Foods and Raw Materials, 2022, vol. 10, no. 1

E-ISSN 2310-9599
ISSN 2308-4057

Research Article
Open Access

https://doi.org/10.21603/2308-4057-2022-1-117-126
Available online at https://jfrm.ru/en

Properties of serum albumin in electrolyzed water

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Received July 07, 2021; Accepted in revised form August 28, 2021; Published online January 31, 2022

Abstract:
Introduction. Electrochemical activation of water controls the physicochemical parameters of aquatic food environment without any reagents. Electrolyzed water affects the properties of macronutrient solutions. The present research studied the effect of anodic and cathodic fractions of electrochemically activated water on protein molecules and their interaction patterns.
Study objects and methods. The study featured bovine serum albumin and its properties in electrochemically activated water with non-standard redox and acidity values. The aqueous solution of bovine serum albumin was studied by viscometry, UV spectrometry, time-of-flight secondary ion mass spectrometry, and electrophoresis.
Results and discussion. By knowing the interaction patterns of electrochemically activated water and protein molecules, food producers can control the properties of biological raw materials. Bovine serum albumin was studied in metastable fractions of electrochemically activated water obtained in the anode or cathode chamber of an electrochemical reactor. Both fractions of electrochemically activated water appeared to modify the properties of bovine serum albumin. The oxidized fraction of electrochemically activated water (anolyte) converted the protein solution into a more homogeneous molecular composition. The solution of bovine serum albumin in the reduced fraction of electrochemically activated water (catholyte) had an abnormally negative redox potential (–800 mV). The aqueous solution of bovine serum albumin in catholyte retained its initial viscosity for a long time, and its level was lower than in the control sample. This effect was consistent with other physicochemical characteristics of the solution.
Conclusion. The research revealed some patterns that make it possible to apply reagent-free viscosity regulation to protein media in the food industry.

Keywords: Electrochemical activation, water, bovine serum albumin, protein-containing food medium, viscosity, molecular mass spectrometry (ToF-SIMS)

Funding: The study was supported by the Russian Science Foundation (RSF), project No. 20-16-00019 "The development of green electrochemistry methods to improve the efficiency of food production: molecular, multicomponent, and cellular biological targets of electrochemically activated aqueous solutions".

Please cite this article in press as: Pogorelov AG, Ipatova LG, Pogorelova MA, Kuznetsov AL, Suvorov OA. Properties of serum albumin in electrolyzed water. Foods and Raw Materials. 2022;10(1):117–126. https://doi.org/10.21603/2308-4057-2022-1-117-126.

INTRODUCTION

Economical and environmentally friendly methods of food processing require novel technological solutions to maintain the high quality of finished products [1–3]. Food science helps understand the impact of innovative approaches on the properties of substances in the production chain, from raw materials and by-products to finished products and waste disposal issues [4–6].

The structure and properties of food ingredients depend on such physical conditions as temperature, pressure, stirring speed, etc., as well as on chemical interactions with other nutrients, e.g. water [7–9]. A targeted effect on the water base can develop the desired characteristics of the semi-finished or finished product [10, 11]. The food industry uses electrochemical activation as a relevant method of reagent-free control of physicochemical and rheological properties.

Electrochemical activation, or electrolysis, is a unipolar electrochemical processing of water or aqueous electrolyte solutions. It occurs in the anode or cathode chamber of a diaphragm or membrane electrochemical reactor [12, 13]. Electrolysis happens as a result of electrochemical and electrical processes in water in a double electric layer of electrodes with a non-equilibrium electric charge transfer. Water is treated with a constant electric current,

and electric potentials exceed its decomposition voltage (+1.25 V). As a result, water passes into a metastable state with non-standard electron activity, redox potential, and other physicochemical parameters. Electrochemically activated water is able to retain this metastable state for a long time and resists the thermodynamic equilibrium with the environment [12].

Metastable compounds with a high oxidizing (anolyte) or reducing (catholyte) ability undergo a series of spontaneous structural, energetic, and chemical transformations and gradually stabilize during storage. They are highly reactive to chemicals and biological objects. Metastable compounds enhance acidic and oxidizing properties of anolyte, as well as the alkaline and reducing properties of catholyte [14–17]. Electrochemical nonequilibrium leads to multiple changes in the reactivity of ions but does not affect their concentration. In electrochemically activated water, the pH values of catholyte and anolyte correspond to the equilibrium concentrations of alkali and acid that exceed the content of salts in this water. The redox values also go beyond the chemical control capabilities for a given electrical conductivity [12].

Electrochemically activated water and its solutions owe their chemical activity to electrically active microbubbles of electrolysis gases. These microbubbles are 0.2–5.0 μm in size, and their concentration can reach 10^6–10^7 mL^{-1}. They are stabilized by uncompensated electric charges at the interface of gas and liquid phases [12, 14, 18].

