Nat. Volatiles & Essent. Oils, 2021; 8(4): 11206-11215

NVEO
Natural Volatiles & Essential Oils

Nanoparticles For Correctiing Food Microflora

Mohammed T. Mahmood [1] , Azeez Z. A.[2] , Marinin A.I.[3] , Krichkovskaya L.V.[4] , Dubonosov V.L.[4] , Lysak P.U.[4]

[1]Dep. of Basic Sci./ College of Nursing/ University of Mosul , Iraq ,

[2]Nineveh Education Directorate,

[3] National University of Food Technologies, (Kiev),

[4]National Technical University (Kharkiv) Ukraine

Abstract

Experimental data show that the presence of SNPs in the nutrient medium makes it possible to quickly suppress the development of bacterial microbiota and intensify the process of accumulation of the producer's biomass. The introduction of SNP inhibits bacterial microbiota and promotes the growth of the biomass of the producer. In thise research, several methods of processing raw materials and , products of fermentation industries with solutions of silver nanoparticles have been developed, to increase the possibility of their safety and finished products due to a decrease in the level of biological infection. In fermentation plants, the development of a bacterial infection can negatively affect the organoleptic properties of the final product. Losses of beer associated with a decrease in quality indicators account for up to 4% of the total volume of manufactured products. Taking into account the peculiarities of beer production, especially in small volumes, this indicator can be significantly higher for small businesses. Considering the fact that in order to be competitive, products must have outstanding organoleptic characteristics, even minor changes in taste, color and smell of beer can lead to the loss of a significant number of customers. The use of colloidal solutions of SNPs to control microbiological contamination of microorganisms can lead to a decrease in product losses.

Based on a study examined the effect of colloidal solutions of SNPs on microorganisms of fermentation industries (yeast, bacteria, filamentous fungi), it has been found, that SNPs are able to effectively inhibit the vital activity of bacterial microorganisms, the process of yeast generation, regardless of the structure of the cell wall of the microorganism and its ability to sporulation. In significant concentrations, SNPs inhibit the vital activity of cultured yeast. The environmental safety of the production of beer, kvass, alcohol - fermentation plants - is largely due to the microbiological safety of raw materials and production, which can be provided by the nanoparticles studied in the article.

Introduction

The development of the modern food industry is closely related to the use of the latest food technologies, one of the priority areas that are nanotechnology. Nanomaterials are complex objects . They can be considered as a special state of matter, since the properties of materials obtained using structural elements of the nanoscale range do not correspond to the properties of a bulk substance. Atoms of nanoparticles have tightly bound structures and are located on the surface of the material. Nanoparticles of different materials have a complex structure, respectively. The specific surface area of the particle itself increases, which indicates that its properties change. [1,2]. Particular attention should be paid to the antimicrobial properties of metal nanoparticles, which are pronounced in relation to pathogenic and technologically harmful microorganisms [3, 4]. In addition, unlike the ions of the same metals, they are non-toxic (at certain concentrations) for mammals [4- 6], due to which they can be used to extend the shelf life of food, as well as harmless to the body antimicrobial drugs of a wide spectrum of action. For instance, silver, as opposed to organic (chemical) preservatives, disinfectants, and antibiotics, is a natural element, which has been successfully used for thousands of years for disinfection, neutralization and preservation of water and food, as well as for medicinal purposes and in modern types of cosmetics [2-4]. Silver is a strong biocide for microbes and viruses (unicellular), and, unlike other metals and agents, at the same time it is much less toxic to multicellular organisms, including warm-blooded [5-7]. Unlike other antiseptics, silver has not only a wide spectrum of antimicrobial activity, but also virucidal and fungicidal properties, while being relatively low toxic to humans. Silver and silver-containing preparations do not pollute nature, they are environmentally friendly, "green" products.

Thus, silver quite closely falls under the concept of an "ideal" preservative, as well as an antiseptic component of various products.

