss of viable cells of 9–13%. The experimental results demonstrate the high efficiency of the encapsulation process, ensuring the preservation of over 80% of probiotic cells within the microcapsules formed from pectin solutions. These data confirm the feasibility of using microencapsulation technology in the development and production of dietary supplements and functional foods enriched with probiotic microorganisms.

The study assessed the survival of viable encapsulated probiotic microorganisms at various storage times: 12 hours, 24 hours, 1 week, 4 weeks, and 8 weeks after the start of the experiment. The results of the study, which examined the viability of encapsulated probiotic microorganisms and the encapsulation efficiency at each storage checkpoint (12 hours, 24 hours, 1 week, 4 weeks, and 8 weeks), are presented in Table 3.

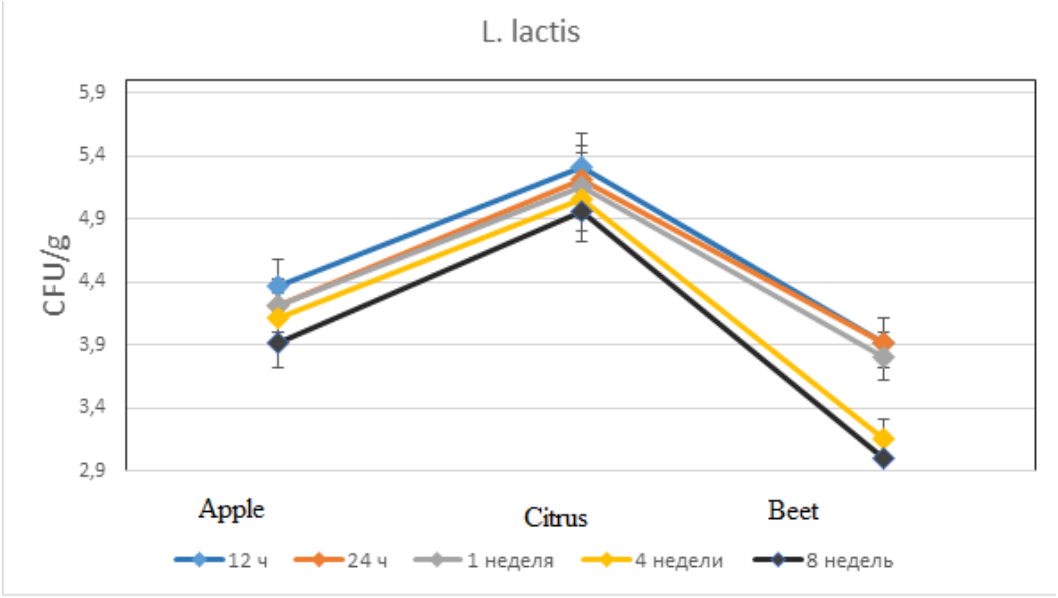
Table 3. Assessment of the growth rate of probiotic microorganisms during storage

