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ORIGINAL ARTICLE

Investigation of the influence of Zinc-containing compounds on the components of the colloidal phase of milk

Andrey V. Blinov [a], Shahida A. Siddiqui [b,c,*], Andrey A. Nagdalian [a,*], Anastasiya A. Blinova [a], Alexey A. Gvozdenko [a], Vladislav V. Raffa [a], Natalya P. Oboturova [a], Alexey B. Golik [a], David G. Maglakelidze [a], Salam A. Ibrahim [d,*]

[a] *North Caucasus Federal University, Pushkina Street 1, 355009 Stavropol, Russia*
[b] *Technical University of Munich Campus Straubing for Biotechnology and Sustainability, Essigberg 3, 94315 Straubing, Germany*
[c] *DIL e.V.– German Institute of Food Technologies, Prof.-von-Klitzing-Straße 7, 49610 D-Quakenbrück, Germany*
[d] *North Carolina Agricultural and Technical State University, E. Market Street, 1601, 24711, Greensboro, NC, USA*

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Photon-correlation spectroscopy;
Acoustic spectroscopy;
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Abstract To solve the problem of insufficient intake of essential macro - and micronutrients into the human body, particularly in the case of the essential trace element Zinc, the possibility of enriching a socially significant product (milk) with various forms of Zinc is considered. The influence of Zinc-containing compounds on the colloidal milk system's dispersed composition and stability, photon correlation spectroscopy methods, acoustic and electroacoustic spectroscopy was established in this research. It has been shown that Zinc lysinatoriboflavinate, is a colloidal and chelated organic form of the essential trace element Zinc, having the most negligible effects on the composition and stability of the dispersed phase particles. This increases the average hydrodynamic radius of the dispersed phase by 5% and the ζ-potential by 10%.

A quantum-chemical simulation of the interaction of milk κ-casein sites with various forms of the essential trace element Zinc in the QChem program was performed using the IQmol molecular

* Corresponding authors at: North Caucasus Federal University, 355009, Pushkina Str., Stavropol, Russian Federation.
Institution: Food Microbiology and Biotechnology Laboratory, 171 Carver Hall, College of Agriculture and Environmental Sciences, North Carolina A & T State University, Greensboro, NC 27411-1064, Tel: 336-285-4860 (O), Fax: 336-334-7239
E-mail addresses: shahidasiddiqui777@gmail.com (S.A. Siddiqui), geniando@yandex.ru (A.A. Nagdalian), ibrah001@ncat.edu (S.A. Ibrahim).
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editor. The mechanism of action of various forms of Zinc on the components of the dispersed system of milk, in particular milk protein (casein), is suggested.

1. Introduction

Zinc (Zn) belongs to essential trace elements (McClung, 2019; Rolles et al., 2018), i.e. vital food components that can exert a high regulatory effect on the organism's processes (Deshpande et al., 2013; Jeejeebhoy, 2009; Reis et al., 2020). Zinc is part of hundreds of enzymes, among which one can distinguish alcohol dehydrogenase, which participates in alcohol transformation reactions, superoxide dismutase, which provides antioxidant protection of the body, carbonic anhydrase, organophosphate hydrolase, and many others (Henkin et al., 1999; Raynes et al., 2011; Valentine et al., 2005; Vallee and Hoch, 1955). Li et al. (2020) noted that Zn is the second most important transition metal in the human body, which plays an important role in cells, including intracellular metabolism, enzymatic catalysis, and neurotransmission. Baltaci et al (2019) pointed out that Zinc plays an important role in the functioning of the thyroid gland.

Thus Zinc participates in many processes in the human body, such as maturation of lymphocytes, reactions of cellular immunity, cell growth, stimulation of immune defence, increase the production of sex hormones, increases the activity of sperm, promotes the proper functioning and development of the male gonads, etc. (King, 2011). An interesting study is presented by Baltaci et al. (2018). The authors found that the combined intake of zinc and melatonin increases the immunity of laboratory animals in breast cancer caused by DMBA, and also significantly increases the percentage of NKT cells

In different countries, the recommended level of Zinc intake varies within different limits. In the UK - from 9.0 to 9.7 mg/day; in Canada - from 9 to 12 mg/day; in the United States, 12 to 15 mg/day; in Japan – 7.2 mg/day, the Netherlands – 14 mg/day; in India – 16 mg/day; in Russia 12–25 mg/day (Andriollo-Sanchez et al., 2005; Arsenault and Brown, 2003; Briefel et al., 2000; Prasad et al., 1993).

As known, insufficient intake of Zinc (hypozincosis) is typical for the population of many countries in the world (Cheboi et al., 2021; Knoell et al., 2019), such as Portugal, Turkey, Panama, Iran, Egypt and others (Palanog et al., 2019), where such clinically pronounced symptoms of hypozincosis as infantilism, hypoasmia, and hypogeisia are registered (Li et al., 2014; Pfaender et al., 2017; Yasuda et al., 2011). Ackland and Michalczyk (2016) claim that Zinc deficiency is the cause of death in more than half a million infants and children under the age of 5 years annually. Hypozincosis can also cause nervous anorexia (Hermens et al., 2020). There is a correlation between insufficient Zinc in the organism and an increase in the body mass index (BMI) (Braun et al., 2017). Also, an inverse relationship is noted between the Zinc content and the development of depressive disorder (Anbari-Nogyni et al., 2020). The occurrence of endemic hypozincosis in the population of many countries is caused by the low content of Zinc in the soil, drinking water and food products (Cardoso et al., 2019).

One of the most common methods to raise the micronutrient deficit is to use functional food products enriched with synergistic micronutrients, and necessary vitamins.

Dairy products are the most suitable for fortification with various trace elements and vitamin supplements. The possibility of using milk as an object for enrichment is due to several factors. Firstly, it has high demand by a broad segment of the population. Secondly, the affinity of the component composition of milk and organic, chelated forms of the essential trace element Zinc for enrichment.

Therefore, the purpose of this study is to study the effect on the properties of the components of the colloidal phase of milk of various forms of the essential trace element Zinc: Zinc lysinatoriboflavinate, Zinc lactate, Zinc asparaginate, Zinc sulfate and the Zinc complex with ethylenediaminetetraacetic acid – Zn-EDTA.

2. Materials and methods

The study was conducted on dairy products of local producers, in particular, whole milk, as well as pasteurized milk with a mass fraction of 3.2% fat of the following manufacturers: Stavropolsky Dairy Plant JSC (Stavropol, Russia), Molochny Rodnik LLC (Pyatigorsk, Russia), Budennovskmolprodukt JSC (Budennovsk, Russia), Adygeysky Molkombinat CJSC (Maykop, Russia), Shepherd Inc. (Nalchik, Kabardino-Balkarian Republic, Russia), Agrocomplex JSC (Krasnodar, Russia). The concentration of Zinc in each of the samples was set at 10 mg/l.

The organic chelated Zinc compounds used in the study were: lactate, asparagine, lysinatoriboflavinate, a complex with ethylenediaminetetraacetic salt (EDTA), and an inorganic compound called – Zinc sulfate.

2.1. Synthesis of chelated forms of the essential trace element Zinc

Synthesis of Zinc lysinatoriboflavinate was performed at the Department of physics and technology of nanostructures and materials on the North Caucasus Federal University faculty. Synthesis of Zinc lysinatoriboflavinate was carried out in the reactor system LR-2.ST (IKA-Werke GmbH & Co. KG, Germany), supplemented by the LAUDA E300 thermostat according to the method described in (Kayshev et al., 2019). L-lysine, Riboflavin and Zinc sulfate were used as starting substances.

During the synthesis of Zinc lactate, the precursors were lactic acid and Zinc oxide, asparaginate – aspartic acid and Zinc sulfate; Zinc complex with EDTA and Zinc oxide.

2.2. Computer simulation

Computer quantum-chemical modelling of the structure of molecules of organic chelated Zinc compounds: lactate, asparaginate, lysinatoriboflavinate, complex with ethylenediaminetetraacetic salt (EDTA), and an inorganic compound – Zinc sulfate.

The molecular models were obtained in the QChem program using the IQmol molecular editor. Information on changes in the surface potential of these protein fragments is also presented. The simulation was carried out before the interaction with Zinc ions and after the formation of Zinc complexes from these sections of the peptide. Simulation parameters: energy: M06, basis: 6-31G, convergence-5, force field-Chemical, where the simulation was performed in vacuum (Gilbert, 2015).