The purpose of this study is to investigate the antimicrobial action of preparations of metal nanoparticles (PNM) on pure cultures of microorganisms. They are considered as typical contaminants in the production and storage of food products, as well as human pathogens, and on the natural microflora of unpasteurized beer to ensure "cold" pasteurization of the latter. In addition, the effect of some stabilizers on the antimicrobial activity of silver NPs was studied as well as characterization of the antimicrobial action of silver NPs in colloidal solutions and determination of the terms of preservation of their antimicrobial activity. To stabilize silver NPs, a surface active agent (surfactant) sodium dodecyl

sulfate (SDS), polymer polyvinylpyrrolidone (PVP) was used. Previously, one of the compounds was used to stabilize colloidal silver solutions - surfactant, polymer or colloidal Ludox silica. In this work, SDS was used for comparison in a ratio of 15: 1 relative to metal; PVP in a molar ratio of 1: 1; and also, , as opposed to the conventional stabilization method, both stabilizers _ SDS and PVP, for which the powders of SDS and PVP were simultaneously dissolved in water by adding silver nitrate to the formed colloidal solution, the total concentration of which was 1.5·104 mol / dm3.

Objects and methods of research

The objects of the study were strains Escherichia coli IEM-1, Bacillus subtilis BT-2, Candida scottii KB-2, Saccharomyces cerevisiae OB-3, Aspergillus niger P-3, Fusarium culmorum T-7, Penicillium chrysogenum F-7, and unpasteurized beer.

For research, we used PNM with particle sizes from 0.7 to 250 nm with different stabilizers. The size distribution and zeta potential of the PNM were determined using a particle size analyzer and zeta potential Zetasizer Nano ZS (Malvern Instruments Ltd, United Kingdom). The measurement results are shown in Fig. 1-6.

The choice of PNM was due to their safety, as well as the effectiveness of action against strains of microorganisms, which is manifested by metal particles outside the nanometer range [8].

Fig.1. Particle size distribution PNM Ag	Fig. 2. Distribution of zeta potential of PNM Ag

Fig. 3. Particle size distribution of PNM Au	Fig. 4. Distribution of the zeta potential of PNM Au

Fig. 5. Particle size distribution of PNM CeO_2	Fig. 6. Distribution of the zeta potential of PNM CeO_2

For the studies used PNM CeO_2, with particle sizes in the range from 3 to 5 nm (Fig. 5) with a zeta-potential value of -23 mV (Fig. 6). The characteristics of the PNM are given in table. 1.

Table. 1. Characterization of preparations of metal nanoparticles.

№ PNM	PNM	Size range PNM, nm	Stabilizer	Concentration of metal (compound), mg / ml

1	CeO_2	3-5	Stabilizer PAA (polyalylamide, 7.0)	3,5
2	CeO_2	3-5	Stabilizer PAA (polyalylamide, 7.0)	2,0
3	Ag	1,5-250	Stabilizer PVP (polyvinylpyrrolidone)	12,5
4	Ag	1,5-250	PVP stabilizer (5,0)	1,5
5	Au	0,7-30	Stabilizer PVP (polyvinylpyrrolidone)	0,2

A number of difficulties arise in the preparation of nanoparticles. It is well known that silver NPs are rapidly oxidized upon contact with air with the formation of an oxide layer around NPs. In addition, NPs easily aggregate in solutions, which leads to a decrease in their activity. Therefore, the issue of stabilizing solutions of silver NPs remains topical up to now.

To determine the antimicrobial properties of PNM by diffusion method into glucose-potato agar (GPA) or meat-peptone agar (MPA), which were poured into Petri dishes in a thick layer (30 ml per dish) and kept in a thermostat at 30 °C for a day, after which a continuous layer of suspension (0.1 ml) of daily test cultures was sown, grown on agar media (bacteria on MPA, yeast on GPA). After inoculation of microorganisms in the medium, four holes 10 mm in diameter were made with a sterile drill, into which the studied PNM were introduced into which the studied PNM was introduced. At the end of incubation (3-4 days), the zones of growth inhibition of test cultures were measured and the lowest concentration of PNM was recorded., which led to a bacteriostatic effect.

