ANTIBACTERIAL EFFECT OF COLLOIDAL SOLUTIONS OF SILVER NANOPARTICLES ON MICROORGANISMS OF CEREAL CROPS

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Abstract: Due to the growing problem of decrease in the quality and biological safety of food raw materials and the food obtained from it, the increase in the measures for grain improvement as one of the main vegetable resources of food productions is a necessary condition in the modern food industry. A perspectively new way of providing biosafety of grain raw materials is the use of silver nanoparticles. The present work provides data on the inhibiting influence of silver colloidal solutions on the bacterial composition of microflora of the most demanded grain crops of wheat and rye. Various antimicrobial efficiency of influence of the chosen colloidal solutions "Adzhenta colloidal silver" and "Colloidal silver concentrate KND-S-K" within 24 and 144 hours after the processing of grain crops has been shown. Various influence of experimentally chosen concentrations of solutions of silver nanoparticles of 0.1 g/dm^3 and 0.075 g/dm^3 on the number of viable cells of grain bacteria has been described. An approximate mechanism of effect of colloidal solutions of silver nanoparticles on the bacterial cells of microorganisms has been stated. A negative role of bacterium *Bacillus subtilis* in grain production has specially been noted. Their identification in grain crops has been performed and measures for effective destruction of them in grain by means of silver nanoparticles have been proposed. The current data can be used for providing biological safety of grain and an effective solution of the problems on its processing for the purpose of manufacturing of quality products.

Keywords: Silver nanoparticles; food production; grain biosafety; *Bacillus subtilis*

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INTRODUCTION

Nanodimensional structures of silver metals are a perspectively new type of materials used in scientific research due to their versatile application in various areas [1, 2].

In comparison with other metals, silver has strongly marked bactericidal and fungicide characteristics. Besides, the use of silver in the form of nanoparticles has allowed to manipulate its size and properties at the nanolevel which resulted in an increase in the antimicrobial potential. As with a decrease in the size of particles the specific phase interface increases and the area of contact of silver with bacteria considerably extends. Thus, it is confirmed that the bactericidal effect of silver nanoparticles depends on their sizes. And even after long processing, the ability of microorganisms to develop resistance to nanosilver [3] hasn't been observed.

The value of silver nanoparticles (SNP) in the food industry – the technologies of application of colloidal systems in the following developments – should specially be noted:

– the manufacturing of packing materials covered with a nanofilm with bactericidal and fungicide properties;
– the use of biodegradable nanosensors to control the temperature and humidity of foodstuffs;
– the addition of emulsion nanosystems in food to increase nutrient availability;
– the development of chelate nanocompounds for more effective delivery of nutrients to cells without a change of color and taste of food;
– receiving nanodisperse amplifiers of taste and aroma;
– the production of nanodimensional powders that make it easier to digest nutrients;
– the use of the nanoparticles capable to take up and remove selectively food contaminants, for example, mycotoxins;
– the development of the antimicrobic nanocomponents providing control for the development of infectious agents of food infections;
– the creation of nanosensors for the identification of vegetable and animal pathogens;

– the use of fluorescent nanoparticles with attached antibodies for the determination of chemicals or food pathogens [4, 5, 6].

Studies in the scope of SNP as an antimicrobial agent when developing antibacterial means against a number of bacteria and filamentous fungi are of great interest in the food industry [7, 8].

It is due, first of all, to the growing problem of a decrease in the quality and biological safety of foodstuffs at all stages of production and storage as a result of high adaptive ability of pathogenic microorganisms to the changing conditions of the environment [9, 10].

Special vigilance is caused, in particular, by practical lack of application of effective and eco-friendly measures for the improvement of grain production. Grain is provided quite well with the nutrients which are a favorable environment for the development of microorganisms under certain conditions. The significant amount of microorganisms negatively influence grain under certain favorable conditions, in other words, they reduce the content of solids in it, provide its pollution, and also poison it with toxic waste products. The contamination of grain by depredators and diseases is revealed during its further processing in the food industry. In this regard, the negative processes in the flour-grinding and baking industries are a serious threat to human health, provide a further decrease in the quality of bakery products. The contamination of grain by infectious agents of the mushroom and bacterial etiology occurs annually to some extent and though the level of contamination is corrected by a number of edaphoclimatic and anthropogenous factors, the harvest shortage can make in some cases up to 30% with a simultaneous decrease in the technological and baking qualities of grain [10, 11].

