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CONSIDERATION OF TECHNOLOGICAL AND SAFETY ASPECTS OF USING ZINC OXIDE NANOPARTICLES FOR INTENSIFYING WHEY FERMENTATION

The study is dedicated to using zinc oxide nanoparticles (ZnO) to intensify the fermentation of whey, an important resource in the food industry. Traditional methods of whey fermentation take a lot of time and require significant resources, reducing their economic efficiency. This study found that the addition of ZnO nanoparticles significantly accelerates the fermentation process. Treating whey with the electro-spark method for 60 seconds allowed achieving the necessary acidity level (160±10 °T) in 18 hours, almost twice as fast as traditional methods, which take up to 36 hours. ZnO nanoparticles also improve the activity of lactic acid bacteria and increase the bactericidal ability of macrophages, which contributes to the overall efficiency of the fermentation process.

The use of ZnO nanoparticles in whey production can significantly improve the efficiency of the technological process, reducing fermentation time and improving the quality of the final product. This opens up new prospects for medium and small enterprises looking to improve the economic efficiency of their operations.

In addition to accelerating fermentation, ZnO nanoparticles have additional advantages in terms of product safety and quality. The study showed that ZnO nanoparticles enhance the antioxidant properties of fermented products, which is important for maintaining their freshness and nutritional value. The high reactivity of ZnO nanoparticles allows them to interact with bacterial membrane receptors, increasing their metabolic activity and resistance to external factors.

Thus, the study demonstrates the significant potential of using ZnO nanoparticles to intensify the whey fermentation process, contributing to more efficient production of food products and ensuring their high quality. This is especially important in modern conditions of limited resources and growing demands for economic efficiency and food safety. The introduction of ZnO nanoparticles into production processes can be a key step in improving fermentation technologies and increasing the competitiveness of food products in the market.

Keywords: *whey, lactic acid, zinc oxide nanoparticles, whey fermentation, intensification, cytotoxicity of nano-ZnO.*

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1. Introduction

Whey is a powerful raw material reserve for the production of food products for mass consumption, as well as for special purposes, in the modern conditions of limited resources of raw milk, due to a number of reasons. It finds use:

– as the main raw material during the production of dry demineralized whey, which has a stable demand in the domestic and foreign markets;
– fresh and fermented drinks based on it;
– as a coagulant during the production of casein and thermo-acid curd cheeses;
– as a food ingredient in the recipes of confectionery, bakery, and meat products;
– as a co-substrate in the production of biogas, etc.

In recent years, the volumes of whey processing have been consistently high, while the processing of sour milk whey has been restrained by a number of technical and technological reasons. This issue is most relevant for operators of the small and medium capacity market, for whom whey processing is more of a problem than an economically attractive direction of milk processing.

Economic and environmental factors encourage an increase in the volume of industrial use of lactic acid whey for food purposes. A promising direction is the production of fermented sour whey with a titrated acidity level of 150–170 °T, which is advisable to use as a coagulant in the production of soft cheeses of thermal acid curdling and a natural acidifier for the dough of rye-wheat bakery products.

The data of the State Statistics Service of Ukraine [1], which indicate a stable positive trend of growth in the volume of production of the specified products, testify in favor of the expediency of considering this direction.

Fermentation of lactic acid whey to achieve the appropriate level of acidity requires time and free production space for its preparation and reservation. This technological

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process can take up to 48 hours and, as a result, the duration of the technological cycle increases and inevitably affects the economic indicators of production as a whole. This fact testifies to the relevance of finding ways to intensify the technological process of manufacturing lactic acid whey.

Technological approaches involving the intensification of lactic acid whey fermentation are, as a rule, limited to the use of various organic acids, food additives and are extremely limited. Then, the improvement of the technology by creating favorable conditions for the growth of lactic acid microflora in the fermented environment deserves attention. This can be achieved by enriching the whey with biologically valuable mineral elements that will act as a nutrient medium for microorganisms and contribute to the growth of the biomass of lactic acid bacteria. This contributes to the intensification of acid formation in the system.

This way is promising, because at the same level as the intensification of the whey fermentation process, it will contribute to the enrichment of food products and products made from it or with its use, with mineral elements.

