POLISH JOURNAL OF FOOD AND NUTRITION SCIENCES

www.pan.olsztyn.pl/journal/
e-mail: joan@pan.olsztyn.pl

Pol. J. Food Nutr. Sci.
2007, Vol. 57, No. 2, pp. 177–184

# OPTIMISATION OF CONDITIONS FOR CAPILLARY ZONE ELECTROPHORESIS (CZE) SEPARATION OF TRICHLOROACETATE EXTRACTS OF SALTED FISH MEAT

*Katarzyna Felisiak*

*Department of Food Science and Technology, Agricultural University of Szczecin, Szczecin*

Key words: capillary electrophoresis, salted Baltic herring, separation parameters, sodium chloride, TCA

Effects of CE parameters and modifying substances on the quality of separation of trichloroacetic (TCA) extracts of salted Baltic herring meat were studied. The optimal separation parameters were as follows: capillary length 36 cm; voltage 20 kV; injection pressure 2 psi*s; capillary temperature 35°C, detection wavelength 200 nm. Buffer enrichment with 20% acetonitrile and 40% methanol resulted in a slight improvement of the separation quality, while 20 mmol/L triethylamine (TEA) produced some improvement, but was a source of an additional peak in field A. Salt concentration 1% was shown to cause the peaks to fuse, to decrease in height, and to disappear completely at the concentration of 5%. Sample purification using solid phase extraction (SPE) and dialysis improved the separation, but was time-consuming or did not ensure that the full composition of the sample would be maintained. Sodium nitrate precipitation of $Cl^-$ ions produced no improvement in separation quality. Reduction in sodium chloride and trichloroacetic acid concentrations to below 1% and about 1.5%, respectively, by diluting TCA extracts with distilled water produced the best results by completely separating the peaks in all fields; the method proved the least time-consuming and ensured that the sample composition remained intact.

## INTRODUCTION

Analysis of non-proteinaceous nitrogen compounds is always associated with sample deproteination with protein-precipitating reagents. Of the many deproteinating reagents, trichloroacetic acid (TCA) is used most frequently (TCA) [Kołakowski, 2005]. At a concentration as low as above 3%, TCA sufficiently deproteinates samples, and is an efficient solvent for non-proteinaceous compounds, including free amino acids, peptides, and polypeptides [Kołakowski, 1973].

Capillary zone electrophoresis (CZE) is a technique making it possible for peptides and amino acids of different loads to be separated in a single run and in a very short time. The high sensitivity and repeatability, low reagent consumption and hence reduced cost of assays, as well as automation of procedures and the user-friendly equipment render CZE very useful in routine analytical work [Michaelsen & Sørensen, 1994; Mucha *et al.*, 1997].

Separation conditions affect the electroosmotic flow, thereby modifying electrophoretic mobility of sample components and separation quality. Depending on sample composition, a set of parameters may improve or worsen the resolution [Oda & Landers, 1997]. Therefore, optimal experimental conditions should be worked out independently for each analytical problem [Dolník, 1996; Messana *et al*., 1997]. Those conditions include the length of the capillary, injection pressure, capillary temperature, and detection wavelength.

A serious drawback of CZE is its sensitivity to high salt content of samples. This is particularly important when salted fish are being analysed; moreover, their tissue salt concentration changes during ripening.

The sample NaCl concentration of about 0.5% NaCl may be tolerated, but as it increases, the peaks become reduced down to complete disappearance at the concentration of about 3% [Shihabi, 1999]. The presence of NaCl reduces the amount of the sample proper, loaded into the capillary, and renders analyte separation more difficult. This effect is particularly intense at electrokinetic injection (the presence of salt affects the electric field strength which makes it possible for the analyte to be transported to the capillary), although it is also observed at hydrodynamic injection. According to some authors, a high salt concentration increases the current, which results in excessive Joule heating and, in consequence, in a poorer separation [Cross & Cao, 1998; Cross & Smairl, 2001]. On the other hand, Song *et al.* [1995] found high NaCl concentrations to produce no significant increase in current intensity and no excessive Joule heat generation.

The present work was aimed at working out optimal parameters (capillary length, current voltage, injection pressure, capillary temperature and detection wavelength) for a direct CZE separation of deproteinated extracts of Baltic herring meat, without the need for any additional analytical procedures such as derivatisation.