An important role in the decrease in the harvest and quality indicators of grain and its derivative products, in particular bread, is played by the infectious agents of bacterioses – sporous bacteria of *Bacillus mesentericus vulgatus Flügge* and *Bacillus subtilis* and other species of this genus [12, 13].

According to the most widespread systematics of phytopathogenic bacteria [14] based on the signs of existence or lack of flagella, Gram staining of colonies and their pigmentation, the genus *Bacillus* which is a part of the *Bacillaceae* family is presented by the most harmful types.

After cooling of bread, in case of low acidity and the temperature above 25°C, the sporous forms of infectious agent begin to develop causing chemical changes in the newly-baked bread. Under the influence of the active amylases emitted by the bacteria in bread the quantity of dextrines which provide the mucilagination of the crumb, loss of its structure and the formation of emptiness increases. Under the influence of proteolytic enzymes of bacteria disintegration products of proteins that give a pungent specific smell to bread are formed. Such bread is not suitable for consumption, nor as animal forage either.

A different degree of contamination by bacteria spores is noted on wheat and corn grain, in soft and hard wheat flour, in bran and other products which are raw materials for bread and bakery products [15, 16, 17].

All the above allows to establish the fact of a huge damage sporous bacteria and other phytopathogenic microorganisms may cause to the food market of the country. The infectious agents of bacterial etiology are wide-spread and have a high level of injuriousness which, along with the decrease in productivity, consists of the contamination of wheat grain providing loss of technological and baking properties in the latter.

Due to this, the main purpose of this work is the study of influence of various concentrations containing silver nanoparticles on the viability of bacterial microbiota of seeds of some grain crops.

OBJECTS AND METHODS OF STUDY

To study the contamination of grain crops, seeds of *Irgin* summer wheat (*Triticum aestivum L.*) and *Krone* winter rye (*Secale cereale*) were used as the objects. The quantitative and qualitative composition of bacterial microflora of grain was studied using the method of electronic microscopy.

To inhibit the processes of microbiological spoilage of grain crops the SNP colloidal solutions "Adzhenta colloidal silver" (the manufacturer is LLC "KorolevFarm", Russia) and the colloidal solution of silver nanoparticles KND-S-K (Scientific and Production Enterprise LLC "Sentoza Faktoring NP", Russia) were used.

Using the method of dynamic laser light scattering dispersion and distribution was studied by the sizes of nanoparticles in the studied colloidal solutions. The test specimens with the volume of 0.7–3.0 ml were placed in the ditch of the device and measurements were taken at a temperature of 23 ± 1°C using the laser particle size analyzer Nanotrac – the modification of Zetatrac ("Microtrac Inc.", the USA) – by means of the Microtrac FLEX software.

The studied SNP colloidal solutions, using the method of dilution by distilled water, brought up to the use rates of 0.075 and 0.1 g/dm^3 which had experimentally been selected taking into account the compliance to normative documents in the tolerant (transferable) content of silver in drinking water (WHO, SanPiN) [18, 19]. Seeds of wheat and rye were processed using the obtained solutions. For this purpose 50 g of seeds and the calculated dose of preparative form of silver colloidal solution taking into account the concentrations of nanoparticles were put into a 150 ml flask. The flask with seeds was stirred up for 2–3 minutes before the full distribution of the preparation on the surface of seeds. Then the processed grain was left for 2 hours after which the test specimens of the studied samples were selected and put into sterile ware in the aseptic conditions excluding the microbial contamination of raw materials and total of bacteria using the method of washed out crops with the dilution degree of 1 : 100 per meat-and-peptone agar (MPA) with the subsequent cultivation at the temperature of 30 ± 1°C for 24–48 hours was determined.

To study the influence of SNP on the bacterial microbiota of grain crops in detail the analysis of contamination of samples was also performed in 24 and 144 hours after processing. The choice of temporary intervals is justified, among other things, by the features of development of microflora of grain crops (by the antagonistic effect of some microorganisms on others).