Microbial cells need a large amount of macro- and microelements: carbon, nitrogen, oxygen, hydrogen, phosphorus, sulfur, potassium, calcium, magnesium, zinc, manganese, sodium, chlorine, molybdenum, silicon, etc. [2]. Zn, Mg and Mn, etc. can be considered as factors of fermentation intensification. They participate in the construction of components of living cells, contribute to the energy exchange and protein synthesis of the microbial cell, are able to activate and stabilize the action of enzymes [3, 4]. Separately, works [5, 6] show improvement in the fermentation activity of brewer's yeast.

The paper [7] shows the positive effect of metal-containing particles of magnesium and manganese with the dominance of the nano-sized state on the fermentation of sour whey.

It was established that the addition of zinc has a positive effect on the physiological function of the *L. plantarum* DNZ-4 strain. At the same time, the antioxidant properties of the zinc-enriched strain increase, including the ability to absorb radicals [4].

The paper [5] describes the positive effect on the viability and fermentation activity of brewer's yeast of their electron-ion processing, which is carried out in the stream during the loading of yeast into the fermentation apparatus. It has been proven that such processing helps to reduce the number of non-viable cells by 26–40 % and accelerate the wort fermentation by an average of 2 days.

It was found that adding zinc nanoaquachelate with a Zn concentration of 0.10 mg/dm^3, obtained by the electrospark method, increases the fermentation activity of beer yeast [6]. At the final stage of fermentation, the amount of released CO_2 was 14 % more than in the control. At the same time, the amount of fermented extract in young beer decreased by 15 % compared to the control, and the main fermentation process was shortened by 1–2 days, depending on the concentration of the initial wort.

In addition to influencing the vital activity of cells of microorganisms, zinc is one of the most important trace elements in the body, which performs three main biological roles: catalyst, structural and regulatory ion [8]. Zinc is one of the most important and irreplaceable trace elements for the vital activity of the human body. In terms of distribution in the human body, it ranks second after iron. Zinc is indispensable for gene expression and metabolism of nucleic acids, and, accordingly, for all processes of cell growth and differentiation. It is a structural component of biological membranes, cell receptors, proteins, and is part of more than 200 enzyme systems that regulate basic metabolic processes. Considering the fact that zinc has a significant effect on the growth and differentiation of cells, its role in various periods of human life – from early childhood to the period of sexual development cannot be underestimated. In addition, the latest studies on the effect of zinc have confirmed that it has the most specific and significant effect on the state of the human immune system. And in the current conditions of the flourishing of viral diseases, in particular, the coronavirus, its importance increases even more.

Whey in its composition contains a whole set of macro- and microelements [8], but the amount of zinc is insufficient to ensure the vital activity of cells of microorganisms and their growth.

Today, in connection with the development of nanotechnology, special attention is paid to zinc and its compounds in the nanoscale state. One of the priority types of nanomaterials are zinc oxide nanoparticles (nano-ZnO), which are used on an ever-increasing scale in various spheres of life. In particular, ZnO nanoparticles (NPs) are widely used in medicine and pharmacology, food industry, agriculture, production of cosmetics and personal hygiene products [9–11].

The above confirms the expediency of enriching whey with zinc to accelerate its fermentation.

The aim of this research is to establish the parameters of the fermentation course of milk whey enriched with zinc, as a result of electrospark dispersion of conductive metal granules. This will allow manufacturers to intensify the production process of sour whey with its further use as a coagulant for soft thermo-acid curdled cheese and a food ingredient for rye-wheat products, as well as establishing safety indicators for the use of zinc nanoparticles.

2. Materials and Methods

The object of research was the technological process of fermentation of whey enriched with zinc oxide nanoparticles in a comparative aspect with the use of $ZnSO_4$ salt.

The subject of research was the quality and safety indicators of fermented lactic acid whey produced with the use of ZnO nanoparticles.

Fermentation was performed on whey obtained by thermal acid coagulation of milk, as it is considered the most receptive medium for the cultivation of lactic acid bacteria. Before fermentation, whey was cleaned of particles of precipitated protein, then it was processed on an experimental periodic electric discharge unit developed by scientists of the National University of Life and Environmental Sciences of Ukraine [12, 13] by dispersing conductive zinc granules in the whey medium. Electrospark processing parameters: capacitor charging voltage – 80–100 V, capacitor capacity – 100 μF, processing time – varied from 30 to 120 s in steps of 30 (*samples 1–4*).