The chemical hardness was calculated using the equation:

$$\eta = \frac{1}{2} \times (E_{LUMO} - E_{HOMO})$$

2.3. Determination of Zinc concentrations

The concentration of Zinc ions in aqueous solutions of Zinc - containing compounds was measured using, aqueous solutions of lysinatoriboflavinate, asparaginate, lactate, Zinc sulfate and Zn-EDTA. The Zinc content in all samples was 10 mg/l. The activity of Zinc ions in each of the studied solutions was measured using a film ion-selective electrode XC-Zn-001 on the pH meter-(ionomer) Expert-001 (NPC Econix-Expert, Russia).

2.4. Methods of investigation of the influence of Zinc containing compounds on the properties of a dispersed phase of milk

In normalized pasteurized milk with a weight of 2.8% protein fraction and 3.2% fat fraction, organic chelated Zinc compounds were inserted during an intensive mixing: lactate, asparagine, lysinatoriboflavinate, a complex with ethylenediaminetetraacetic salt (EDTA), and an inorganic compound – Zinc sulfate. Then the obtained samples of fortified dairy products were kept for 1 h for the complete dissolution of all reagents and examined on the DT 1202 (Dispersion Technology Inc., New-York, USA) and Photocor complex spectrometers (Antek-97 LLC, Moscow, Russia).

The dispersed phase composition (average hydrodynamic radius of particles, size distribution, the polydispersity of particles of aqueous solutions of milk) was determined by photon-correlation spectroscopy on a multifunctional dynamic and static light scattering spectrometer Photocor Complex. Measurements were made following Russian state standard GOST R 8.774106 ("State system for ensuring the uniformity of measurements. (2011) State verification schedule for measuring instruments of alternating current from $1 \cdot 10^{-8}$ to 100 A in the frequency range from $1 \cdot 10^{-1}$ to $1 \cdot 10^{6}$ Hz GOST R 8.767-2011 - T84.8," n.d.). Computer processing of the spectroscopy data array was performed using DynaLS software (Antek-97 LLC, Moscow, Russia).

The following measurement parameter values have been used:

- the angle of the photodetector position is 150 degrees;
- the number of measurements in one cycle is 100;
- the wavelength of laser radiation is 650 nm;
- measurement cycle duration is 1 s.

The particle diameter and ξ-potential of the dispersed phase of milk were studied by acoustic and electroacoustic spectroscopy at the DT-1202 facility.

We used the following measurement parameter values:

- the frequency of ultrasonic radiation is 3–100 MHz;
- the number of measurements in one cycle is 10.

3. Results and discussion

To justify the necessity for the fortification of milk and dairy products fortification in general with the trace element Zinc, this essential trace element in milk samples taken from various manufacturers was analyzed. The results are as shown in Fig. 1.

The trace element Zinc content in these producers' milk samples varies within 2.00 ± 0.35. The highest content of 2.35 mg/kg was observed in milk of Agrocomplex JSC (Krasnodar, Russia), and the lowest was 1.94 mg/kg – in the milk of Molochny Rodnik (Pyatigorsk, Russia).

Also, the recommended rate of consumption of dairy products by the population in terms of milk is 325 kg for 1 person per year following the Recommendations on rational consumption standards of food products (Kubicová et al., 2019) that meet modern requirements of healthy nutrition (approved by order of the Ministry of health of the Russian Federation dated August 19, 2016 №. 614 ("Order M.H. the Russian Federation dated august 19, 2016," n.d.).

Detection of insufficient content of the trace element Zinc in the studied milk samples based on the analysis of the data presented in Fig. 1 could lead to the necessity of enriching milk with Zinc to the level of 5–10 mg/kg.

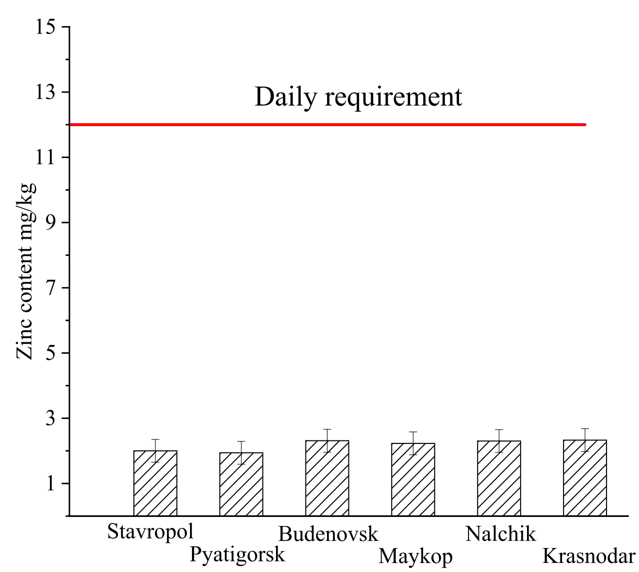


Fig. 1 Content of the essential trace element Zinc in milk from the following manufacturers: Stavropolsky Dairy Plant JSC (Stavropol, Russia), Molochny Rodnik LLC (Pyatigorsk, Russia), Budennovskmolprodukt JSC (Budennovsk, Russia), Adygeysky Molkombinat CJSC (Maykop, Russia), Shepherd Inc. (Nalchik, Kabardino-Balkarian Republic, Russia), Agrocomplex JSC (Krasnodar, Russia).

The following organic chelated Zinc compounds were considered: lactate, asparagine, lysinatoriboflavinate, a complex with ethylenediaminetetraacetic salt (EDTA), and an inorganic compound – Zinc sulfate, whose molecular models are presented in Fig. 2. The molecular models were obtained in the QChem program with the IQmol molecular editor.

Samples of milk enriched with various forms of Zinc, were examined using an acoustic and electroacoustic spectrometer DT 1202 and a photon-correlation spectrometer Photocor complex. Experimental data obtained by photon-correlation spectroscopy are shown in Figs. 3-5.

As a result, it was found out that the distribution of dispersed phase particles in milk samples is unimodal, the

