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Physicochemical and Antibacterial Properties of Carrageenan and Gelatine Hydrosols and Hydrogels Incorporated with Acidic Electrolyzed Water

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Abstract: The article focuses on investigation of the effects of usage of acidic electrolyzed water (AEW) with different sodium chloride concentration (0.001%, 0.01%, and 0.1%) for the preparation of carrageenan and gelatine hydrosols and hydrogels. To determine physiochemical properties of hydrosols, the pH, oxidation-reduction potential (ORP), available chloride concentration (ACC) and rheological parameters such us gelation and flow temperatures were measured. The samples were also characterized using Fourier transform infrared spectroscopy (FT IR) and texture profile analysis (TPA). Additionally, the article contains an analysis of antibacterial activity of carrageenan and gelatine hydrosols incorporated with acidic electrolyzed water, against *Staphylococcus aureus* and *Escherichia coli*. The FT IR spectra demonstrated that the structure of gelatine and carrageenan are not significantly affected by electrolyzed NaCl solution components. Furthermore, TPA analysis showed that the use of AEW did not cause undesirable changes in hydrogels layer. The microbiological analysis confirmed inhibition of bacterial growth by hydrosols to about 2.10 log reduction. The results showed that the range of reduction of microorganisms depends on the type AEW used. This might be explained by the fact that the lowest pH and the highest ACC values of hydrosols were obtained for samples with the longest period of exposure to electrolysis (10 min) and the highest amount of NaCl (0.1% w/v). These results suggest that hydrogels and hydrosols incorporated with AEW may be used for food preservation.

Keywords: Fourier transform infrared; microbiology; carrageenan; gelatine; texture profile analysis; gelation and flow temperatures

1. Introduction

Microbial spoilage causing deterioration of meat quality and loss of weight through surface evaporation are important subjects in the meat industry [1]. Various techniques have been proposed to eliminate or reduce bacterial population on meat and meat products. Many of these methods result in chemical and physical changes to the processed meat such as deviation in color, odor, and texture [2].

Packaging combined with natural active compounds is an alternative way to improve quality of processed foods. In recent years, much attention has been paid to the development of antimicrobial active systems by means of incorporating antimicrobial substances onto the coatings of food packaging, which would help to improve food safety and prolong shelf life [3]. An example of good

carrier of antimicrobials that is used in food production are hydrocolloids which is an important group of functional additives characterized by texture-forming and stabilizing properties.

Hydrocolloids are hydrophilic polymers, of vegetable, animal, microbial or synthetic origin, that have many hydroxyl groups and may be polyelectrolytes. Nowadays, they are widely used as film-forming solutions to perform and control the texture, flavor, and shelf-life of foods. Hydrocolloidal materials, *i.e.*, proteins and polysaccharides, are widely used to obtain edible films and coatings [4].

Carrageenans have a special position among the hydrocolloids applied in meat processing. Carrageenan is characterized by its thickening and gelling properties which allow to modify rheological properties of meat products [5]. Carrageenan is an anionic polysaccharide extracted from marine red algae and chemically it is a linear polymer, sulfated galactan, composed of alternating disaccharide repeating units of 3-linked β-D-galactopyranose (G units) and 4-linked α-D-galactopyranose (D units) or 4-linked 3,6-anhydro-α-D-galactopyranose (DA units). Depending on the number and position of sulfate groups, three main carrageenans have been identified: iota (ι)-, kappa (κ)- and lambda (λ)-carrageenan [6] Carrageenan is biocompatible, biodegradable, nontoxic, cheap and gel forming. Oligosaccharides from κ-carrageenan possess antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Saccharomyces cerevisiae*, *Pseudomonas citronellolis* and *Mucor* species [7].

Gelatine coatings are thin, flexible, and transparent biodegradable materials for which there are many applications in food processing, packaging, and drug recovery, among others [3]. Gelatine is a protein compound obtained from denatured collagen. Gelatine is a mixture of proteins derived from acid or alkaline hydrolysis of collagen [8]. It has rheological properties of thermo-reversible transformation between sol and gel form. The quality of gelatine is correlated with its physical, chemical and structural characteristics. The most important physical properties of this hydrocolloid are gel strength and viscosity. Gelatine has excellent biocompatibility, biodegradability, non-immunogenicity, and can be modify at amino acids level [8].

Acidic electrolyzed water (AEW) is produced by the electrolysis of a weak sodium chloride solution. During electrolysis the weak sodium chloride solution dissociates into AEW which has pH values of 2–3, an oxidation-reduction potential of >1100 mV and an active chlorine content of 10 to 90 ppm. Alkaline electrolyzed water has a pH of 10–13 and an oxidation-reduction potential of −800 to −900 mV. AEW possesses strong antibacterial properties and has been used as a sanitizer in the food industries of many countries. The safety of using AEW has been reported in the literature [9]. Studies have shown that acidic electrolyzed water exhibited strong bactericidal activity against many foodborne pathogens, including *Salmonella enteritidis*, *Listeria monocytogenes, Campylobacter jejuni*, and *Escherichia coli* O157:H7 [10].

The hydrosols and hydrogels incorporated with AEW may have antimicrobial properties and could be used as substituents for synthetic disinfection agents. The treatment with hydrosols and hydrogels incorporated with acidic electrolyzed water could be a way of achieving microbial stability as well as safer food products and allow obtaining an environmentally friendly packaging material.

