Vol. 7, No. 2, 2013 Chemical Technology

Orest Kondratovych, Iryna Koval, Volodymyr Kyslenko, Liliya Shevchuk, Lesia Predzumirska and Natalia Maksymiv

WHEY DISINFECTION AND ITS PROPERTIES CHANGED UNDER ULTRASONIC TREATMENT

Lviv Polytechnic National University,
12, St. Bandery str., 79013 Lviv, Ukraine;
orest.kondartovych@gmail.com

Received: February 02, 2012 / Revised: September 13, 2012 / Accepted: March 18, 2013

© Kondratovych O., Koval I., Kyslenko V., Shevchuk L., Predzumirska L., Maksymiv N., 2013

Abstract. The effect of ultrasonic cavitation on the vital activity of microorganisms in lactowhey has been investigated. The influence of different gases on the process proceeding and oxygen role in the oxidation of whey organic components under the influence of ultrasound has been determined. The destruction of protein macromolecules takes place in whey chemical structure after ultrasonic treatment. The destruction has been investigated and branched amino and carboxyl groups in whey peptides have been observed.

Keywords: lactowhey, ultrasound, protein, decontamination, *b*-lactoglobulin, pH.

1. Introduction

Whey is used as the source of protein to stabilize food emulsions and dispersions. It is a product containing proteins in the amount of 0.5–1.5 %. The proteins include casein (10 % of the total amount), *b*-lactoglobulin (7–12 %), α-lactalbumin (2–5 %), albumin, immunoglobulins and proteozopeptones. Moreover, whey contains lactopherrin, enzymes and other components. Whey proteins are the most valuable parts of milk proteins due to the presence of scarce and indispensable amino acids (lysine, tryptophane, methionine, threonine and cystein) in them. Therefore their usage for the food purposes is of great practical importance.

Amino acids of some fractions of whey proteins have the same qualitative composition but their quantitative compositione is different. Whey protein has all indispensable amino acids. Their amount satisfies or exceeds the amount of amino acids in the "ideal protein" (except aromatic, sulphur-containing ones and valine). The non-protein nitrogeneous compounds present in the whey are: free amino acids, urea, creatine, creatinine and purine bases). The amount of free amino acids is small and depends upon the whey type. This fact is connected with the deeper hydrolysis of milk proteins under the action of lactic acid bacteria and lactic acid as well [1].

In spite of the greatest content of *b*-lactoglobulin (BLG) in the whey, it is a poor substrate for many ferments of the food industry. Protein decomposition under the action of enzymes makes it a very strong food allergen [2]. However its protein nature is a sufficiently good medium for the microorganisms growth; thus time of BLG water solutions storage and consequently whey storage is very small. Ultrasound (US) is an effective method for homogenization of foodstuffs containing proteins, oils and solid structural elements [3]. At the same time ultrasonic treatment is an important method for disinfection of drinking water and water contained in food, *e.g.* milk, juice, *etc.* [4-6].

The effect of ultrasound on the foam- and emulsion-forming of wheat fiber increases with the increase of its electrophoretic and rheological properties [7]. Under proteins ultrasonic treatment the volume of foam and its stability slowly increase with the increase of ultrasound power and become strongly pronounced at 100 % capacity.

Sukmanov V. *et al.* [8] carried out investigations concerning the rheological properties of eggs. The experimental conditions were: initial concentration of bacteria of intestinal bacillus group within the range of 108 CCO/ml (cells of conventional organism), the ultrasonic treatment of 20, 30 and 40 W, and 200 MPa at US frequency of 25 kHz for 400 s (100 s of the time is a period of pressure increase and decrease) and complex treatment: ultrasound + high presser (US + HP). The results show that eggs US-treatment within the mentioned range of power increases the temperature twice. The HP treatment ensures eggs sterilization after 350 s and complex treatment – after 300 s.

