Capillary Zone Electrophoresis Separation of Hydrolysates Obtained from Food Industry By-products

Ryszard AMAROWICZ[1], Przemysław ZDUŃCZYK[1] and Ewa FLACZYK[2]

[1]Department of Food Science, Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Olsztyn, Poland; [2]Department of Human Nutrition and Food Technology, Agricultural University of Poznan, Poznan, Poland

Abstract

Amarowicz R., Zduńczyk P., Flaczyk E. (2004): **Capillary zone electrophoresis separation of hydrolysates obtained from food industry by-products**. Czech J. Food Sci., **22**: 121–124.

Enzymic hydrolysates were obtained from cracklings (CEH and CEH*) using alcalase. Acid hydrolysates were prepared from cracklings (CAH) and chicken feathers (FAH). The degree of hydrolysis (DH) of CEH and CEH* were 14 and 15.1%, respectively. CAH, its Sephadex G-25 fraction (CAH*) and FAH were characterised by DH of 53.8%, 47.8% and 46.2%. The electrophoreograms of enzymic hydrolysates were characterised by one high and sharp peak and several not base line separated peaks. More single and sharp peaks were observed on the electrophoreograms of acid hydrolysates. Migration times of the majority of peptides present in enzymic hydrolysates ranged between 4 and 6 min.

Keywords: capillary zone electrophoresis; hydrolysates; cracklings; chicken feathers

Capillary zone electrophoresis is one of the most widely used mode of the high performance capillary electrophoresis (HPCE) due to its simplicity of operation and its versatility (Heiger 1992). Many of the applications of Capillary zone electrophoresis (CZE) have been for peptides and proteins a significant success has been achieved in peptide mapping using this technique. In peptide mapping, a protein is enzymically or chemically cleaved into smaller peptides and subsequently separated. The analysis is primarily qualitative and is used to detect differences between hydrolysates (Schwartz & Prichett 1994).

In our previous study, the antioxidative activity of the hydrolysates obtained from food industry by-products was evaluated by spectroscopic methods and the oxidation stability of lard with the addition of hydrolysates was tested using a *Rancimat* and an *Oxidograph* apparatus set at 110°C (Flaczyk *et al.* 2003). We also observed the angiotensin I-converting enzyme (ACE) inhibitory activity of the above mentioned hydrolysates (Amarowicz *et al.* 2004).

The goal of the present study was to verify a facile capillary electrophoresis as a method for the separation of peptides in protein hydrolysates obtained from food industry by-products.

MATERIAL AND METHODS

Materials. Cracklings and feathers from chickens were obtained from the local food industry in Poznan (Poland). The content of total nitrogen (N_{tot}) in the cracklings and feathers was determined to be 13.1% and 14.1%, respectively.

Enzymic hydrolysis. Two enzymic hydrolysates from cracklings (CEH and CEH*) were prepared using Alcalase 2.4 L (Novo Nordisk Company, Bagsverd, Denmark; the declared activity of 0.6 Anson

units (AU) per gram). The hydrolysis was carried out in a 250 ml thermostatic vessel equipped with a stirrer and a pH-meter (Denver Instr., Model 15, USA), set at a temperature of 55 ± 1°C for 1 h at pH 8.5; the enzyme/substrate ratio was 0.15 AU/g (Shahidi *et al.* 1995). The sample of defatted powdered cracklings (10 g) was suspended in 100 ml distilled water. After the addition of *Alcalase*, the pH of solution was continuously controlled and adjusted with 4M NaOH. After hydrolysis, the enzyme was inactivated by heating of the mixture at 80°C for 5 min. The non-hydrolysed material was precipitated from the solution using chloroform and then removed by centrifugation. The pH of the supernatant was adjusted to 7, and the hydrolysate was lyophilised (Heto FD3, Denmark).

