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APPLICATION OF THE CONDUCTOMETRIC METHOD FOR DIFFERENTIATION OF PROTEINS AND PEPTIDES

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Key words: electrolytic conductivity, proteins, peptides, enzymatic and acid hydrolysis of proteins

The aim of this work was to use the conductometry method for the differentiation of the chemically- and enzymatically-modified food proteins. The protein extracts of soybean, bean pea, maize and rice seeds, 3 varieties "Tonacja", "Nawra" and "Sukces" of wheat, as well bovine serum albumin, pepsin trypsin and acid protein hydrolyzates were investigated. Additionally, proteins were glycated by glucose. It was found that differences of the electrolytic conductivity between native proteins and their hydrolyzates were statistically significant; the electrolytic conductivity of the water protein solutions was directly proportional to the concentration of protein. It was also proved that the concentration of glucose in the protein solution modified its electrolytic conductivity. Moreover, statistically significant differences were observed between native and glycated proteins. Finally, it was stated that the electrolytic conductivity can be a tool for the differentiation of the native and modified proteins.

INTRODUCTION

The conductometry is the analytical method used both in research laboratories as well as in industry [De Diego *et al.,* 2001; Kostyra *et al.,* 1981; Kuyucak & Chung, 1994; Martos *et al.,* 1999; Yung & Berezansky, 1995]. The theoretical assumption of this method is the fact that the electrolytic conductivity is the result of the motion of anions and cations. Because proteins and peptides are electrolytes, measurement their conductivity can be used for the prediction of their electric state. The molecular state of a protein and peptide mixture is the result of their interactions. It can change under the influence of physicochemical factors. The detection of the differences between the electrical states of proteins, peptides or their complexes can be a useful tool in programming the future analytical procedures. Hence, the conductometric measurements of water solutions of proteins and peptides and alcohol solutions of peptides could be recognized as a screening system of the differentiation of proteins and peptides.

The aim of this work was to use the conductometric method for the differentiation of native proteins derived from the same and different varieties of plants, and proteins modified by glycation and enzymatic hydrolysis.

MATERIALS AND METHODS

The protein extracts of defatted soybean, bean, pea, maize and rice, seeds, 3 varieties "Tonacja", "Nawra" and "Sukces" of wheat (Production and Experimental Station, Bałcyny, Poland), bovine albumin (Sigma), pepsin (Sigma) and trypsin (Sigma) protein hydrolyzates were used in the experiments. The conductometric measurements were performed with a conductometer EC 215 (Hanna Instruments Company).

Extraction of albumin and globulin mixture. Pea, soybean, bean, rice and maize meals (1 g) were suspended in 25 mL of the following solution: 0.5 mol/L NaCL and 0.25 mol/L ascorbic acid, and homogenized (homogenizer MPW-309) for 5 min at a room temperature. Proteins were extracted for 1 h at 4°C with constant mixing with a magnetic laboratory stirrer (Membrane, MWCO – 3500 g) against deionizated water at 4°C, frozen at -20°C and lyophilized.

The mixture of wheat albumins and globulins was extracted at 20°C as follows: 7 g of the meal was suspended in 200 mL of deionized water. Proteins were extracted for 2 h at 4°C. The mixture was centrifuged at 9000 g for 15 min. The extract was dialysed against deionized water for 48 h at 4°C and then lyophilized.

Determination of protein. The content of protein in the protein extracts was determined with the Bradford method, according to the procedure described by Kłyszejko-Stefanowicz [1999].

Pepsin hydrolysis. Soybean, pea, maize, rice and bean protein lyophilizates (30 mg) were suspended in 9 mL of deionized water and adjusted to pH 2.0 with 1 mol/L HCL. A portion of 0.9 mg of pepsin (1.360 U/mg, Sigma) dissolved in 1 mL of deionized water was added to the protein solution. The hydroly-

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sis was performed for 2 h at 37°C, next the solution was neutralized to pH 7.0 with 1 mol/L NaOH, concentrated under reduced pressure (Evaporator, type 350, Unipan, Poland) at 40°C and dissolved in methanol to the final volume of 30 mL.