The foaming ability of egg-white was investigated as well. A membrane structure with air bubbles is formed as a result of whisking. The considerable increase of foaming ability (by 4.5–5 % regardless of HP value in the range of 100–400 MPa and HP-treatment time) is explained by homogenizing effect under US action. The homogenization favors proteins and adipose matter distribution in the egg and thus increases the foaming ability.

Fatal effects of ultrasound (20 kHz frequency used) on the functional properties of whey proteins like solubility and foaming ability were investigated in [9]. They are caused by the increasing temperature during the sample treatment. Using ultrasound of 40 kHz frequency had less effect on protein properties. The best results were obtained after treatment for 15 min compared with that for 30 min. Ultrasound treatment with 500 kHz frequency did not affect the foaming properties. Conductivity decreased for all samples after US-treatment with 40 and 500 kHz frequency. The temperature of the model protein solution increased after each US-treatment. Therefore, the compliance of the determined optimal conditions for whey US-treatment is the incontestable fact to increase the disinfection efficiency and storage time of the product.

2. Experimental

In our experiments we used whey after milk fermentation and casein and fat separation. Whey was preserved for seven days at 275–276 K in a refrigerator. The initial concentrations in whey were: 5.8–6.4 % (BLG); 0.03–0.05 % (fat); 1.5–3.8 % (lactose); 0.8–1.2 % (lactic acid) and 6.1–6.3 % (solids). Whey was diluted four times before its ultrasonic treatment.

The number of microorganism colonies in the sample was determined by deep sowing of 1 cm^3 of the dispersion in a Petri dish with nutritious medium followed by the incubation in a thermostat at 310 K for 48 h.

Coefficient of oxygen demand (COD) was determined by standard bichromate method [10].

Whey pH was measured using laboratory ionomer I-160MI. 0.1 N solution of hydrochloric acid and 0.06 N solution of sodium hydroxide were used for the titration of whey solutions. Accuracy of pH measuring was ± 0.02.

Ultrasound transducer UZDN-2T with operating frequency of 22 kHz and capacity of 35 W was used for microorganism dispersions. The experiments were carried out at T = 298 K and $P = 1 \cdot 10^5$ Pa in the medium of oxygen, helium and argon.

3. Results and Discussion

Investigation of ultrasound treatment on microorganism number (MN) in whey shows (Fig. 1) that in Ar and He media the number of viable cells in the volume unit of the whey sharply decreases during 30 min and then gradually decreases during an hour and a half. In the oxygen medium MN increases during 30 min because aerobic bacteria are present in the whey and further oversaturation by O_2 and US-treatment results in bacteria inactivation. The number of microorganisms depends on time and conditions of the whey storage both for the initial whey as well as for the whey treated by ultrasound. This phenomenon can be connected with the formation of stable forms of microorganism cells, for example possible formation of spores, as well as stabilization of micro-organisms by the protein particles present in the whey.

The rate of microorganism inactivation depends on the initial number of cells (from 217000 (Ar+US) to 40000 (O_2+US) cells/ml) and increases with the increase their number. Therefore MN/MN_0 ratio is given in Fig. 1 for the obviousness. The effect of gas nature on the microorganism stability under US-treatment may be estimated by the effective rate constant. It is calculated in accordance with the first order kinetic equation (1):

$$\ln C_t = \ln C_0 - kt \tag{1}$$

$$\text{Thus, } k = \frac{1}{t} \ln \frac{C_0}{C_t} \tag{2}$$

Numerical value of the rate constant k may be determined if we obtain the linear dependence within $\ln C_t - t$ coordinates. The investigated process is of the first order and $\mathrm{tg}\alpha = -k$.

The determination of k and n values is based on the results of Eq. (3) integration:

$$C_A = C_{A_0} \cdot e^{-kt} \text{ at } n = 1 \tag{3}$$

or

$$\ln \frac{C_A}{C_{A_0}} = -kt \tag{4}$$

Fig. 2 shows that in the different gases media all kinetic data lie on the straight line in semilogarithmic coordinates. Since in the oxygen medium there is MN conglomeration for the first 30 min, the effective rate constant is calculated only at the stage of microorganisms inactivation. The correlation coefficient of straight lines is equal to 0.87–0.95 and effective rate constants are equal to $4.87 \cdot 10^{-4}$; $2.19 \cdot 10^{-4}$ and $2.12 \cdot 10^{-4}$ s^{-1} for helium, argon and oxygen, respectively.