Determination of the antimicrobial properties of PNM in the suspension of the studied cultures was carried out according to the method, in which in the initial suspension of the studied test-cultures of bacteria and yeast grown on agar media (bacteria - MPA, yeast – GPA) within 15, 24 and 72 hours, the number of living cells was determined by the Koch method (colony-forming units, KUO / ml). Next, the suspension of test cultures was introduced into test tubes (3 ml), the calculated volumes of PNM were

added and kept for 0; 0.5; 1 and 24 hours, at the optimum temperature necessary for the growth of these cultures. After the time of each exposure, the number of living cells was determined by the Koch method.

Cell survival was determined as the ratio of the number of living cells in the treated PNM in the samples to the number of cells in the original suspension as a percentage.

Antimicrobial effect of PNM on the microflora of food products (unpasteurized beer) were determined by introducing them into a test sample with a volume of 3 ml. The duration of exposure was 0; 0.5; one; 24; 168 and 504 h. As a control, we used beer without PNM, which was kept in a thermostat. Sowing was carried out on MPA and wort agar (WA) to determine changes in the amount of bacterial and yeast microflora.

Results and Discussion

It was found that with an increase in the concentration of PNM, their antimicrobial effect increases. This pattern can be observed under the action of the PNM CeO2 (preparation № 1, Tab. 1) on cells E. coli IEM-1 and PNM Ag (preparation № 5, Tab. 1) - on B. subtilis BT-2 (with an increase in PNM concentrations from 0.5 to 7.5 mg / l, the growth retardation zones of test-cultures increased from 0 to 12 mm). The most effective was PNM Ag (preparation № 4, Tab. 1), which was characterized by a wide spectrum of bactericidal action on strains of microorganisms (Fig. 7).

Fig. 7. Comparative effect of preparation № 4 (PNM Ag) on daily cultures of microorganisms. Initial cell concentration (KUO / ml): S. cerevisiae OB-3–2.10^8; B. subtilis BT-2–2.10^7; E. coli IEM-1–3.10^8

As the dependence shows (Fig. 7), PNM acted on both bacterial and yeast cells PNM CeO2 (preparations № 2, № 3, Tab. 1) and PNM Au (preparation № 6, Tab. 1) did not show antimicrobial effect on test-cultures at all concentration ranges.

It was found that PNM did not show a bactericidal effect on micromycetes (A. niger P-3, F. culmorum T-7 и P. chrysogenum F-7) and yeast C. scottii KB-2. It is likely that a higher concentration of drugs is needed to inhibit the growth of these microorganisms.

Further studies were carried out on test-cultures S. cerevisiae OB-3 and B. subtilis BT-2. The choice of these cultures is due to the fact, that Saccharomycetes are very common in the food industry. (e.g. yeast in the production of bread, beer), and potato stick is a food contaminant and can form heat-

resistant spores. The study was carried out by introducing the maximum concentration of PNM - 5 mg / l (preparations № 3, № 5, № 6, Tab. 1).

It was found that PNM CeO2 (preparation №. 2, Tab. 1) does not have an antimicrobial effect on B. subtilis cells BT-2, but inhibits S. cerevisiae OB-3 by 50 % after an hour of exposure, and after 24 hours – 92 % (Fig. 8). PNM Au (preparation № 5, Tab. 1) began to act immediately, but over time, the number of living cells of both cultures increased almost to the initial level (Fig. 8). PNM Ag turned out to be the most effective: it showed the greatest bactericidal effect on B. subtilis BT-2 - after an hour of exposure, 100 % death of bacteria was observed (Fig. 8).