Electrochemically activated water and its solutions have non-standard physicochemical parameters of pH and redox potential, which makes them biologically active [13, 18, 19]. Electrochemically activated water solutions of both low and high molecular weight compounds differ from similar solutions of non-electrolyzed water [12, 16].

Electrochemically activated water and its solutions behave differently in technological processes. For instance, electrochemically activated water and ultrapure water are known to affect apricot protein extraction [20]. At the same pH = 9.5, electrochemically activated water had a better extraction efficiency than ultrapure water. Foaming ability and stability of the electrochemically activated water emulsions were 11.17% and 36.33 min, whereas in the ultrapure water samples they were 4.75% and 23.88 min, respectively. Electrochemically activated water had a more ordered secondary structure than ultrapure water. The ordered structures of α-helix and β-sheet were 7.5 and 60.2%, while the disordered structures and random turns were 8.4 and 23.8%, respectively. The extraction method increased the yield of the product, minimized the structural degradation, and improved the functional properties of apricot protein [20].

Electrochemical activation proved an effective means of extracting protein from canola meal [21]. Under the electric field, the cathode chamber produced an alkaline solution from a sodium chloride (NaCl) solution. The alkaline solution had better extractive properties compared to the samples subjected to chemical alkalization. The extracted proteins had a better extractability, composition, and secondary structure. The concentration of NaCl was 0.01–1 M, electroactivation time – 10–60 min, current – 0.2 and 0.3A. The experiment was conducted in a three-chamber cell separated by ion-exchange membranes.

The resulting solutions underwent an extraction procedure. The maximal protein extract of 34.32 ± 1.21% occurred when the electrolyzed solution was generated at 0.3A, regardless of the activation time. The standard extraction (pH 7–10) yielded 31.18 ± 1.89% proteins under the same conditions. The Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) showed that the electrophoretic profiles of electrolyzed protein concentrates and isolates differed from those obtained with the conventional method. The Fourier Transform Infrared Spectroscopy (FTIR) showed significant differences in the secondary structures of proteins depending on the pH and salt concentration. The electrochemically activated samples had a lower denaturation [21].

Electric field can change the properties of aqueous protein solutions due to their electrical conductivity and the chemical structure of polyampholytic polyelectrolytes. Their amino acid units have ionogenic side groups, and their acidic groups alternate with basic ones, which provides macromolecules with specific electric, configuration, and hydrodynamic properties [22]. Molecular conformation, volume, and rheology depend on the concentration of the polyelectrolyte in the solution, e.g. temperature, pressure, low molecular weight substances, pH value, etc. [23–27].

Animal blood proteins can serve as an example of such relationships. Serum proteins have a lot of beneficial nutritional properties, which makes them part of many food formulations. A globular molecule of bovine serum albumin consists of several hundred amino acid residues. Its three-dimensional structure is labile, mobile, and sensitive to exogenous factors [23, 26, 28]. A bovine serum albumin solution contains protein fragments of different dimensions. Its monomers and aggregates are in a state of dynamic equilibrium, and the weight of polypeptides increases as the albumin concentration in the solution rises [26].

The dissolution of crystalline albumin depends on the contact time of the phases: it can change its conformation, develop intermolecular bonds, or destroy them. The structure of albumin solutions and their surface properties depend on the pH of the solution and the pH value of the isoelectric point. The closer to the isoelectric point, the more turbid the solutions are and the lower their viscosity gets. This phenomenon can be explained by the minimal energy of electrostatic repulsion between the side chains of albumin molecules

and the molecules themselves. The resulting aggregates are denser, more compact, and larger in size. They have less effect on the flow and increase light scattering. During structuring, the turbidity and viscosity of the solutions change nonlinearly, depending on the protein concentration [24, 27].

The surface activity of albumin increases together with proton concentration. In an acidic environment, more non-polar groups emerge on the surface of the molecule than in a neutral or slightly alkaline environment. Obviously, the surface activity of albumin molecules is minimal at physiological pH values [27].

Denaturation and aggregation of serum protein isolates depend on the pH of the medium. This effect is widely used in food technology. When acidity pH drops to 1, it leads to the denaturation of bovine serum albumin with a conformational transition. This process is caused by the loss of the tertiary structure, which occurs as the polypeptide chain of the bovine serum albumin molecule unfolds and the aggregates increase in size [26].

A strong alkaline environment has a more pronounced texturing effect, e.g. 2N NaOH solution with a pH of 12.4 ± 0.4 or alkaline electrolyzed water with a pH of 11.5 ± 0.4. In an acidic environment, the effect is less pronounced, e.g. 2N HCl with a pH of 2.0 ± 0.2 or acidic electrolysis water with pH 2.5 ± 0.2 [29]. Albumin is a polyelectrolyte with a high conformational mobility. In an electrochemically activated solution, it should be sensitive both to the acidity of the solution and its redox potential. The present research objective was to study the effect of electrochemically activated water on the properties of serum albumin in protein solutions.