The range of effective rate constants of the microorganisms inactivation is specified by the experimental conditions. Since the conditions were constant (pressure, temperature, capacity, and US frequency) and gas medium was different we may assert that gas nature affects the reaction rate. Therefore it was advisable to investigate the effect of gas nature on the oxidation rate of organic components present in the whey.

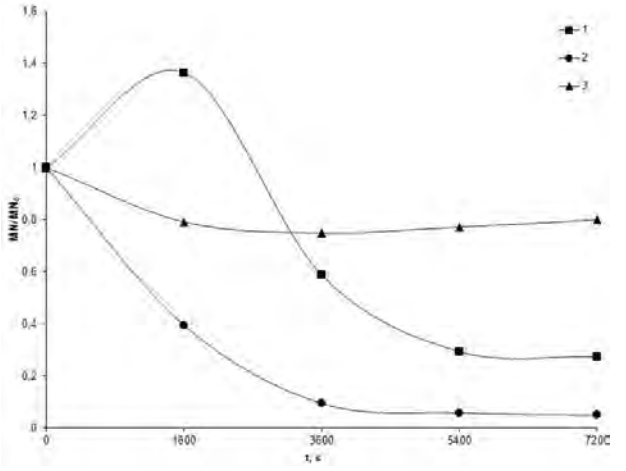

Fig. 1. Dependence of the relative microorganism number on whey US-treatment in oxygen (1), helium (2) and argon (3) media

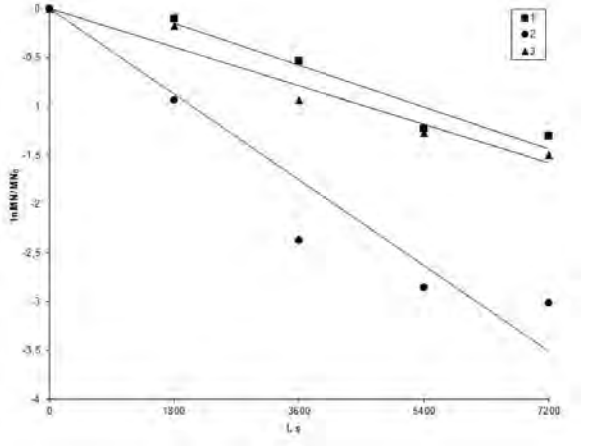

Fig. 2. Semilogarithmic anamorphous of the kinetic curves of microorganism inactivation under whey US-treatment in oxygen (1), helium (2) and argon (3) media

Investigation of the organic components oxidation was carried out using the coefficient of oxygen demand (COD). The analysis (Fig. 3) shows that the greatest effect on the organic compounds oxidation occurs in the presence of helium. Oxygen usage in the ultrasonic field is ineffective for short time but the increase of treatment time slightly increases the purification efficiency. After 1 h the efficiency is 41.41 % and then, after 30 min it is increased by 30 %. Helium has the highest purification efficiency (92.45 %), argon – 77.73 % and oxygen – 72.73 %.

Under US-treatment the protein molecule undergoes various chemical and physico-chemical transformations. The change depends on the structure of protein side and end-groups, as well as on the nature of gas bubbled into the medium. The investigation of US effect on the molecule of blood hemocyanine in the presence of air showed that the mentioned molecule is split into the identical components formed while using denaturated compounds [11]. However, some hemocyanine fragments formed under US-treatment lose their ability to interact between each other. It means that side- and end-groups of the protein molecule, which are responsible for its ability to reactivate, undergo chemical transformations and render the reversibility of hemocyanine ultrasonic dissociation impossible.

During the investigations of the serum protein structure the probability of protein molecules collision in the ultrasonic field sharply increases resulting in the molecules interaction between each other and formation of salt-like bonds: $–COOH + –NH_2 = –COONH_3$.