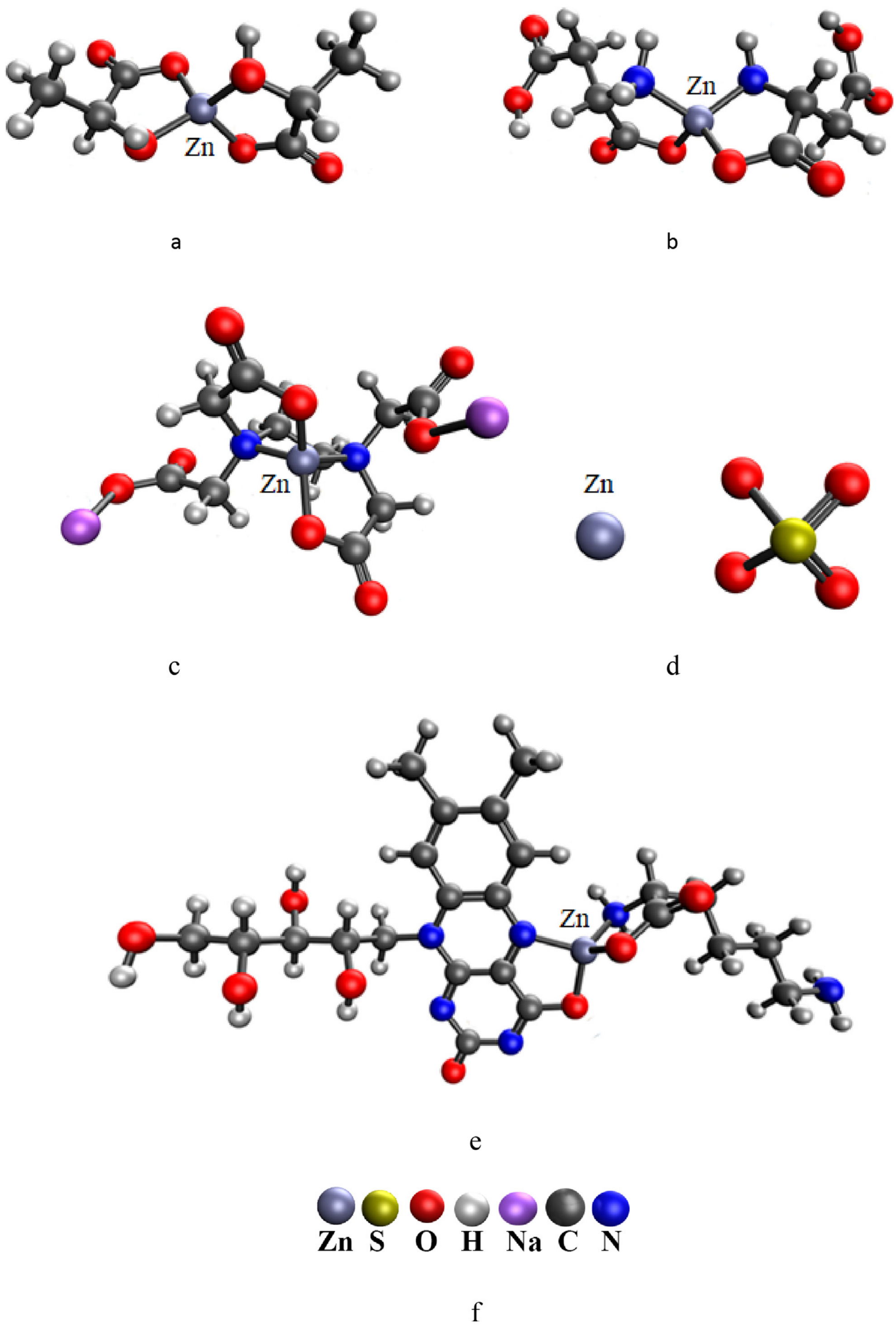


Fig. 2 Models of molecules of Zinc -containing compounds: a) Zinc lactate, b) Zinc asparaginate c) Zinc chelate complex molecule with EDTA, d) Zinc sulfate, e) lysinatoriboflavinate Zinc sulfate f) models legend.

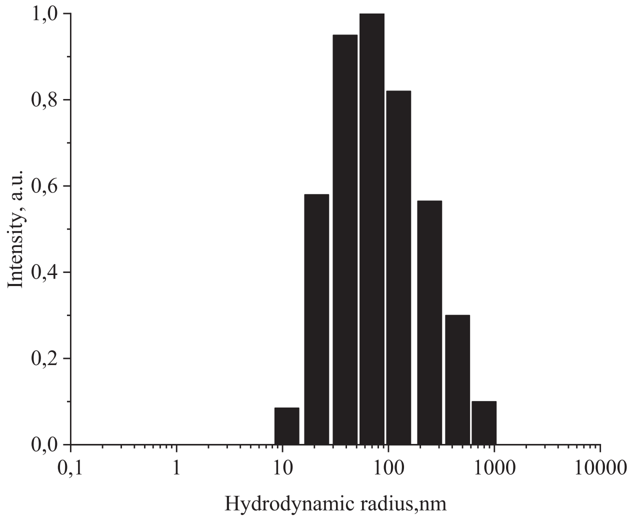


Fig. 3 Histogram of the distribution of particles of the dispersed phase of native milk according to photon-correlation spectroscopy data.

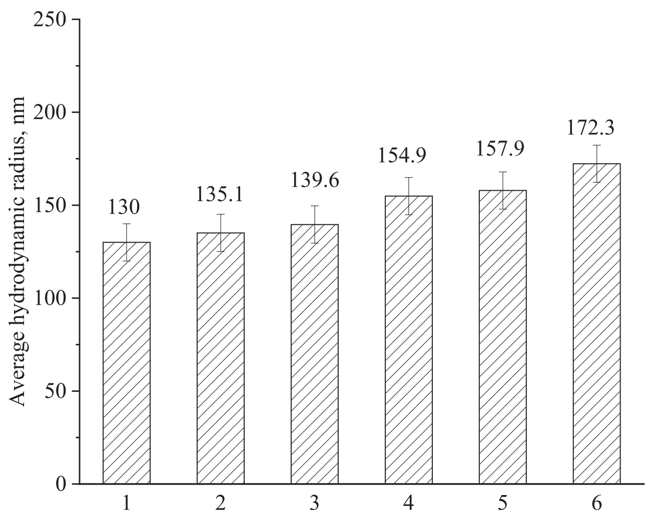


Fig. 4 Average hydrodynamic radius of dispersed phase particles of milk samples: 1-without impurities, 2-enriched with Zinc lysinatoriboflavinate, 3- *Zn*-EDTA, 4- Zinc asparaginate, 5- Zinc lactate, 6- Zinc sulfate.

particles have moderate polydispersity and an average hydrodynamic radius of about 130–160 nm.

Trough the analysis of histograms of the distribution of particles of the dispersed phase (Fig. 3) of milk, it was found that only one particle fraction is present in the sample, that is, the distribution is unimodal, the particles have moderate polydispersity and an average hydrodynamic radius about 130–160 nm. The appearance of only one fraction on the histogram is because the distribution is quantitative, and as it is known, in the dispersed phase of milk, the protein component predominates in quantity, which is mainly formed from casein micelles.

As a result of analysis and processing of distribution histograms of all milk samples enriched with various Zinc compounds, histograms showing the effect of various

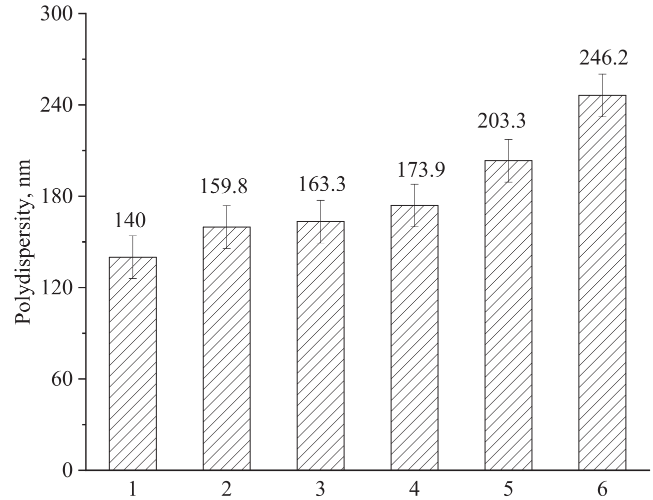


Fig. 5 Polydispersity of particles of the dispersed phase of milk samples: 1-without impurities, 2-enriched with Zinc lysinatoriboflavinate, 3- *Zn*-EDTA, 4- Zinc asparaginate, 5- Zinc lactate, 6- Zinc sulfate.

Zinc-containing compounds on the average hydrodynamic radius and polydispersity of colloidal phase particles of milk were obtained, shown in Figs. 4 and 5.

The average hydrodynamic radius of the dispersed phase particles of the initial normalized pasteurized milk is about 130 nm with a polydispersity of 140 nm. A minor change in the composition of disperse phase in milk is observed in the sample containing a triple Zinc-containing colloidal complex – Zinc lysinatoriboflavinate. Here the average hydrodynamic radius of particles is 135 nm with a polydispersity of 160 nm. The most significant changes in the sample containing the Zinc sulfate particles have an average hydrodynamic radius of 172 nm with a polydispersity of 250 nm.

Data on the composition, stability and properties of the dispersed phase of milk samples enriched with various Zinc compounds were also obtained using the acoustic and electroacoustic spectrometer DT 1202. The results are shown in Figs. 6 and 7.

It was found that the histograms of the particle distribution of the dispersed phase of the initial milk sample contain two particle fractions, which indicates that the distribution is bimodal, with an average diameter of the first fraction of 270 nm and the second is 5500 nm, where the first fraction is protein and the second is fat (Zhao et al., 2013). During the analysis of Fig. 6, it was established that the addition of lysinatoriboflavinate of Zinc in milk does not increase the diameter of the particles of the protein fraction, compared with the complex of Zinc with EDTA, aspartic acid and lactate-ion. The usage of that substances as a source of essential trace element Zinc for the enrichment of milk contributes to an increase of the particle diameter of the protein fraction to 290; 300; 320 nm, respectively.

