INFLUENCE OF THE WHEY TYPE ON COMPOSITION AND PROPERTIES OF ITS MINERALIZATES

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Abstract: Physical and chemical properties of whey mineralizates obtained from unsalted cheese whey, curdy whey and casein whey treated by electrodialysis. The extent of electrodialysis treatment of whey on the structure of its dispersed phase was studied by the photon-correlation spectroscopy method. The considerable effect of electrodialysis treatment on the dispersed structure of whey, on stability of whey proteins that form the basis of the dispersed phase of whey was defined. These variations may significantly affect the organoleptic and technological properties of demineralized whey, its shelf life and biological value. It's been established that the demineralization causes significant changes to the specific electrical conductivity and the active acidity of both whey and whey mineralizates. Their physical and chemical properties were studied with the following methods: potentiometry, conductometry, stalagmometric method, viscometry, refractometry. The elemental and phase composition were studied by a range of advanced methods, such as X-ray phase analysis, scanning electron microscopy, energy dispersive X-ray fluorescent microanalysis, infrared spectroscopy. It's revealed that the main crystalline phases of whey mineralizates are the potassium and sodium chlorides, calcium and magnesium phosphates, calcium sulfate and carbonate. The results of infrared spectroscopy allowed identification of the lactate, citrate, sulfate and phosphate ions in the structure of whey mineralizates. The ultimate composition of whey mineralizates is represented by such chemical elements as *Cl*, *Ca*, *Na*, *Mg*, *K*, *S*, *P*, *O*, *Al*, *Si* and *N*. The correlation between the composition and properties of whey mineralizate and initial milk whey is established.

Keywords: Whey mineralizates, infrared spectroscopy, X-ray phase analysis, scanning electron microscopy, energy-dispersive microanalysis

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INTRODUCTION

Electrodialysis treatment of whey is found to be the most effective method of targeted control of its mineral composition and acidity [1, 2]. It should be noted that the whey electrodialysis does not significantly affect the qualitative and quantitative properties of whey proteins, lactose content, and the content of vitamins in demineralized whey; in the meantime, its technological and organoleptic properties considerably improve [3].

The process of whey electrodialysis desalination or demineralization ends in demineralized whey and salt concentrate, namely, the whey mineralizate. The demineralized whey (especially when dry) is used in food production for children and for special purposes; in confectionery and bakery; for meat products; in pharmaceutical industry, etc. [1–5]. On the other hand, the whey mineralizates have not found the practical use. As shown in works [6, 7], whey mineralizates may be used as the basis for washing and disinfecting agents used in dairy enterprises. In this regard, the study of physical and chemical, surface-active properties and the composition of whey mineralizates, as well as the impact caused by the type of initial whey to such parameters are quite relevant.

OBJECTS AND METHODS OF THE STUDY

The targets of this research are the milk whey and whey mineralizates obtained by electrodialysis of unsalted cheese whey, curdy whey and casein whey. Milk whey was demineralized by the *ED-mini* electrodialysis device (manufactured by *MEGA JSC*, Czech Republic) using *RALEXAMH-PES* anion exchange membranes and *RALEXSMH-PES* cation exchange membranes. Samples were treated under the electrodialysis until 90% demineralization was achieved. Parameters of electrodialysis included the following: voltage $U = 12.5$ V; membrane area – 0.14 m^2; steam of membranes – 10; diluate flow – 70 l/h; temperature $t = 22.0 \pm 2$°C. The volume of experimental whey mineralizates was produced at the International R&D "Electro-and Baromembrane Technologies" Laboratory of MEGAProfiLine LLC (Stavropol Territory, Stavropol).

The composition and properties of milk whey mineralizates were studied by laboratories of the Applied Biotechnology Department of the Institute of Living Systems and of the Department of Nanomaterial Technology of the Institute of Electric Power Engineering, Electronics and Nanotechnologies,

Federal State Autonomous University of Higher Education North-Caucasian Federal University, as well as by the R&D Laboratory "Physical Methods of Research and Analysis" of the Center for Collective Use of Scientific Equipment (Stavropol Territory, Stavropol).

The structure of the milk whey discontinuous phase was assessed by the photon-correlation spectroscopy method at the *Photocor Complex* unit (by Antek-97 LLC, Russia) [8]. The array of spectroscopic data was processed using the *DynaLS* software. The method of conductivity measurements at the EXPERT-002 conductometer [9, 10] was applied to measure the specific conductivity (SC) of milk whey and its mineralizates. The active acidity of whey and mineralized whey was determined by potentiometric method; the density was measured by the hydrometer [11]; the limiting wetting angle was measured by the sessile drop method that is the direct measurement of the angle by the shape of a drop on the solid surface; the surface tension was measured by stalagmometric method [12]; the kinematic viscosity was measured by the glass capillary viscosimeter VPZh-1 (Technocom NPO, Russia) [13]; the refractive index was measured by the refractometer; the titrated acidity of whey mineralizates was determined by titrimetry as per the procedure [14]. The structure of mineralizates the availability of certain vibrations of atomic group bonds was determined by the infrared spectroscopy (IR spectroscopy) using the IR Fourier spectrometer of FSM 1201 model listed with the No. 18895-99 in the National Register of Measuring Equipment of Russia. The phase composition of dry residues of whey mineralizates was evaluated by X-ray phase analysis by the method of powder diffraction at the *PANanytical Empyrean* X-ray diffractometer (manufactured by *PANalytical BV*, the Netherlands) as per the procedure [15]. The elemental composition of whey mineralizate dry residues was investigated using the energy-dispersive (elemental) analysis at the *MIRA-LMH* scanning electron microscope with the element determination system *AZtecEnergy Standart/X-max 20 (standard)* (manufactured by *Tescan*, Czech Republic) [16].