To study the qualitative composition of microflora, microscopic preparations (fixed dabs painted according to Gram - to study bacterial morphology) were made of the colonies of the microorganisms differing from each other in cultural properties.

The spore-forming bacteria of the genus *Bacillus* were determined using the technique described in GOST ISO 21871 [20]. Initially, inoculation, first, of a liquid enriched medium (tryptone soy polymixil broth (TSPB) with the established volume of initial sample of suspension of 10 ml in the pasteurized washouts from the samples at the rate of 1 : 100 was consistently performed. The duration of incubation was 48 h at a temperature of 30°C. Then, a transfer to a solid nutrient medium (PEMBA) and incubation for 18–48 h at 37°C for the further analysis of Petri dishes to check the presence of the colonies which, according to the characteristics, can correspond to presumptive *Bacillus* bacteria was performed. Microbiological medium from HiMedia Laboratories, India, Merck Ltd., SRL Pvt., Ltd., Mumbai were used to perform the studies.

RESULTS AND DISCUSSION

According to TR TS 021/2011 certain complexes of microorganisms of grain crops (Table 1) [21] are normalized.

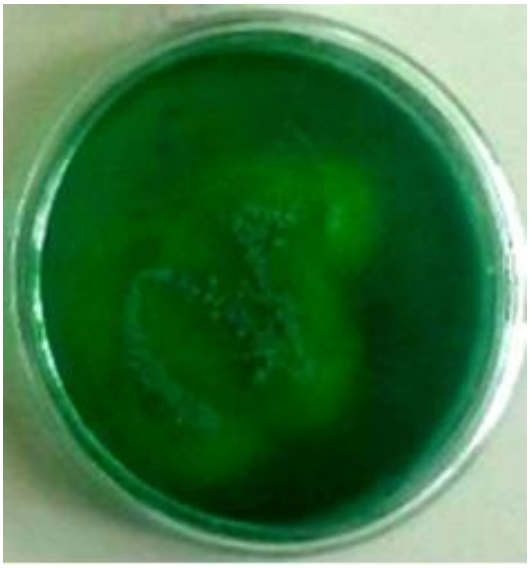
The determination of content of microorganisms of the studied grain has shown compliance with the normalized indicators, except the indicator of the total plate count. According to the obtained data, the indicator was 9.0×10^3 CFU/g and 7.0×10^3 CFU/g for wheat and rye respectively with the norm of 5.0×10^3 CFU/g.

When studying the qualitative composition of microbiota of wheat and rye seeds it has been established that the bacterial complex is presented by: Gram-negative bacteria *Erwinia herbicola*, *Escherichia coli* and *Pseudomonas fluorescens* and Gram-positive spore-forming bacteria of the genus *Bacillus* (presumably *Bacillus subtilis*), *Clostridium perfringens* and gram-positive cocci.

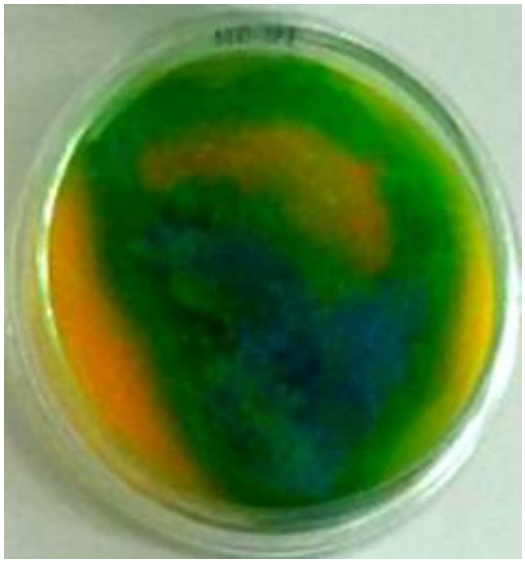
The results of the experiments according to the method [20] of identification of bacteria of the genus *Bacillus* in the composition of microfloras of grain crops show the existence of this type of microorganisms in wheat seeds and the absence of them in rye seeds (Fig. 1). True to form, the colonies that correspond to the representatives of bacteria of the genus *Bacillus* (Fig. 1a) have sizes from 2 mm to 5 mm, uneven edges with branchings on a smooth glass surface, a whitish-gray color in the center and a blue/turquoise background, and also a deposit aureole (the egg yolk reaction) up to 5 mm wide.