Acid hydrolysis. Acid hydrolysis was carried out using 4.75M HCl at 110°C for 4 h (cracklings) or 12 h (feathers), at the material to HCl solution ratio of 3:7. After hydrolysis, the solutions were neutralised and adjusted to pH 5.7 with sodium carbonate powder. The mixtures were shaken with activated charcoal (1.8% (m/v) during heating for 15 min at a temperature of 60–70°C), filtered through Whatman No. 3 filter paper, stored at 4°C for 4 weeks, and finally spray dried. A Mini Spray Dryer B-191 (Büchi, Switzerland) was used for this purpose. Five hundred millilitres of sample were spray-dried over a period of 30 min at the inlet temperature of 160°C; the outlet temperature of 70°C was employed. Hydrolysates were marked as CAH (prepared from cracklings) and FAH (prepared from feathers). CAH* was separated from CAH using gel filtration on a Sephadex G-25 column (2.8 × 50 cm) with distilled water as the mobile phase.

Chemical analysis. The content of total nitrogen (N_{tot}) in the hydrolysates was determined using the Kjeldahl method. α-Amino nitrogen (N_{NH_2}) (DH) of the hydrolysates were determined using the TNBS (trinitrobenzenesulphonic acid) method (Panasiuk *et al.* 1998). The degree of hydrolysis (DH) was calculated as the ratio of α-amino nitrogen (N_{NH_2}) liberated during hydrolysis to total nitrogen (N_{tot}) and was expressed in percent. All results are reported as the mean of triplicate.

Capillary zone electrophoresis (CZE). CZE of the hydrolysates investigated was carried out using a Beckman CE instrument with UV diode array detection. The buffer employed for CZE consisted of 100mM boric acid, adjusted to pH 8.3 with 1M NaOH. An uncoated fused-silica capillary (70 μm i.d. × 67/60 cm) was used. The separation was performed at 20 kV. The sample concentration was 2 mg/ml. The capillary temperature was 40°C. The photodiode detector was set at 214 nm.

RESULTS AND DISCUSSION

The chemical composition of the hydrolysates is presented in Table 1. The highest content of N_{tot} was found with CEH and CEH*. These hydrolysates revealed to have the lowest level of N_{NH_2} (1.72% and 1.69%) and the lowest degree of hydrolysis (DH). The content of N_{tot} in CAH was 7.94% and the DH was approximately 54%. CAH* (Sephadex G-25 fraction separated from CAH) was compared to CAH, the content of N_{tot} was slightly higher, but that of DH – lower. The DH for CEH and CEH* were in the range of values given in literature for various protein sources hydrolysed by *Alcalase* (Ponnampalam *et al.* 1987; Kim *et al.* 1990; Gwiazda *et al.* 1994; Hoyle & Merritt 1994; Shahidi *et al.* 1994).

Table 1. Chemical characteristics of the investigated hydrolysates

Hydrolysate	N_{tot} (%)	N_{NH_2} (%)	DH (%)
CEH	**12.32**	**1.72**	**14.0**
CEH*	11.21	1.69	15.1
CAH	7.94	3.75	53.8
CAH*	8.90	4.27	47.8
FAH	8.21	3.79	46.2

The electrophoreograms of enzymatic hydrolysates were very similar (CEH and CEH* in Figure 1), which confirmed a good reproducibility of the enzymic process used for hydrolysis. They were characterised by one high and sharp peak (migration time of 4.06 min) and several not base line separated peaks. Migration times of the majority of peptides present in the enzymic hydrolysates ranged between 4 and 6 min. For CAH*, the last peak was slightly shifted on the electrophoreogram.

More single and sharp peaks were observed on the electrophoreograms of acid hydrolysates. Three main peaks were observed at migration times between 4 and 6 min. The difference between CAH and its fraction CAH* separated by means of Sephadex G-25 column chromatography was