RESULTS AND DISCUSSION

Physical and chemical properties of whey and whey mineralizates were studied at the first stage. The process of milk whey electrodialysis allowed obtaining dependences of specific conductivity (SC) and active acidity of curdy whey, cheese whey, casein whey and whey mineralizates on demineralization level. The results are shown graphically below. Fig. 1 shows the dependence of the specific conductivity of milk whey on the degree of its demineralization.

As shown by the data analysis in Fig. 1, prior to the demineralization process, the initial milk whey had the values of specific conductivity as follow: the largest value obtained for the casein whey with SC = 9.54 ± 0.50 mS/cm, followed by the curdy whey with SC = 7.19 ± 0.55 mS/cm and the lowest value for the cheese whey with SC = 5.17 ± 0.52 mS/cm. The specific conductivity of all the whey samples studied in process of electrodialysis treatment decreased on a straight-line basis. The process of demineralization of milk whey was completed as the specific conductivity value was reached of about 1–1.5 mS/cm that corresponded to 90% of demineralization.

Fig. 2 shows the dependence of specific conductivity of whey mineralizates on the extent of whey demineralization.

Analysis of dependencies shown in Fig. 2 shows that the specific electrical conductivity of whey mineralizates increases as the extent of whey demineralization rises due to ion transition entering the dispersion medium of milk whey and to the salt concentrate influenced by the electromotive force (EMF). Upon completion of electrodialysis treatment, the casein whey mineralizate has the greatest specific conductance (SC) followed by the curdy and cheese whey that correlates with ion concentrations and SC of initial whey.

In parallel with SC measurement, the active acidity (*pH*) was measured for both whey of various types and whey mineralizates. Fig. 3 shows the results of *pH* measurement of milk whey as it is demineralized.

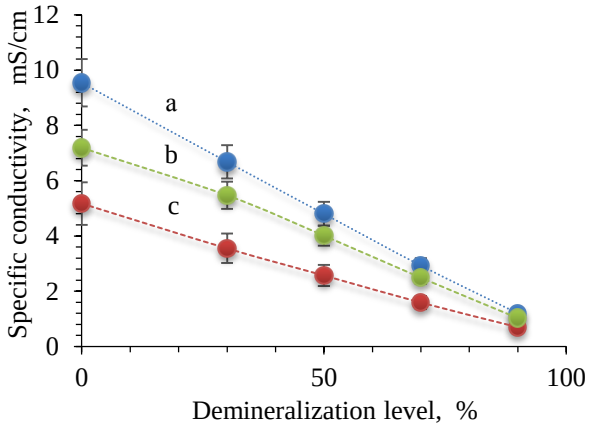


Fig. 1. Dependence of specific conductivity of milk whey on the extent of its demineralization: (a) casein, (b) curdy, (c) cheese.

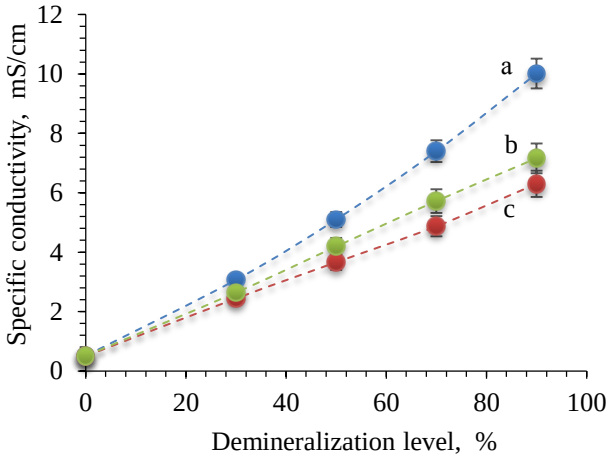


Fig. 2. Dependence of specific conductivity of whey mineralizates on the extent of whey demineralization: (a) casein, (b) curdy, (c) cheese.

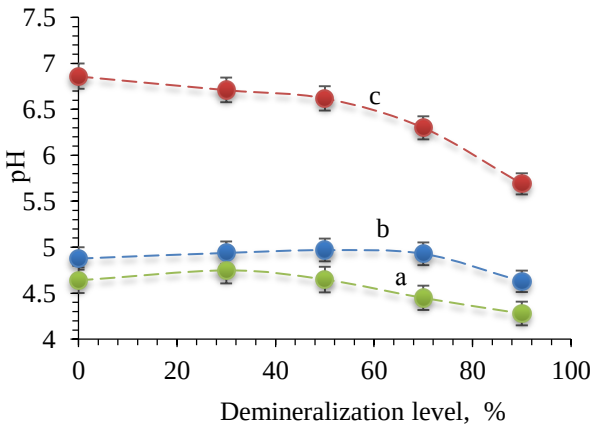


Fig. 3. Dependence of the milk whey active acidity on the extent of its demineralization: (a) casein, (b) curdy, (c) cheese.