This study was conducted to determine whether acidic electrolyzed water increased the antibacterial effect of carrageenan and gelatine hydrosols and how AEW influenced mechanical and physicochemical properties of the obtained hydrosols and hydrogels.

2. Experimental Section

2.1. Apparatus

Electrolyzed salt solutions (acidic electrolyzed water (AEW)) were generated using a water generator (own design batch type generator, equipped with two titanium electrodes coated with 0.6 um layer of platinum) by membrane electrolysis of diluted salt solutions (0.001%, 0.01%, and 0.1%) for various periods of time (0, 5 and 10 min). The diluted aqueous solutions of sodium chloride (analytical quality, POCH) were added to both the anode and the cathode chambers (2 L

in each chamber) of the generator. AEW was collected from the anode side of the water generator. Non-electrolyzed sodium chloride solutions at concentrations of 0.001%, 0.01% and 0.1% were used as a reference.

2.2. Material

Gelatine from porcine skin (180 Bloom) was purchased from Weishardt (Graulhet, France) and κ-carrageenan was obtained from Sigma-Aldrich (Poznan, Poland).

2.3. Preparation of Hydrosols

Gelatine/Carrageenan, as a base component, was dissolved in (non-)electrolyzed sodium chloride solutions in concentration shown in Tables 1 and 2 by stirring (IKA, Staufen, Germany, RW 20 digital) at 300 rpm at 60 °C for 60 s. The final concentration of gelatine/carrageenan hydrosols was 2.5% (*w*/*v*).

Table 1. Physicochemical properties of carrageenan hydrosols dissolved in (non-)electrolyzed sodium chloride solutions.

	Variants	Concentration of NaCl (N) (%)	Electrolysis Time (E) (min)	pH	ORP (mV)	ACC (mg/L)
Carrageenan (C)	CN0.001E0	0.001	0	$9.20^{g} \pm 0.00$	$98.57^{c} \pm 0.09$	$0.00^{a} \pm 0.00$
	CN0.001E5		5	$8.29^{f} \pm 0.01$	$107.60^{d} \pm 3.42$	$0.00^{a} \pm 0.00$
	CN0.001E10		10	$8.24^{e} \pm 0.00$	$72.80^{a} \pm 1.01$	$0.00^{a} \pm 0.00$
	CN0.01E0	0.01	0	$9.24^{h} \pm 0.00$	$114.97^{e} \pm 0.28$	$0.00^{a} \pm 0.00$
	CN0.01E5		5	$7.72^{d} \pm 0.01$	$133.77^{f} \pm 4.08$	$1.11^{b} \pm 0.31$
	CN0.01E10		10	$7.12^{c} \pm 0.02$	$89.47^{b} \pm 0.88$	$3.19^{c} \pm 0.35$
	CN0.1E0	0.1	0	$9.26^{h} \pm 0.01$	$134.07^{f} \pm 0.35$	$0.00^{a} \pm 0.00$
	CN0.1E5		5	$3.82^{b} \pm 0.00$	$134.50^{f} \pm 2.00$	$6.14^{d} \pm 0.24$
	CN0.1E10		10	$2.59^{a} \pm 0.02$	$89.47^{b} \pm 0.88$	$8.20^{e} \pm 0.30$

$^{a–h}$ Means with different superscript letters in the same column are significantly different ($p \leqslant 0.05$) according to the ANOVA.

Table 2. Physicochemical properties of gelatine hydrosols dissolved in (non-)electrolyzed sodium chloride solutions.

	Variants	Concentration of NaCl (N) (%)	Electrolysis Time (E) (min)	pH	ORP (mV)	ACC (mg/L)
Gelatine (G)	GN0.001E0	0.001	0	$5.87^{g} \pm 0.01$	$249.30^{c} \pm 0.60$	$0^{a} \pm 0.00$
	GN0.001E5		5	$5.84^{f} \pm 0.01$	$222.03^{a} \pm 0.58$	$0^{a} \pm 0.00$
	GN0.001E10		10	$5.66^{d} \pm 0.01$	$225.20^{a} \pm 0.57$	$0^{a} \pm 0.00$
	GN0.01E0	0.01	0	$5.82^{e,f} \pm 0.00$	$253.37^{c} \pm 1.13$	$0^{a} \pm 0.00$
	GN0.01E5		5	$5.67^{d} \pm 0.01$	$237.33^{b} \pm 2.12$	$1.12^{b} \pm 0.12$
	GN0.01E10		10	$5.34^{c} \pm 0.01$	$236.80^{b} \pm 2.95$	$1.51^{c} \pm 0.09$
	GN0.1E0	0.1	0	$5.80^{e} \pm 0.01$	$260.77^{d} \pm 0.12$	$0^{a} \pm 0.00$
	GN0.1E5		5	$4.73^{b} \pm 0.02$	$327.90^{f} \pm 1.46$	$1.87^{d} \pm 0.07$
	GN0.1E10		10	$4.53^{a} \pm 0.01$	$312.40^{e} \pm 1.93$	$2.19^{e} \pm 0.06$

$^{a–h}$ means with different superscript letters in the same column are significantly different ($p \leqslant 0.05$) according to the ANOVA.