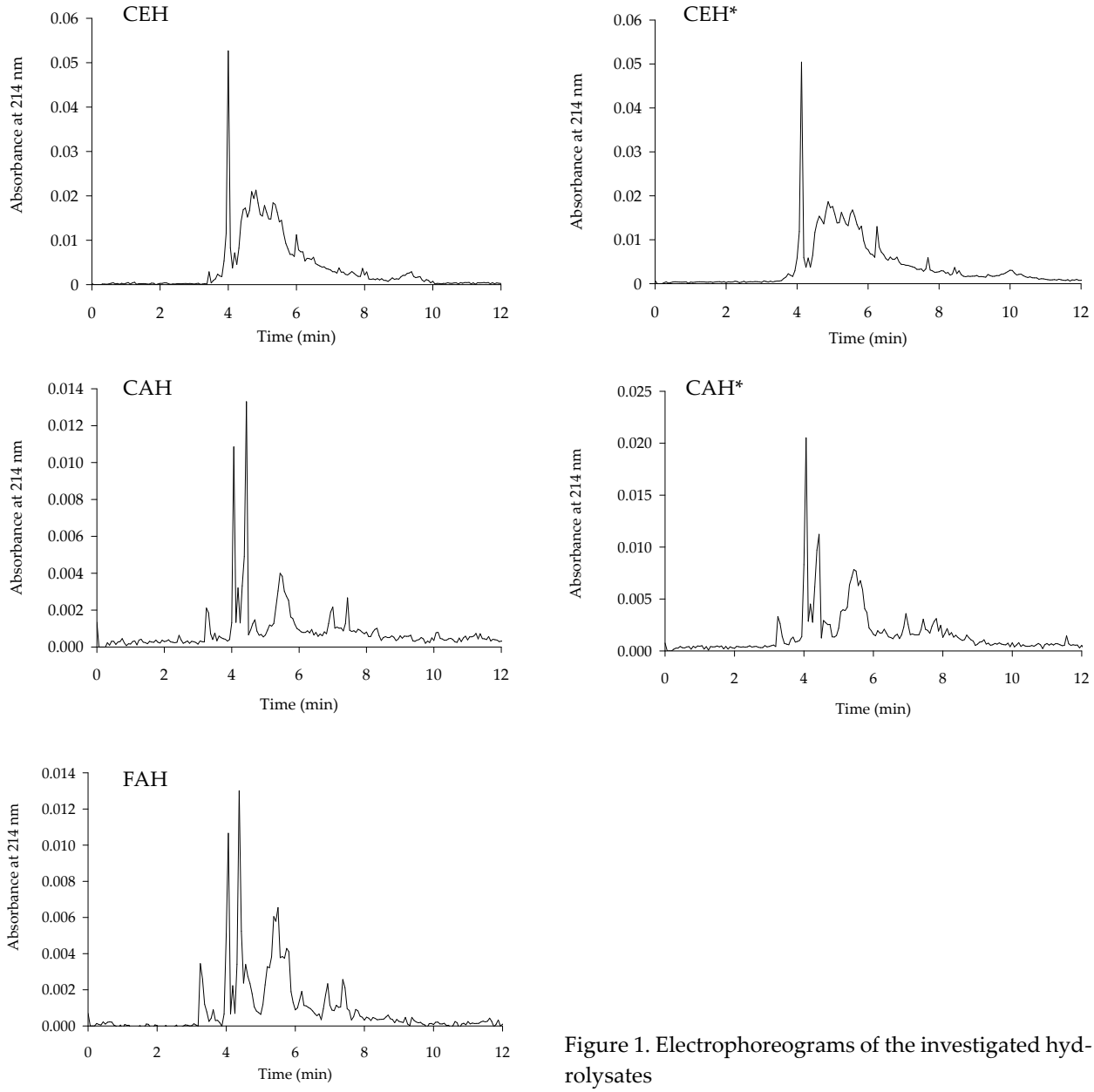

Figure 1. Electrophoreograms of the investigated hydrolysates

found in the case of peaks with the migration times of 4.06 and 4.44 min. CAH was characterised by a high peak with the migration time of 4.44 min and a smaller peak with the migration time of 4.06 min. On the electrophoreogram of CAH*, the relation of the highest of these peaks was reversed. The electrophoreogram originated from FAH possessed intensive peaks with the migration time longer than 5 min. The coefficient of variation of 8 separated CE analyses of CEH calculated for a peak with the retention time of 4.06 min was 2.25. These results confirmed a good reproducibility of the method applied.

In our previous study (Flaczyk *et al.* 2003), protein hydrolysates obtained from food industry by-products were analysed by reversed-phase HPLC in the gradient system of water and acetonitrile containing 0.1% of trifluoroacetic acid. CZE method provides a shorter time of separation than HPLC. The duration of HPLC analysis was 40 min. The majority of compounds (i.e., small peptides or amino acids) were eluted between 10 and 20 min. Chromatogram of enzymic hydrolysate was characterised by many poorly separated peaks originating from peptides. No base line separation was achieved.