As per the analysis of dependencies shown Fig. 3, the active acidity values of tested whey species were as follow prior to demineralization: casein whey $pH = 4.64 \pm 0.11$; curdy whey $pH = 4.88 \pm 0.12$ and cheese whey $pH = 6.86 \pm 0.10$. These active acidity values are well correlated with published data and are associated with processes to obtain these types of milk whey [2, 5]. For example, to produce the casein whey, the protein is coagulated using the hydrochloric acid (pH << 7), adding the *HCl* solution to the source milk until the isoelectric point of casein is reached (pH = 4.6) [17]. When producing curdy whey, the protein coagulation is associated with vital activity of lactic acid bacteria resulting in the large amount of lactic acid which is the weak electrolyte (pH < 7). The production of cheese whey is associated with the use of enzymatic processes that do not have a significant effect on acid-base properties of milk.

By the results of dependencies analysis as shown in Fig. 3, it was found that the active acidity of whey types tested decreases during demineralization. The greatest changes in whey pH are reported at the about 50% demineralization. This is apparently due to the process of water molecule decomposition on the membrane surfaces when this level of demineralization is reached under the effect of electric forces. This results in the release of the certain amount of H^+ protons that determine the value of active acidity of the medium, and as a result, pH decreases. OH^- ions released during the water molecule decomposition have no significant effect on the whey active acidity as they are removed in process of further desalting, which may be related to the process technique due to the selective permeability of membranes used relative to hydroxide ions.

Fig. 4 shows dependences of whey mineralizate active acidity on the extent of whey demineralization. It was found that the process of electrodialysis treatment results in the decrease of mineralizate active acidity of casein and curdy whey due to hydrochloric and lactic acid penetrated therein, respectively.

The active acidity of the cheese whey mineralizate varies insignificantly and is within $6 \le pH \le 7.5$; this is apparently due to the order of ion passing to the mineralizate. It is possible that at the beginning of electrodialysis, the ions with acidic properties pass into the mineralizate to explain the decrease in pH, followed by ions of basic properties. At the end of electrodialysis treatment, the active acidity of mineralizates is within: in acidic area - for casein and curdy mineralizates ($pH = 4.01 \pm 0.21$ and $pH = 5.05 \pm 0.22$, respectively), in the slightly acidic area - for cheese mineralizate ($pH = 5.92 \pm 0.25$).

Data were also obtained on the influence of extent of the whey electrodialysis treatment on the structure of its dispersed phase. The average hydrodynamic radius of particles of the whey dispersed phase was found by the photon correlation spectroscopy. Figure 5 shows the histogram of particle size distribution of the dispersed phase of initial curdy whey sample prior to electrodialysis treatment, distribution histograms of the disperse phase particles for the cheese and casein wheys are similar.

The analysis of results indicates that curdy, cheese and casein whey samples have a single dispersed phase with the average hydrodynamic radius of about 130 ± 50 nm. Quite monodisperse distribution should be noted for the dispersed phase of milk whey by particle sizes.

The distribution histograms of the average hydrodynamic radius of particles of the disperse phase of curdy, cheese and casein whey at 90% demineralization are shown in Fig. 6 a, b, c.

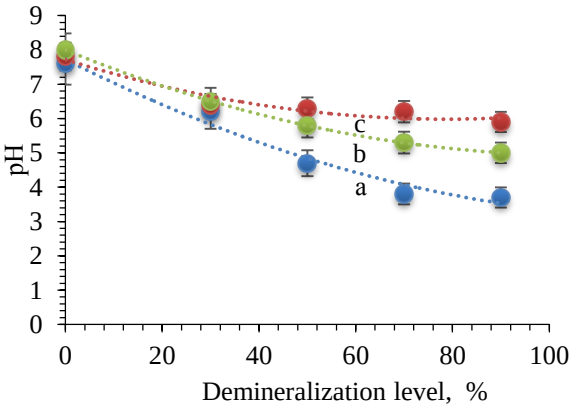


Fig. 4. Dependence of whey mineralizate active acidity on the extent of whey demineralization: (a) casein, (b) curdy, (c) cheese.