Storage period	Composition of the capsule	Average CFU/g value according to densitometry indicators	Average CFU/g value based on 10th dilutions
12 o'clock	Apple pectin 2%, L. lactis	(4.36± 0.12)*10 ^{8}	(4.68±0.13)*10 ^{8}
	Apple pectin 2%, L. planturum	(6.97± 0.18)*10 ^{8}	(6.66±0.19)*10 ^{8}
	Citrus pectin 2%, L. lactis	(5.31± 0.15)*10 ^{8}	(5.67±0.16)*10 ^{8}
	Citrus pectin 2%, L. planturum	(8.19±0.22)*10 ^{8}	(8.28±0.21)*10 ^{8}
	Beet pectin 2%, L. lactis	(3.91± 0.12)*10 ^{8}	(3.69±0.12)*10 ^{8}
	Beet pectin 2%, L. planturum	(4.36± 0.12)*10 ^{8}	(5.04±0.14)*10 ^{8}
24 hours	Apple pectin 2%, L. lactis	(4.21± 0.12)*10 ^{8}	(4.44±0.13)*10 ^{8}
	Apple pectin 2%, L. planturum	(6.97± 0.20)*10 ^{8}	(6.84±0.21)*10 ^{8}
	Citrus pectin 2%, L. lactis	(5.21± 0.15)*10 ^{8}	(5.58±0.16)*10 ^{8}
	Citrus pectin 2%, L. planturum	(8.07± 0.21)*10 ^{8}	(8.07±0.22)*10 ^{8}
	Beet pectin 2%, L. lactis	(3.91± ±0.13)*10 ^{8}	(3.61±0.13)*10 ^{8}
	Beet pectin 2%, L. planturum	(4.36± 0.12)*10 ^{8}	(4.92±0.14)*10 ^{8}
1 week	Apple pectin 2%, L. lactis	(4.21± 0.12)*10 ^{8}	(4.56±0.13)*10 ^{8}
	Apple pectin 2%, L. planturum	(6.9 ±0.18)*10 ^{8}	(6.63±0.19)*10 ^{8}
	Citrus pectin 2%, L. lactis	(5.16 ±0.13)*10 ^{8}	(5.34±0.15)*10 ^{8}
	Citrus pectin 2%, L. planturum	(8.02 ±0.20)*10 ^{8}	(7.89±0.22)*10 ^{8}
	Beet pectin 2%, L. lactis	(3.81 ±0.11)*10 ^{8}	(3.39±0.11)*10 ^{8}
	Beet pectin 2%, L. planturum	(4.26 ±0.12)*10 ^{8}	(4.77±0.14)*10 ^{8}
4 weeks	Apple pectin 2%, L. lactis	(4.11 ±0.11)*10 ^{8}	(4.23±0.12)*10 ^{8}
	Apple pectin 2%, L. planturum	(6.26 ±0.18)*10 ^{8}	(6.69±0.20)*10 ^{8}
	Citrus pectin 2%, L. lactis	(5.06 ±0.14)*10 ^{8}	(4.98±0.15)*10 ^{8}
	Citrus pectin 2%, L. planturum	(7.87 ±0.21)*10 ^{8}	(7.5±0.21)*10 ^{8}
	Beet pectin 2%, L. lactis	(3.16 ±0.09)*10 ^{8}	(3.36±0.10)*10 ^{8}
	Beet pectin 2%, L. planturum	(4.06 ±0.11)*10 ^{8}	(4.08±0.12)*10 ^{8}
8 weeks	Apple pectin 2%, L. lactis	(3.91 ±0.12)*10 ^{8}	(3.66±0.12)*10 ^{8}
	Apple pectin 2%, L. planturum	(6.01 ±0.18)*10 ^{8}	(6.03±0.19)*10 ^{8}
	Citrus pectin 2%, L. lactis	(4.96 ±0.13)*10 ^{8}	(4.77±0.14)*10 ^{8}
	Citrus pectin 2%, L. planturum	(7.22 ±0.20)*10 ^{8}	(7.56±0.22)*10 ^{8}
	Beet pectin 2%, L. lactis	(2.97 ±0.09)*10 ^{8}	(2.97±0.10)*10 ^{8}
	Beet pectin 2%, L. planturum	(3.96 ±0.11)*10 ^{8}	(3.72±0.12)*10 ^{8}

Reliability of differences	L. lactis	12 hours	$p < 0.001$	$p < 0.001$
		24 hours	$p < 0.001$	$p < 0.001$
		1 week	$p < 0.001$	$p < 0.001$
		4 weeks	$p < 0.001$	$p < 0.001$
		8 weeks	$p < 0.001$	$p < 0.001$
	L. planturum	12 hours	$p < 0.001$	$p < 0.001$
		24 hours	$p < 0.001$	$p < 0.001$
		1 week	$p < 0.001$	$p < 0.001$
		4 weeks	$p < 0.001$	$p < 0.001$
		8 weeks	$p < 0.001$	$p < 0.001$

Note: CFU/g values were compared between experimental groups cultured with different pectin types and concentrations over time. Repeated- measures analysis of variance (RM ANOVA) was used to assess the influence of pectin type, pectin concentration, and shelf life on changes in viable microorganism counts.