Pepsin hydrolysis of the crude extract of wheat proteins (CEP) was performed as follows: 0.2 g of wheat albumin and globulin mixture were suspended in 50 mL of deionized water and adjusted to pH 2.0 with 1 mol/L HCl; next 6.1 mg of pepsin (1.360 U/mg) dissolved in 1 mL of deionized water were added. The hydrolysis was stopped by the incubation of the solution in a water bath at 100°C for 5 min. The hydrolyzate was dialyzed against deionized water for 72 h at 4°C.

Trypsin hydrolysis. A CEP (0.2 g) was suspended in 50 mL of deionized water and adjusted to pH 8.0 with 1 mol/L NaOH. A portion of 2.0 mg of trypsin (1.120 BAEE U/mg, Sigma) was dissolved in 1 mL of deionized water, added to the solution and incubated for 2 h at 37°C. The hydrolysis was stopped by the incubation at the solution in water bath at 100°C for 5 min. The hydrolyzate was dialyzed against deionized water for 72 h at 4°C.

Partial acid hydrolysis. Glycated CEP (0.1 g) was suspended in 4 mL of 3 mol/L HCL and transferred to a glass vial. The vial was filled with nitrogen and drowned. The hydrolysis of proteins was performed at 37°C for 7 days. The hydrolyzate was dialyzed against deionized water for 3 days at 4°C in order to remove HCL. The solution was centrifuged at 9000 g for 15 min before conductivity measurements.

Glycation of wheat proteins. CEP (0.133 g) were suspended in 40 mL of deionized water (pH adjusted to 7.4 with 0.1 mol/L NaOH), containing 0.2 g glucose and 4 mg azide sodium for 7 days at 37°C. Then the sample was incubated for 7 days at 37°C. Finally, the solution was dialyzed for 3 days at 4°C in order to remove glucose and salt, and lyophilized. The control sample was the solution of the crude wheat proteins without glucose.

Measurements of electrolytic conductivity. Electrolytic conductivity of all investigated solutions according to the amounts described above was determined with an EC 215 conductometer (Hanna Instruments Company) at 25°C. The measurements were repeated three times.

SDS-PAGE electrophoresis. A 3 mg dose of the CEP was dissolved in 0.4 mL of Tris buffer (62.5 mmol/L, pH 6.8) containing of 2% SDS, 10% glycerol, 5% of mercaptoethanol and 0.002% of bromophenol blue. The probe was heated at 100°C for 5 min. The electrophoresis was performed according to Laemmli [1970] using 15% polyacrylamide gel. The electropherograms were visualized with 0.1% of Coomassie Brilliant Blue R-250.

Measurements of electrolytic conductivity. Glycated CEP (0.1 g) was dissolved in 30 mL of deionized water with the addition of 0.125; 0.250 and 0.500 g of glucose. The electrolytic conductivity was determined after 0, 1, 2 and 3 h.

Statistical analysis. The mean values and standard deviations of conductivity were calculated.

RESULTS AND DISCUSSION

The contents of protein in soybean, pea, faba bean, rice and maize protein extracts are presented in Figure 1. Generally, these results are in agreement with literature data [Sikorski *et al*., 1994; Huebner *et al*., 1990]. It is known that the composition of different protein fractions differs in terms of quality and quantity. This means that they should be characterised by different susceptibility to enzymatic and acid hydrolysis. In consequence, they should differ in the electric state which should be shown by the measurement of the electrolytic conductivity. Results of electrolytic conductivity measurement of the water solutions of the pepsin hydrolyzates of the investi-

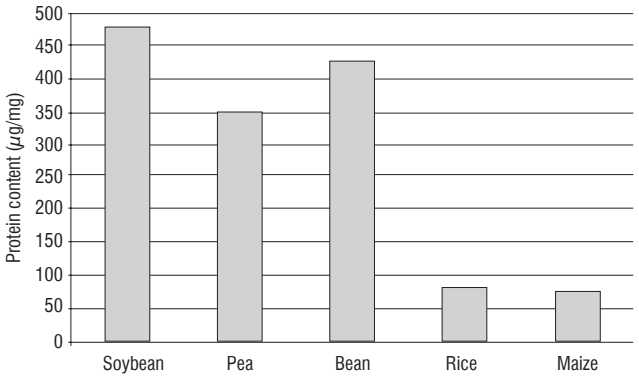

FIGURE 1. Content of protein in soybean, pea, bean, rice and maize protein extracts, expressed in μg/mg lyophylizate.