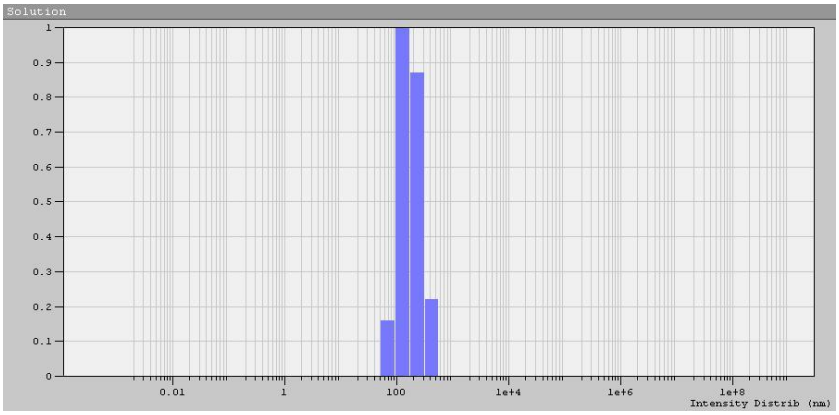
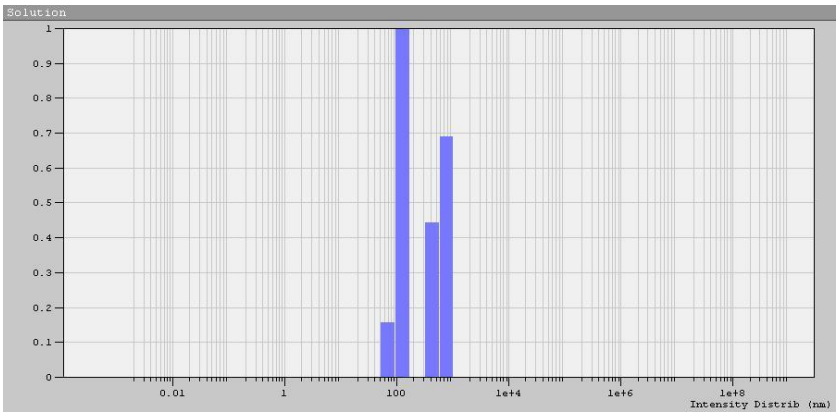
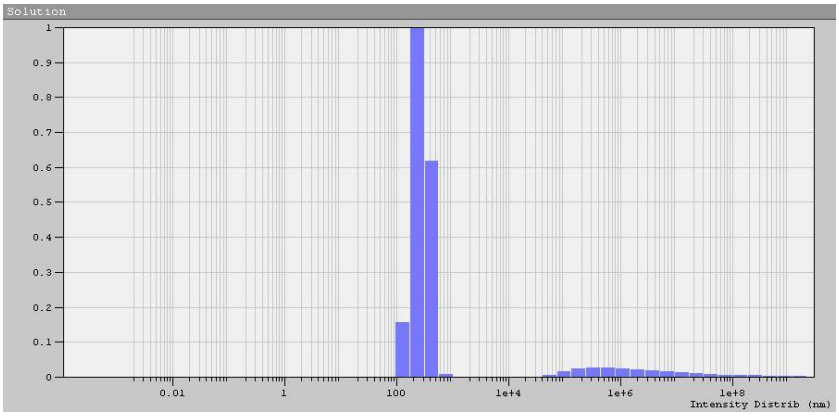


Fig. 5. Histogram of distribution of hydrodynamic radii of particles of the curdy whey disperse phase.



(a)



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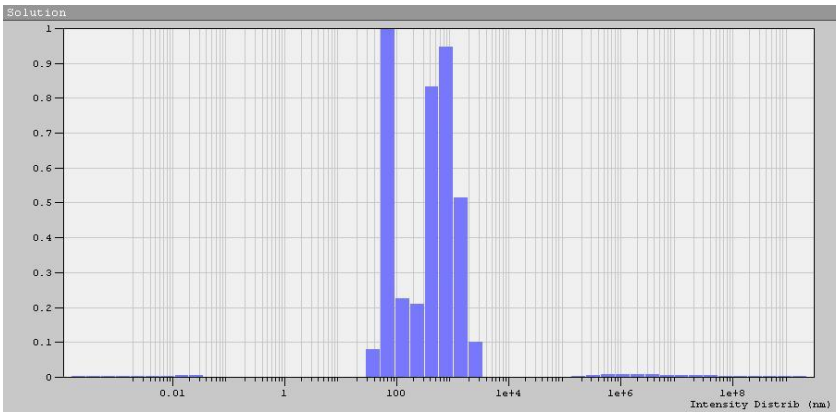

(c)

Fig. 6. Distribution histograms of the hydrodynamic radii of particles of the whey dispersed phase at 90% demineralization: (a) curdy, (b) cheese, (c) casein.

As per the analysis of histograms shown in Fig. 6 a, b, c, at 90% demineralization the second fraction occurrence was reported in all three milk whey samples and its content in curdy and casein whey is signify.cant totaling over 50%; the content of the second fraction is about 5% in the cheese whey.

At the second stage of the study, data were obtained on physical and chemical properties of whey mineralizates as shown in Table 1.

The analysis of data shown in Table 1 indicates that values of such physical and chemical properties as density, wetting angle, surface tension, kinematic viscosity and refractive index are similar for all mineralizates tested and do not depend on the origin of raw stock. The active acidity of the medium, specific electrical conductivity and titratable acidity depend on the ionic composition of whey mineralizates and, in turn, they correlate with the ionic composition of the raw stock (curdy, cheese and casein whey).

At the third stage of the study, whey mineralizate samples were dried and tested by IR spectroscopy, X-ray phase analysis and scanning electron microscopy. Fig. 7 shows the IR spectra obtained.

As shown by the IR spectra explanation [18, 19] of dry residues of whey mineralizates, bands are seen in the area of valence vibrations that may be associated with *-OH, -CH* and *-NH* groups forming part of mineralizates [18, 19]; comprising mineralizates; the area of deformation vibrations is represented by bands typical for bonds present in lactate, citrate, sulfate and phosphate ions.

Due to the fact that certain compounds with ionic bonds comrising mineralizates are optically transparent in the IR spectrum area, the X-ray phase analysis was used to identify them and to define their structure. The resulting X-ray diffraction patterns are shown in Fig. 8.

As per the analysis of diffractograms, main phases of curdy, cheese and casein whey mineralizates are the potassium and sodium chlorides, as well as calcium and magnesium phosphates, calcium sulphate and carbonate. Main components of the crystalline phase of whey mineralizates are described in Table 2 [20].