2.4. Hydrosols Characterization

2.4.1. Physicochemical Properties

The oxidation-reduction potential (ORP) and pH of hydrosols were measured with pH/ORP meter (Seven Multi™ model S40 Mettler Toledo) using an ORP electrode (Inlab Redox Pro) and pH

electrode (Inlab Routine Pro), respectively. Available chlorine concentration (ACC) was determined by the iodometric method [11].

2.4.2. Fourier Transform Infrared Spectroscopy (FT IR)

The spectral measurements were performed in The Laboratory of Vibrational Spectroscopy belonging to The Faculty of Chemistry at Wroclaw University of Technology. The middle-infrared spectra (4000–400 cm^{-1}) were collected on a Fourier transform, Bruker VERTEX 70 V vacuum spectrometer equipped with an air-cooled DTGS detector (Ettlingen, Germany). The gelatine or carrageenan samples were placed on the diamond crystal of the ATR accessory. The spectral data were recorded at the resolution of 2 cm^{-1} with 64 scans collection and further elaborated using Bruker OPUS software.

2.4.3. Rheological Measurements

Rheological measurements were performed with a RheoStress 6000 rheometer (Thermo Scientific, Karlsruhe, Germany) operating in oscillatory mode, with a strain of 5% and frequency of 1 Hz. These conditions were checked to stand in the linear viscoelastic region. Storage modulus G′ and loss modulus G″ were recorded as a function of temperature. 1 mL of gelatine/carrageenan hydrosols (immediately after mixing) was applied onto the measurement plate. A cone/plate geometry with a cone of 0.52 mm was used and hydrosols evaporation was prevented by the use of paraffin oil around measured area. The results of G′ and G″ were obtained in two stages—during cooling from 80 to 12 °C and by heating from 12 to 80 °C. Temperature ramps of ± 1.2 °C· min^{-1} were applied. Determination of the equilibrium G′ = G″ for evaluation of variability of both modules as a function of temperature specifies the conditions of sol–gel phase transition (stage I) and gel–sol (stage II). The gelation and flow temperatures of hydrosols were obtained from the point of intersection of curves. All tests were performed using cone sensor (C60/1° Ti L) and measuring plate (TMP60 Steel 18/8) in CS mode. The measuring device was running on RheoWin Job Manager version 4.00 software (Haake, Vreden, Germany).

2.4.4. Mechanical Properties

Texture profile analysis (TPA) was performed using Zwick Roell Z010, type: Z6FD1 equipped with a head measuring load up to 100 N. The gels were formed in 4 °C for 24 h and removed from the glass flask, cut into cylinders of 23 mm length and 15 mm diameter and then subjected to an instrumental texture profile analysis (TPA) similar to that described by Lau [12]. The gel samples were placed between parallel flat plate fixtures fitted to a TA.XT2 Texture Analyzer (Stable Micro Systems, Surrey, UK) interfaced with a microcomputer. All measurements were made on gels equilibrated to 10 °C. The gels were compressed twice at 70% deformation and relaxation time with 30 s. The results are reported as the means of triplicate tests. Textural parameters such as hardness, cohesiveness, springiness, gumminess and chewiness can be obtained. Textural parameters considered in the present study were defined as follows [12].

- Hardness: The peak force during the first compression cycle.
- Cohesiveness: The ratio of the area under the first and second compression.
- Springiness: the distance the sample was compressed during the second compression to the peak force, divided by the initial sample height, reported as a percentage.
- Gumminess: Hardness × cohesiveness.
- Chewiness: Gumminess × springiness.

2.4.5. Antibacterial Activity

The following bacterial strains (Gram-positive and Gram-negative) were used for the testing in this study: *Staphylococcus aureus* (PCM 2602) and *Escherichia coli* (PCM 2560). These strains of

microorganism, obtained from the culture collections of Institute of Immunology and Experimental Therapy (Polish Academy of Sciences in Wroclaw) were maintained in Tryptic Soy Broth (Sigma Aldrich, Poznan, Poland) at 37 °C for 18 h. Suspensions of tested organisms were prepared in concentrations of 6.0 × 10^8 colony-forming units (CFU)/mL by adjusting the turbidity equivalent to McFarland 2.0 standard, and the final concentrations were confirmed by agar plating. The same bacterial suspension for each bacterial species was tested throughout the investigation. To determine the antibacterial activity of experimental hydrosols, 10 mL of the suspensions were added to 90 mL of hydrosols prepared as described previously and exposed for 10 min. After 10 min treatment, bacterial suspension containing 10^7 CFU/mL was diluted several times to obtain a dilution of 10^1 CFU/mL and then 1 mL of final two dilutions was transferred to triplicate nutrient agar plates. Antibacterial activity was performed using the viable plate count method. Plates were incubated at 37 °C for 24 h for each tested strains. Only plates with 30–300 colonies were counted. The results are presented as log reduction and were calculated according to Equation (1):

$$\text{Log reduction} = \log_{10}(N_0) - \log_{10}(N) \quad (1)$$

where N_0 is the number of viable microorganisms before treatment; and N is the number of viable microorganisms after treatment.

2.5. Statistical Analysis

The experiments were conducted in triplicate (three independent experiments analyzed in triplicate). The effects of two independent categorical variables such as time of electrolysis and concentration of sodium chloride were evaluated. Data were analyzed using a 2-way factor analysis of variance (ANOVA) using Statistica 10 (StatSoft, Cracow, Poland). Differences between means were established by Duncan Test with 5% significance.