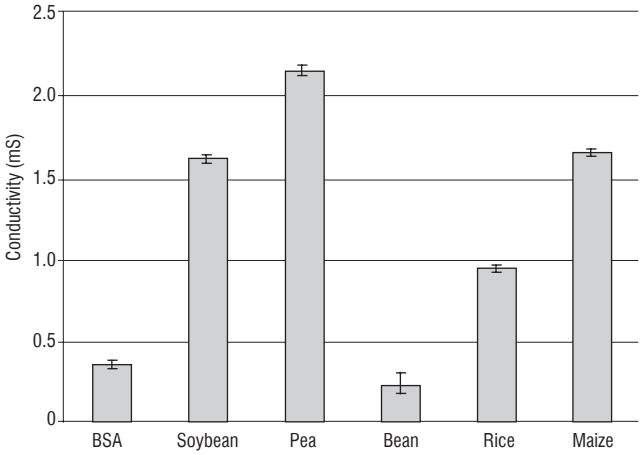

FIGURE 2. Electrolytic conductivity of pepsin hydrolyzates of the soybean, pea, bean, rice and maize protein extracts.

gated proteins are shown in Figure 2. From these data there result two basis facts. Firstly, the differences between the electrolytic conductivity of the pepsin hydrolyzates of the investigated proteins were statistically significant. And secondly, the electrolytic conductivity of the pepsin protein hydrolyzates was not in direct proportion to the content of protein in the protein extracts. The data obtained prove that the measurement of the electrolytic conductivity of the enzymatic protein hydrolyzates can be used for the differentiation of their electric states, which proves their different peptide composition and the susceptibility to the enzymatic hydrolysis. Analysing the electrolytic conductivity of the protein hydrolyzates it is

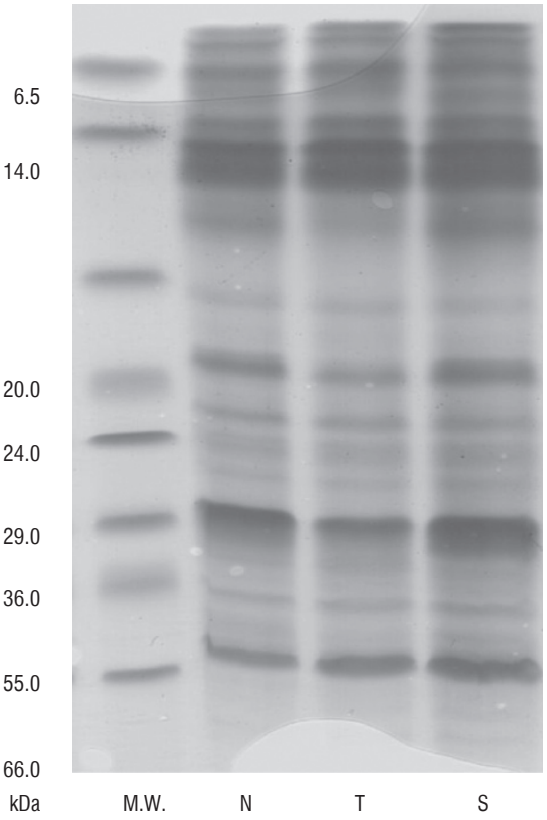

FIGURE 3. Electrophoregram of the crude extract of proteins
M.W. – molecular weight standard, N - cv. "Nawra", T – cv. "Tonacja", S– cv. "Sukces"

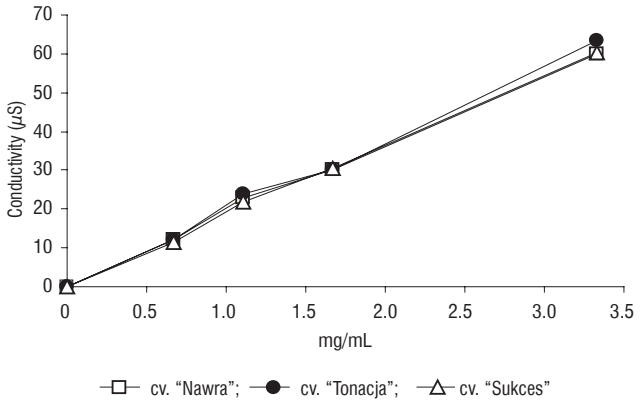

FIGURE 4. Relationship between the electrolytic conductivity of the crude extract of proteins and their concentrations.

worth remembering that some peptides with low molecular weights are lost during dialysis. These results are in agreement with our earlier investigations [Kostyra *et al.,* 1981].