Table 1. Physical and chemical properties of whey mineralizates

Type of initial milk whey	Physical and chemical properties							
	Active acidity of the medium, *pH*	Density, ρ, kg/m^3	Wetting angle, θ, °	Surface tension, σ, mN/m	Titrable acidity, T°	Kinemtic viscosity, β, 10^{-6}·m^2/s	Refractive index, *n*	Specific conductivity, $\varkappa$, mS/cm
Curdy	5.05	1.005	96	76	16	0.904	1.334	7.16
Cheese	5.92	1.004	97	78	14	0.907	1.334	6.30
Casein	4.01	1.006	95	77	19	0.903	1.334	10.01

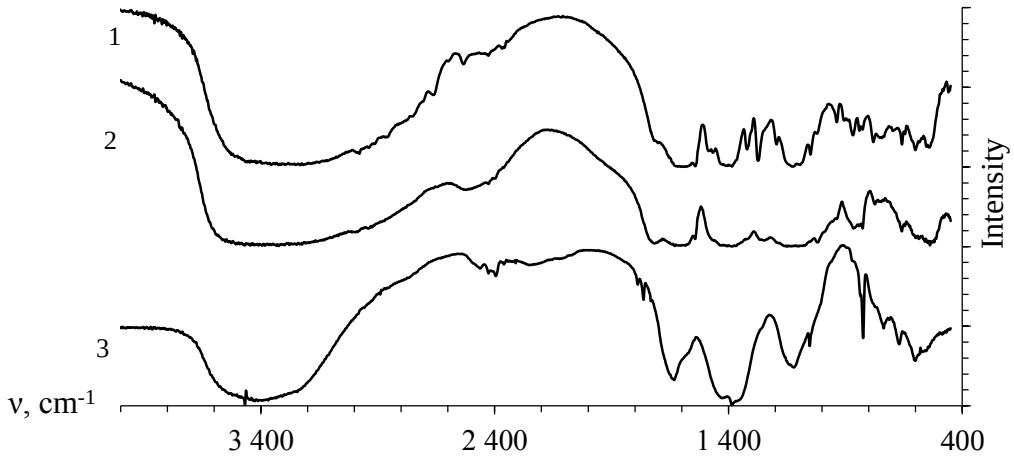

Fig. 7. IR spectra of dry residues of whey mineralizates: (1) cheese whey, (2) curdy whey, (3) casein whey.