A)



B)

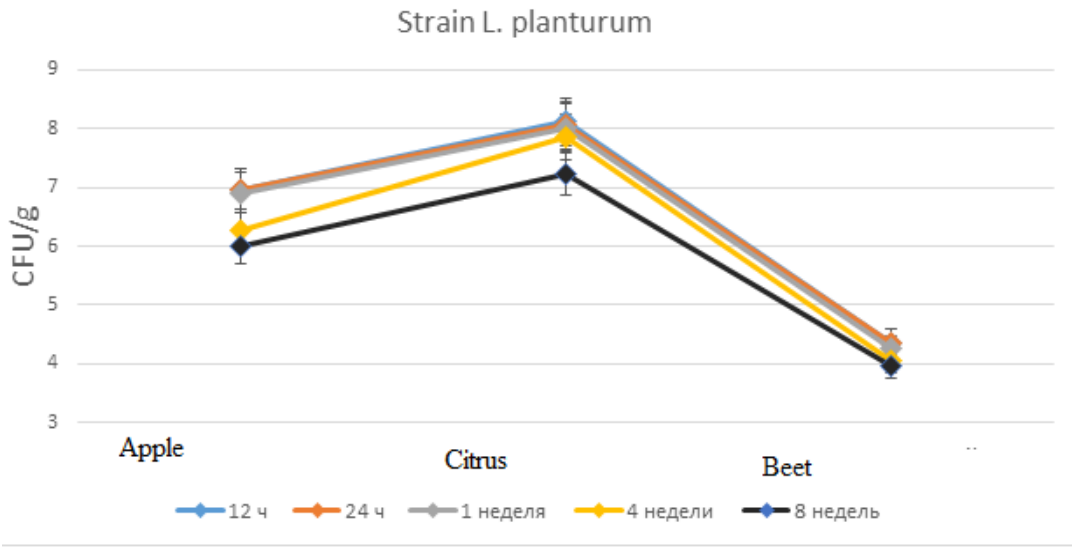


Figure 2. Effect of pectin type on growth dynamics and viability of probiotic strains *Lactococcus lactis* (a) and *Lactiplantibacillus plantarum* (b) during storage (RM ANOVA)

The results of the analysis of variance (RM ANOVA) confirmed statistically significant differences ($p < 0.05$) in the growth dynamics and viability of probiotic strains Lactococcus lactis and Lactiplantibacillus plantarum when using different types of pectin.

The most pronounced effect was observed for samples containing citrus pectin, which demonstrated the highest cell preservation after 8 weeks of storage (87.41% and 88.81%, respectively). The differences between citrus and apple/beet pectins were significant ($p < 0.001$), indicating that pectin type plays a key role in determining microbial stability during encapsulation and storage.

The RM ANOVA results demonstrate that the type of pectin has a statistically significant effect ($F > 5$, $p < 0.05$) on the growth and survival rates of probiotic cultures, confirming the effectiveness of using citrus pectin as a protective matrix.

Conclusion. The study found that microencapsulation of probiotic microorganisms in pectin-based matrices significantly increases their stability and survivability during storage. Microcapsules produced using citrus pectin exhibited optimal protective properties, ensuring 87.41% and 88.81% survival *of Lactococcus lactis* and *Lactiplantibacillus plantarum*, respectively, after 8 weeks of storage. The structure and degree of methoxylation of pectin were shown to significantly influence encapsulation efficiency and probiotic cell stability. These results confirm the potential of pectin polysaccharides, particularly citrus pectin, for the development of dietary supplements and functional foods with extended shelf lives and high biological value.

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