Table 1. Microbiological standards of grain safety

Parameters	Norm
Total plate count, CFU/g, not more than	5.0×10^3
Escherichia coli group bacteria, not permitted in the mass of the product (g)	0.01
Mold, CFU/g, not more than	50.0
B. cereus, not permitted in the mass of the product (g)	0.1
Pathogenic microorganisms, including salmonellas, are not permitted in the mass of the product (g)	25.0



(a)



(b)

Fig. 1. Visual analysis of presumptive bacteria of the genus *Bacillus* of microflora: (a) of wheat seeds, (b) of rye seeds.

Lack of the colonies that correspond to bacteria of the genus *Bacillus* bacteria in the analysis of bacterial microbiota of rye seeds (Fig. 1b) is explained probably by various susceptibility of plants to this complex of microorganisms, and also by the factors of the external environment of cultivation of seeds [22, 23, 24]. Besides, the rye grain taken for the study differed initially from wheat grains by lower contamination by microorganisms as a result of the initial analysis of content of microorganisms.

As for cultural and morphological features, it is possible to draw a conclusion that this kind of bacterium belongs to *Bacillus subtilis*. This culture forms flat, dry colonies of dense consistence with a specifically white granular deposit that can easily be taken off from the agar. The diameter is 2.5 mm. The edges are almost straight or slightly rugged. Other types of colonies have not been noted.

To confirm the colonies that correspond to representatives of bacteria of the genus *Bacillus subtilis* it has been also established using the microscopy method that the bacteria allocated by us from the microbiota of wheat seeds correspond to this kind. These are Gram-positive thin sticks of 3–5×0.9 microns, they are separate and have the form of threads or chains. They may contain ellipsoidal or cylindrical spores the diameter of which is not higher than the width of a microbic cage (Fig. 2).

On the basis of the obtained data on the quantitative and qualitative composition of microorganisms of the studied grain crops a need of their processing by antimicrobial means to prevent a microbiological damage has been determined.

The establishment of antibacterial activity of SNP solutions depending on the concentration and duration of processing of grain crops was the following stage of work, the obtained data are presented in Table 2.

Proceeding from the basic data on the content of the total viable count in raw grain crops it is possible to note that the least antibacterial effect of SNP can be observed after 2 hours of processing of grain of wheat and rye regardless of the concentrations and the type of colloidal solutions. At the same time, it is known from the studies performed earlier that the access of SNP to the cellular wall is complicated with a high concentration of bacterial cells, and a smaller amount of particles is adsorbed on its surface which provides a decrease in the biocidal effect [25].

It was shown during the subsequent quantitative analysis of bacterial microflora of grain that after 24 hours of processing the colloidal solutions of nanoparticles with the concentration of 0.1 g/dm^3, in general, show a deeper effect against the complex of microorganisms of grain crops in comparison with the lower concentration of SNP of 0.075 g/dm^3.

At the same time it should be noted that the control of the samples of grain processed by SNP after 24 hours has shown almost full compliance with the norms of TR TS 021/2011 [21]. Thus, it is obvious that SNP colloidal solutions are capable to inhibit effectively the development of bacterial microbiota of grain crops.

Analyzing the quantity of microorganisms in the grain processed after 144 hours it is possible to come to a conclusion that the influence of SNP is already insignificant in comparison with the previous period of processing (2–24 h).

At the same time, estimating the antimicrobial effect of colloidal solutions of the chosen concentrations after 144 hours by comparison of their inhibiting influence on microorganisms, it is fair to note that SNP solutions with the concentration both of 0.075 g/dm^3 and 0.1 g/dm^3 have the highest effect in both chosen preparations.

The obtained data have also been presented in the form of diagrams (Fig. 3 a–d).