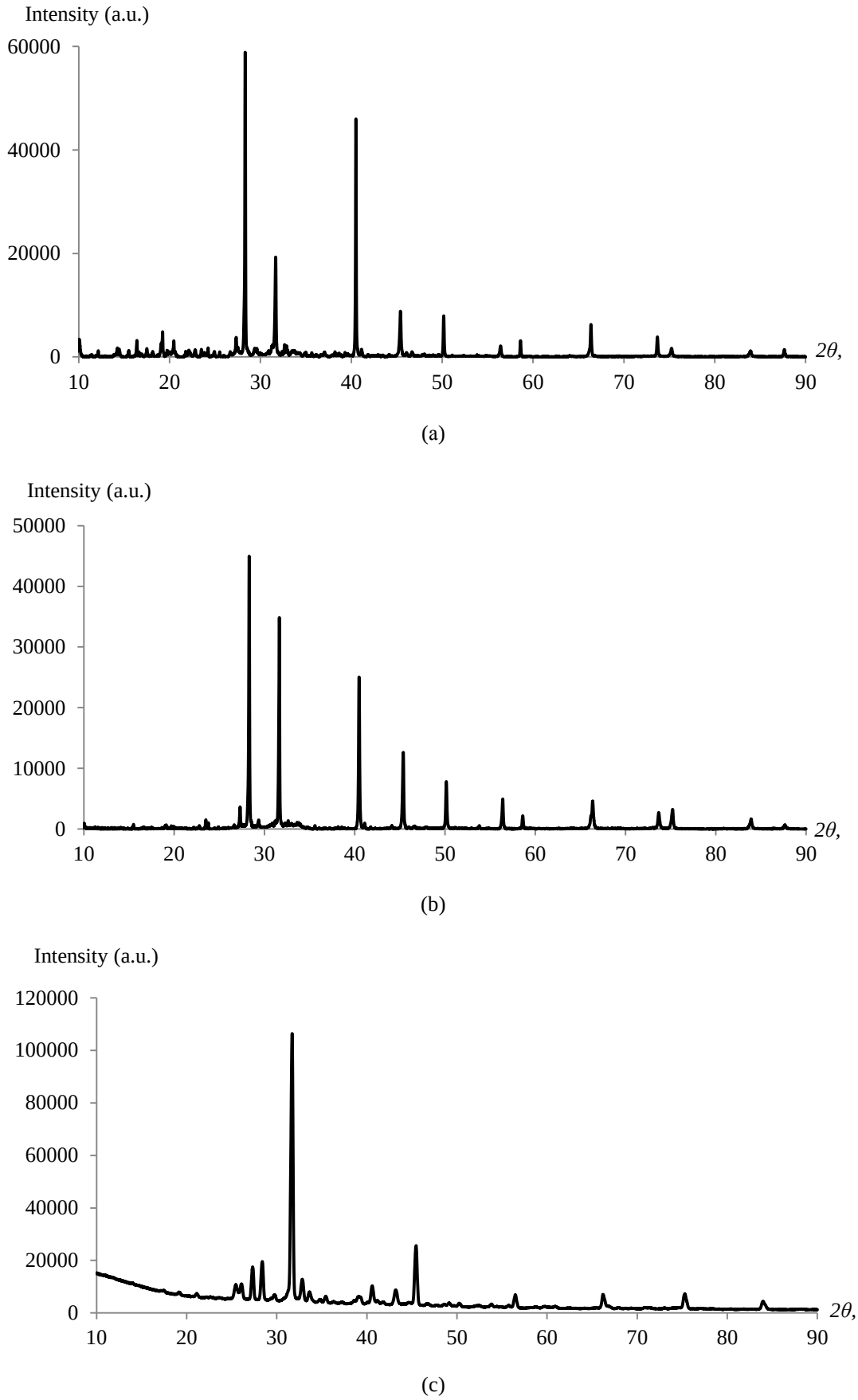

Fig. 8. Diffractograms of dry residues of mineralizates of various whey: (a) cheese whey mineralizate; (b) curdy whey mineralizate; (c) casein whey mineralizate.

Table 2. Description of main components of the whey mineralizate crystalline phase

Compound	Formula	Molecular mass	Type of crystal lattice	Space group	Elementary cell parameters
Potassium chloride	*KCl*	74.5	Cubic	*Fm-3m*	a = b = c (*Å*):6.2917 Alpha = Beta = Gamma (°):90.0000
Sodium chloride	*NaCl*	58.5	Cubic	*Fm-3m*	a = b = c (Å):5.6418 Alpha = Beta = Gamma (°):90.0000
Calcium sulphate	$CaSO_4$	136	Orthorhombic	*Bmmb*	a(Å):6.9920 b(Å):6.9990 c(Å):6.2400 Alpha = Beta = Gamma (°):90.0000
Calcium carbonate	$CaCO_3$	100	Orthorhombic	*Pmcn*	a(Å):4.9653 b(Å):8.0088 c(Å):5.7847 Alpha = Beta = Gamma (°):90.0000
Calcium orthophosphate	$Ca_3(PO_4)_2$	310	Orthorhombic	*R3c*	a (Å):10.3633 b (Å):10.3633 c (Å):37.2581 Alpha (°):90.0000 Beta(°):90.0000 Gamma(°)120.0000
Magnesium orthophosphate	$Mg_3(PO_4)_2$	262	Triclinic	*P-1*	a (Å):8.5120 b(Å):8.9820 c (Å):9.3200 Alpha (°):116.3400 Beta (°):91.5000 Gamma(°):114.4900

Whey mineralizate samples were tested by the scanning electron microscopy at the scanning electron microscope *MIRA-LMH* with the unit structure determination system *AZtecEnergy Standart/X-max 20* (*standard*) by *Tescan* Company. Previously, the whey mineralizate sample elements were studied by the energy-dispersive microanalysis. The *EDX* spectra obtained are shown in Fig. 9.

Mathematical processing of *EDX*-spectra helps to specify components of whey mineralizates as shown in Table 3.

As per the analysis of data shown in Table 3, curdy, cheese and casein whey mineralizates contain such elements as *Cl, Ca, Na, Mg, K, S, P, O, Al, Si* (nitrogen (*N*) is also found in the cheese whey mineralizate). The highest content of *Cl* is found in the casein whey mineralizate due to the hydrochloric acid used to produce it. The content of *Ca* and *P* in mineralizates of curdy and casein whey is several times higher than in that of cheese whey. The reason is as follows: the calcium phosphate in the casein and curdy whey is mainly ionic and is easily removed treated by electrodialysis and easily passes into mineralizates; the calcium phosphate is colloidal in the cheese whey of $pH \approx 7$, so it is hardly removed during electrodialysis. The content of *K, Na, Mg, S* in all milk whey mineralizates is fairly similar. In milk and whey, these elements form the ion-salt balance being not actively involved in colloidal system stabilization; these elements easily transform to mineralizate under the electrodialysis treatment of the milk whey.

It should be noted that it is not feasible to define the percentage of carbon by this method due to specific conditions for sampling, where a thin layer of carbon (5–10 nm) is applied onto the sample surface.