STUDY OBJECTS AND METHODS

Sample preparation. The research featured bovine serum albumin BSA 100 (Merck, Sigma-Aldrich). Preparations with casein proteins and instant food gelatin were used as control (Dr. Oetker, OOO Oetker, Russia, TU 20.59.60-011-42450906-2018).

The research involved UV spectrometry, time-of-flight secondary ion mass spectrometry (ToF-SIMS), and electrophoresis of an 1% protein aqueous solution, which was then diluted with water or electrochemically activated water at a ratio of 1:4. Fractions of electrolyzed water, catholyte (pH 8.2, redox –800 mV), and anolyte (pH 2.2, redox +800 mV) were obtained in a fresh drinking water purification unit by means of direct electrochemical action in diaphragm modular electrochemical cells (LLC Delfin Aqua, Russia). Artesian water (pH 7.2, redox +360 mV) from the city water supply served as control. In the viscosity test, electrolyzed water with a negative redox value was obtained using an Izumrud-K1 installation (NPO Ekran OJSC, Russia). Tap water passed through a number of stages:

1. Anode chamber of a flow-type electrochemical module. Here the water was disinfected due to peroxide and chlorine-oxygen compounds, then saturated with oxygen and ozone to kill microorganisms and oxidize organic impurities.

2. Reaction-flotation reactor. It removed coagulated products of anodic treatment from electrolytically obtained microbubbles of oxygen and ozone.

3. Heterophase catalytic reactor. The procedure removed active chlorine compounds and produced active oxygen compounds.

4. Cathode chamber. Here the residual ions of iron, copper, magnesium, etc. were converted into insoluble hydroxides, which were then removed in the flotation and electrokinetic reactors. During the cathodic treatment, molecular hydrogen and free hydroxyl groups entered the water and gave it a negative redox value and antioxidant properties.

The electrochemically activated water had pH 7.3 and redox –223 mV, while for the initial water these values were 7.3 and +190 mV, respectively. The acidity (pH) and redox potential of the solution were measured using a SevenExellence S470 multivariable device (METTLER TOLEDO, Switzerland) with a pH electrode (Inlab Routine Pro, Mettler Toledo, Switzerland) and a redox electrode (Inlab Redox Pro, Mettler Toledo, Switzerland). Depending on the concentration of bovine serum albumin, its pH value ranged 7.2–7.6 for electrolyzed water and 7.9–8.6 for tap water.

UV spectrometry. Spectrometry was used to define the effect of electrolyzed water on aqueous protein solutions. Its optical density was recorded in the absorption spectrum of the sample in the ultraviolet region at a wavelength of 235 and 280 nm using a Shimadzu UV-2401PC spectrophotometer (Japan).

Time-of-flight secondary ion mass spectrometry (ToF-SIMS). To obtain samples for mass spectrometry, 2 μL of bovine serum albumin solution in water or water fractions were applied to a clean glass substrate. Its surface was covered with a conductive ITO-indium tin oxide film (Sigma-Aldrich). After drying in an stream, the sample was transferred to the chamber of a ToF-SIMS 5 secondary ion mass spectrometer (ION-ToF GmbH, Germany). The preparation was ionized with a 200 nm beam of primary Bi^{3+} ions at 30 keV. After 70 ns of exposure, secondary ions were registered (~ 80 μs) and the beam moved to the next point. The primary ion irradiation did not exceed 5×10^{12} ions/cm^2. The principal component analysis helped to assess the differences between the obtained mass spectra [30, 31].

Measuring the kinematic viscosity of protein solutions. This parameter was measured using an Ostwald VPZh-4m capillary viscometer (VPZh-4m viscometer, LABTECH LLC, Russia) at 20°C. The viscosity value was calculated as follows:

$$V = \frac{(g*K*t)}{9.8} \quad (1)$$

where V is the kinematic viscosity of the liquid, mm^2/s; K is the constant of the viscometer, mm^2/s^2; t is the flow time, sec; g is the gravity acceleration, 9.8 m/s^2.