3. Results and Discussion

3.1. Physicochemical Properties

The characteristics of physicochemical properties of prepared carrageenan and gelatine hydrosols including pH, oxidation–reduction potential and available chlorine concentration used for treatment are presented in Tables 1 and 2. The pH of sols containing AEW was significantly lower than in tested sols with non-electrolyzed NaCl solutions. The lowest pH value was observed for CN0.1E10 carrageenan hydrosol (2.59) and GN0.1E10 gelatine hydrosols (4.53). No available chlorine was detectable in sols dissolved in non-electrolyzed chloride solutions. The highest ACC amount was observed for CN0.1E10 (8.20 mg/L) and GN0.1E10 (2.19 mg/L) hydrosols. The ACC concentration depended on the amount of NaCl added [13]. It is in agreement with the results obtained by Brychcy *et al.* [2] for AEW solutions. Increasing electrolysis time in AEW production from 5 to 10 min significantly affected the pH value and ACC concentration. Brychcy *et al.* [2,14] observed the same dependence in ORP value.

3.2. Fourier Transform Infrared Spectroscopy (FT IR)

In case of gelatines, the natural polymers extracted from collagen, the Fourier transform infrared spectroscopy (FT IR) focuses mainly on peptide bands named Amide A and Amide I–III [15–18]. In the spectroscopic analysis of carrageenan, the characteristic vibrations of polysaccharides and those of sulfate groups are frequently considered [19]. The main purpose of presented spectroscopic research is to detect any possible changes in the structure of gelatine or carrageenan upon dissolution in the various solutions of regular and electrolyzed NaCl.

In order to trace the effects of NaCl concentration and electrolysis, three spectra of respective natural polymer are compared in Figure 1 (gelatine) or Figure 2 (carrageenan).

In both figures, there are presented FT IR spectra of the three samples with specified properties, variants: N0.001E0, N0.001E10 and N0.1E10 (see description in Tables 1 and 2).

In case of gelatine (Figure 1), the Amide A band, resulting from intense IR absorptions of N–H (and water O–H) stretching vibrations, is observed as medium and broad band centered at about 3300 cm^{-1}.

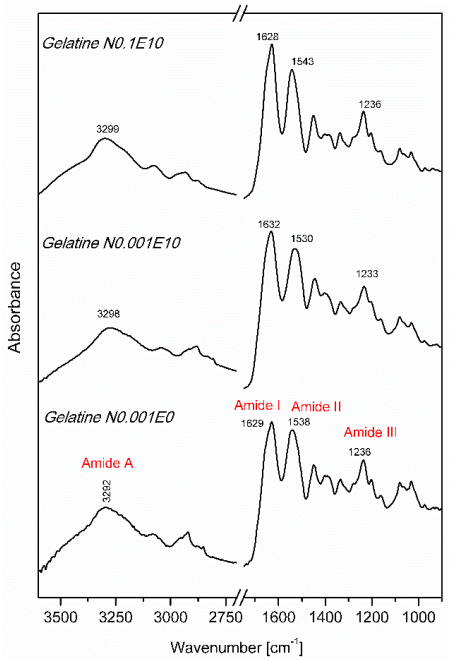

Figure 1. The Fourier transform infrared spectra of Gelatine N0.1E10, Gelatine N0.001E10 and Gelatine N0.001E0 (see description in text). Only discussed bands are labeled.

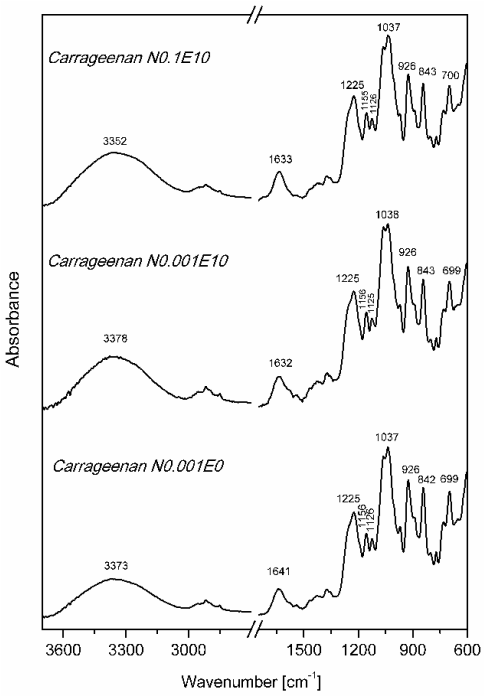

Figure 2. The FT IR spectra of Carrageenan N0.1E10, Carrageenan N0.001E10 and Carrageenan N0.001E0 (see description in text). Only discussed bands are labeled.

