Betulin nanosuspension as a promising raw material for the production of long-term storage bread

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Abstract. The gist of this article boils down to the betulin nanosuspension as a promising raw material for the production of long-term storage bread. The methodology for obtaining stable finely dispersed nanosuspension of betulin, including its transfer to a water-soluble state, the introduction of emulsifiers that increase the sedimentation stability of the suspension is scientifically substantiated. The stability and possible shelf life of betulin nanosuspensions were investigated. It is proved that the introduction of betulin nanosuspension with emulsifiers of various nature improves the structural and mechanical characteristics of wheat flour bread. Betulin nanosuspension has been proven to inhibit potato bread disease and increase the shelf life of wheat flour. The bread sample using nanosuspension of betulin with lecithin emulsifier possessed optimal characteristics. According to the analysis of the dynamics of storage of bread from wheat flour with betulin nanosuspension, a shelf life of 7 days was determined.

1. Introduction

The production of bakery products of long-term storage is one of the trends in modern bakery aimed at solving the country’s food security problem, including uninterrupted supply of bread to the population living in areas with severe climatic conditions, as well as in crisis and emergency situations. In recent years, there has been a steady tendency in Russia to reduce the production of non-durable bread [1-10].

In addition, with the entry into force of the law of November 28, 2018 No. 446 - of the Federal Law «On Amendments to Article 5 of the Federal Law» «On the Development of Agriculture» and the Federal Law «On the Basics of State Regulation of Trade Activities in the Russian Federation», business entities, engaged in trading activities for the sale of food products through the organization of a trading network, and business entities engaged in the supply of food products to trading networks cannot conclude agreements containing conditions for the return of food products not sold after a certain period, for which the expiration date is set to thirty days inclusive, which significantly affects the competitiveness of the enterprise as a whole [11].

In the production of bakery products by manufacturers, special attention is paid to the production of products with useful properties. These products are of great importance in a wholesome and healthy diet. They satisfy not only the physiological needs of a person, but also perform preventive and therapeutic functions: they activate the body’s defenses and normalize metabolism. In this context, betulin may be a promising component.

Betulin is a triterpenoid of a number of lupan contained in the birch bark along with betulinic acid, betulonm. Betulin is not inferior in its properties to pharmaceuticals than tanites and ihydroquercetins,
and also has diverse - lipid-lowering, hypocholesterolemic, antioxidant, antiseptic activity. Despite the obvious promise, at the moment betulin is used in the food industry, including bread baking, extremely few.

2. **Purpose of the study**

The purpose of this research was to prepare nanosuspension based on betulin with a particle size of not more than 10 μm, having high digestibility and biological activity, as well as an analysis of the dynamics of the quality indicators of bread, based on nanosuspension of betulin, during its storage.

3. **Problem statement**

The main reason for the small use of betulin in the Russian Federation is its insolubility in water, which significantly reduces the possibilities of food technologists. Betulin is practically insoluble in water, and its solubility in ethanol is not more than 4.3 g / 100 g at 80 °C [9]. As you know, the possibility of using betulin in powder form is limited by the complexity of its distribution in the volume of the test. In addition to inhibiting potato bread disease, betulin also has adaptogenic and immunomodulatory effects, so its introduction into the diet is advisable [12]. The use of betulin microparticles with a size of a fraction of a micron will significantly increase the efficiency of its use, including increasing its manufacturability when added to bulk food products such as flour, powdered sugar, etc., increasing its solubility, bioavailability and digestibility in view of the increased contact area with him.

In the gastrointestinal tract system betulin function is close to the action of bile acids, the mechanism of which includes colloid-chemical effects, including micellation, dispersion, and formation of complexes with lipids [11].