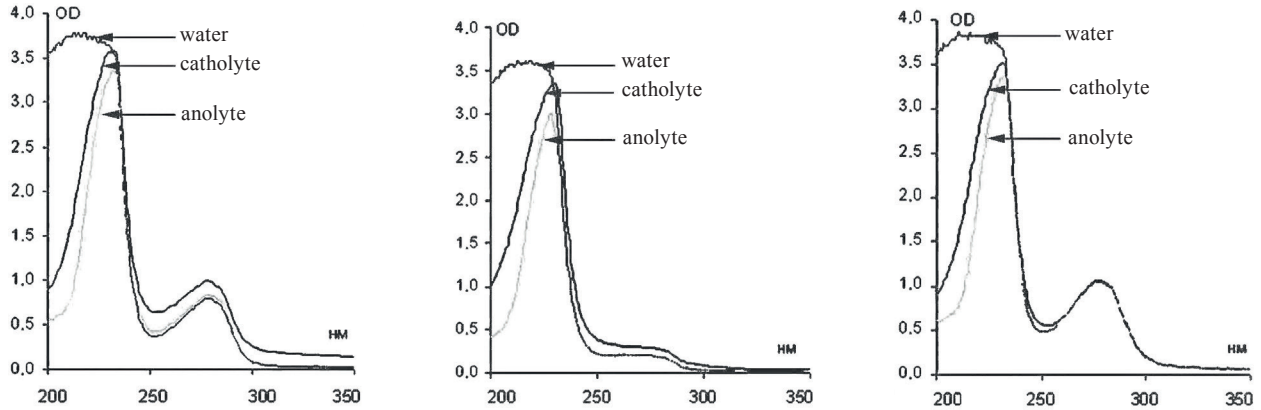


Figure 1 Nonspecific UV absorption spectra of 0.20% solutions of bovine serum albumin (a), food gelatin (b), and casein (c) in water or electrochemically activated water fractions (catholyte, anolyte)

The protein content in the diluted solution was 0.01, 0.05, 0.1, and 0.2%; in the concentrated solution – 1, 3, 5, and 10%.

Protein electrophoresis. This parameter was measured using standard methods and the following ingredients. Acrylamide 8% separating gel contained 0.375 M Tris HCl (pH 8.8), 0.1% PSA (ammonium persulfate 0, 0.1% DS-Na (Dodecylsulfat Na-salz), and 0.01% TEMED (tetramethylethylenediamine). Acrylamide 5% focusing gel included 0.125 M Tris HCl (pH 6.8), 0.1% PSA, 0.1% DS-Na, and 0.01% TEMED. The electrode buffer included 0.025 M Tris HCl (pH 8.3) and 0.19 M glycine.

The protein was diluted in buffer: 2% DS-Na, 10% glycerin, 5% 2-mercaptoethanol, 0.004% bromophenol blue, 0.063 M Tris HCl, pH 6.8. After that, it was boiled in a 100% water bath for 5 min. The solution was applied to the gel, where electrophoresis was carried out at 20 mA for 2 h. The resulting preparation was stained with Cumassi R 250.

RESULTS AND DISCUSSION

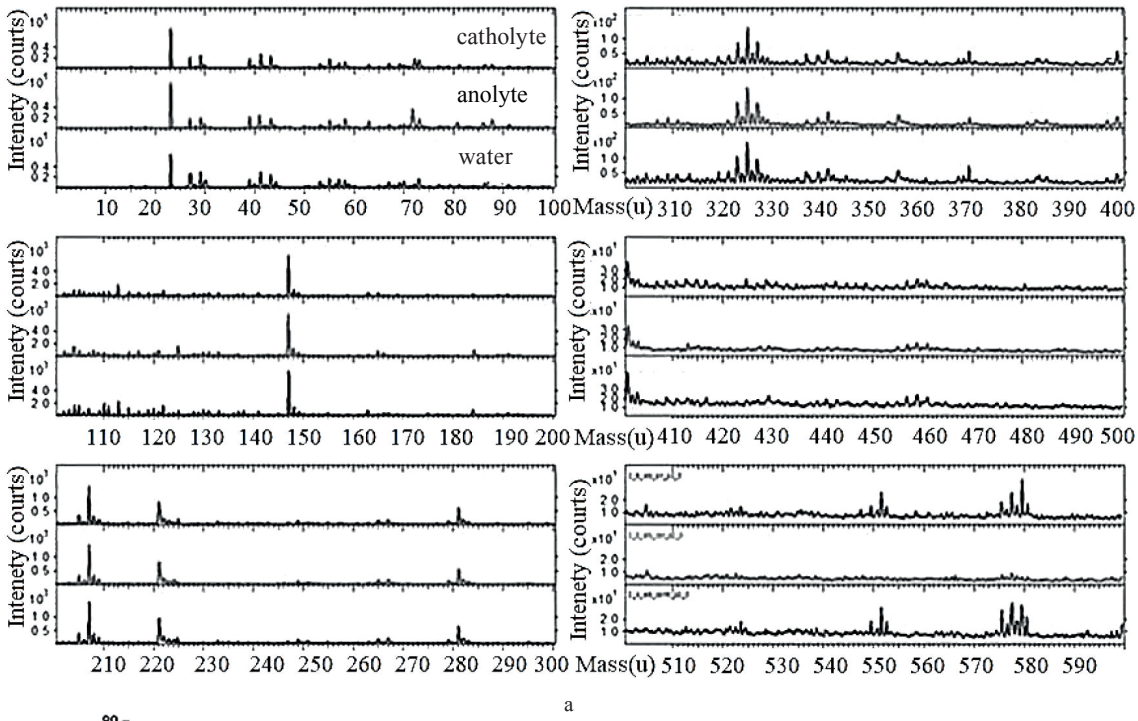
UV spectrometry. The method of UV spectrometry of aqueous solutions was used to study the effect of electrochemically activated water on protein. The UV spectrum was not specific for biomolecule solutions, but it made it possible to perform a comparative analysis of integral changes in the sample. The UV spectrometry test featured bovine serum albumin, food gelatin, and casein (Fig. 1).