Amide I and Amide II bands are two major absorptions of the protein infrared spectrum. The Amide I band observed at about 1630 cm^{-1} is mainly associated with the C=O group stretching vibration. Amide II results mainly from the N–H bending vibration and from small contribution of the C–N stretching vibration. This band is observed for gelatine between 1530 and 1543 cm^{-1}. The Amide III vibration (peak at about 1235 cm^{-1}) is more complex, resulting from the in-phase combination of the C–N stretching and the N–H bending vibrations with considerable contributions of backbone and side-chain vibrations [16,17,20]. For three spectra measured here, the largest wavenumber difference found for Amide II bands (13 cm^{-1}). It may be due to hydrogen bonds formed between NH_2 groups and Cl^- ions. Assuming the smallest amount of Cl^- ions in sample N0.001E10 (part of Cl^- ions was oxidized during electrolysis), it may be observed that Amide II band position goes along with the Cl^- concentration. In conclusion, all three spectra are very similar indicating that the structure of gelatine is not significantly affected by electrolyzed NaCl solution components, as chlorine or others.

The carrageenan spectrum is dominated by intense absorptions in the fingerprint region (1225–700 cm^{-1}). Most of the bands observed there are specific for polysaccharide vibrations, but there are also bands resulting from sulfate group vibrations. The highest wavenumber band of ester sulfate group is observed at 1225 cm^{-1}. The most intensive band at 1037 cm^{-1} is due to the combination of C=O and S=O modes. The C–O stretching vibrations of 3,5-anhydro bridges are observed as strong band at 926 cm^{-1}. Similar origin (C–O–C α(1,3)) stretching vibration [19,21,22] has the 699 cm^{-1} absorption. The marker band at 842 cm^{-1} results from vibrations of C–O–S group and determines here the *kappa*-conformer [21,22]. The wavenumbers and relative intensities of the above bands are very similar for all three carrageenan samples investigated here. For all major bands observed below 1300 cm^{-1}, the maximum wavenumber difference is 1 cm^{-1}. Such spectral similarity confirms that different NaCl concentrations, as well as electrolysis products, do not change the structure of carrageenan.

3.3. Rheological Measurements

The dependence of storage and loss modulus on temperature is shown in Figures 3 and 4. The gelatinization and flow temperatures of carrageenan and gelatine hydrosols were obtained from the point of intersection of storage and loss modulus curves. The lowest gelatinization temperature of gelatine hydrosols was observed for samples combined with 0.1% of sodium chloride solution. No significant differences of gelatinization temperature were verified in gelatine hydrosols that contained electrolyzed and non-electrolyzed NaCl solution. The study of Cuvelier *et al.* [23] demonstrates that gelatinization temperature for the gelatine hydrogels is in the range 26.4–32.6 °C and these values are highly dependent on the concentration of gelatine. In our study, the results were obtained at a level of 19.64–20.73 °C, although the concentration of gelatine was constant (2.5%). It is probably caused by different salt concentration, which was also confirmed by Chatterjee and Bohidar [24]. The lowest gelatinization temperature of carrageenan hydrogels was observed for samples produced with non-electrolyzed 0.001% sodium chloride solution. Huang *et al.* [25] observed that usage of different type of carrageenan and cations has an impact on gelatinization temperature. The usage of more concentrated salt solution resulted in higher gelatinization temperatures of experimental carrageenan hydrosols. Increasing electrolysis time from 5 to 10 min has no impact on sol–gel transition temperature of carrageenan hydrosols.

The highest flow temperature (31 °C) of gelatine hydrosols was observed for GN0.001E0 variant and the lowest (28.27 °C) gel–sol temperature was observed for variant of hydrosol incorporated with 0.1% NaCl subjected to 10 min of electrolysis (GN0.1E10). AEW caused a significant decrease of flow temperature in experimental gelatin samples. Liquidification was significantly decreased from 31.0 °C (GN0.001E0) to 28.3 °C (GN0.1E10). Cuvelier *et al.* [23] demonstrated that the gel–sol temperature is also dependent on the concentration of used gelatine and is in the range of 20–32.5 °C [23] while Jones [26] established the range of 27–34 °C. It was observed significantly higher flow temperature for CN0.1E10 than for CN0.001E0 hydrosol. Carrageenan hydrogels contained

0.1% of sodium chloride solution subjected to electrolysis for 10 min, were characterized by the highest (62.48 °C) transition temperature. These results indicate that increasing salt concentration and electrolysis time significantly increases flow temperature of obtained carrageenan hydrosols.

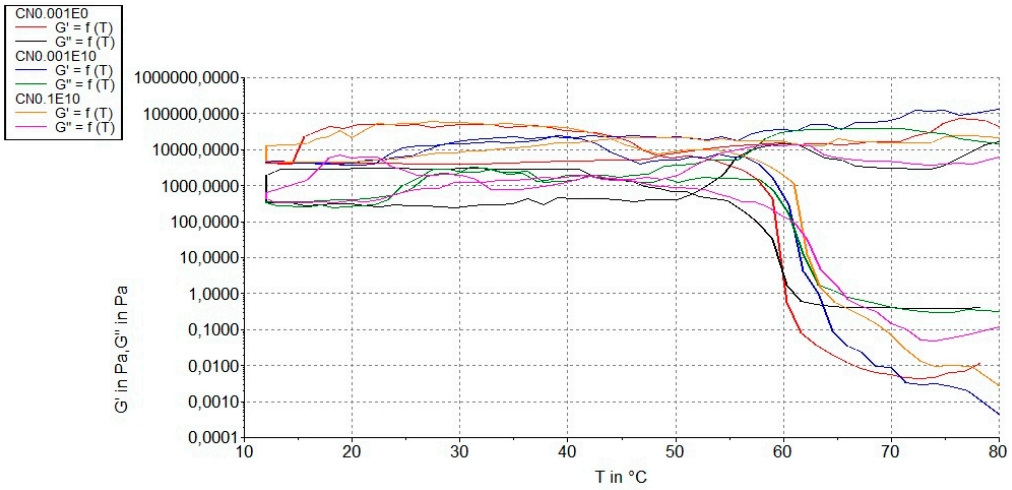

Figure 3. Storage and loss modulus of samples CN0.001E0, CN0.001E10 and CN0.1E10 as a function of temperature.