Fig. 8. Survival of daily cells of B. subtilis BT-2 and S. cerevisiae OB-3 with the addition of PNM. Initial cell concentration (KUO / ml): B. subtilis BT-2 (24 h) – $4{,}6.10^3$; S. cerevisiae OB-3 (24 h) – $3{,}5.10^2$

Since PNM had an effective effect on pure cultures of microorganisms, then in further experiments it was decided to investigate their effect already when introduced directly into the product. We used unpasteurized beer in which PNM was added Ag, Au, CeO_2 at a concentration of 5 mg / l and PNM Ag/Au in different proportions. It was found that different PNMs are more active at different times: PNM short-term (up to 24 hours)– Au, Ag/Au; PNM of early (up to 1 hour) and late (after 168 hours) action – CeO_2; PNM of prolonged action (0-504 h)– Ag. This phenomenon can be explained by a change in the species microflora of beer during storage. At the end of the exposure, PNM Ag turned out to be the most effective. He, unlike PNM Ag/Au (80/20), showed weaker antimicrobial activity at the beginning of the experiment, but after exposure decreased to 16 % of bacterial and yeast flora 41 % (Tab. 3), and when compared with the action of PNM CeO2 and PNM Au, it retained activity throughout the entire exposure time.

Table 3. Antimicrobial effect of PNM on the microflora of unpasteurized beer.

PNM	Cell survival (%) microflora	
	bacterial	yeast
Au (№ 6)	95±5,0	93±4,7
CeO_2 (№ 3)	76±3,8	91±4,6
Ag (№ 5)	84±3,7	59±2,9

Note: The initial concentration of cells after 504 h - 1.2 10^5 (KUO / ml). 60/40; 80/20 - ratio of PNM Ag / Au, respectively (%)

As a result of studies of the bactericidal action of PNM Ag, Au, CeO2 on the microflora of food and intermediate products at a concentration of 0.5-7.5 mg / l it was found that, they exhibit antimicrobial action against E. coli IEM-1, B. subtilis BT-2 и S. cerevisiae OB-3 (growth retardation zones up to 12, 19, 0.45 mm), but do not inhibit micromycetes (A. niger P-3, F. силтогит T-7 и P. chrysogenum F-7) and yeast C. scotti KB-2. At the same time, PNM Ag turned out to be the most effective, which alone or in a composition with PNM Au inhibits the growth of bacteria and yeast by almost 100 % after an hour of exposure., reduces by 20-30% the contaminating (bacterial and yeast) microflora of unpasteurized beer, effectively acts on resistant spore culture of B. subtilis BT-2.

As already mentioned above for the stabilization of silver NP used surface active agent (surfactant). When using polyvinylpyrrolidone polymer (PVP) come into opposition to two properties of this compound. PVP is a nonspecific antidote to silver. At the same time, it has long been known that even insignificant additions of PVP significantly increase the stability of colloidal silver solutions. Samples were subjected to microbiological examination: colloidal solution of silver NPs with PVP stabilizer. During the experiments, control samples were also investigated in parallel at the appropriate concentrations: silver nitrate solution ($AgNO_3$) and PVP stabilizer solution. The studies were carried out using test microorganisms Staphylococcus aureus ATCC 6538, Escherichia coli K12 NCTC 10538, Pseudomonas aeruginosa ATCC 15442 and Candida albicans ATCC 10231. Test samples were examined after 1, 2, 4 and 24 hours, and also 15 and 30 days to find out the terms of preservation of the antimicrobial activity of solutions at room temperature storage. The antimicrobial properties of colloidal solutions of Ag NPs were studied using the suspension method.

First of all, attention was drawn to the almost complete absence of antimicrobial activity in a solution of colloidal silver with its stabilization by PVP over the observation period, with the exception of the effect on E. coli - after 24 hours of exposure, a reduction of> 5.06 l g was achieved. Minor additions of PVP significantly increased the stability of colloidal silver solutions. Such results were obtained by many authors when working with nanoparticles [10-12]. At the same time the authors also indicate the presence of bactericidal action of silver NP solutions stabilized with PVP [11-13]. Positive results obtained from the experimental selection of stabilizers, made it possible to start studying the antimicrobial action of solutions of silver NPs stabilized with a binary mixture, which should increase the positive effect, depending on the type of microorganisms and the determination of the shelf life.