References

Amarowicz R., Flaczyk E., Wanasundara J., Pegg R.B. (2004): Hydrolysates with angiotensin I-converting enzyme (ACE) inhibitory activity obtained from food industry by-product. Pol. J. Food Nutr. Sci. (submitted).

Flaczyk E., Amarowicz R., Korczak J. (2003): Antioxidant activity of protein hydrolyzates from by-products of the food industry. J. Food Lipids, **10**: 129–140.

Gwiazda S., Wyrzykowska A., Wasylik K. (1994): Comparison of selected methods for determining the degree of hydrolysis of rapeseed proteins. Pol. J. Food Nutr. Sci., **44**: 165–173.

Heiger D. (1992): High Performance Capillary Electrophoresis – An Introduction. Hewlett-Packard GmbH, Waldbronn, Germany.

Hoyle N.T., Merritt J.H. (1994): Quality of fish protein hydrolysates from herring (*Clupea harengus*). J. Food Sci., **59**: 76–79.

Kim S.Y., Park P.S.-W., Rhee K.C. (1990): Functional properties of proteolytic enzyme modified soy protein isolate. J. Agr. Food Chem., **38**: 651–656.

Panasiuk r., Amarowicz r., Kostyra k., Sijtsma l. (1998): Determination of α-amino nitrogen in pea protein hydrolysates: A comparison of three analytical methods. Food Chem., **62**: 363–367.

Ponnampalam R., Goulet G., Amiot J., Brisson G.J. (1987): Some functional and nutritional properties of oat flours as affected by proteolysis. J. Agr. Food Chem., **35**: 279–285.

Schwartz H., Prichett T. (1994): Separation of Protein and Peptides by Capillary Electrophoresis: Application to Analytical Biotechnology. Beckmann Instruments Inc., Fullerton, CA, USA.

Shahidi F., Synowiecki J., Balejko J. (1994): Proteolytic hydrolysis of muscle proteins of harp seal (*Phoca groenladica*). J. Agr. Food Chem., **42**: 2634–2638.

Shahidi F., Han X.Q., Synowiecki J. (1995): Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). Food Chem., **53**: 285–293.

Received for publication January 12, 2004
Accepted after corrections March 15, 2004

Souhrn

Amarowicz R., Zduńczyk P., Flaczyk E. (2004): **Separace hydrolyzátů získaných z vedlejších produktů potravinářského průmyslu pomocí kapilární zonové elektroforézy.** Czech J. Food Sci., **22**: 121–124.

Byly připraveny enzymové hydrolyzáty z odpadní kůže (cracklings) (CEH a CEH*) při použití alkalasy, a dále kyselé hydrolyzáty z odpadní kůže (CAH) a z kuřecího peří (FAH). Stupeň hydrolýzy (DH) byl 14 % v případě CEH a 15,1 % v případě CEH*. Stupeň hydrolýzy u CAH byl 53,8 %, u jeho frakce získané pomocí Sephadexu G-25 (CAH*) 47,8 % a u FAH byl 46,2 %. Pro elektroforeogramy enzymových hydrolyzátů byl charakteristický výskyt jednoho vysokého ostrého peaku a několika dalších peaků nerozdělených na základně. Elektroforeogramy kyselých hydrolyzátů vykázaly větší počet jednotlivých ostrých peaků. Migrační časy většiny peptidů přítomných v enzymových hydrolyzátech se pohybovaly mezi 4 až 6 minutami.

Klíčová slova: kapilární zonová elektroforéza; hydrolyzáty; odpadní kůže (cracklings); kuřecí peří

Corresponding author:

Doc. Dr. Ryszard Amarowicz, Polska Akademia Nauk, Instytut Rozrodu Zwierząt i Badań Żywności, Zakład Chemii Żywności, ul. Tuwima 10, P.O. Box 55, 10-718 Olsztyn, Polska
tel.: + 89 523 26 75, fax: + 89 524 01 24, e-mail: amaro@pan.olsztyn.pl