The next experiment was carried out using the protein extracts from three Polish varieties of wheat with protein content: 10.7% (Navra), 9.7% (Sukces) and 9.7% (Tonacja), respectively (Figure 3). Similar protein contents in all wheat varieties suggest their considerable similarity in respect of the qualitative composition of proteins. This assumption was confirmed by SDS-PAGE electrophoresis. The protein electrophoregrams of all three wheat varieties are practically the same. This means that the molecular weights and the electric charges of the individual protein fractions are very similar. If

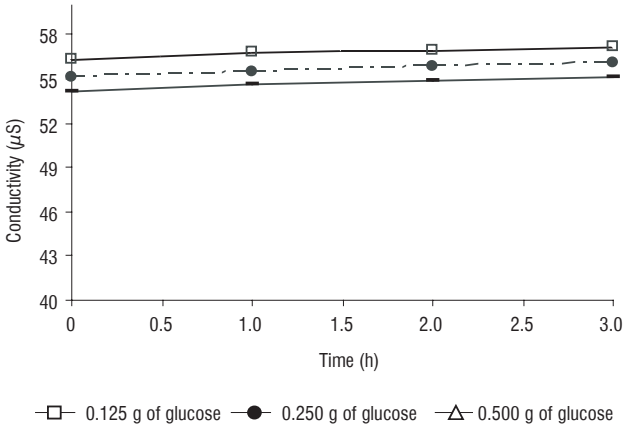

FIGURE 5. Influence of glucose addition on the electrolytic conductivity of the crude extract of proteins.

this assumption is correct, then the electrolytic conductivity of the CEP should be also very similar. The changes of the electrolytic conductivity in the relation to the concentration of the CEP are presented in Figure 4. The electrolytic conductivity of all albumin and globulin mixtures was almost the same. It was interesting that this electrolytic conductivity was proportional to the concentration of proteins. It was also proved that the concentration of glucose in the protein solution modifies its electrolytic conductivity which suggests the reaction of glycation between glucose and free amine groups of proteins present in the solution (Figure 5). This fact suggests the possibility of determining the concentration of water soluble proteins by the conductometric method.

Figure 6 presents the electrolytic conductivity of the CEP before and after pepsin and trypsin hydrolysis. On the basis of these results, it can be stated that the electrolytic conductivity of the pepsin and trypsin hydrolyzates is lower than that of differentiating proteins and their enzymatic hydrolyzates by the measurement of the electrolytic conductivity.

The next experiment concerned the differentiation of the native and glycated proteins by the measurement of their electrolytic conductivity. Figure 7 presents the electrolytic conductivity of the albumin and globulin mixture before and after glycation. Differences of the electrolytic conductivity were only observed for the glycated CEP and their acid

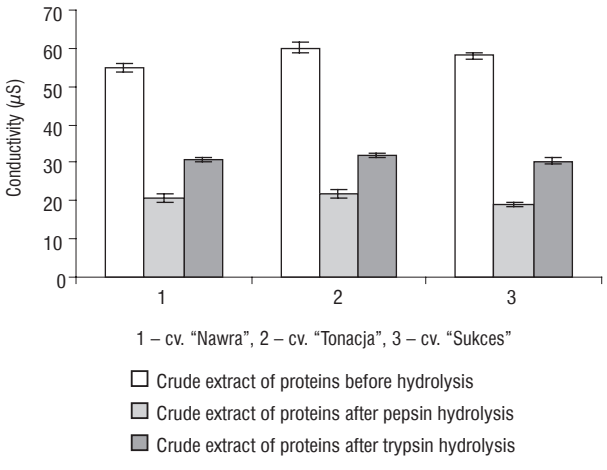

FIGURE 6. Electrolytic conductivity of the crude extract of proteins before and after pepsin and trypsin hydrolysis.