The increase of proteins molecular mass is observed at even short expositions. With the increase of treatment time the salt-like bonds of enlarged molecules are easily destructed. The presence of the enlarged protein particles is observed in the case of whey alkaline reaction [11].

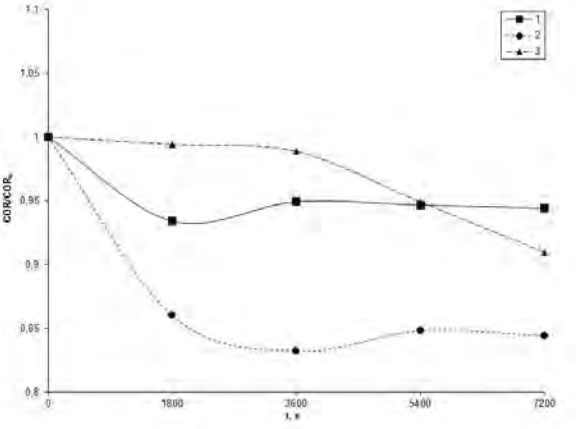

Fig. 3. Coefficient of oxygen demand *vs* time under whey US-treatment in oxygen (1), helium (2) and argon (3) media

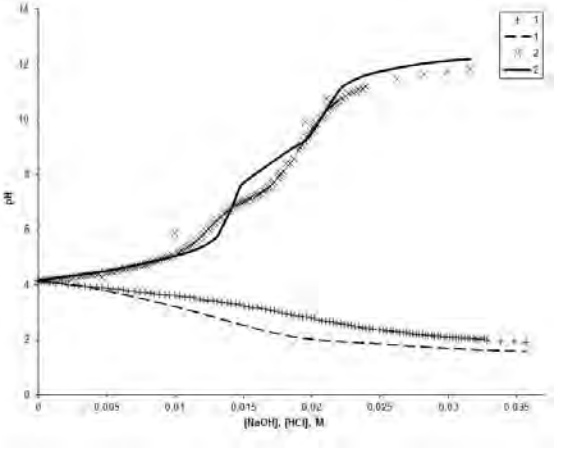

Fig. 4. Curves of whey potentiometric titration by hydrochloric acid (1) and sodium hydroxide (2). Experimental data are denoted by dots, theoretical curves are calculated in accordance with Eqs. (4)-(8) for whey solution

Destruction of whey proteins macromolecules was investigated using potentiometric titration of the functional end-groups. Initial whey pH changes from 4.1 to 4.6 and depends on the obtaining and preserving conditions. Typical curves of whey potentiometric titration by sodium hydroxide and hydrochloric acid are presented in Fig. 4.

Two areas are observed on the curve of whey titration by acid: the first area in the pH range of 2.5–3 corresponds to the titration of carbonic acids salts with amines followed by amine chlorides formation, and the second area in the pH range < 2 corresponds to the change of HCl concentration. The curve of whey titration by sodium hydroxide has three areas: the first one – till pH 7 – corresponds to neutralization of free carboxyl groups of proteins, the second one corresponds to the decomposition of amine salts with carboxyl groups in the range from 7 to 10 and the third area corresponds to the change of the sodium hydroxide concentration. These curves show that whey peptides contain many branched amines and carboxyl groups in their macromolecules. Theoretical curves of NaOH and HCl concentrations dependence on pH were calculated for the estimation of dependence of protein macromolecules functional end-groups on the change of pH during titration. β-Lactoglobulin is the basic peptide component of whey with molecular mass of 18400 g/mol and the amino acid content is represented in Table [12-14].