Betulin is a hydrophobic substance, therefore, an important problem is to increase the hydrophilicity of the molecule with the formation of an adsorption shell on its surface, which gives the suspension the necessary stability, as well as further blocking the process of aggregation of betulin particles and the formation of a precipitate, increasing the stability of the suspension. The following requirements were imposed on the suspension: 1) the size of the particles of betulin was not more than 10 μm 2) the stability of the suspension during storage at a temperature of 20 ± 5 °C for at least 6 months. 3) the suspension should not impair the consumer properties of wheat flour bread, but, on the contrary, contribute to the improvement of organoleptic characteristics, increase the shelf life of wheat flour bread for at least 7 days and suppress the potato bread disease.

4. **Research questions**

We considered 2 methods of producing a suspension - dispersion: by mechanical action (ultrasonic grinding, the use of magnetostrictive and electrostrictive emitters; etc.) and condensation, based on the addition of a solution of betulin in a solvent that mixes well with a dispersion medium with intensive mechanical stirring with using electric mixers and rotary pulsation apparatus. Finally, the second method was chosen, as the most appropriate for the tasks and allowing to obtain a stable suspension.

To obtain a suspension of betulin, the following steps were used:

- Translation of betulin into a water-soluble state, for which its dispersion and compositions of the contacting phases were changed. It was revealed that with a decrease in the particle size of betulin below 30 nm, betulin crystal aggregates are not formed, and 5–10 betulin molecules are able to form only incomplete amorphous clusters. Hydrophilization of the surface of a betulin molecule leads to an increase in its wettability and sedimentation stability of the system, and micronization of betulin contributes to the emergence of a larger specific surface, which leads to an increase in free surface energy. All suspensions have kinetic and aggregative instability, as they are heterogeneous systems. Sedimentation stability is the most important characteristic of a suspension, affecting the distribution of particles in the entire volume of the system.
The introduction of components that increase the sedimentation stability of the suspension, i.e. its ability to maintain the distribution of particles throughout the dispersed system. For this purpose, sodium chloride and emulsifiers of various nature were used to slow down the interaction between betulin molecules, their aggregation during suspension formation and after.

Emulsifiers were added during the stabilization process of the mixing phase to form a uniform, stable suspension. It is noted that emulsifiers helped prevent recrystallization during product cooling. At the moment, the list of emulsifiers for the food industry that are approved for use in the food industry is quite extensive. When choosing an emulsifier were guided by its emulsifying properties, availability, cost, ability to influence the rheological characteristics of the test [15].

Four variants of model samples of the suspension with stabilizers (emulsifiers) approved for use in food production were prepared. The main characteristics of the emulsifiers used are shown in table 1.

| № in order | Name                      | The code | Additive Characteristic                                      |
|------------|---------------------------|----------|-------------------------------------------------------------|
| 1          | Sorbitan Monostearate     | E 491    | Used as a structure-forming and stabilizing component, emulsifier, particle anti-aggregator. |
| 2          | Calcium Stearoyl-2-Lactylate | E482    | It has good emulsifying properties, increase the specific volume and porosity of the dough, extend the shelf life of bakery products. |
| 3          | Fatty Acid Polyglycerides Esters | E 475    | It is soluble in hot water, ethanol, helps to densify the structure of bread and its porosity |
| 4          | Lecithin                  | E 322    | Antioxidant, emulsifier, increases the plasticity of the dough, has emulsifying properties, increases the shelf life of bakery products. |

5. Research methods
Stage 1. Dissolution of betulin in a mixture of ethyl alcohol and glycerol in a ratio of 70:30 for 20 minutes with vigorous stirring on a magnetic stirrer at a speed of 500 rpm. Glycerin (E 422) is a viscous liquid with a sweetish taste, is an approved food additive, and has a very low hygroscopicity. According to the Stokes formula, the sedimentation rate is directly proportional to the difference in the density of the phase and the medium. Depending on this indicator, particles of the dispersed phase can settle (d1 > d2) or float (d2 < d1). If d1 = d2, the system is stable and the suspension is stable. Betulin particles settle at a rate inversely proportional to the viscosity of the system. Therefore, the inclusion of a viscous component (glycerol) increases the sedimentation stability of the dispersion system and prevents the aggregation of particles into clusters.