In contrast to photon-correlation spectroscopy, acoustic spectroscopy also provides information about the influence of Zinc compounds on the fat fraction of milk. As a result, it was found that the addition of lysinatoriboflavinate, lactate, Zinc asparaginate and Zinc complex with EDTA to milk does not affect the particle size of the fat fraction of milk. The most significant changes in the dispersed composition of the colloid

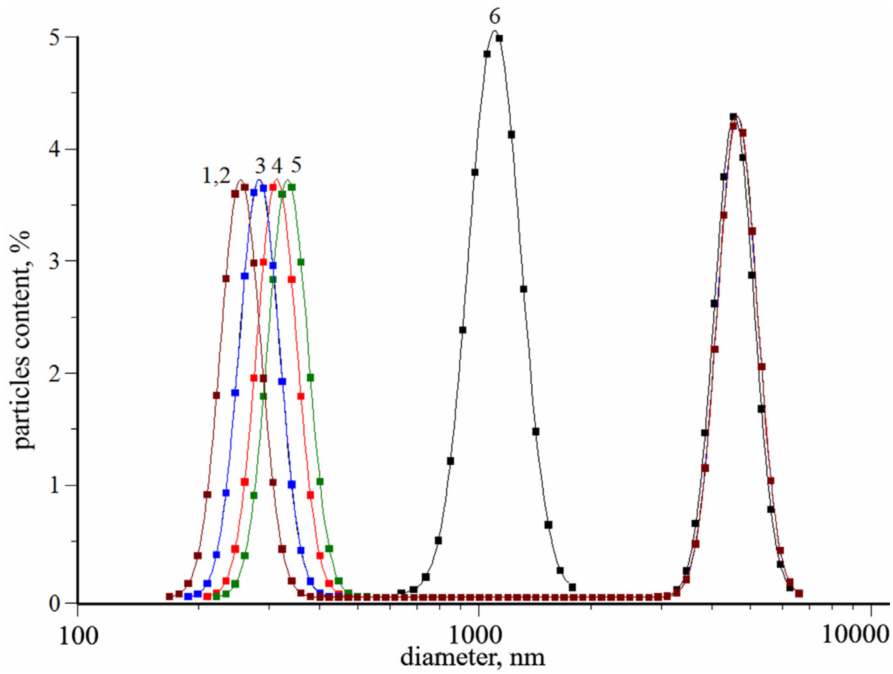


Fig. 6 Histograms of the distribution of particles of the dispersed phase of milk samples: 1-without impurities, 2-enriched with Zinc lysinatoriboflavinate, 3-*Zn*-EDTA, 4- Zinc asparaginate, 5-Zinc lactate, 6-Zinc sulfate.

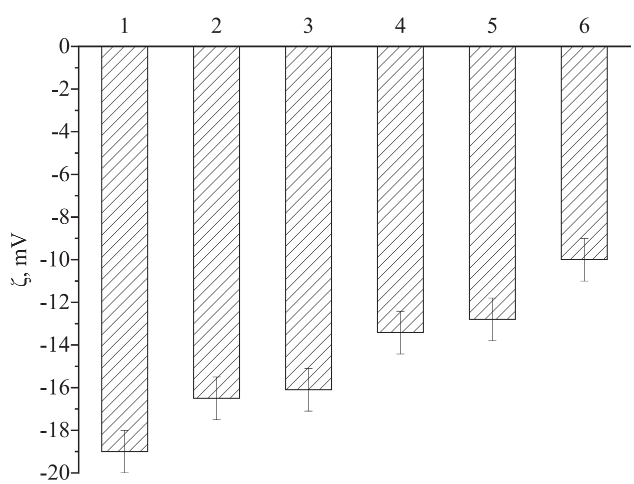


Fig. 7 ζ-potential of dispersed phase particles of milk samples: 1-without impurities, 2-enriched with Zinc lysinatoriboflavinate, 3-*Zn*-EDTA, 4- Zinc asparaginate, 5- Zinc lactate, 6- Zinc sulfate.

phase of milk are observed while using Zinc sulfate. In this sample, the distribution is unimodal and the average diameter is about 1000 nm.

Then, using electroacoustic spectroscopy, information about the influence of various Zinc -containing compounds on the electrokinetic potential (ζ-potential) of particles of the dispersed phase of milk samples shown in Fig. 7 was obtained.

It was found that the ζ-potential of particles of the dispersed phase of the initial milk sample is 19 mV, and after adding Zinc -containing compounds to the milk, the ζ-potential of particles increases. The smallest changes are observed in the case of adding Zinc lysinatoriboflavinate, when using which the ζ-potential of particles of the dispersed phase of milk increases to −16.5 mV, and the largest – when using Zinc sulfate to −10 mV.

As it's known, the ζ-potential of particles is a measure that characterizes the stability of the colloidal system, that is, the greater the charge of the particles, the greater their resistance to electrostatic coagulation. The ζ-potential of milk dispersed phase particles is due to the presence of negative *C*-terminal sites *of* κ-casein on the surface of casein micelles, and carboxyl groups on the surface of fat balls.

In milk, Zinc mainly binds to milk proteins (Milačič et al., 2012). According to the analysis of the literature data, casein micelles consist of submicelles with a diameter of 10–15 nm, which are aggregates of the main casein fractions (a_S-, β-, and κ-) (De Kruif et al., 2012; Dezhampanah et al., 2017; Hamza et al., 2010; Kayshev et al., 2019; Liu and Guo, 2008). The polypeptide chain of casein coagulates in submicellar in such a way that most groups are hydrophobic core and hydrophilic are located on the surface of submicelles. The hydrophilic part contains negatively charged acid groups of aspartic, glutamic (COO^-) and phosphoric acids (PO_3^{3-}). The hydrophilic properties of submicelles and micelles are enhanced by the outward-oriented glycomacropeptide κ-casein, which is mostly located on the surface of the submicelles (Horne, 2006). Submicelles with a low content of κ-casein are located inside the micelles, and those with a high content are located on the surface. The peptide part of the κ-casein glycomacropeptides contains a large amount of hydroxyaminoacids (serine and threonine), glutamic and aspartic acids, and the carbohydrate part contains free carboxyl groups of sialic acid (Addeo and Mercier, 1977).

By the latest ideas about the structure of casein micelles, its surface has a "hair layer" that extends into the dispersed medium at 10–15 nm and prevents the micelles from approaching. Micelles have a porous structure, which causes the penetration of micelles, water and enzymes.

The dissociation of the carboxyl groups of glutamic, aspartic, and sialic acids present on the surface of the micelles, as well as the phosphate groups, gives the micelles a negative charge, which causes the forces of electrostatic repulsion between the colloidal particles.

Taking into account theoretical and practical ideas about the structure of casein micelles, the interaction of Zinc ions with negatively charged groups in the *C*-terminal region of casein is considered. For this purpose, computer quantum-chemical modelling of negatively charged fragments of the *C*-terminal site of κ-casein containing carboxyl, hydroxyl groups and phosphoric acid residues was carried out. 7 fragments with the following amino acid sequences were considered: *Ala-Ser(P)-Pro, Leu-Ser-Arg, Pro-Thr-Arg, Ser-Asp-Val, Ser-Cys-Gln, Arg-Tyr-Pro, Ile-Glu-Ser*, and sialic acid. The simulation was carried out before the interaction with Zinc ions and after the formation of Zinc complexes from these sections of the peptide. The models are shown in Figs. 8-11 and the Supplementary.

The simulation showed that the negative charge in the fragments of the *C*-terminal site of the κ-casein under