Next, the processed milk whey was pasteurized at a temperature of (76±2) °C, cooled to a temperature of (37±1) °C and fermented with pure cultures of *Lactobacillus acidophilus*. The fact that they are characterized by a high level of acid formation (the limit of acid formation) over 300 °T. This, in turn, is of significant importance during the processing of whey with an elevated pH level due to the accumulation of metal particles. At the same time, this culture is an active antagonist against foreign microbiota and can ensure the safety of the finished product. Fermentation

was carried out at a temperature of (37±1) °C until the desired level of acidity (160±10) °T was reached.

The control was fermented whey produced without the use of electrospark processing of raw materials (hereinafter referred to as *the control*).

To compare the effect of zinc in the nano- and micro-sized state with the effect of Zn ions on the fermentation of milk whey, $ZnSO_4$ salt was added before fermentation in an amount corresponding to the amount of Zn enriched in the whey as a result of electrospark dispersion for 60 s (*sample 5*).

To determine the effect of electrospark treatment on the acid-forming properties of *Lbc. acidophilus*, the titrated acidity of milk whey was determined after every 6 hours of fermentation at a temperature of (37±1) °C.

To determine the dispersion characteristics and cytotoxicity of ZnO particles on the electric discharge complex, a colloidal solution of zinc was obtained by dispersing granules of this metal in a medium of deionized water (conductivity 0.001–0.003 mS/cm). The unit scheme is shown in Fig. 1.

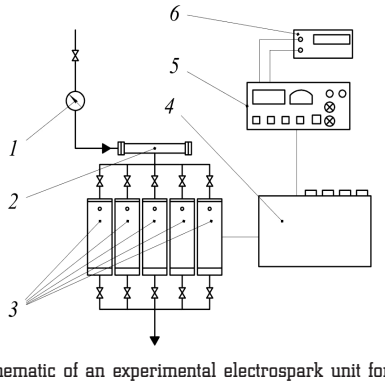

Fig. 1. Schematic of an experimental electrospark unit for obtaining colloidal zinc solution: *1* – manometer; *2* – collector for distribution of deionized water between reaction chambers; *3* – discharge reaction chambers with a conductive layer of zinc granules; *4* – discharge pulse generator; *5* – control panel; *6* – control and measuring device

The main element of the laboratory technological complex is a discharge pulse generator *4* with a pulse frequency of 0.2–2.0 kHz and a discharge circuit inductance of 1 μH. The power part of the installation is built on a three-sided element base, a capacitor is used as an energy store.

The zinc granules to be dispersed were placed on the bottom of the discharge chamber *3* with a volume of up to 1000 cm^3 between the main electrodes made of the appropriate metal.

The concentration of zinc was determined by the method of optical emission spectrometry with inductively coupled plasma (OES-ICP), Optima 210 DV device manufactured by PerkinElmer (USA).

The statistical size distribution of zinc particles and the electrokinetic potential (ζ-potential) were investigated by dynamic light scattering on a Malvern Zetasizer Nano ZC analyzer (Malvern Instruments Ltd, UK).

Cytotoxicity of ZnO NPs was determined by alternative methods of in vitro toxicological studies, namely: their effect on blood plasma proteins was evaluated by the method of studying their denaturation. At the same time, conformational changes in the structure of proteins were determined after incubation of human albumin and immunoglobulin (Biopharma, Kyiv) with ZnO·NPs and Zn ions ($ZnSO_4$) in vitro.