Stage 2. The resulting suspension was heated to 40 °C in order to distill off the solvent (ethanol) on a RE 52AA rotary evaporator (Shaanxi NEV brand, China). The action of the rotary evaporator is based on lowering the boiling point of the solvent by creating a reduced pressure in its system using a water-jet or vacuum pump. This approach allows you to remove the solvent from the solution at a lower temperature, avoiding adverse reactions that can occur when the mixture is heated [13].

Stage 3. An aqueous solution was prepared, including sodium chloride as an anti-aggregator of particles and one of the emulsifiers (table 1). Dosages were chosen experimentally by varying the mass of NaCl in accordance with the data of scientific sources, the emulsifier in accordance with the manufacturer's recommendations and the requirements of the Technical Regulation of the Customs Union 029.

To prepare a suspension with predetermined properties, the process of feeding the internal phase with the necessary adjustment of the flow rate is very important in order to prevent the aggregation process and the formation of multiple nucleation centers and adhesion of the resulting betulin microparticles.
For this purpose, a flowing microreactor brand Mr-Lab-VS (Germany) from borosilicate glass was used, the internal volume of 1 ml, the diameter of the channels with a feed system of 0.1-20 ml / min. 1 mm, microreactor dimensions 115x60x6 +/- 0.5 mm with the supply of one reagent [15].

The betulin solution was slowly, with a feed rate of 0.1 ml / min, introduced into the solution of the external phase heated to 70 °C while stirring intensively with an IKA dispersant (speed from 8 to 24 thousand m / s, linear speed up to 20 m / s, shear loads 60 000 1 / m). The suspension of betulin was microscopic, a photograph is shown in figure 01.

Figure 1. Photographs of betulin nanosuspension under a microscope (polarizing optical microscope "Polar 3 with magnification a) 2000 b) 1000x.

Figure 1 shows that a homogeneous heterogeneous amorphous-structural suspension with a particle size of 0.05 to 0.1 μm (50-100 nm) was obtained. Thus, since the obtained betulin particles have sizes in all three dimensions from 1 to 100 nm, this suspension can be called nanosuspension [14].

As a result of testing the proposed technology for producing nanosuspension in order to achieve the optimal set of properties and selecting affordable and low-cost components, the composition of the betulin nanosuspension options for further research was found.

Next, stability and possible storage periods of betulin nanosuspensions were investigated, for which 4 bottles of each type of suspension were packaged in brown light-proof glass bottles with hermetically screwed caps with a volume of 100 cm³ and placed in a climate chamber “Binder KBF 240” (Binder, Germany) and kept at a temperature of 55 °C for 720 hours.

Nanosuspensions with emulsifier sorbitan monostearate during storage had a lower degree of stability. According to the tests, the obtained betulin nanosuspensions, packaged in brown glass bottles of light-protective material with hermetically screwed caps, can be stored at a temperature not lower than 0 °C and not higher than 20 °C in for two years (24 months).

Comparison of the analysis results obtained when determining the mass concentration of betulin before testing the stability of the emulsion, during the tests and after their completion, was carried out by checking the statistical significance of their difference. The calculations performed using the F-criterion showed the absence of statistically significant differences between the variances of the series of analyzes, since the numerical values of their ratios do not exceed the value of the F-criterion for a given probability of 95% and degrees of freedom (F95,2,2 = 19,2). Comparison of the average values of the results of the suspension analysis during testing and in front of them using the Student criterion (t95.4 = 2.78) showed the absence of a statistically significant difference between the results.