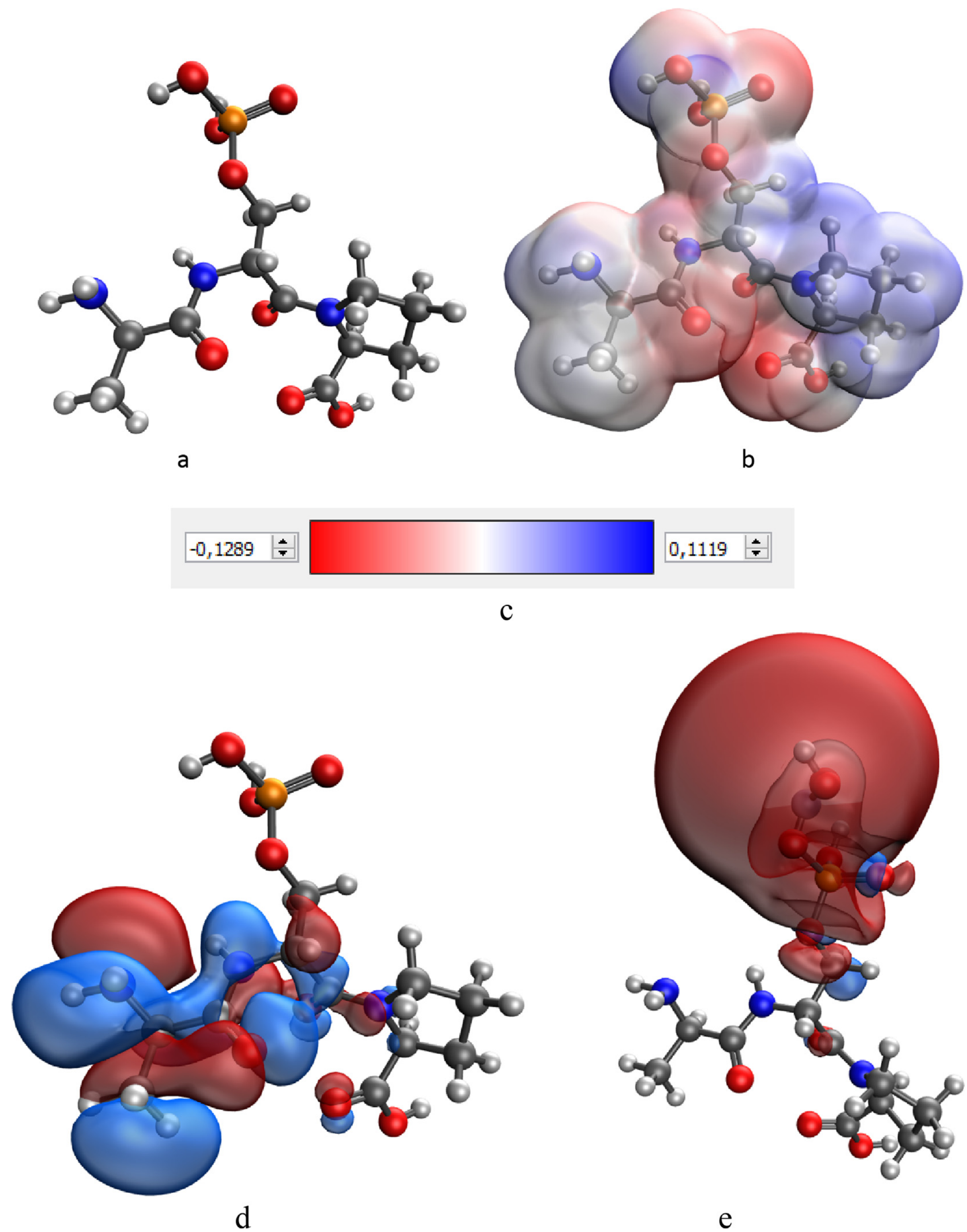


Fig. 8 Model of the *Ala-Ser(P)-Pro* κ-casein segment containing the phosphoric acid residue: (a), electron density distribution (b), electron density distribution gradient (c) highest occupied molecular orbital HOMO (d) and lowest unoccupied molecular orbital LUMO (e).

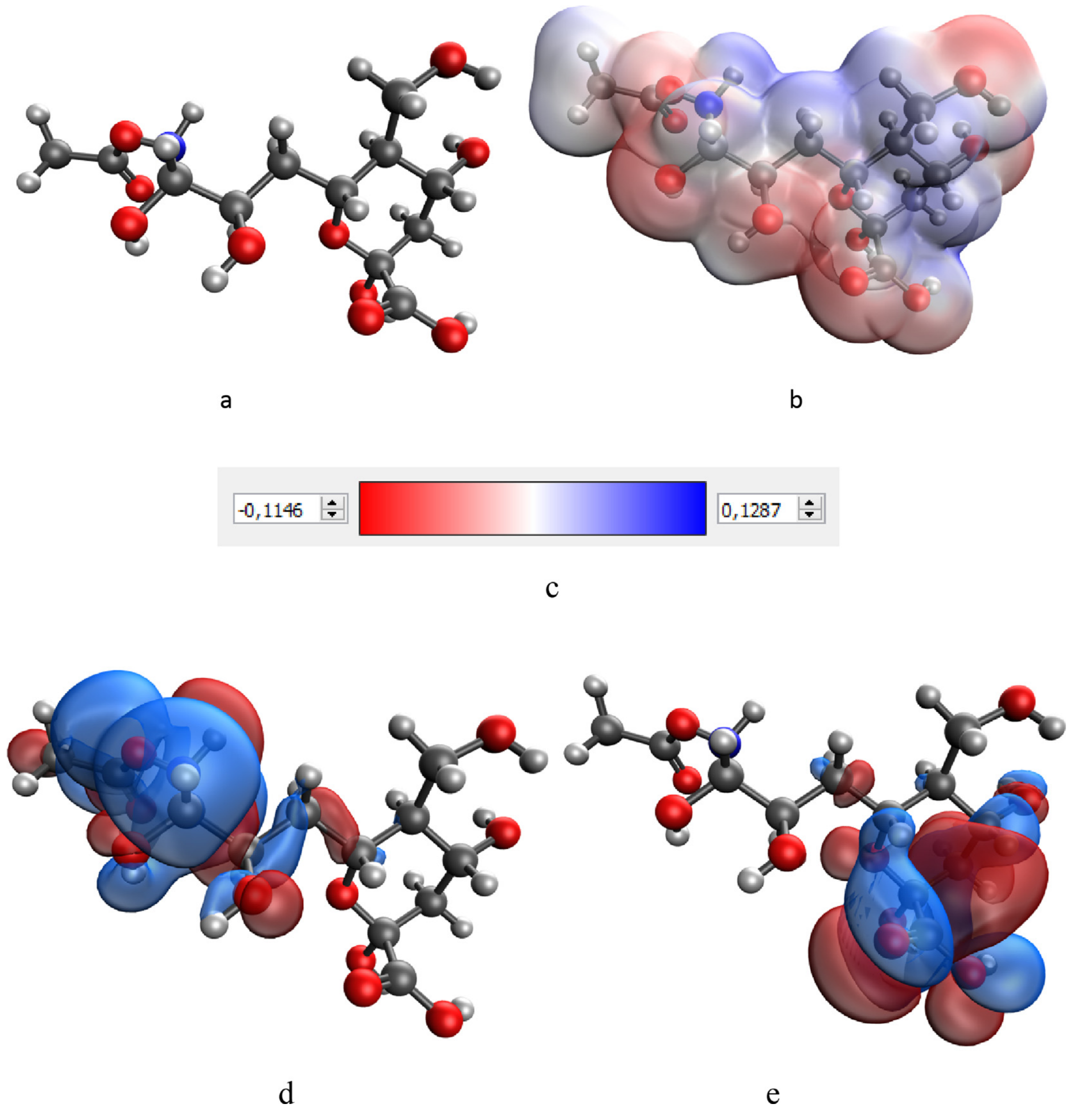


Fig. 9 Model of a sialic acid molecule: (a), electron density distribution (b), electron density distribution gradient (c) highest occupied molecular orbital HOMO (d) and lowest unoccupied molecular orbital LUMO (e).

consideration is concentrated on the oxygen atoms belonging to the carboxyl, hydroxyl groups and phosphoric acid residues. The interaction of positively charged Zinc ions with the most negative sphere of the fragments of this protein leads to a significant redistribution of the electron density and a change in the spatial configuration.

Data on the total energy of the κ-casein segments before and after interaction with Zinc ions were also obtained, the results are presented in Table 1.

A significant decrease in the energy of the molecular system of negatively charged segments of κ-casein when positively charged Zinc ions are attached to them is noticed. This indicates the most likely energetically favourable mechanism of interaction of Zn^{2+} ions with κ-casein, leading to the loss of negative charge on the surface of the micelles and to further coagulation of the micelles and destabilization of the entire milk system (Kayshev et al., 2019).

According to the references, HOMO and LUMO are the main orbitals describing the chemical stability of the system. HOMO is directly related to the concept of "ionization potential". LUMO – with the concept of "electron affinity" (Gvozdenko et al., 2020).

Based on the data obtained, the value of the chemical hardness (η) was calculated. Chemical hardness characterizes the stability of the system (Ghammamy et al., 2014). As a result of data analysis, the following molecular complexes were found to be the most stable: *Ala-Ser (P)-Pro*-Zn, *Ile-Glu-Ser*, and sialic acid-Zn.