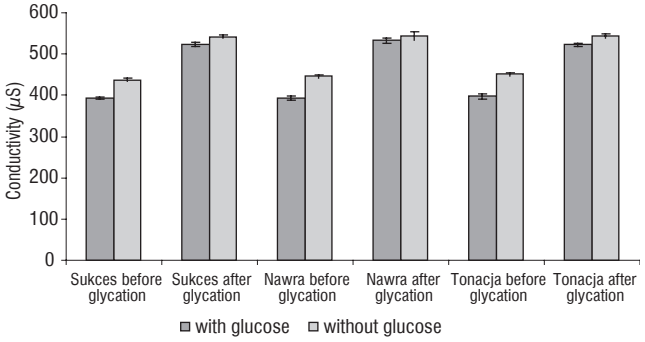

FIGURE 7. Electrolytic conductivity of the crude extract of proteins before and after non-enzymatic glycosylation.

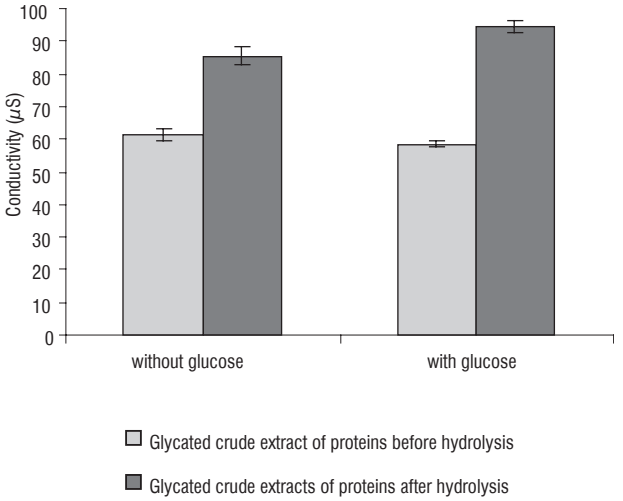

FIGURE 8. Influence of partial acid hydrolysis on electrolytic conductivity of glycated crude extract of proteins.

partial hydrolyzates are shown in Figure 8. Similarly to the enzymatic hydrolyzates, the acid partial hydrolyzates of the glycated CEP were characterised by different electrolytic conductivity in comparison with the glycated proteins. On the basis of the above results it can be stated that enzymatic and acid hydrolyzates of the native and modified proteins can be differentiated by the measurement of the electrolytic conductivity. This means that the suitable standardized measurement of the electrolytic conductivity of the native and modified proteins and their enzymatic or acid hydrolyzates can be used for the determination of the changes caused by the physical and chemical agents.

CONCLUSIONS

1. The electrolytic conductivity of the pepsin hydrolyzates derived from the crude extract of proteins was statistically significantly different.

2. The electrolytic conductivity of the water protein solutions was directly proportional to the concentration of protein.

3. The concentration of glucose in the protein solution influences its electrolytic conductivity, which suggests the glycation reaction proceeding.

4. The electrolytic conductivity of the native and glycated proteins was statistically significantly different.

5. The native as well as chemically- and enzymatically-modified proteins can be differentiated by the electrolytic conductivity.

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ZASTOSOWANIE KONDUKTOMETRII DO RÓŻNICOWANIA BIAŁEK I PEPTYDÓW

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Celem pracy było zastosowanie konduktometrii do różnicowania chemicznie i enzymatycznie zmodyfikowanych białek żywności. Badano ekstrakty białek soi, grochu, fasoli, kukurydzy, ryżu i 3 odmian pszenicy, albuminę surowicy krwi wołowej oraz hydrolizaty kwasowe i enzymatyczne tych białek. Dodatkowo białka poddawano glikacji za pomocą glukozy. Stwierdzono statystycznie istotną różnice pomiędzy przewodnictwem elektrolitycznym hydrolizatów pepsynowych badanych ekstraktów. Wykazano, że przewodnictwo elektrolityczne wodnych roztworów białek jest proporcjonalne do ich stężenia. Dowiedziono, że obecność glukozy podczas enzymatycznej hydrolizy białek wpływa na ich przewodnictwo elektrolityczne, co sugeruje zachodzenie reakcji glikacji pomiędzy glukozą i wolnymi grupami aminowymi białek. Wykazano także statystycznie istotne różnice w przewodnictwie elektrolitycznym natywnych i zglikolizowanych białek.