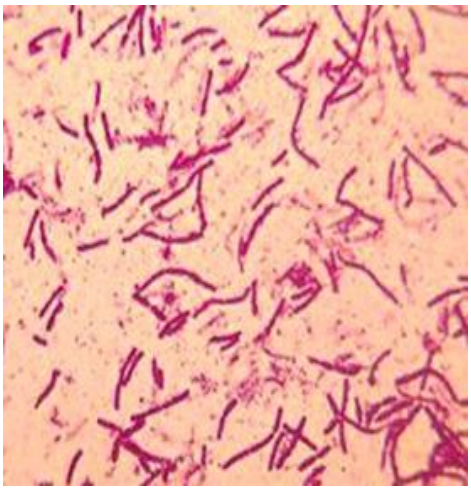


Fig. 2. Microscopic analysis of bacteria of the genus *Bacillus subtilis.*

Table 2. Antimicrobial activity of various concentrations of colloidal solutions of nanoparticles

Cultivation time	Total viable microorganisms in wheat, (CFU/g)				Total viable microorganisms in rye, (CFU/g)			
	Adzhenta		KND-S-K		Adzhenta		KND-S-K	
	0.1 g/dm^3	0.075 g/dm^3	0.1 g/dm^3	0.075 g/dm^3	0.1 g/dm^3	0.075 g/dm^3	0.1 g/dm^3	0.075 g/dm^3
2 h	$8\cdot10^3$	$7\cdot10^3$	$9\cdot10^3$	$8\cdot10^3$	$7\cdot10^3$	$6\cdot10^3$	$7\cdot10^3$	$7\cdot10^3$
24 h	$5\cdot10^3$	$5\cdot10^3$	$6\cdot10^3$	$5\cdot10^3$	$4\cdot10^3$	$5\cdot10^3$	$6\cdot10^3$	$6\cdot10^3$
144 h	$4\cdot10^3$	$3\cdot10^3$	$5\cdot10^3$	$5\cdot10^3$	$3\cdot10^3$	$4\cdot10^3$	$5\cdot10^3$	$4\cdot10^3$