For the experiment, protein solutions were prepared in a 0.9 % NaCl solution with a final protein concentration of 1 mg/cm^3. The protein solution was carefully mixed with a solution of metal NPs in a 1:1 ratio, incubated for 2 hours at 37 °C. For each protein, a series of studies was performed: *1 test tube*: 1 cm^3 of protein + 1 cm^3 of 0.9 % NaCl (negative control); *2 test tubes*: 1 cm^3 of protein + 1 cm^3 of 0.1 M HCl in 0.9 % NaCl (positive control); *3–7 test tubes*: 1 cm^3 of protein + 1 cm^3 of a solution of ZnO and $ZnSO_4$ nanoparticles with a concentration of 0.45; 0.25; 0.13; 0.056; 0.028 mg/cm^3 by metal. The optical density of the experimental samples was measured in relation to the negative control on a spectrophotometer PV 1251 C at a wavelength of 450 nm. According to the test results, the degree of denaturation of blood proteins x (%) was determined according to the formula:

$$x = \frac{OD_{t.s.}}{OD_{p.c.}} \cdot 100\ \%,$$

where $OD_{t.s.}$ – optical density of the test sample; $OB_{p.c.}$ – optical density of the positive control.

In order to assess the effect of ZnO nanoparticles and $ZnSO_4$ salt on the functional state of phagocytic cells, a study will be conducted on macrophages isolated from the peritoneal exudate of rats, in the reaction of nitroblue tetrazolium reduction (HBT test).

To obtain peritoneal macrophages, an animal anesthetized with sodium ethamin was injected intraperitoneally with 5 cm^3 of medium 199 to wash out cells from the abdominal cavity. After isolation, the cells were washed twice with medium 199, and the total number of living cells in the suspension was counted using the vital dye trypan blue in a Goriaev chamber. Cell suspensions with a viability of at least 95 % were used in the experiment. Cells at a final concentration of $5 \cdot 10^6$ cells/cm^3 were placed in a 96-well flat-bottom plate for cell cultures at 100 μl per well. Solutions of ZnO and $ZnSO_4$ nanoparticles were added to the experimental wells in the following zinc concentrations: 1.13; 0.56; 0.28; 0.14 and 0.07 mg/cm^3, incubated for 1 hour in a thermostat at a temperature of 37 °C in the presence of CO_2. The level of influence of NPs and zinc ions on the bactericidal activity of peritoneal macrophages was assessed by the ability to produce reactive oxygen species («respiratory burst») in the HBT test. The cell suspension was transferred to a glass slide, after fixation with methanol it was stained with 0.2 % neutral red. The percentage of cells containing dark blue formazan granules (HBT-positive cells) was counted under a microscope [14].

The results of experimental studies were subjected to statistical processing implemented using standard Microsoft Office software packages.

3. Results and Discussion

At the first stage, the dispersion characteristics of the obtained ZnO particles were investigated. Dispersion analysis established that the obtained colloidal zinc solution contained particles in the size range from 40 to 300 nm with an average size of 101.3±5.0 nm (Fig. 2).

About 50 % of ZnO particles are in the nanoscale range, which contributes to increasing the biological availability and functional-technological efficiency of the valuable mineral element.

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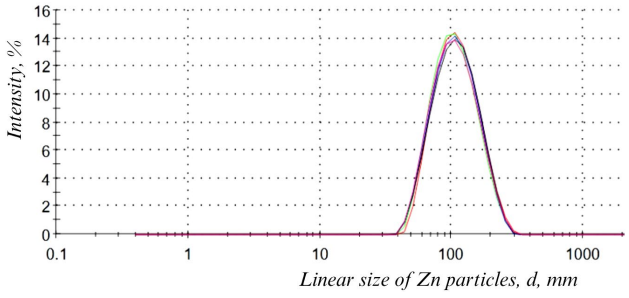

Fig. 2. Distribution curves of linear sizes of ZnO particles

The ζ-potential of the colloidal solution was –15.4±6.12 mV, and the polydispersity index was 0.101. The indicated indicators make it possible to claim that it is sedimentation resistant.

It should be noted that with an increase in the processing time of 90–120 s, the proportion of the microfraction in the samples increased.

It was found that after electrospark processing of milk whey for 30–120 s in the electric discharge complex, the zinc content in the test samples increased by 1.5–3.2 times, depending on the duration of the processing. It was found that due to the saturation of the whey with metal-containing particles, the pH level of the samples increased. At the same time, a direct dependence of the increase in pH on the treatment time was found.

Electrospark treatment of whey for 30–90 s in an electric discharge complex with conductive zinc granules did not have a significant effect on organoleptic parameters, however, after 120 s treatment, negative taste and smell descriptors appeared.