Figure 1 demonstrates that the obtained absorption spectra were identical for all the proteins in the experiment. Unlike the conventional water solutions, the solutions of electrochemically activated water fractions had a lower optical density, and their absorption peak was in 235–280 nm. All the samples of electrochemically activated water had a slightly higher absorption level of the protein solution in catholyte. These changes were more obvious in the solution of biochemically pure albumin (Fig. 1a) than in the samples of food gelatin (Fig. 1b) and casein (Fig. 1c). The UV spectrometry demonstrated the modification of the protein in the solution of electrolyzed water fractions. The time-of-flight molecular mass spectrometry (ToF-SIMS) provided additional data on the state of albumin in the solutions.

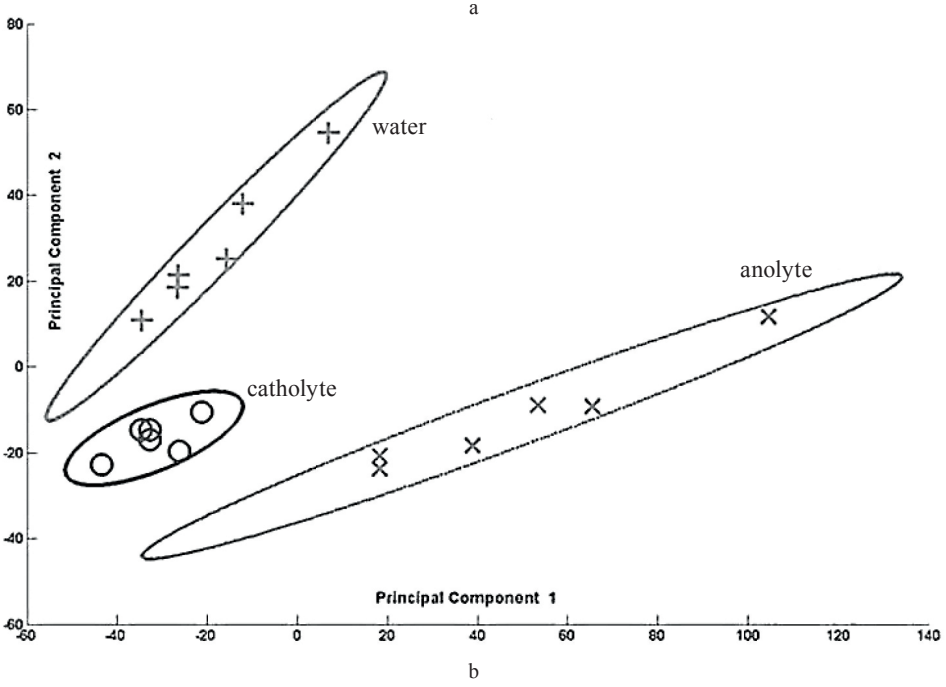
Time-of-flight secondary ion mass spectrometr (ToF-SIMS). This method was used to perform the molecular analysis of protein samples. A droplet of each solution was dried on a cover glass in a stream of clean air. ToF-SIMS provided information about chemical composition, molecular orientation, surface order, chemical bonding, and purity. Mass spectra of each preparation were compared using various data classification techniques. The principal component analysis is one of the most popular techniques used in mass spectrometry. It features the most intense peaks in mass spectra and provides a 95% confidence interval [30, 31].

Briefly, the program received 20 principal components: the higher the component number, the more variation in the data it reflected. Such a number of coordinates was unnecessary, so the space of the first two components was used to analyze the similarity of the samples. All the samples of biological macromolecules in this research underwent the same ToF-SIMS preparation procedure and the same principal component analysis. Figure 2 shows the results of the molecular mass spectrometry.