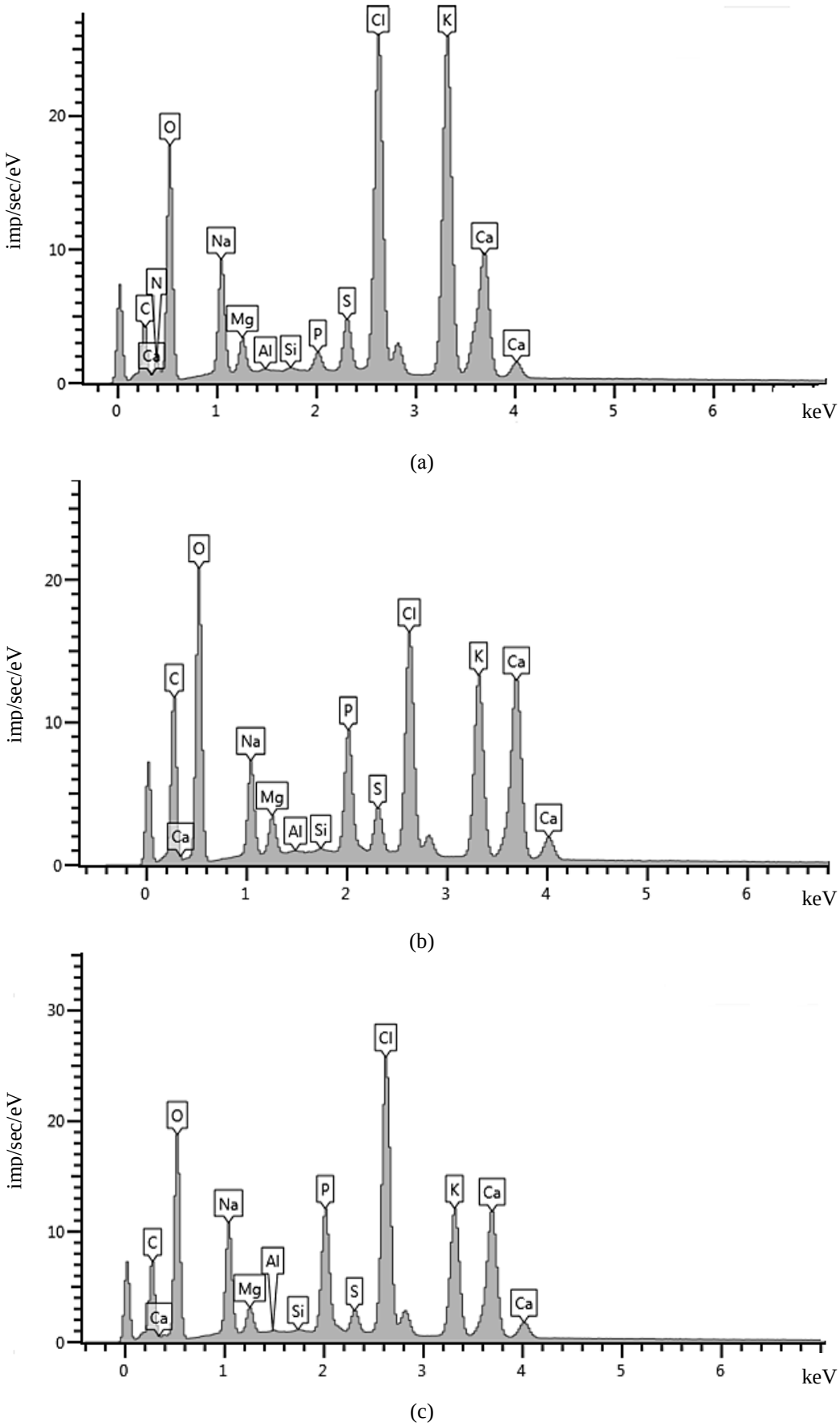

Fig. 9. *EDX*-spectrum of whey mineralizates: (a) cheese whey mineralizate; (b) curdy whey mineralizate; (c) casein whey mineralizate.

Table 3. Unit structure of milk whey mineralizates

Unit	Content of elements in milk whey mineralizates,%		
	casein	curdy	cheese
N	–	–	8.54
O	45.76	51.14	45.01
Na	8.41	6.05	6.20
Mg	1.57	1.87	1.43
Al	0.07	0.07	0.07
Si	0.09	0.10	0.09
P	6.13	4.94	0.64
S	1.20	1.92	1.80
Cl	15.79	10.32	12.99
K	9.92	11.04	11.72
Ca	11.06	12.55	7.52

Figures 10–12 show the test results of dry residues micro-structure of casein, cheese and curdy whey mineralizers obtained by scanning electron microscopy.

As per the analysis of SEM micro-images of whey mineralizate samples as shown in Figures 10–12, the microstructure strongly depends on the origin of the raw material, namely the whey. Polycrystalline formations of quite higher polydispersity are found in all milk whey mineralizates. The crystallite size in the casein whey mineralizate sample is 2 to 200 μm, of cubic shape that is relevant to the structure of potassium and sodium chlorides. Polycrystalline formations sized about 2 to 50 μm comprising of plates ("scales") are found in samples of both cheese and curdy whey mineralizates.

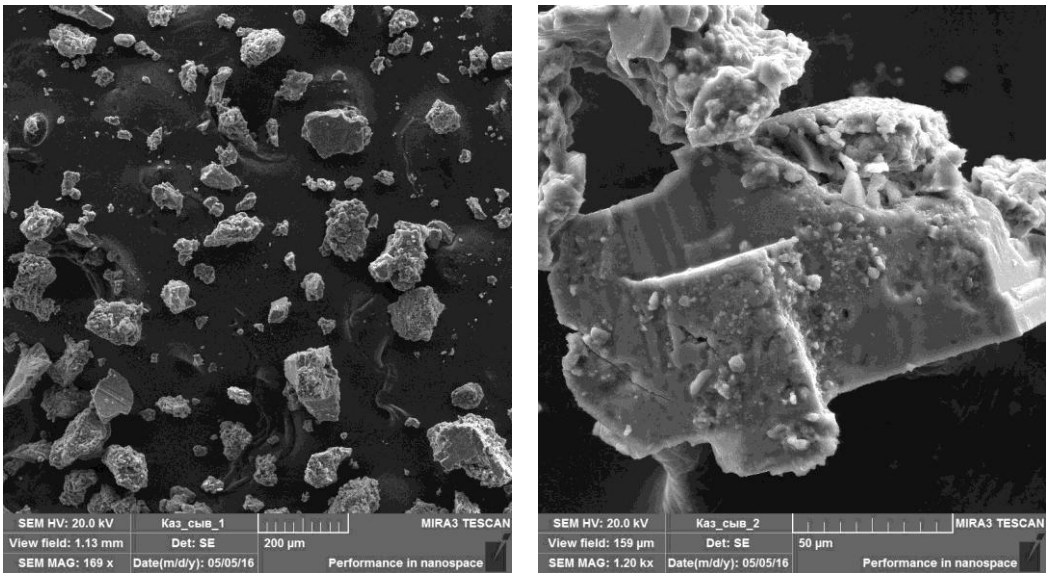

Fig. 10. SEM micro-images of the dry residue of casein whey mineralizate.