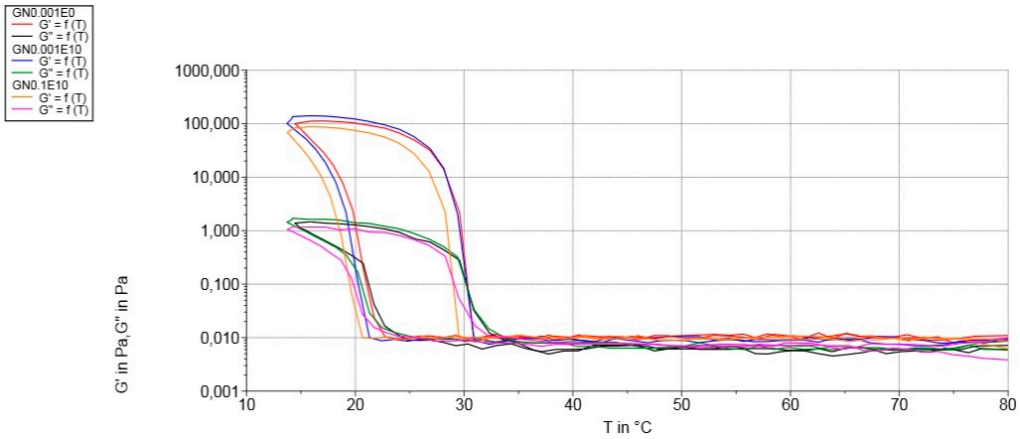

Figure 4. Storage and loss modulus of samples GN0.001E0, GN0.001E10 and GN0.1E10 as a function of temperature.

3.4. Mechanical Properties

Table 3 presents the instrumental TPA attributes. The carrageenan gels incorporated with 0.01% sodium chloride solution showed the highest hardness values compared with all samples. The prolongation of electrolysis time from 5 to 10 min in AEW production had no influence on hardness of obtained carrageenan gels. The decrease in the hardness of carrageenan gels in an acidic pH could be due to partial hydrolysis of the polysaccharide [27].

In the case of carrageenan gels, the springiness varied from 0.25 to 0.38. The lowest value of springiness (0.25) was observed for CN0.1E10 carrageenan gel. There were no significant differences in the springiness for other samples. Springiness of gelatine gels does not differ significantly, except for GN0.01E10, which had the highest value (0.49) of this parameter. According to Rahman and Al-Mahrouq [28], if springiness is high, it requires more mastication energy in the mouth.

Table 3. Values of Texture Profile Analysis (TPA) of experimental carrageenan and gelatine hydrosols.