Organoleptic and physico-chemical parameters of betulin nanosuspensions are shown in table 2.

| Index                          | Value                          |
|--------------------------------|-------------------------------|
| Appearance                     | A thick, impurity-free liquid  |
| Colour                         | White with a grayish tint     |
| Smell                          | Without smell                 |
| Transparency                   | Not transparent               |
| pH                             | 6-7                           |
| molar concentration of nanoobjects, not less | 0.1 mmol / dm3 |
| average size of nanoobjects contained in nanosuspension, not more than | 100 microns |
Colloidal stability

| Density at a temperature of (20 ± 0.5 °C), g/cm³ | Stable |
|-----------------------------------------------|--------|
| 1.1-1.2                                       |        |

The resulting suspensions are an opaque thick white liquid. According to the data obtained, it was decided that it is advisable to continue studying the effect of betulin nanosuspension with emulsifiers: calcium stearoyl-2-lactylate, polyglycerol fatty acid esters, lecithin.

Prototypes of bread from wheat flour, prepared in accordance with GOST 27669-88 "Baking wheat flour. Laboratory test baking method" with 3 options for betulin nanosuspension (sample No. 1 - nanosuspension betulin + Stearoyl-2-calcium lactylate, sample No. 2 - nanosuspension betulin + esters of polyglycerides of fatty acids, sample No. 3 - nanosuspension betulin + lecithin) for storage and assessed the quality of bread by organoleptic and physico-chemical indicators. A shelf life of 10 days was established according to MUK 4.2.1847-04 "Sanitary and epidemiological assessment of the justification of shelf life and storage conditions of food products" with a safety factor of 1.3.

Samples of bread were stored in plastic packaging at a temperature of 22 ± 2 °C, recording changes in quality indicators [14, 16]. Evaluation of the freshness of the products was carried out on a point scale taking into account Q. When assessing the total points are divided into categories as follows: “very fresh” - 100.0-80.0, “fresh” - 79.9 - 60.0, “moderately stale” - 59.9 - 40.0, “stale” - 39.9 - 20.0, “very stale” - below 19.9 points. The degree of infection of potato disease, according to the developed descriptive point scale.

As a result of studies of the compressibility of the crumb of the samples, it was found that after 7 days in the test samples, the compressibility of the crumb decreased by 55%, 43.7%, 37.6%, while the control for the sample by 73%. Research data is presented in figure 2.

![Figure 2. Freshness of the studied samples during storage in comparison with the control sample during storage, score.](image)

The freshness of bread is determined by quality indicators such as crumbiness, crumb hydrophilicity, amount of shrinkage, change in crumb moisture, and the amount of aroma-forming compounds.

6. Findings

An organoleptic assessment of the quality of bread showed that with the addition of nanosuspension of betulin, the crumb porosity improves, it becomes more developed, uniform, tender and elastic, retains freshness for seven days compared to the control (2 days), signs of staling appear on the fourth day of storage.

Studies have shown that crumb crumbiness increases during storage; after 120 hours, it amounted to 12% in the control sample, from 6 to 8% in samples with betulin nanosuspension. The amount of drying in the samples with nanosuspension of betulin was significantly lower than in the control sample.

According to the results of the study, it can be assumed that the introduction of betulin, which has emulsifying properties on its own [15], enhanced by the introduction of emulsifiers, make up a complex including an emulsifier molecule with an amylose fraction of starch. In all experimental samples with a suspension of betulin, stable resistance to the disease of potato bread disease is maintained under
provocative conditions for 72 hours. In control bread samples, signs of a disease of potato bread disease began to appear after 48 hours.

Sample No. 3 (wheat flour bread with betulin + lecithin nanosuspension) possessed the best storage performance. Thus, the application of betulin nanosuspension is effective for suppressing potato bread disease.

7. Conclusion
A technique is proposed for producing finely dispersed nanosuspension of betulin, which includes ethyl alcohol, betulin, emulsifier, sodium chloride, food glycerin. A dosage of nanosuspension in an amount of 2% by weight of flour corresponds to the recommended daily dose of betulin and maintains stable resistance to potato bread disease under provocative conditions for 72 hours.