At the next stage, the course of the fermentation process of the obtained samples of milk whey enriched with ZnO particles was studied in comparison with the control and whey enriched with $ZnSO_4$ (Fig. 3).

It was established that a gradual increase in titrated acidity during the fermentation of whey fermented with pure *Lbc. Acidophilus* cultures was characteristic of all experimental samples.

However, it should be noted that in *samples 1–2* enriched with ZnO nanoparticles as a result of electrospark treatment for 30–60 s, the increase in titrated acidity (ΔT) over time was more intense compared to *the control* and *sample 5* (Fig. 3). Thus, after 24 hours of fermentation in experimental *samples 1–2*, the titrated acidity increased to 150–171 °T depending on the processing time; in *samples No. 5* – up to 132 °T. Whereas in *the control* at the same time this indicator was only 121 °T.

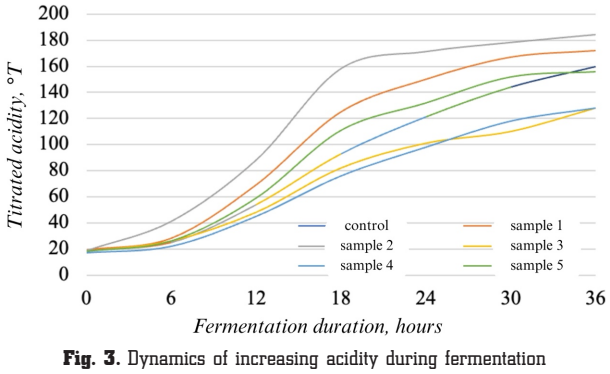

Fig. 3. Dynamics of increasing acidity during fermentation of milk whey enriched with ZnO·NPs, compared to the control and the addition of $ZnSO_4$ salt

It was noted that with the extension of the duration of electrospark treatment to 90–120 s (*samples 3–4*), fermentation occurred more slowly, which can be explained by the increase in the number of metal particles in the micro-sized range and, as a result, the shift of pH in the whey to the alkaline side.The culture of lactic acid microorganisms *Lbc. acidophilus* has the highest acid-forming activity showed in milk whey treated in a discharge chamber with a conductive layer of Zn granules for a treatment duration of 60 seconds.

It was found that whey enriched with ZnO nanoparticles (*sample 2*, 60 s treatment) acquired the appropriate acidity (160±10 °T) after an average of 18 h, while whey with $ZnSO_4$ added after 28–30 h, and *the control* after 32–36 h of fermentation.

Numerous scientific studies show that nanoparticles have special physicochemical characteristics that distinguish them from substances in their normal state. Nanoparticles are able to pass through biological barriers within the body, penetrate the internal environment of the cell or affect membrane receptors, initiating an immune response. The increased reactivity and biological activity of nanoparticles determine the relevance of studying their properties and behavior when they enter the human body [15].

It is known that nanoparticles, after entering the blood, lymph, gastric juice or any other biological fluid, interact with proteins [15, 16]. Possessing high surface energy, nanoparticles can destroy the covalent bonds of high-molecular proteins, as well as adsorb them on their surface, forming a so-called «crown». The latter is determined by the electrostatic interaction of charged groups of protein molecules, as well as their high affinity to the surface of nanoparticles. As a result of mutual influence, the properties of nanoparticles and proteins themselves change. The main proteins forming the «crown» of nanoparticles are albumin, immunoglobulins, complement factors, fibrinogen, and apolipoproteins. Coating of nanoparticles with these proteins largely determines their further fate – distribution between tissues and organs, rate of removal from the body, opsonization (phagocytosis with the participation of membrane receptors). Proteins and other organic substances increase the solubility of nanoparticles (for example, ZnO, CdSe, iron and aluminum oxides), but nanoparticles can also affect protein molecules, causing their aggregation, oxidizing side groups, reducing enzymatic activity, or changing their conformation [16].

Using the method of optical emission spectrometry with inductively coupled plasma, it was established that the concentration of zinc in the obtained colloidal solution was 0.045 mg/cm^3. For further studies of the cytotoxicity of ZnO particles and the effect of their concentration on this indicator, the original colloidal solution was diluted 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:100.