An increase in the ζ-potential of particles of the dispersed phase of milk and its approximation to zero shows a drop in the stability of particles caused under the Hardy-Schulze law, which coagulates the ability of oppositely charged ions, which in this case are Zn^{2+} ions, the appearance of which in solution is caused by the dissociation of Zinc compounds (Fig. 12).

The process of dissociation of Zinc compounds is considered by the example of a complex of Zinc with EDTA and Zinc sulfate:

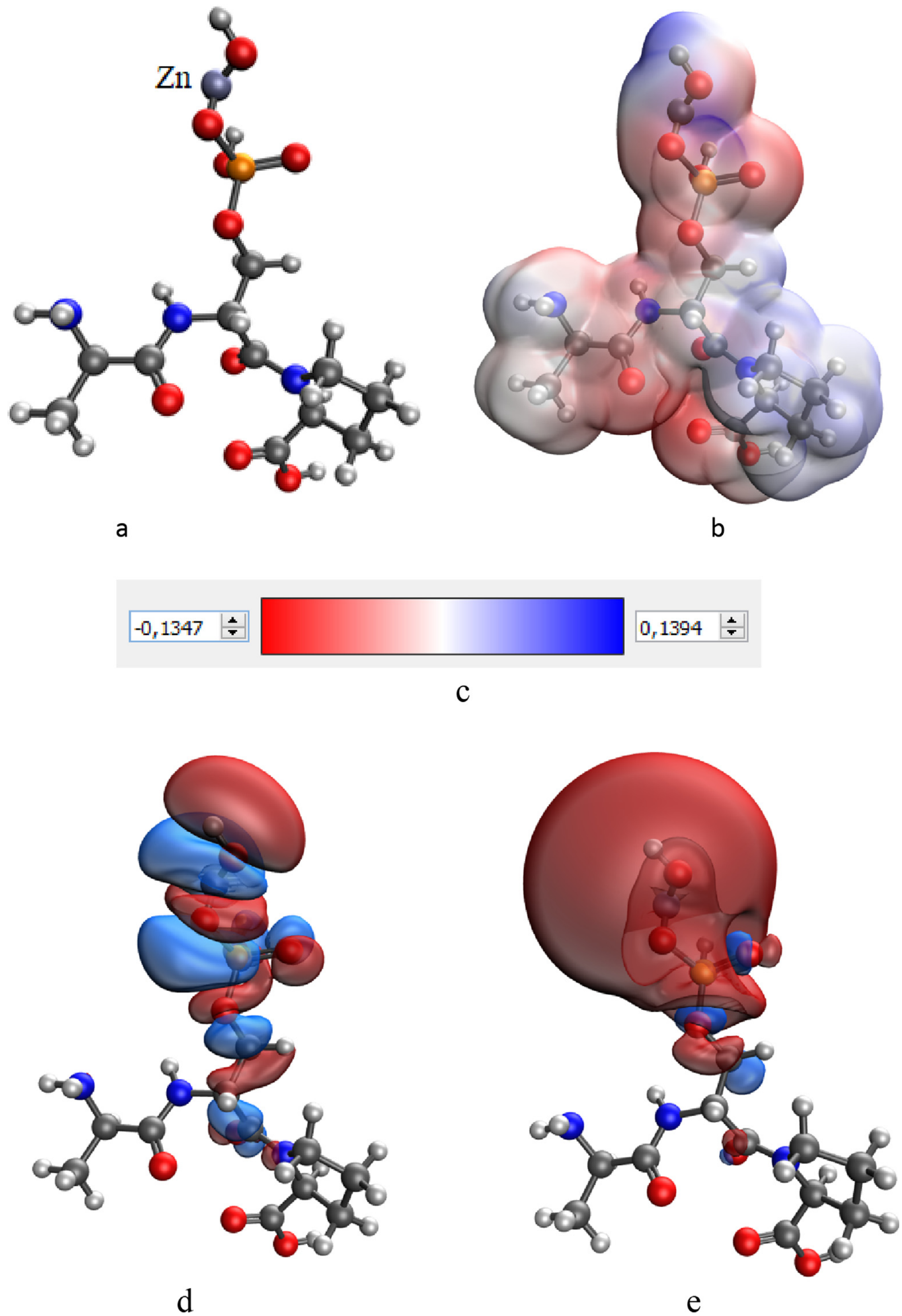


Fig. 10 Model of the interaction between *Ala-Ser(P)-Pro* κ-casein segment containing phosphoric acid and a Zinc atom: the model of the molecular complex (a), the electron density distribution (b), the gradient of the electron density distribution (c) highest occupied molecular orbital HOMO (d) and lowest unoccupied molecular orbital LUMO (e).

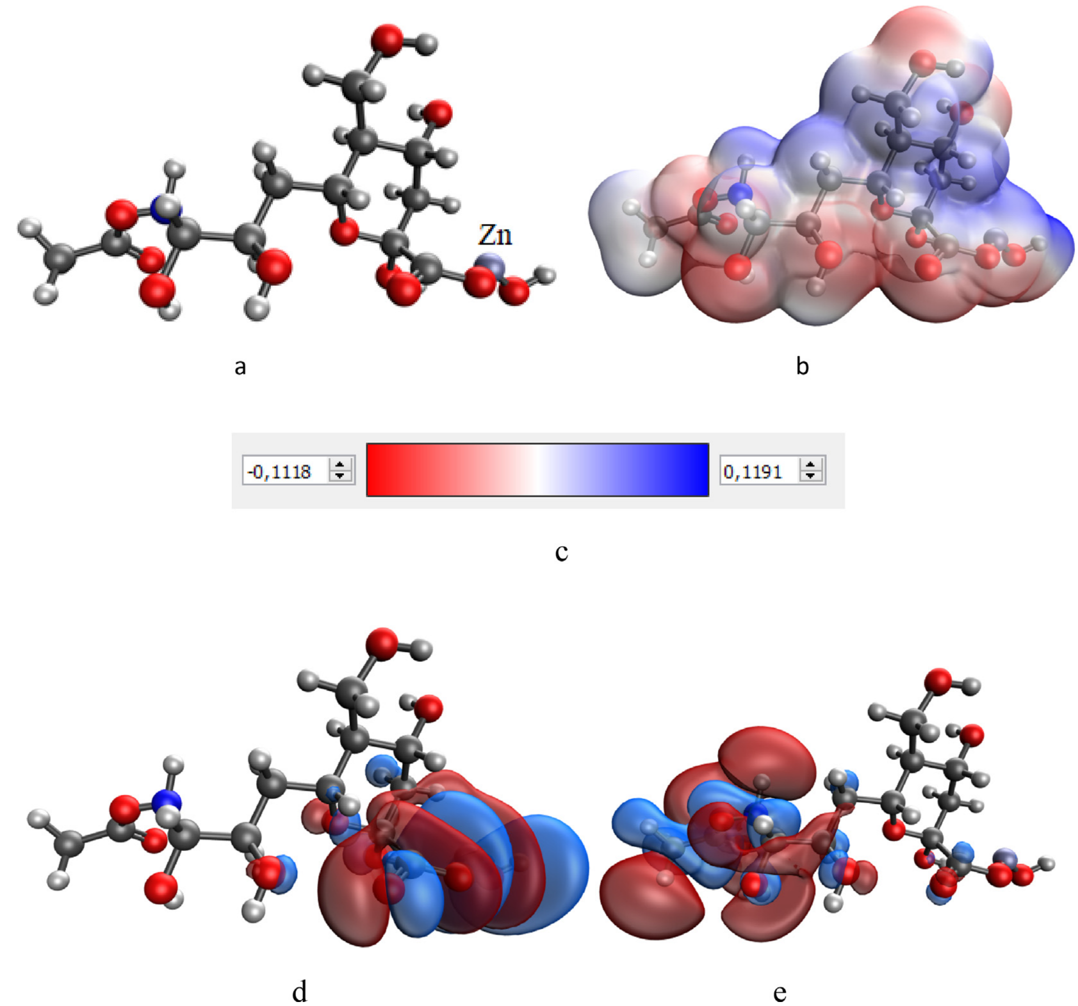
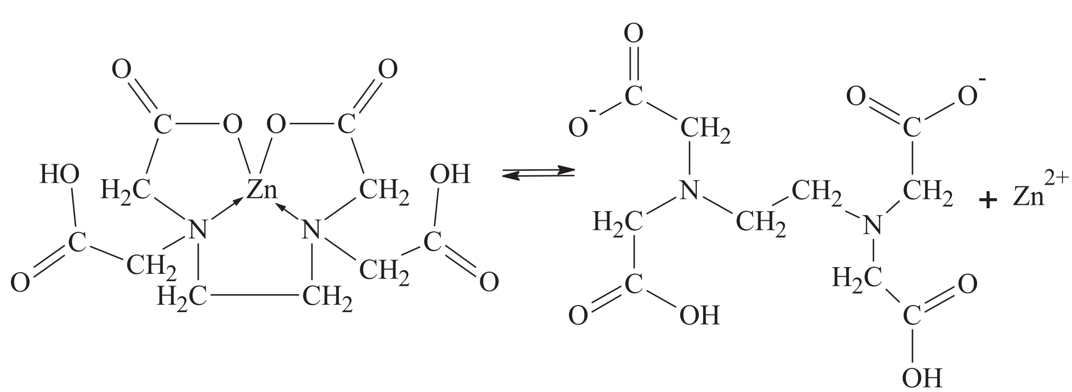