Hydrocolloid	Variants	Hardness (H)	Springiness (S)	Gumminess (G)	Chewiness (CH)	Cohesiveness (C)
Carrageenan	CN0.001E0	93.63 ^{c} ± 4.84	0.36 ^{b} ± 0.02	3.62 ^{c} ± 0.11	1.30 ^{bc} ± 0.08	0.04 ^{ab} ± 0.01
	CN0.001E5	74.63 ^{b} ± 4.48	0.36 ^{b} ± 0.01	3.28 ^{bc} ± 0.22	1.24 ^{bc} ± 0.06	0.04 ^{ab} ± 0.01
	CN0.001E10	61.77 ^{ab} ± 5.80	0.28 ^{ab} ± 0.06	2.33 ^{ab} ± 0.32	0.68 ^{a} ± 0.24	0.04 ^{ab} ± 0.01
	CN0.01E0	101.93 ^{c} ± 4.11	0.33 ^{ab} ± 0.01	3.29 ^{bc} ± 0.54	1.29 ^{bc} ± 0.20	0.03 ^{a} ± 0.01
	CN0.01E5	76.47 ^{b} ± 4.08	0.36 ^{b} ± 0.03	4.24 ^{c} ± 0.42	1.56 ^{c} ± 0.28	0.06 ^{c} ± 0.00
	CN0.01E10	71.83 ^{b} ± 4.31	0.38 ^{b} ± 0.03	3.28 ^{bc} ± 0.27	1.28 ^{bc} ± 0.20	0.05 ^{bc} ± 0.00
	CN0.1E0	65.37 ^{b} ± 3.04	0.37 ^{b} ± 0.01	3.90 ^{c} ± 0.32	1.57 ^{c} ± 0.01	0.05 ^{bc} ± 0.01
	CN0.1E5	48.60 ^{a} ± 1.99	0.34 ^{ab} ± 0.02	2.24 ^{a} ± 0.13	0.78 ^{ab} ± 0.09	0.05 ^{bc} ± 0.00
	CN0.1E10	63.20 ^{ab} ± 7.68	0.25 ^{a} ± 0.05	1.78 ^{a} ± 0.16	0.46 ^{a} ± 0.12	0.03 ^{a} ± 0.01
Gelatine	GN0.001E0	15.43 ^{c} ± 1.77	0.32 ^{ab} ± 0.05	0.62 ^{a} ± 0.26	0.19 ^{a} ± 0.14	0.06 ^{a} ± 0.01
	GN0.001E5	12.42 ^{bc} ± 1.57	0.34 ^{ab} ± 0.00	0.45 ^{a} ± 0.01	0.18 ^{a} ± 0.00	0.05 ^{a} ± 0.01
	GN0.001E10	12.80 ^{bc} ± 1.96	0.34 ^{ab} ± 0.05	0.37 ^{a} ± 0.02	0.13 ^{a} ± 0.03	0.04 ^{a} ± 0.01
	GN0.01E0	11.38 ^{bc} ± 1.15	0.34 ^{ab} ± 0.02	0.36 ^{a} ± 0.03	0.11 ^{a} ± 0.00	0.04 ^{a} ± 0.00
	GN0.01E5	9.50 ^{abc} ± 1.85	0.40 ^{abc} ± 0.05	0.92 ^{ab} ± 0.01	0.41 ^{ab} ± 0.03	0.07 ^{a} ± 0.02
	GN0.01E10	13.55 ^{bc} ± 2.88	0.49 ^{c} ± 0.07	1.34 ^{b} ± 0.50	0.73 ^{b} ± 0.36	0.09 ^{a} ± 0.01
	GN0.1E0	8.39 ^{ab} ± 2.54	0.31 ^{ab} ± 0.00	0.34 ^{a} ± 0.04	0.10 ^{a} ± 0.01	0.08 ^{a} ± 0.01
	GN0.1E5	4.68 ^{a} ± 1.22	0.29 ^{a} ± 0.05	0.33 ^{a} ± 0.03	0.10 ^{a} ± 0.02	0.08 ^{a} ± 0.02
	GN0.1E10	11.95 ^{bc} ± 2.38	0.46 ^{bc} ± 0.05	0.71 ^{a} ± 0.04	0.30 ^{a} ± 0.01	0.10 ^{a} ± 0.04

All TPA values are mean ± standard deviation of three replicates (n = 3); $^{a–c}$ Means with different letters in the same column are significantly different.

Chewiness is a function of water activity and increased with the increase of solids content. The use of electrolyzed NaCl solution at the highest concentration (0.1%) caused significant differences compared with gels incorporated with non-electrolyzed 0.1% of NaCl. No statistically significant differences were observed in chewiness between the experimental gelatine gels. The only exceptions were GN0.01E10 gels, characterized by 1.34 of chewiness level.

The highest level of gumminess (1.57) was observed for CN0.1E10 sample. The usage of 0.1% of electrolyzed sodium chloride solution caused significant decrease of hydrogels gumminess in comparison to the application of non-electrolyzed salt solution. Gelatine gels contained 0.01% sodium chloride solution subjected to electrolysis by 10 min showed the highest gumminess values compared with all samples. In the cases of other samples there were no significant differences in the gumminess for different types of used AEW ($p < 0.05$).

There were no statistically significant differences in the cohesiveness of the other variants of obtained carrageenan and gelatine gels. The only exceptions were CN0.01E5 gels, which showed higher cohesiveness compared to the gel with non-electrolyzed sodium chloride solution at the same concentration of salt (CN0.01E0) and gels with lower cohesiveness (CN0.1E10) comparing to the CN0.1E0.

3.5. Antibacterial Activity

Antibacterial activity of both carrageenan and gelatine hydrosols incorporated with acidic electrolyzed water against *Staphylococcus aureus* and *Escherichia coli* is presented in Figures 5 and 6. The referencing hydrosols (with non-electrolyzed sodium chloride solution) showed no activity against any tested microorganisms. The highest reduction of *Staphylococcus aureus* and *Escherichia coli* was observed after treatment with carrageenan (1.80 and 1.59 log reduction, respectively) and gelatine (2.10 and 1.56 log reduction, respectively) hydrosols incorporated with 0.1% of electrolyzed sodium chloride solution subjected to electrolysis for 10 min. Because of the lack of information in the literature about the antimicrobial effects of hydrosols with AEW, our results were compared with those found for only AEW treatment. Usage of CN0.1E10 and GN0.1E10 hydrosols resulted in the highest reduction of tested microorganisms, which is probably caused by the highest concentration of active chlorine (8.15 mg/L for carrageenan sols and 2.22 mg/L for gelatine sols) in these hydrosols.

In accordance with this study results, Kim *et al.* [29] described that the treatment with acidic electrolyzed water containing 10 mg/L of free chlorine reduced bacterial growth such as *Escherichia coli*, *Bacillus cereus*, and *Listeria monocytogenes*. Park *et al.* [30] also showed that AEW treatment causes decreasing in population of *S. aureus* from 8.04 $\log_{10}$CFU$\cdot$ mL^{-1} to 3.9 $\log_{10}$CFU$\cdot$ mL^{-1}. The active factors responsible for the bactericidal activity of AEW against bacterial cells are chlorine-related substances, such as chlorine, hypochlorous acid and hypochlorous ion [9,10]. Low value of pH in CN0.1E10 and G0.1E10 sols probably affects the outer membranes of bacterial cells and allows hypochlorous acid to enter the cells of bacteria more productively [31]. Our previous study of AEW revealed that the logarithmic reductions are correlated with the bacterial strains, electrolysis time, presence of sodium chloride in electrolysis process and this research showed that it depends also on the kind of hydrosols used. Despite the strong antibacterial activity of AEW treatment, it has low cytotoxicity; therefore, it is minimally invasive to tissue. AEW has also less adverse impact on the environment and user's health because its breakdown produces only saline and traces of chlorine gas [25]. Acidic electrolyzed water is also less expensive than most traditional disinfection methods [32] and possess no difficulties with regard to transporting and storing potentially hazardous chemicals [33].