The results of measuring the optical density of albumin and immunoglobulin G after incubation with nano=ZnO and $ZnSO_4$ are presented in the Tables 1, 2.

The obtained results of the study of the optical density of the experimental samples in comparison with the control indicate that the degree of protein denaturation depended on the concentration of the studied compounds. The most toxic NPs·ZnO and $ZnSO_4$ in relation to immunoglobulin and albumin were at doses of 0.45 mg/cm^3 and 0.225 mg/cm^3, while doses of 0.014; 0.007 and 0.004 mg/cm^3 of both zinc preparations had no significant effect on the protein structure.

Table 1

Study of denaturation of human blood plasma proteins after incubation with ZnO nanoparticles

Zn concentration, mg/cm^3	Units of optical density	Immunoglobulin denaturation in the presence of ZnO·NPs, %	Units of optical density	Albumin denaturation in the presence of ZnO·NPs, %
0.450	0.928±0.005	63.75±0.29	0.574±0.002	27.63±0.23
0.225	0.483±0.008	33.17±0.45	0.315±0.001	15.16±0.14
0.113	0.180±0.001	12.34±0.03	0.311±0.001	14.97±0.14
0.056	0.123±0.002	7.90±0.08	0.164±0.001	7.90±0.07
0.028	0.115±0.001	7.76±0.17	0.129±0.002	6.23±0.05
0.014	0.100±0.001	6.87±0.05	0.150±0.004	7.21±0.13
0.007	0.079±0.001	5.43±0.06	0.079±0.001	3.81±0.07
0.004	0.069±0.001	4.72±0.04	0.062±0.002	3.00±0.05
0.9 % NaCl (negative control)	0.052±0.001	–	0.064±0.002	–
0.1 N HCl (positive control)	1.455±0.03	100	2.077±0.018	100

Table 2

Results of determination of denaturation of human blood plasma proteins after incubation with $ZnSO_4$ salt solution

Zn concentration, mg/cm^3	Units of optical density	Immunoglobulin denaturation in the presence of ZnO·NPs, %	Units of optical density	Albumin denaturation in the presence of ZnSO, %
0.450	0.773±0.010	53.10±0.66	1.142±0.001	54.92±0.49
0.225	0.311±0.002	21.35±0.13	0.816±0.007	39.30±0.57
0.113	0.215±0.005	14.78±0.33	0.786±0.007	37.85±0.43
0.056	0.134±0.002	9.19±0.10	0.355±0.003	17.11±0.15
0.028	0.092±0.001	6.06±0.29	0.149±0.005	7.19±0.31
0.014	0.067±0.002	4.60±0.01	0.055±0.001	2.64±0.04
0.007	0.054±0.001	3.69±0.04	0.039±0.001	1.89±0.04
0.004	0.056±0.001	3.80±0.02	0.038±0.001	1.81±0.02
0.9 % NaCl (negative control)	0.052±0.001	–	0.064±0.002	–
0.1 N HCl (positive control)	1.455±0.03	100	2.077±0.018	100

ZnO nanoparticles were more active in relation to immunoglobulin, while the effect of Zn ions on the structure of both proteins was the same.

The conducted studies proved that after in vitro incubation of peritoneal macrophages with ZnO·NPs at a concentration of 1.13 mg/cm^3 and 0.56 mg/cm^3 for 24 hours, a significant increase in the HBT test indicator was observed (by 62.5 % and 28.5 % compared to control). After incubation of macrophages with $ZnSO_4$ at a concentration of 1.13 mg/cm^3 and 0.56 mg/cm^3 for 24 hours, an increase in the HBT test was observed (by 34.7 % and 13.2 % compared to the control). Incubation of macrophages with smaller doses of ZnO nanoparticles and ions of this metal in $ZnSO_4$ salt (0.07–0.28 mg/cm^3) did not significantly affect the activation of the «respiratory burst» in macrophages (Table 3).

Analyzing the obtained results, it can be concluded that nano-ZnO in the studied concentrations somewhat stimulated the bactericidal ability of macrophages to a greater extent than Zn ions. Among the investigated concentrations, the dose of 1.13 mg/cm^3 turned out to be the most effective from the point of view of antibacterial effect.