Table

Content of amino acids of whey and their dissociation constants

Amino acid	Content, g/100g of protein	Dissociation constants, l/mol			
		Acid groups		Amine groups	
Tryptophane	2.1	$4.169\cdot10^{-4}$	-	$4.07\cdot10^{-10}$	-
Isoleucine	6.1	$1.74\cdot10^{-10}$	-	$2.09\cdot10^{-12}$	-
Tyrosine	3.0	$3.98\cdot10^{-9}$	-	$1.58\cdot10^{-12}$	-
Phenylalanine	3.4	$2.19\cdot10^{-5}$	-	$1.29\cdot10^{-12}$	-
Proline	6.1	$2.29\cdot10^{-11}$	-	$8.91\cdot10^{-13}$	-
Leucine	10.4	$1.82\cdot10^{-10}$	-	$2.14\cdot10^{-12}$	-
Valine	5.8	$1.91\cdot10^{-10}$	-	$1.95\cdot10^{-12}$	-
Lysine	9.2	$2.95\cdot10^{-11}$	-	$1,51\cdot10^{-12}$	$8.91\cdot10^{-6}$
Methionine	2.1	$6.17\cdot10^{-10}$	-	$1.91\cdot10^{-12}$	-
Cistein	2.3	$4.57\cdot10^{-11}$	$7.24\cdot10^{-9}$	$7.24\cdot10^{-13}$	-
Alanine	4.9	$1.35\cdot10^{-10}$	-	$2.24\cdot10^{-12}$	-
Arginine	2.8	$3.31\cdot10^{-13}$	-	$1.05\cdot10^{-12}$	$1.10\cdot10^{-5}$
Histidine	2.0	$6.76\cdot10^{-10}$	-	$6.31\cdot10^{-13}$	$1.00\cdot10^{-8}$
Threonine	6.8	$1.95\cdot10^{-3}$	-	$2.4\cdot10^{-10}$	-
Serine	5.2	$1.55\cdot10^{-10}$	-	$1.58\cdot10^{-12}$	-
Glycine	2.0	$1.32\cdot10^{-10}$	-	$2.24\cdot10^{-12}$	-
Aspartic acid	10.7	$1.0\cdot10^{-10}$	$1.26\cdot10^{-4}$	$9.77\cdot10^{-13}$	-
Glutamic acid	18.8	$4.47\cdot10^{-10}$	$3.09\cdot10^{-5}$	$2.0\cdot10^{-12}$	-

For the calculations we took into consideration only branches of amino acids functional groups (lysine, cistein, arginine, histidine, aspartic acid. and glutamic acid) and two functional groups of amino acids with different dissociation constants for one protein macromolecule with the molecular mass of 1840 g/mol. Concentration of carboxyl and amine groups ions was calculated in accordance with the equations:

$$[-COO^-] = K_{id}([COOH]_o - [H^+])/[H^+] \quad (5)$$

$$[-NH_3^+] = K_{jd}([NH_2]_o - [OH^-])/[OH^-] \quad (6)$$

$$[H^+] = 10^{-pH} \quad (7)$$

$$[OH^-] = 10^{(pH-14)} \quad (8)$$

Ion concentrations were calculated in the pH range from 1 to 13 with the step of 0.01 and using balance of cations and anions in a solution in accordance with the equation:

$$\sum_{i=1}^{n}[Anion]_i - \sum_{j=1}^{m}[Cation]_j = d \quad (9)$$

Minimum δ value allows to obtain the pH solution with the error of ±0.01.

Typical theoretical curve of pH dependence on NaOH and HCl concentrations is represented in Fig. 4. The curve shape corresponds to the shape of experimental curve, but plateau at pH close to 7 on the experimental curve corresponds to plateau at pH 8 on the theoretical curve. The reason may be polymeric structure of proteins because protein macromolecule changes its conformation at pH 7. The ionized amino groups of macromolecule external globules with pH < 7 are exchanged for ionized carboxyl groups with pH > 7.

Calculations show that only amine or carboxyl groups with the dissociation constant higher than 10^{-7} affect the pH solution because the groups with dissociation constants lower that 10^{-7} are completely hydrolyzed. The comparison of experimental and theoretical titration curves of whey by hydrochloric acid (Fig. 4) shows that the carboxyl groups concentration in solution is higher than those in proteins. Evidently, it is connected with the presence of lactic acid formed from lactose during cheese production by ferment method.