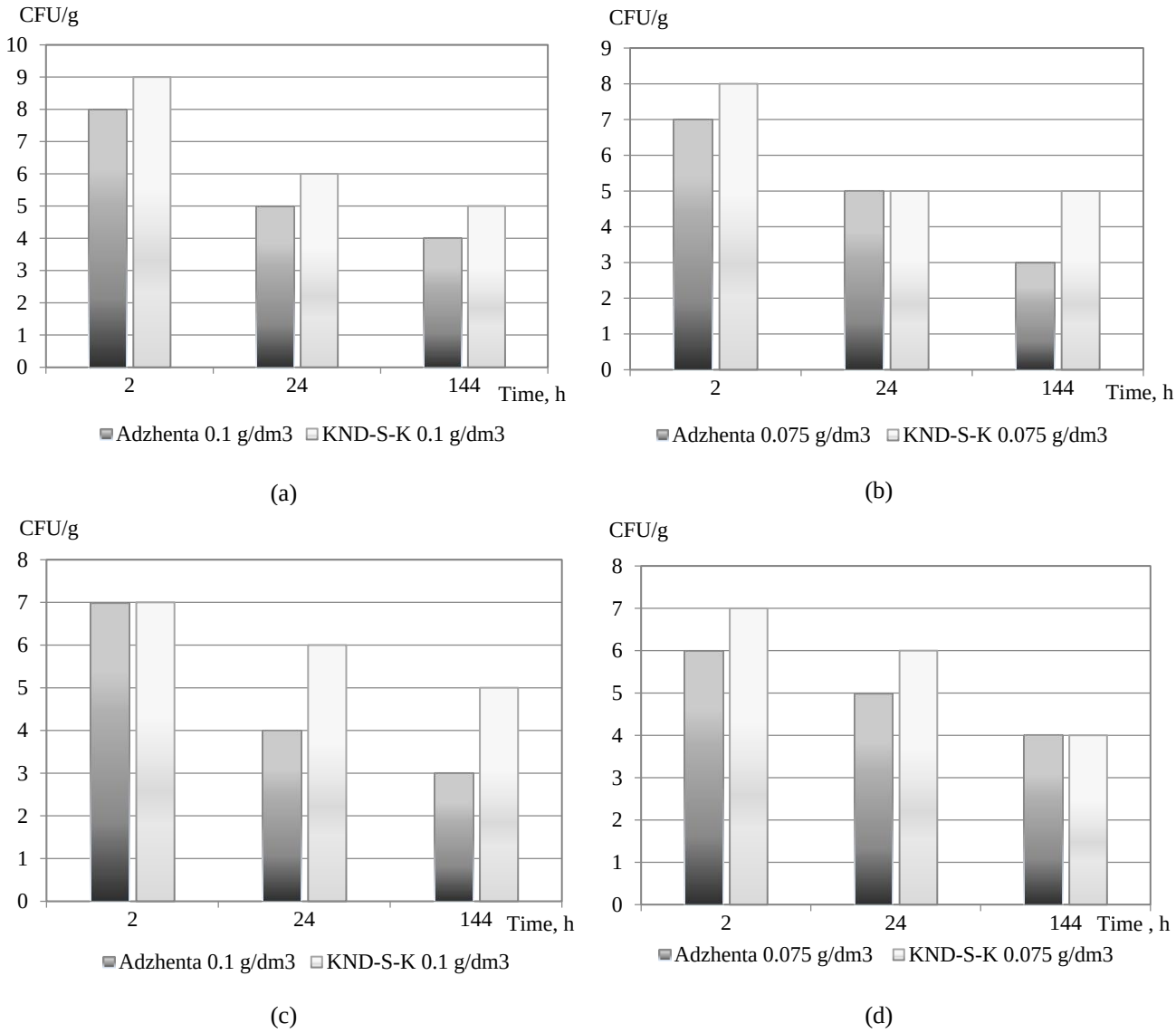


Fig. 3. Diagram of antibacterial activity of silver nanoparticles of various preparations on microorganisms: (a) of wheat with the concentration of 0.1 g/dm^3; (b) of wheat with the concentration of 0.075 g/dm^3; (c) of rye with the concentration of 0.1 g/dm^3; (d) of rye with the concentration of 0.075 g/dm^3.

At the same time, it is necessary, based on the performed studies, to emphasize quite a high antimicrobial influence of Adzhenta colloidal silver after 24 hours of processing in all the samples of grain in comparison with the solution of silver nanoparticles KND-S-K. Most likely, various ways of obtaining and stabilizing SNP in the solution of the used preparations are the reason for that. It is well known that atoms of metals have a high chemical activity which remains in the clusters and nanoparticles, which are formed of atoms, with a large number of atoms which can provide a sharp decrease in their activity [26, 27].

The SNP solutions of colloidal degree of dispersion of KND-S-K stabilized by arabic gum, chitosan and sulfoamber acid with the particles of 6–20 nanometers (Fig. 4) have a high antimicrobial effect, but in many respects depend on the physical and chemical factors of the environment due to the chemical method of stabilization [28, 29]. Chemically, Adzhenta colloidal silver is silver nitrate and is produced without any chemical processes, with the use of an electromagnetic charge which suspends silver particles of 5–15 nanometers (Fig. 5) in water with the formation of solution. Therefore, most likely it shows a more considerable effect.

Besides, as it is reported from the literary data [26, 30], the mechanism of inhibition of SNP is directly related to their size (less than 100 nanometers) and the largest surface area to volume. It is known that with a decrease in the size of particles the specific phase interface and, therefore, the comparative concentration of active silver increases which provides an increase in the area of contact of silver with bacteria.

At the stage of study of influence of SNP colloidal solutions on the presence of a bacterium of the genus *Bacillus subtilis* in the processed wheat sample, the results of the performed study also specify that under the influence of SNP there is destruction of a bacterial cell after 24 hours of cultivation in both chosen preparations. The effect of colloidal solutions was already shown in both cases with the concentration of 0.075 g/dm^3.