References
[1] Zhang Yao, Li Dandan, Yang, Jin Na Zhengyu and Xu Xueming 2018 Comparison of dextran molecular weight on wheat bread quality and their performance in dough rheology and starch retrogradation LWT 98 39-45
[2] Zhang Yao, Guo Lunan, Xu Dan, Li Dandan and Xu Xueming 2018 Effects of dextran with different molecular weights on the quality of wheat sourdough breads Food Chemistry 256 373-9
[3] Luo Denglin, Wu Ruoyan, Zhang Jie, Zhang Kangyi and Li Xuan 2018 Effects of ultrasound assisted dough fermentation on the quality of steamed bread Journal of Cereal Science 83 147-52
[4] Licciardello F, Giannone Virgilio, Del Nobile Matteo Alessandro, Muratore Giuepp, and Pasqualone Antonella 2017 Shelf life assessment of industrial durum wheat bread as a function of packaging system Food Chemistry 224 181-90
[5] Frauenlob Johannes, Moriano Maria Eletta, Innerkofler Ute, D'Amico Stefano and Schoenlechner Regine 2017 Effect of physicochemical and empirical rheological wheat flour properties on quality parameters of bread made from pre-fermented frozen dough Journal of Cereal Science 77 58-65
[6] Mellado-Ortega Elena and Hornero-Méndez Dámaso 2017 Effect of long-term storage on the free and esterified carotenoids in durum wheat (Triticum turgidum conv. durum) and tritordeum (Tritordeum Ascherson et Graebner) grains Food Research International 99 (2) 877-90
[7] Cho Seong-Woo, Kang Chon-Sik, Seok Ko Hyeon, Baik Byung-Kee and Park Chul Soo 2018 Influence of protein characteristics and the proportion of gluten on end-use quality in Korean wheat cultivars Journal of Integrative Agriculture 17(8) 1706-9
[8] Gänzle Michael G and Zheng Jinshui 2018 Lifestyles of sourdough lactobacilli – Do they matter for microbial ecology and bread quality? Journal of Food Microbiology In press corrected proof Available online
[9] Rizzello Carlo Giuseppe, Verni Michela, Bordignon Stefano, Graaglia Valerio and Gobbetti Marco 2017 Hydrolysate from a mixture of legume flours with antifungal activity as an ingredient for prolonging the shelf-life of wheatbread Food Microbiology 64 72-82
[10] Tebben Lauren, Shen Yanting and Li Yonghui 2018 Improvers and functional ingredients in whole wheat bread: A review of their effects on dough properties and bread quality Trends in Food Science & Technology 81 10-24
[11] Khalikov R M and Nigamatullina G B 2015 Transformation of amylose and amylopectin macromolecules in the technological processing of starch granules of plant materials in the food industry Nauka-rastudent.ru 01(013-2015) (Electronic resource URL: http://nauka-rastudent.ru/13/2315/
[12] Vorobyova O A and Melnikova N B 2015 Development and validation of methods for analyzing the components of the pharmaceutical composition of betulin and thymol in pumpkin seed oil CUCURBITA PEPO International Journal of Applied and Basic Research 10(2) 295-303
[13] Pankratieva N A and Zavorokhina N V 2017 Modeling bread recipes with high nutritional value and improved rheological properties *Apk of Russia* **24**(5) 1227-33

[14] Pankratieva N A, Zavorokhina N V, Shkolnikova M N, Selivanov N I and Chepelev N I 2018 Development of recipes for flour products with increased shelf life and increased nutritional value *Bulletin of KrasGAU* **4**(139) 191-6

[15] Zavorokhina N V, Pankratieva N A and Goncharova N A 2019 New technologies for the production of wheat bread long-term storage under the conditions of new industrialization *Advances in Social Science Education and Humanities Research* **240** 62-5

[16] Zavorokhina N V, Pankratieva N A and Goncharova N A 2019 Improving preservation methods consumer characteristics of bread long-term storage *International Scientific Conference Fifth Technological Order: Prospects for the Development and Modernization of the Russian Agro-Industrial. Complex* 36-41