The addition of lactic acid with the concentration of 0.015 mol/l and dissociation constant of $3.89 \cdot 10^{-4}$ l/mol into whey allows to describe the experimental titration curve (Fig. 5).

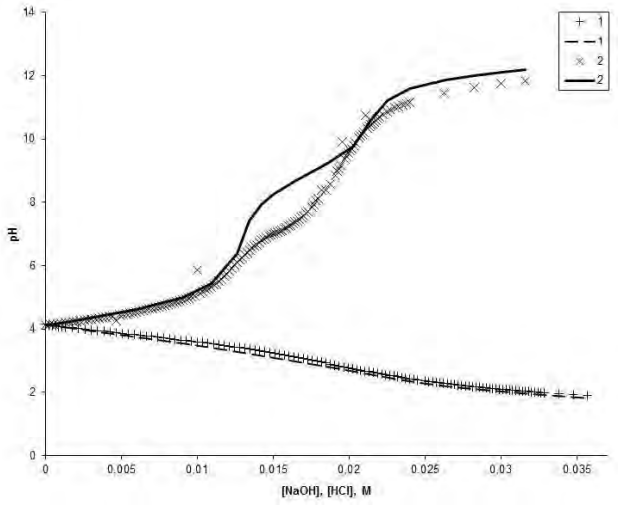

Fig. 5. Curves of whey potentiometric titration by hydrochloric acid (1) and sodium hydroxide (2). Experimental data are denoted by dots, theoretical curves are calculated in accordance with Eqs. (5)-(9) for the whey containing lactic acid

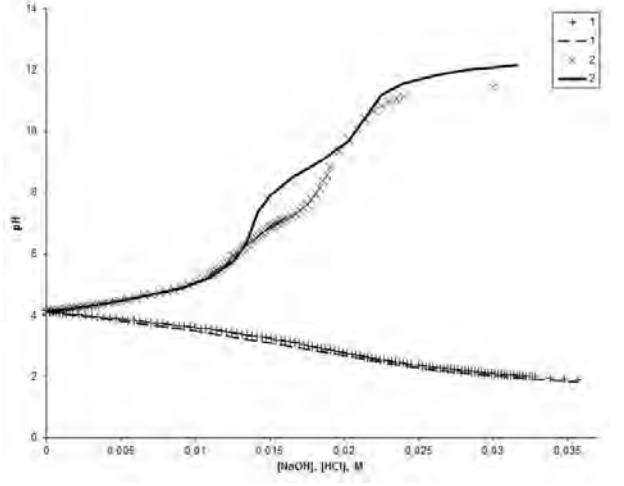

Fig. 6. Curves of potentiometric titration of ultrasound treated whey by hydrochloric acid (1) and sodium hydroxide (2). Experimental data are denoted by dots, theoretical curves theoretical curves are calculated in accordance with Eqs. (5)-(9) for the whey containing lactic acid

The curve shape of whey potentiometric titration after 0.5 h of ultrasonic treatment indicates the presence of enlarged protein particles and probability of destruction occurred in the protein molecule in the alkaline medium. The changes take place in a period of the most intensive COD decrease for the first 30 min in the medium of the most efficient gas (Fig. 6). The curve length in the pH range 4–7 is greater than that in Figs. 4 and 5. It means that the new amine and carboxyl groups are formed under US-treatment.

The concentration of new carboxyl and amino groups is equal to 0.0020 mol/l and calculated from theoretical and experimental groups indicated in Figs. 5 and 6. Molecular mass of destructed protein macromolecules and destruction degree of protein macromolecules may be calculated taking into account that the initial concentration of whey macromolecules is $7.9 \cdot 10^{-4}$ mol/l. The concentration of protein macromolecules after ultrasonic treatment is equal to 0.0028 mol/l (the sum of macromolecules initial concentration and new carboxyl or amine groups concentration). The molecular mass of destructed protein macromolecules is equal to:

$$MM_d = MM_i \cdot [Macromolecule]_i / [Macromolecule]_d \quad (10)$$

where MM_d and MM_i are the molecular masses of the initial and destructed protein; $[Macromolecule]_i$ and $[Macromolecule]_d$ are concentrations of macromolecules peptide before and after ultrasonic treatment.