Conclusion:

1. Antimicrobial action of native silver nanoparticles and in coalition with surfactant (polyvinylpyralidone) has been shown on microorganisms Staphylococcus aureus ATCC 6538, Escherichia coli K12 NCTC 10538, Pseudomonas aeruginosa ATCC 15442 and Candida albicans and yeast C. scotti KB-2. ATCC.

2. The bactericidal action of PNM Ag, Au, CeO2 on the microflora of food products and intermediates was shown at their concentration of 0.5-7.5 mg / l and it was found that they exhibit antimicrobial action against E. coli IEM-1, B. subtilis BT-2 и S. cerevisiae OB-3.

References

1. The study of the antimicrobial activity of colloidal solutions of silver nanoparticles prepared using food stabilizers / G. Balandin, O. Suvorov, L. Shaburova. D. Podkopaev, Yu. Ustinova, G. Ermolaeva// Journal of Food Science and Technology. - 2015. - T. 52. - Issue 6 - C. 3881-3886.

2. Comparative Evaluation of Antimicrobial Activity of Silver Nanoparticles / D. Podkopaev, L. Shaburova, G. Balandin, O. Kraineva, N. Labutina. O. Suvorov, Yu. Sidorenko // Nanotechnologies in Russia. - 2014. - № 9. - C. 93-97.

3. Головин Ю.И. Нанотехнологическая революция стартовала / Ю.И. Головин // Природа. – 2004. – №1. – С. 25–36.

4. Sharma V.K. Silver nanoparticles: green synthesis and their antimicrobial activities / V.K. Sharma, R.A. Yngard, Y. Lin // Adv. Colloid. Interface Sci. – 2009. – V. 145, № 1–2. – P. 83–96.

5. Non-cytotoxic silver nanoparticle-polysaccharide nanocomposites with antimicrobial activity / A. Travan, C. Pelillo, I. Donati, [et. al.] // Biomacromolecules. – 2009. – V. 10, № 6. – P. 1429–1435.

6. Sukhina M.A., Shelygin Yu.A., Piyadina A.Yu., Feldman N.B., Ananyan M.A., Lutsenko S.V. The inhibitory and destructive action of the silver nanoparticle preparation on biofilms formed by clinically relevant microorganisms. / Koloproktologia. 2019;18(3(69)):56-70.

7. Борисович В.Б. Застосування наночасток Ag, Cu, Zn у лікуванні ран / В.Б. Борисович // Здоров'я тварин і ліки. – 2008. – №3. – С. 14–15.

8. Препараты серебра: вчера, сегодня, завтра / [Поляченко Ю.В., Щербаков А.Б., Усатенко А.В., Повх Г.В.]. – К.: МАУП, 2007. – 66 с.

9. Мосин О.В. Золото и нанотехнологии: [Электронный ресурс] / О.В. Мосин. – Режим доступа: URL: http://www.o8ode.ru/article/water/nanotechnology/nanogold.htm. –

10. Синтез и свойства наночастиц серебра: достижения и перспективы / Ю.А. Крутяков, А.А. Кудринский, А.Ю. Оленин и др. // Успехи химии. — 2008. — Т. 77, № 3. — С. 242269.

11. Onestep, sizecontrollable synthesis of stable Ag nanoparticles / Ch. Tian, B. Mao, E. Wang et al. // Nanotechnology. — 2007. — Vol. 18. — P. 285.

12. Препарати срібла: вчора, сьогодні і завтра / О.Б. Щербаков, Г.І. Корчак, О.В. Сурмашева та ін. // Фармацевтичний журнал. — 2006. — № 5. — С. 55-67.

13. Ребрикова Н.Л. Исследование действия наносеребра и нитрата серебра на рост микроскопических грибов. Перспективы использования наносеребра для защиты реставрационных материалов / Н.Л. Ребрикова // Immu nopathology, Allergology, Infectology. — 2010. — № 1. — С. 224