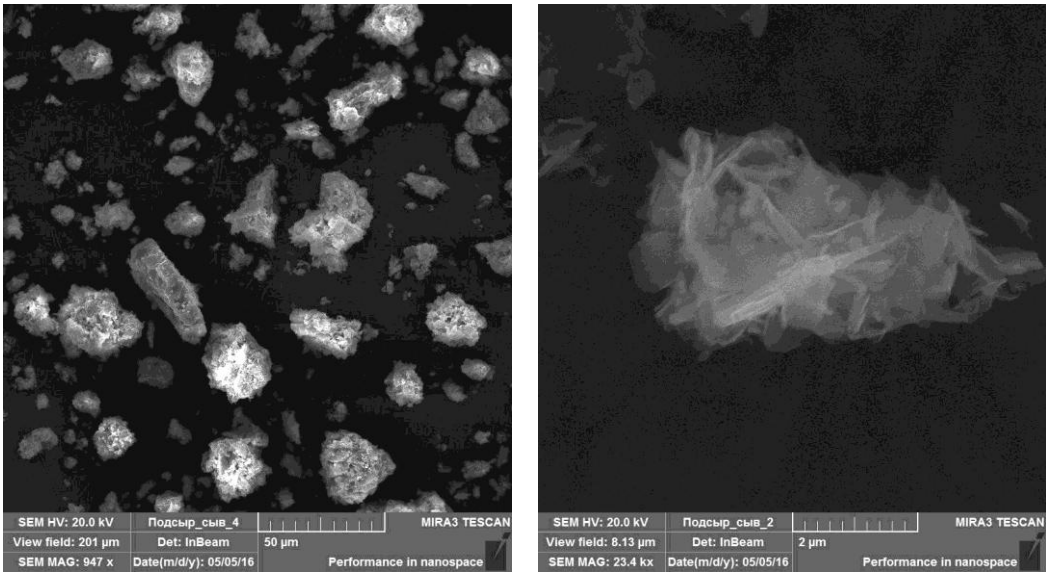

Fig. 11. SEM-micro-images of the dry residue of the cheese whey mineralizate.

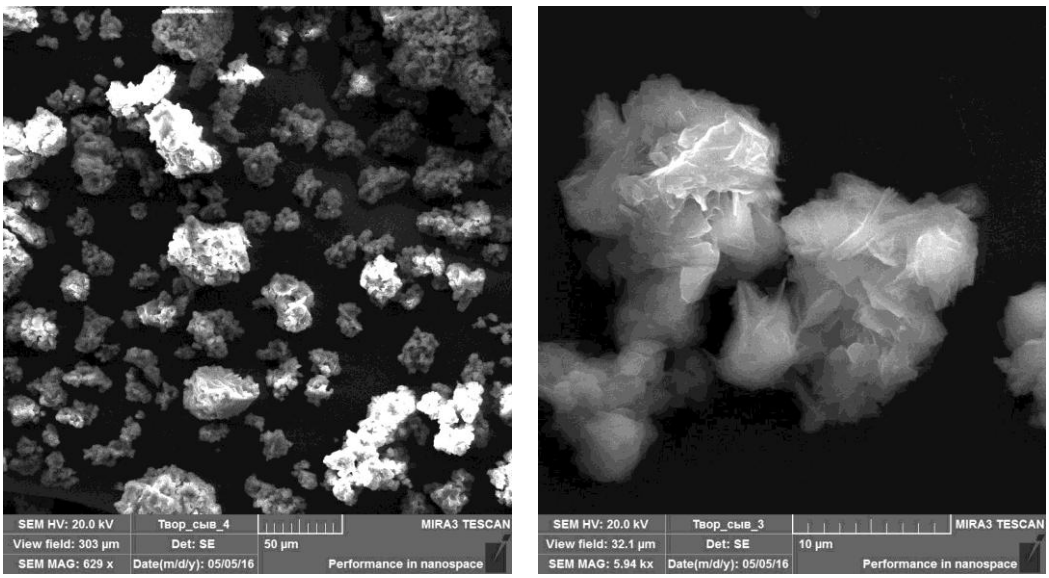

Fig. 12. SEM micro-images of the dry residue of curdy whey mineralizate.

CONCLUSIONS

Resulting from studies above, we may conclude on the considerable impact of electrodialysis treatment on the dispersed composition of milk whey and, consequently, on the stability of whey proteins as the basis of the milk whey dispersed phase. These variations may significantly affect the organoleptic and technological properties of demineralized whey, its shelf life and bioavailability. The study of the impact of nature and ion concentration on the particle consolidation process of the milk whey dispersed phase remains urgent and requires further studies.

We can further conclude that the type of dairy raw material considerably impact the physical and chemical properties and structure of whey mineralizates.

ACKNOWLEDGEMENTS

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