The molecular mass of destructed protein macromolecule is calculated in accordance with Eq. (10) and is equal to 5126 g/mol. The destruction degree is equal to 3.4 and under US-treatment for 0.5 h more than three peptide macromolecules are formed from one protein macromolecule.

4. Conclusions

Whey ultrasonic treatment is an effective method of product disinfection and allows to double its storage time. The kinetics of microorganism destruction in whey is described by first order kinetic equation. Whey US-treatment destructs the polymers, increases the number of amino and carboxyl groups due to the protein hydrolysis and does not causes evident oxidation of organic compounds. The polypeptides molecular mass decreases from $17 \cdot 10^3$ to $5 \cdot 10^3$ g/mol under US-treatment.

References

[1] Kerelin Ja. and Repin B.: Biohimicheskaya Ochistka Stochnyh Vod Predpriyatiy Pishchovoy Promyshlennosti. Pishchevaya promyshlennost, Moskwa 1974.

[2] Stanic D., Stojadinovic M., Sancho A. and Ćirkovic-Velickovic T.: Conference COST 928. Naples 2010, O4.5.
[3] Suslick K. and Kirk-Othmer S.: Encyclopedia of Chemical Technology; 4th edn., V. 26. J. Wiley & Sons, New York 1998.
[4] Kalumuck K., Chahine G., Hsiao C-T. and Choi J-K.: Fifth International Symposium on Cavitation (CAV2003). Japan, Osaka 2003, 1.
[5] Ashokkumar M., Lee J., Kentish S. and Grieser F.: Ultrasonics Sonochemistry, 2007, **14**, 470.
[6] Gera N. and Doores S.: J. Food Sci. Vol., 2011, **76**, M111.
[7] Zhang H., Claver I., Zhu K.-X. and Zhou H.: Molecules, 2011, **16**, 4231.
[8] Sukmanov V. and Dekan O.: Obladnannya ta Techn. Harchovyh Vyrobnyctv, 2010, **23**, 62.
[9] Jambraka A., Mason T., Lelas V. *et al.*: J. Food Eng., 2008, **86,** 281.
[10] Eshvort M.: Titrametricheskye Metody Analiza Organicheskykh Soedineniy. Khimia, Moskwa 1972.
[11] Margulis M.: Osnovy Zvukokhimii (Khimicheskie Reakcii v Akusticheskykh Polyakh). Vyshaya Shkola, Moskwa 1984.
[12] Bounous G., Batist G. and Gold P.: Clinical and Investigated Medicine, 1989, **12**, 154.
[13] Skurikhin I.: Khimicheskyi Sostav Pishevykh Poduktov. Spravochnik. Agropromizdat, Moskwa 1987.
[14] Severin E.: Biokhimiya. Uchebnik dlya VUZov. GEOTAR-MED, Moskwa 2004.

ДЕЗІНФЕКЦІЯ СИРОВАТКИ ТА ЗМІНА ЇЇ ВЛАСТИВОСТЕЙ ПІД ДІЄЮ УЛЬТРАЗВУКОВОГО ВИПРОМІНЕННЯ

Анотація. *Досліджено вплив ультразвукової кавітації на життєдіяльність мікроорганізмів, що містяться в молочній сироватці. Визначено вплив різних газів на проходження процесу дезінфекції та окиснення органічних речовин, що містяться у молочній сироватці. Показано, що в хімічній структурі сироватки після ультразвукової обробки відбувається деструкція білків. Досліджено деструкцію макромолекул білка і виявлено розгалужені аміно- і карбоксильні групи в пептидах сироватки.*

Ключові слова: *молочна сироватка, ультразвук, білок, знезараження, β-лактоглобулін, pH.*