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Вивчення взаємодії антимікробних пептидів, зокрема граміцидину S, з клітинними мембранами

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Study of the Interaction of Antimicrobial Peptides, in Particular Gramicidin S With Cell Membranes

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AMPs are short peptides, mostly less than 100 amino acids in length, which are generally characterized by total positive charges and high proportions of hydrophobic residues. As a result, they tend to establish nonspecific interactions with negatively charged phospholipids, such as phosphatidylglycerol, which are particularly abundant in microbial membranes, causing increased permeability, leakage of cytoplasmic components, and cell death. The cationic charge of the peptides leads to their repeated accumulation next to the negatively charged surfaces of gram-negative (outer membrane) or gram-positive (cell wall) bacteria. Cationic antimicrobial peptides can have low cellular interaction, which leads to undesirable interaction with non-target cells. To solve this problem, liposomal containers are used to facilitate controlled delivery of the active substance. The addition of substances such as cholestyrene and cardiolipin to their composition is aimed at improving the quality of encapsulation and release of the peptide into the vesicle membrane and its stability. By controlling the concentration of cardiolipin during liposome formation, it is possible to model the level of their potential at the hydrodynamic shear limit (zeta potential). To analyze such effects, we used the antimicrobial peptide Gramicidin (GS), a short peptide antibiotic derived from the bacterium Bacillus brevis, consisting of 15 L- and D-amino acid residues. Our experimental data, obtained by light scattering and fluorescence microscopy, confirm this. According to the available data, GS has antibacterial effects within the concentration range from 3 to 12.5 μg/ml, an increase in concentration up to 18.7 μg/ml results in cytotoxic effects on cell culture, and at a concentration of 35.2 μg/ml, the hemolysis occurs, particularly of red blood cells.

We hypothesized that different liposome compositions could affect the quality of drug absorption and release. The study used L929 cells cultured in two different forms - in a monolayer incubated with liposomes of different lipid composition loaded with HS and in the form of spheroids (3D culture) incubated with HS at concentrations of 10, 25, 50 and 75 μg/ml. During the experiment, the adhesive properties and cell viability were analyzed using the FDA/EB staining test. The results showed that liposomes loaded with the peptide at concentrations of 10 and 25 μg/ml did not significantly affect cell viability, but treatment with liposomes loaded with GS at concentrations of 50 and 75 μg/ml led to a decrease in the indicator. When liposomes of different composition were incubated with GS at concentrations of 50 and 75 μg/ml, the adhesive properties of cells also decreased. The fusion of the cell monolayer at these concentrations was in the range of 67–75% (compared to the control, a decrease of about 17–25% was observed). A similar effect, but more pronounced, could be observed with 3D cell cultures.

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Сезонна динаміка зміни точки замерзання натурального молока як критерій визначення можливої кількості доданої води

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Seasonal Dynamics of Changes in the Freezing Point of Natural Milk as a Criterion for Determining the Possible Amount of Added Water

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Determining the freezing point of natural milk produced in Ukraine in different seasons is crucial. Without these values, assessing the degree of its impurity with water is challenging while maintaining high quality and safety standards.

The study was carried out in the production conditions of the Testing Center of the Livestock Farming Institute of the National Academy of Agrarian Sciences, accredited by the National Accreditation Agency of Ukraine, under the requirements of DSTU ISO/IEC 17025: 2006 as a basic organization of the metrological service of the Ministry of Agrarian Policy and Food of Ukraine. The naturalness of milk (water may be added) was evaluated according to DSTU 7671:2014 using an infrared analyzer Bentley 150 manufactured in the USA. The relationship between the indices was proved using correlation analysis.

When discussing the differences in the freezing point of milk, it is important to note that the season of the year had a slight effect on this index, with fluctuations ranging from –0.532°C to –0.572°C. The average annual value of freezing of natural milk during the seven-year evaluation, for $n > 74$ thousand, is -0.555 ± 0.001°C. Concurrently, the correlation coefficients between the freezing point and the mass fraction of dry matter in milk demonstrated an upward trend from winter to summer, with a subsequent decline in autumn. In particular, the highest and average strength between these indices was observed in summer, with the highest coefficient of determination ($r = +0.660$, $R = 43.56$). The correlation between these traits was almost similar in winter and autumn (respectively $r = + 0.560$, $R = 31.36$ and $r = + 0.550$, $R = 30.25$), and the lowest was in spring ($r = + 0.520$, $R = 27.04$).

The data obtained from the freezing point tests with 10% water added to milk demonstrate the significance of assessing the corresponding index to determine the degree of water adulteration.

As part of the registration of deviations of the freezing point of natural milk established by monitoring studies from the standard values according to DSTU 3667 as amended and from EU requirements, it was demonstrated that in the winter-spring period they were higher by 7.1–7.5%, in summer and autumn this figure decreased to 6.0 and 6.2%, and depending on EU requirements, the registered values were accompanied by an increase of 7.5, respectively; 7.9; 6.4 and 6.6 %. To determine the degree of milk adulteration with water, the freezing point of natural milk -0.555 ± 0.001°C determined in the studies should be used. Alternatively, the cryoscopic method can be used on samples that are one hundred percent natural. Accordingly, the reference freezing point of natural cow milk is –0.555°C, which can be used as a criterion for calculating the possible amount of added water.