Figure 2a illustrates a typical mass spectrum of bovine serum albumin dissolved in water or electrolyzed water fractions. Figure 2b clearly demonstrates a significant difference between all three samples. The catholyte-treated protein showed significant heterogeneity compared to the control and especially the anolyte-treated sample. Anolyte treatment had a focusing effect on the protein samples, if compared to the heterogeneous group of samples obtained from the albumin solution in catholyte or water. The change in the mass spectrum may be due to the development of new



a



b

Figure 2 ToF-SIMS analysis of bovine serum albumin samples in conventional water (control) and electrochemically activated fractions (anolyte, catholyte): (a) ranges of molecular weights: bottom mass spectrum – control (water), medium – after catholyte treatment, upper – after anolyte treatment; (b) ellipses – 95% confidence areas for n = 6 measurements for each group

peaks and/or a change in the intensity of similar peaks. The results of molecular analysis statistically confirmed the UV spectrometry data (Fig. 1a): electrochemically activated water fractions really modified the albumin. The total change in the physicochemical properties of protein could change in the rheological properties (viscosity) of the solution.

Kinematic viscosity of bovine serum albumin. The viscosity of aqueous solutions of hydrocolloids is an important characteristic of food systems. For instance, the viscosity of protein solutions is one of the most serious problems when highly concentrated protein formulations or milk powder. The viscosity of food systems is controlled both by physical methods and by additives. A small amount of such low molecular weight additives as salt reduces the viscosity that results from electrostatic repulsion and attraction. Arginine hydrochloride (ArgHCl) is known to act as a chaotropic agent. It destroys the network of hydrogen bonds between water molecules, thus suppressing hydrophobic

attraction and clustering, which can reduce the viscosity of the solution [32].

Hydrodynamic cavitation served as a technological tool to reduce the viscosity of serum protein concentrate before spray drying. Whey protein concentrate (31% dry matter) underwent various hydrodynamic cavitation treatments. The samples were tested for viscosity during 14 days of storage. The enthalpy of denaturation was estimated using differential scanning calorimetry, while the particle size was measured using dynamic light scattering. The hydrodynamic cavitation treatment appeared to reduce the viscosity by 7–8%, and this effect remained constant for 14 days of storage. According to the particle size distribution, the destruction of aggregates decreased the number of large particles and thus caused the drop in viscosity [33].

The viscosity of protein solution depends not only on the size, but also on the shape, morphology, and structure of the particles. For instance, flow behavior of partially denatured serum protein aggregates showed a complex dependence on the microstructural morphology of particles, their concentration, and shear rate [24]. Even though the protein content in the solution was the same, particles with an open fibrillar/tubular structure had a higher viscosity than compact aggregates. Rough and uneven particles appeared to form solutions of higher viscosity than smooth particles of the same size. Serum proteins of various sizes and denaturation degrees produced solutions of different viscosity, probably, as a result of interactions between protein aggregates. Partial denaturation technology could control the structure of serum protein aggregates to achieve specific viscosity characteristics [24].

Protein is a biopolymer. Hence, the viscosity of its solution depends not only on its properties and concentration, but also on the solvent. Temperature, pH, impurities, and dissolved gases affect the viscosity of solutions. For example, negative redox potential can affect the quality and interaction efficiency of dissolved macromolecules [34, 35].

This part of the experiment featured electrolyzed water with a standard acidity value (pH ~ 7.3) but extremely low redox potential (–223 mV) at the starting point. The redox potential value of tap water in the control sample remained constant (+190 mV) at 20°C during 24 h. However, the redox potential value of electrolyzed water (–223 mV) gradually increased as the metastable state relaxed. The highest relaxation rate occurred in the first 6 h after treatment, and then the process slowed down. After 24 h, the redox potential reached +69 mV, which was much lower than in the control sample. As the concentration of albumin kept growing from 0.01 to 0.2%, the relaxation rate increased gradually. After bovine serum albumin dissolved, the redox potential index increased from –194 to –162 mV. When the albumin concentration reached 1–3%, the effect intensified abruptly and reached plateau at –90 mV. Adding bovine serum albumin speeded up the recovery of the redox potential of the electrolyzed water. The kinetics of the process depended on the concentration. After 24 h, the maximal value was +125 mV at 0.2% of bovine serum albumin, which was much lower than the redox potential of the control sample (+190 mV).

The results clearly showed the dependence of the redox potential of electrochemically activated water solutions on albumin concentration. Such interactions may affect the kinematic viscosity of the solution: the molecular conformation change and/or intermolecular bonds are distorted. The structure of water in the electrochemical reactor changes, thus resulting in a negative redox potential of the water. These changes can also affect the behavior of macromolecules, i.e. solubility, interaction, conformation, repulsion or attraction, as hydrogen, hydrophobic, or electrostatic non-covalent bonds get stronger or weaker [36, 37].

Figure 3 shows the changes in the kinematic viscosity of the solutions of tap water and electrolyzed water at different concentrations of bovine serum albumin from 1 to 10%.