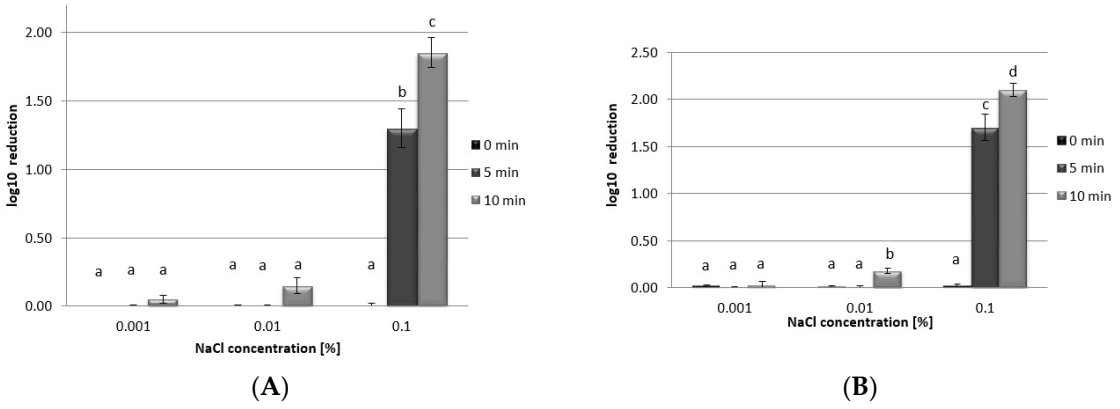

Figure 5. Inactivation of *Staphylococcus aureus* by carrageenan (**A**) and gelatine (**B**) hydrosols. Values with different letters (a–d) within the same concentration differ significantly ($p < 0.05$)

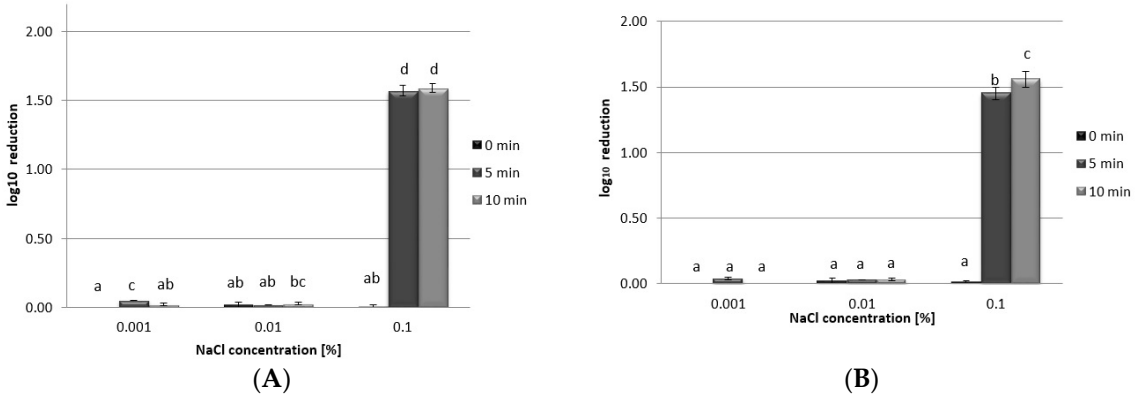

Figure 6. Inactivation of *Escherichia coli* by carrageenan (**A**) and gelatine (**B**) hydrosols. Values with different letters (a–d) within the same concentration differ significantly ($p < 0.05$)

4. Conclusions

The analysis of the physicochemical properties showed that hydrosols/hydrogels incorporated with AEW are suitable for food application. The results of rheological analysis indicate that increasing salt concentration and duration of electrolysis significantly increase gel–sol temperature of obtained

carrageenan hydrosols. Spectral similarity of FT IR showed that various salt concentrations and durations of electrolysis do not influence the carrageenan and gelatine hydrogels. The usage of acidic electrolyzed water in the production of carrageenan and gelatine hydrosols and hydrogels has not caused undesirable changes in their chemical and textural properties. The obtained results showed that the antibacterial activity strictly depends on values of pH and ACC concentration.

The electrolysis facilitates greater reduction of examined microorganisms only at the highest concentration of salt. AEW-containing hydrosols are characterized by greater effectiveness in inactivation of *Staphylococcus aureus* than hydrosols incorporated with non-electrolyzed sodium chloride solutions. This study reports that application of AEW with biopolymers is an effective method to significantly reduce the presence of *Staphylococcus aureus* and *Escherichia coli.* The studied method is universal and can find application in a wide range of products as an innovative system of food preservation.

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