The obtained data are consistent with the results of other researchers [17], who tested the antibacterial activity of nano-ZnO with a size of ~13±2 nm and ZnO salts against five pathogenic bacteria by the disk diffusion method. The authors of [17] also confirmed that ZnO·NPs had greater bactericidal activity than zinc ions in ZnO oxide. As for the mechanisms of the antibacterial action of ZnO·NPs, the authors assume that one of the main ones is the photocatalytic formation of hydrogen peroxide. The inhibition of the growth of microorganisms is also affected by the penetration of ZnO nanoparticles into the bacterial membrane and its subsequent destruction.

Table 3

Indicators of HBT test in rat macrophages after in vitro incubation with nano-ZnO and $ZnSO_4$ solutions

Concentrations, mg/cm^3	The form of zinc-containing compounds	
	Colloidal solution containing ZnO nano- and microparticles	$ZnSO_4$ salt
0	14.4±2.3	14.4±2.3
0.07	14.1±0.5	13.9±0.1
0.14	14.4±0.5	15.4±0.5
0.28	17.8±0.6	15.0±1.4
0.56	18.5±0.5	16.3±0.2
1.13	23.4±0.8	19.4±0.1

Practical significance. The conducted studies reveal the prospects of using ZnO nanoparticles to intensify the process of acidification of whey, followed by its use as a coagulant in the production of soft cheeses or a natural acidifier in the recipes of rye-wheat bread.

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Limitations of research. Considering that the electric discharge unit for obtaining zinc nanoparticles is an experimental technological complex of periodic action, it is advisable to implement it at enterprises with a small volume of whey processing.

The influence of martial law conditions. Unfortunately, the war in Ukraine is holding back the industrial implementation of the obtained results and scaling at medium and large capacity enterprises.

Prospects for further research. The subject of further research is the improvement of the technology of soft cheeses and rye-wheat bread, the study of quality indicators and the establishment of technological process parameters by using fermented whey enriched with zinc nanoparticles.

4. Conclusions

Dispersion characteristics of ZnO particles obtained by electrospark dispersion of zinc granules were studied. The colloidal solution contained ZnO particles in the size range from 40 to 300 nm with an average size of 101.3±5.0 nm, and was sedimentation resistant. About 50 % of ZnO particles are in the nanoscale range.

Physico-chemical and organoleptic indicators of milk whey before and after its electrospark treatment in an electric discharge chamber with a conductive layer of zinc granules were determined:

– an increase in the zinc content was established (1.8–4.1 times) depending on the duration of processing (30–120 s);

– an increase in pH was established depending on the duration of treatment;

– the absence of significant changes in organoleptic properties after electrospark treatment within 30–60 s was proven.

The intensification of the fermentation of lactic acid whey as a result of its enrichment with ZnO nanoparticles has been proven. The duration of fermentation until titrated acidity (160±10 °T) was almost halved compared to traditional whey and whey with the addition of $ZnSO_4$ salt. The resulting effect is due to an increase in the amount of a trace element important for the nutrition of lactobacilli cells.

The safety indicators of milk whey enriched with ZnO nanoparticles were studied by alternative methods of in vitro toxicological studies:

– it was established that the cytotoxicity of zinc nanoparticles and $ZnSO_4$ salt depended on the concentration of the studied compounds;

– the greatest toxicity in relation to immunoglobulin and blood plasma albumin was manifested in the presence of ZnO and $ZnSO_4$ in doses of 0.225–0.45 mg/cm^3;

– it was proven that the dosage is 0.014; 0.007 and 0.004 mg/cm^3 of both zinc preparations had no significant effect on the structure of blood plasma proteins;

– ZnO NPs in the studied concentrations stimulated the bactericidal ability of macrophages to a somewhat greater extent than Zn ions. Among the investigated concentrations, the dose of 1.13 mg/cm^3 turned out to be the most effective from the point of view of antibacterial effect.

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Conflict of interest

The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

Financing

The study was conducted without financial support.

Data availability

The manuscript has no associated data.

Use of artificial intelligence

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

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