Electrochemical treatment of tap water decreased the viscosity of the solution at all the concentrations of bovine serum albumin (Fig. 3). The tap water solution became more viscous over time, while the electrolyzed water with the same concentration of albumin remained almost the same. The viscosity of the solution usually

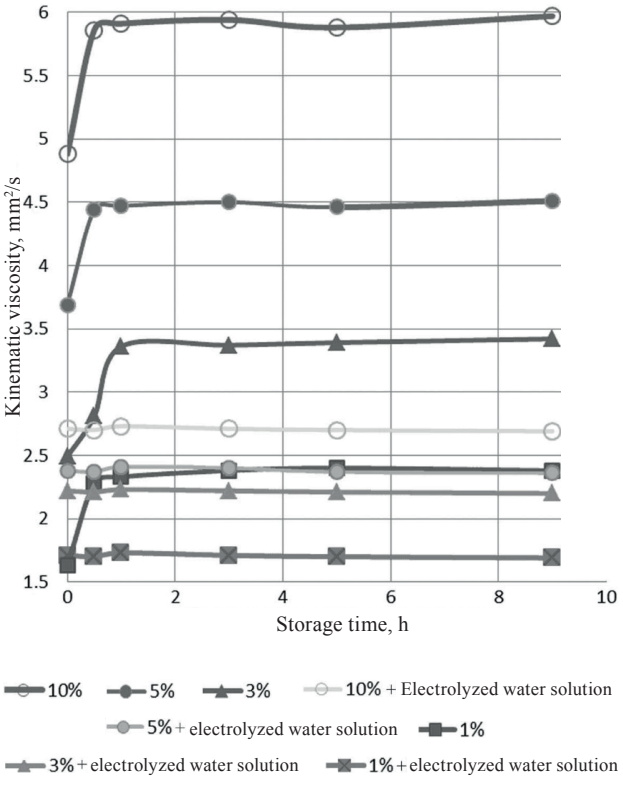


Figure 3 Kinematic viscosity of water solution vs. electrochemically activated water solution at different concentrations of bovine serum albumin, %

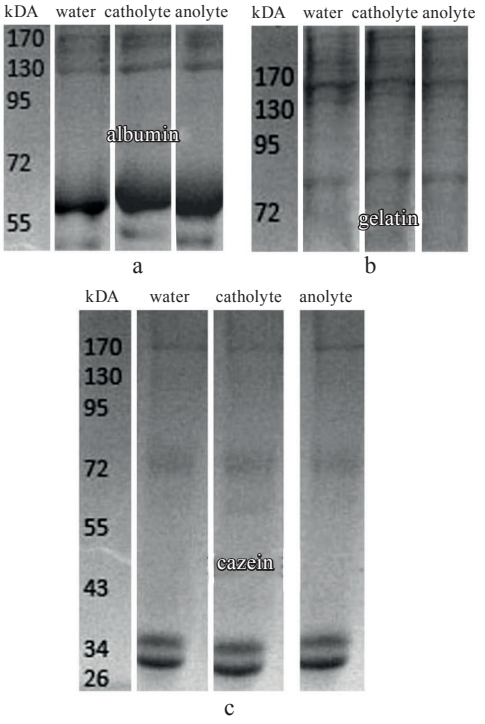


Figure 4 Gel electrophoresis of protein solutions in water or in the fraction of electrochemically activated fractions (catholyte, anolyte): (a) albumin, (b) gelatin, (c) casein

increases together with the increase in the protein content, but electrolyzed water reduced the initial value of this parameter, especially in the 10% solutions of bovine serum albumin. This effect might have resulted from the increased electrostatic repulsion between albumin molecules. At an isoelectric point of pl 4.9 and pH 7.3–8.6, the total charge of the protein became negative due to additional dissociation of carboxyl groups caused by albumin molecular conformation.

Both the protein monoproduct sample (albumin) and the food protein composition samples (gelatin and casein) changed when dissolved in the electrolyzed water fractions. The observed effects might be a consequence of changes in the structure of the protein and/or its fragmentation. Figure 4 shows the results of gel electrophoresis.

Protein electrophoreses differed (Fig. 4), but the solutions of the same protein in water or electrolyzed water fractions showed no significant differences. A high performance liquid chromatography (HPLC) confirmed this observation. The data of gel electrophoresis differed from the results of molecular analysis (ToF-SIMS) because these two methods are based on different physical principles. Electrophoresis features proteins and their large fragments in an electric field while mass spectrometry registers amino acid ions and small peptides. The decrease in molecular weight under the action of electrolyzed water was insignificant, but it could still affect the peptide structure both in the oxidized (pH 2.2, redox +800 mV) and reduced (pH 8.2, redox –800 mV) fractions of electrolyzed water. When proteins dissolved in the anodic and cathodic fractions of purified drinking water, they got neither fragmented nor structurally changed.