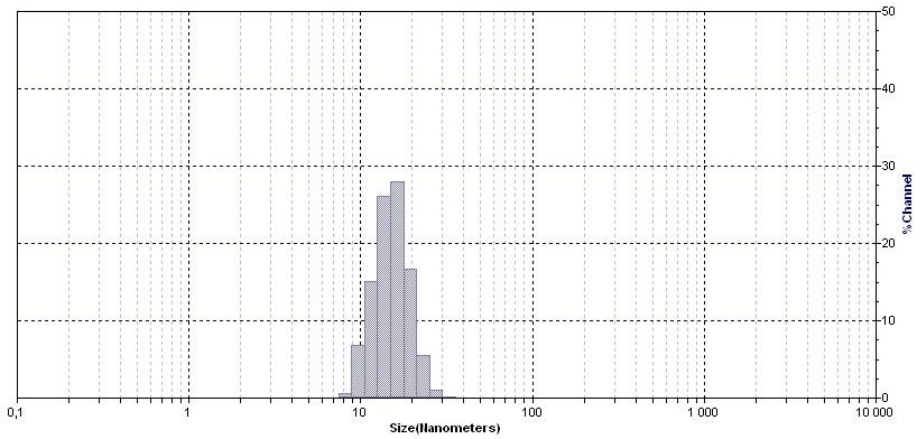


Fig. 4. Histogram of distribution of nanoparticles by size in the KND-S-K colloidal solution.

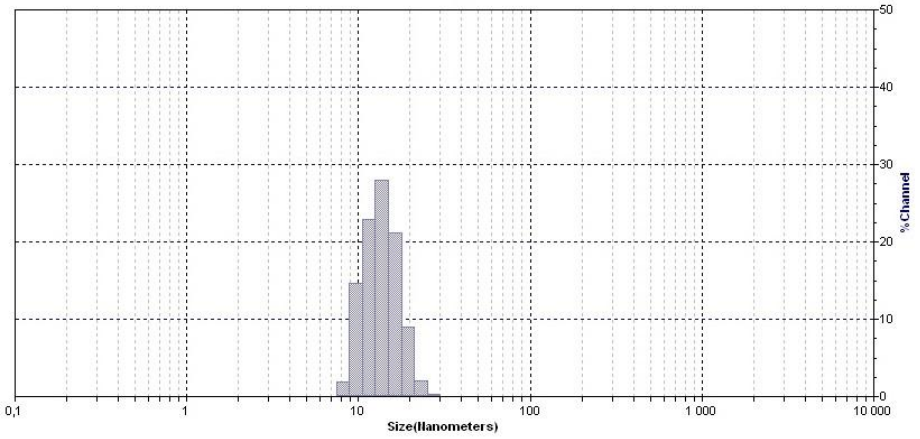


Fig. 5. Histogram of distribution of nanoparticles by size in the Adzhenta colloidal solution.

Thus, based on the results of the performed studies, it is possible to draw a conclusion that the addition of the preparation "Adzhenta colloidal silver" into the grain mass with the concentration of SNP of 0.1 g/dm^3 will allow after 24 hours to prevent the development of microbial contamination, will increase the period of storage and the quality of grain, and also provide the inhibition of further development of spore-forming forms of bacteria of the genus *Bacillus subtilis.* The processing of grain mass using the solution of silver nanoparticles KND-S-K of 0.1 g/dm^3 within 24 hours will also favorably effect the inhibition of bacterial microbiota of grain crops.

CONCLUSION

The performed study was developed to show the efficiency of antibacterial preparations of a new generation – nanodimensional silver dispersions for the food industry.

As a result of the done work, a method of application of preparations of silver nanoparticles to reduce the microbial contamination in the grain processing and baking industry providing an increase in the microbiological purity of grain for the purpose of providing biological safety has been offered.

Antibacterial properties of nanoparticles of Adzhenta colloidal silver and KND-S-K on the basis of understanding of their effect to prove their efficiency against various pathogenic microorganisms of grain crops have been studied.

Various results of effect of SNP preparations of the colloidal degree of dispersion (KND-S-K, Adzhenta) depending on the concentrations in the solution have been obtained and the preferable modes of processing of grain crops have been chosen. On the basis of results of the studies biostatic concentrations of SNP preparations have been determined.

The efficiency of their application against significant pathogenic bacterial microorganisms of grain productions has been proved (in particular, against *Bacillus subtilis*).

It has been established on the basis of study of influence of SNP colloidal solutions on the bacterial microbiota of grain of wheat and rye that SNP are capable to inhibit effectively the activity of the bacterial microorganisms that contaminate grain raw materials.

Finally, the conclusions provided in this work will help us to apply SNP colloidal solutions to inhibit the growth of bacterial microorganisms and reduce considerably the level of microbiological contamination of grain crops.

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