Fig. 11 Model of interaction between a sialic acid molecule and a Zinc atom: molecular complex model (a), electron density distribution (b), electron density distribution gradient (c) highest occupied molecular orbital HOMO (d) and lowest unoccupied molecular orbital LUMO (e).



$$ZnSO_4 \rightarrow Zn^{2+} + SO_4^{2-}$$

As a result of the dissociation of Zinc compounds, negatively charged anions and positively charged Zn^{2+} cations are released into the aqueous medium. It is important to note that the dissociation of complex compounds into ions is characterized by a constant of instability, which shows the ratio of the concentrations of ions formed during dissociation to the concentration of non-dissociated molecules

of the complex:

Table 1 Results of quantum chemical calculations.

Complex	E, kcal/mol	HOMO	LUMO	η
Ala-Ser(P)-Pro	−1537.43	–	–	–
Ala-Ser(P)-Pro -Zn	−3389.74	−0.205	−0.052	0.153
Leu-Ser-Arg	−1293.01	–	–	–
Leu-Ser-Arg -Zn	−3144.23	−0.150	−0.024	0.126
Pro-Thr-Arg	−1127.18	–	–	–
Pro-Thr-Arg -Zn	−2979.48	−0.140	−0.029	0.111
Ser-Asp-Val	−1159.67	–	–	–
Ser-Asp-Val -Zn	−3011.97	−0.182	−0.057	0.125
Ser-Cys-Gln	−1573.50	–	–	–
Ser-Cys-Gln -Zn	−3424.73	−0.167	−0.072	0.095
Arg-Tyr-Pro	−1444.10	–	–	–
Arg-Tyr-Pro -Zn	−3296.93	−0.056	−0.023	0.033
Ile-Glu-Ser	−1238.25	–	–	–
Ile-Glu-Ser -Zn	−3090.53	−0.189	−0.042	0.147
Sialic acid	−1275.54	–	–	–
Sialic acid -Zn	−3127.80	−0.166	−0.020	0.146

E – energy of the molecular system, HOMO – highest occupied molecular orbital, LUMO – lowest unoccupied molecular orbital, η – value of the chemical hardness.

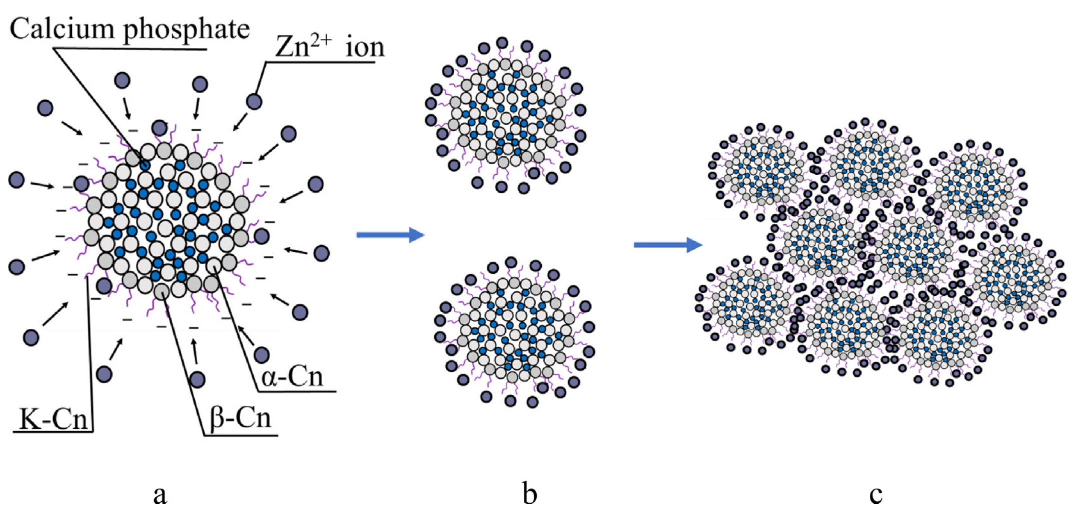


Fig. 12 Diagram of the process of coagulation of casein micelles under the action of Zinc ions: a) diagram of the structure of the casein micelle, b) loss of the surface charge of the casein micelle, c) aggregate of casein micelles.

$$K_{[Zn-EDTA]} = \frac{[Zn^{2+}] \cdot [EDTA^{2-}]}{[Zn - EDTA]} \quad (2)$$

Therefore, the lower the instability constant of the complex, the stronger the complex and it is less subjected to the process of dissociation. As a result, fewer Zinc ions are released into the water environment.

At the next stage, studies were conducted to determine the concentration of Zinc ions in aqueous solutions of Zinc - containing compounds. The results are shown in Fig. 13.

It was found that the concentration of Zinc ions in an aqueous solution of Zinc sulfate is 10 mg/l, that is, this inorganic Zinc compound dissociates completely. In aqueous solutions of asparaginate and Zinc lactate, the concentration of Zn^{2+} ions is about 0.15 mg/l, and in solutions of Zinc lysinatoriboflavinate and Zn-EDTA – 0.05 mg/l. In this connection, for the enrichment of milk and dairy products, it is necessary to use compounds with a small instability constant and, as a result, a source of a small amount of Zinc ions, which will not cause coagulation and aggregation of casein micelles. Such as a chelate complex of Zinc with EDTA and Zinc lysinatoriboflavinate.

The Zn-EDTA complex has an instability constant = 10^{-16}, and therefore, in the dispersion medium of milk, the molecules of this compound are in the non-dissociated state, forming a molecular solution, which is schematically reflected in Fig. 14.

It is important to note that EDTA is toxic in (Grčman et al., 2001; Hempe and Cousins, 1989; O'Dell et al., 1964; Oberleas et al., 1966). In particular, (Hempe and Cousins, 1989) compared the efficiency of the Zn-methionine complex and EDTA for the absorption of Zinc ions by the body. It was found that both the Zn-methionine complex and EDTA reduce the efficiency of Zinc absorption. The toxic effect of EDTA on the plant caused by increased phytoextraction of heavy metals from the soil was shown in (Grčman et al.,

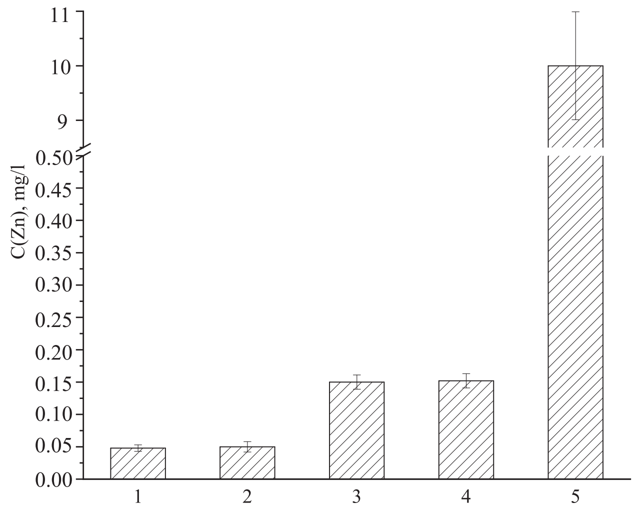


Fig. 13 Free Zinc content according to ion-selective measurements in aqueous solutions of Zinc -containing compounds: 1- Zinc lysinatoriboflavinate, 2- *Zn*-EDTA, 3- Zinc asparaginate, 4- Zinc lactate, 5- Zinc sulfate.