The effect of the anodic and cathodic fractions of electrolyzed water on the properties of serum albumin confirmed the prospects of the targeted use of electrochemical activation in the food industry as a means of condition monitoring.

The research results correlated with other studies that state the importance of water for reagent-free control of protein quality in the food industry. According to [38], polyphosphate (50%) can be partially replaced with alkaline electrolyzed water (1.25 g/L sodium tripolyphosphate, 0.3 g/L sodium metapolyphosphate, 0.4 g/L sodium polyphosphate, pH = 11.4). The replacement improved the quality of catfish fillet: its weight and water retention capacity increased. A higher phosphate content had a similar result (2.5 g/L sodium tripolyphosphate, 0.6 g/L sodium metapolyphosphate, 0.8 g/L sodium polyphosphate, pH = 9.0). However, the experiment established no change in hardness and elasticity. The test samples improved in color and oxidation resistance, though [38].

Electrochemical activation proved to be an effective sustainable technology to produce acidic and alkaline (anolyte and catholyte) extraction solutions that could replace hydrochloric acid and sodium hydroxide. For example, a combination of electrolyzed water and ultrasonic treatment improved the efficiency of extracting proteins from sea krill [39]. Unlike a similar combined method with deionized water, the electrolyzed water method reduced NaOH consumption by 30.9% w/w. Electrochemically activated water with a negative redox potential –(800–900 mV) showed good antioxidant properties, which protected the active groups of proteins (carbonyl, sulfhydryl) from oxidation. Ultrasonic treatment provided an additional increase in the extraction yield, raised the solubility, reduced the particle size, changed the structure, and improved the functional properties of krill proteins, e.g. emulsifying and foaming capacity, foam stability, etc. [39].

A combination of electrochemically activated water, isoelectric precipitation, as well as isoelectronic precipitation and electrochemically activated water treatment (IP-EWT) provided a high recovery rate (≥ 50%) of protein concentrate from heat stabilized defatted rice bran [40]. The protein fraction contained 65.1 wt% protein and had a high amino acid value (76.6%). A Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis and an immunoblotting analysis showed no signs of allergenic rice protein or heavy metals in the protein fractions. The combined IP-EWT process was environmentally friendly. It yielded highly concentrated and safe protein from plant materials without enzymes or chemicals, e.g. organic solvents, buffering agents, surfactants, etc.

Electrochemically activated water proved effective in the extraction of dry material from soybean meal [41]. Solutions of anolyte and catholyte had a

high ability to extract proteins, carbohydrates, and especially minerals. The extracted proteins had a well-balanced amino acid composition, which meant they could serve as ingredients in various functional foods. Electrochemically activated water gives the food industry an important alternative to chemical reagents. In future, it can become an effective tool for functional modification of proteins [41, 42].

CONCLUSION

The present research involved viscometry, UV spectrometry, time-of-flight mass spectrometry of secondary ions, and electrophoresis of bovine serum albumin. All the methods confirmed a multifaceted effect of the anodic and cathodic fractions of electrochemically activated water on the structure and properties of protein in aqueous solutions. The protein monoproduct (serum albumin) was subject to modification when interacting with fractions of electrolyzed water. The oxidized fraction of electrochemically activated water (anolyte) made the protein solution more homogeneous in terms of molecular composition. The research registered a significant unified effect of anolyte with a high concentration of hydrogen peroxide on the disulfide bond of amino acid residues, e.g. cysteine.

However, the mechanism of action of the reduced fraction of electrochemically activated water (catholyte) still remains unclear. The catholyte has a pronounced antioxidant activity, but the activity of antioxidants in biological systems can be studied only by the compensation of oxidative stress to the normal level of the redox potential of the medium (~ 360 mV). The catholyte-based solutions of bovine serum albumin had an abnormally negative potential (–800 mV), which was not induced under physiological conditions or pathological changes. Unlike the control samples, the experimental samples with electrochemically activated water retained the initial viscosity for a long time. Their viscosity was lower than that of the protein solution in non-electrochemically activated water. This effect was consistent with other physicochemical changes.

The obtained patterns revealed good prospects for reagent-free control of protein food media in technological processes. Less food additives and technological aids during processing means the possibility to modify the functional properties of protein food ingredients, e.g. texturing isolates. Electrochemically activated water can serve as a water base for liquid protein-fortified products. The method helps maintain the desirable viscosity, consistency, and sensory properties of functional foods.

CONTRIBUTION

A.G. Pogorelov supervised the project and proofread the final manuscript. L.G. Ipatova wrote and improved the manuscript. M.A. Pogorelova obtained and analyzed the data. A.L. Kuznetsov interpreted the data. O.A. Suvorov designed the research concept.

CONFLICT OF INTEREST

The authors claim there is no conflict or interests regarding the publication of this article.

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