2001). Unlike the Zn-EDTA complex, Zinc lysine riboflavinate has a high biological value, since it is a chelate complex between the essential trace element Zinc and biologically active substances-vital micronutrients: vitamin B2 (riboflavin) and the essential amino acid L-lysine (Anastassiadis, 2007; Bacher et al., 2000; Lee and Tian, 2015; Pinto and Zempleni, 2016; Powers, 2003).

It should also be noted that Zinc lysinatoriboflavinate in the dispersion medium of the milk forms a colloidal solution. Studies conducted using photon-correlation spectroscopy have shown that the average hydrodynamic radius of Zinc lysinatoriboflavinate micelles is 150 nm (Blinov et al., 2019). Zinc lysinatoriboflavinate micelles have a negative charge, which is confirmed by the measurement of the ζ-potential (-31.6 mV). The approximate scheme of the structure of micelles of Zinc lysinatoriboflavinate is presented in Fig. 15a.

The negative charge of the micelles is due to the presence of a hydrophilic ribitol residue in the riboflavin molecule, which is part of Zinclysinatoriboflavinate. As can be seen from Fig. 15b, the ribitol residue contains 4 hydroxyl groups, on which, according to computer quantum chemical modelling, a negative charge is concentrated.

As a result, Zinc lysinatoriboflavinate is the optimal form of the essential trace element Zinc for the enrichment of dairy products as compared to other forms of Zn has the least impact on the properties of the dispersed phase of milk, due to electrostatic repulsion of the micelles of Zinc lysinatoriboflavinate from the components of the dispersion medium of milk. This process is shown schematically in Fig. 16.

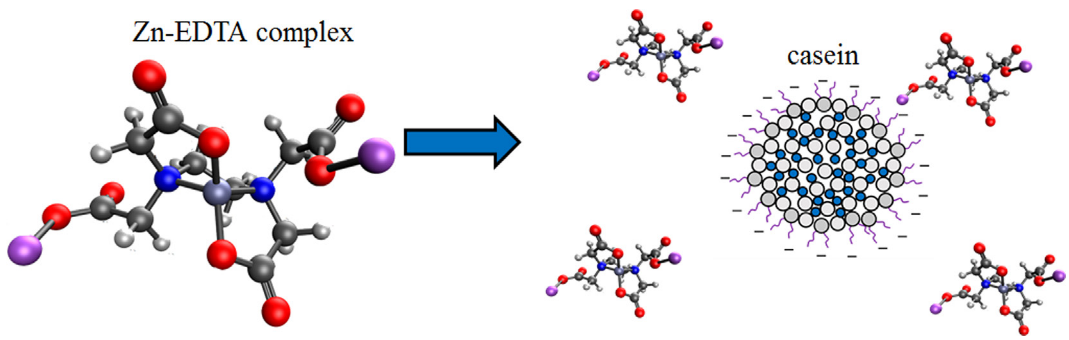


Fig. 14 Diagram of the state of dispersion of casein micelles in the presence of Zn-EDTA molecules.

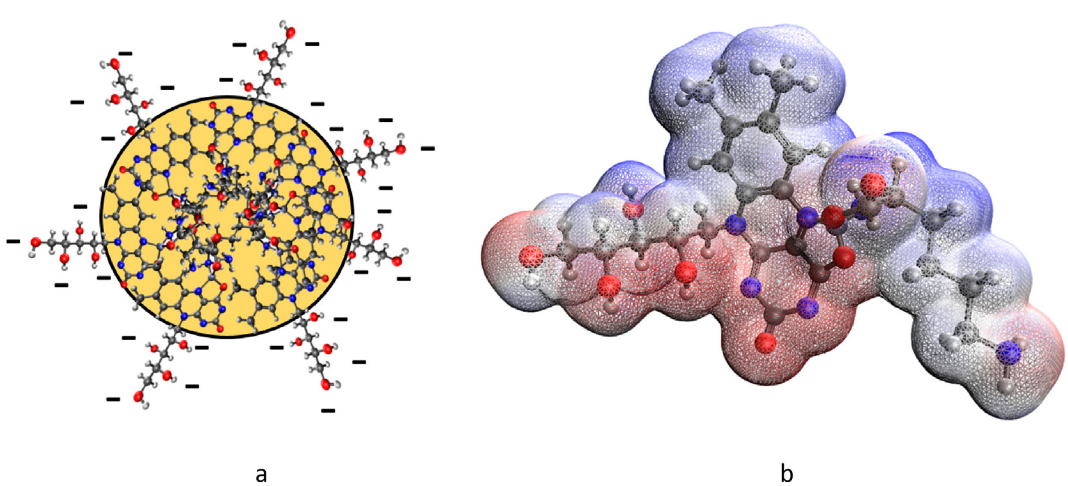


Fig. 15 Structure diagram of the Zinc lysinatoriboflavinate micelle (a) electron density distribution in the Zinc lysinatoriboflavinate molecule (b).

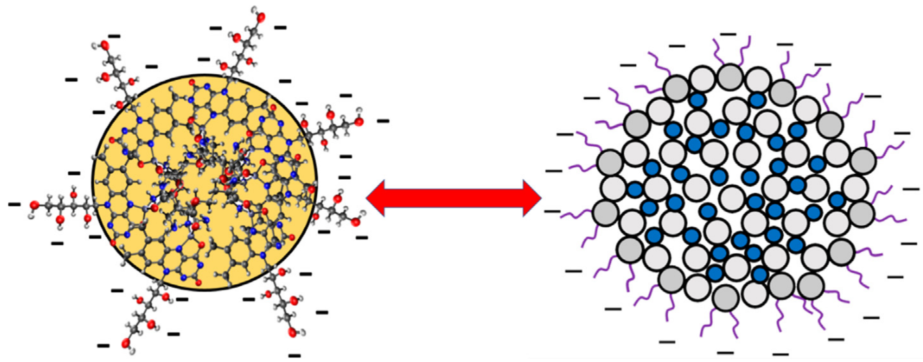


Fig. 16 Scheme of electrostatic repulsion of Zinc lysine riboflavin micelles and the main milk protein-casein.

For a more detailed analysis and determination of the types of interaction of zinc with milk components, it would be better to additionally use the IR spectroscopy, Raman spectroscopy and NMR spectroscopy methods. However, then we would have to separate all the components into separate parts and examine them separately. Since milk is a dynamic system, when divided into components, its properties will change. In this case, it will be applicable only to certain milk products, but not for milk as a single multicomponent, multiphase system. Therefore, in this work we considered the influence of Zn on the whole milk system, in the form in which it enters production for further processing. In this regard, the data obtained in the work are valid only for milk. Work with dairy products is planned in future scope of this research.

4. Conclusion

Based on the conducted studies, it is necessary to use compounds with a small constant instability to enrich milk and dairy products. As a result, they become the sources of a small amount of free Zinc ions, which will not cause coagulation and aggregation of casein micelles.

The developed colloidal chelate form of Zinc lysinatoriboflavinate is the optimal form of the essential Zinc element compared to other Zinc forms. It has the least effect on the properties of the dispersed phase of milk. It was found that the least influence on the hydrodynamic radius of particles of the dispersed phase of milk samples is exerted by Zinc lysinatoriboflavinate, which increases the hydrodynamic radius by 5 nm when enriched. This was confirmed by electroacoustic spectroscopy, as a result of which data was obtained showing that the enrichment of milk with Zinc lysinatoriboflavinate caused the smallest increase in the ζ-potential, which characterizes the greatest stability of particles. It is important to note that all considered dependencies of the hydrodynamic radius, polydispersity, and ζ-potential of particles of the dispersed phase of milk samples correlate with Zinc content ions in aqueous solutions of compounds with which the milk samples were enriched. This was confirmed by electroacoustic spectroscopy, as a result of which data were obtained showing that the enrichment of milk with Zinc lysinatoriboflavinate caused the smallest change in the ζ-potential, which characterizes the greatest stability of particles. It is important to note that all the considered dependences of the hydrodynamic radius, polydispersity, and ζ-potential of particles of the dispersed phase of milk samples correlate with the content of Zinc ions in aqueous solutions of used Zinc-compounds with which the milk samples were enriched. The obtained results are confirmed by computer quantum-chemical modelling.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.arabjc.2021.103229.

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