Author's address for correspondence: Katarzyna Felisiak, Agricultural University of Szczecin, Department of Food Science and Technology, ul. Papieża Pawła VI 3, 71-459 Szczecin, Poland; fax: (48 91) 42 50 432; e-mail: stypko@tz.ar.szczecin.pl

## MATERIALS AND METHODS

**Raw materials**. The study involved Baltic herring (*Clupea harengus* L.) from the spring stock, assigned to commercial grade S and D, with a small addition (3–5%) of individuals from the autumn stock, eliminated from each batch. The fish were caught with gill nets off Dziwnów (fishing ground designation DANUTA-2).

The fish were delivered to the laboratory on ice, in 25 kg capacity polystyrene foam boxes (Atlantic Styro A/S), according to the Polish Standard [PN-55/A-86761], 1–2 days after capture. On delivery, the fish were mostly under retreating *rigor mortis*.

**Additional raw materials.** When preparing samples for assays, the following additional raw materials were used: edible salt (non-iodinised), grade II, PKWiU 14.40.10-00.11 (Kłodawa Salt Mines P.P.); and 10% alcohol vinegar (P.P.H.U. "FRUKTUS" s.c.)

**Reagents.** *a) used in sample deproteination:* trichloroacetic acid, analytical grade (POCh); acetonitrile, spectrophotometry grade (J.T. Baker); silver nitrate, analytical grade (POCh); silver sulphate, analytical grade (POCh); *b) used in sample purification or separation and in preparing standard solutions:* methanol, analytical grade (POCh); 98% ethanol, analytical grade (POCh); 37-38% HCl, analytical grade (POCh); triethylamine, HPLC grade (Sigma); 0.1 mol/L phosphate buffer, pH 2.5 (Bio-Rad); and *c) amino acid and peptide standards* (mostly manufactured by Merck, Reanal, and Sigma), chromatographic grade.

**Preparation and storage of samples.** After removing the ice and discarding sub-sized or mechanically damaged individuals, the fish were headed using the straight cut, gutted, and washed.

The carcasses were washed in running water and placed, dorsal side up, on perforated trays and left for 20 min for dripping. The dried carcasses were placed in 2 L glass jars which were then filled with 14% edible salt solution with 0.7% acetic acid and chilled to 8°C, following the procedure described by Kołakowski & Bednarczyk [1997]. The fish weight in each sample was 1150±0.5 g, the brine to fish weight ratio amounting to 1:1. The jar contents were gently mixed so that no myofibrillar protein extraction would ensue and the jars were closed with glass tops and stored at 8±1°C in cold room.

Samples for assays were collected after 14 days of storage. The carcasses and brine were transferred to large funnels lined with polyamide fibre ("dederon") filtering cloth and left until the liquid and solid fractions were fully separated (*ca*. 10 min at room temperature).

The salted herring carcasses were filleted, skinned, and minced in a 3 mm aperture diameter EM-1 meat grinder ("Mesko", Skarżysko-Kamienna); the mince was mixed and collected in a plastic container. The mince is henceforth termed "the salted Baltic herring meat".

**Preparation of meat TCA extract.** A 50 g sample of minced meat was weighed on a technical scale to 0.1 g and placed in a jar of an MPW 102 homogeniser ("Mechanika Precyzyjna", Warszawa); 200 mL of 5% trichloroacetic acid (TCA) were added and the jar content was homogenised for 30 s at 13000 rpm. The homogenate was left to stand for 15 min and the homogenisation was repeated; after 30 min, the homogenate was filtered through a Whatman paper No. 389 filter (intermediate filtration rate) to a dry dark glass bottle.

**Preparation of amino acid standard solutions**. Standard amino acids were weighed out to 0.0001 g on an analytical balance and dissolved separately, in groups arranged by solubility, in water or in a small amount of 3 mol/L hydrochloric acid. The solutions were quantitatively transferred to 100 mL measuring flasks and brought to volume with 80% ethyl alcohol. Each standard solution obtained contained 0.1 mol/L of an amino acid and was stored in a dark place at room temperature.

**Preparation of standard peptide solutions.** Standard peptides were weighed out as above and dissolved separately in 1 mol/L HCl, followed by dissolution in 0.1 mol/L phosphate buffer of pH 2.5 (Bio-Rad), diluted 10 times with bidistilled water. The solutions obtained were quantitatively transferred to 100 mL measuring flasks and brought to volume with diluted phosphate buffer. The solutions were stored frozen.

**Capillary electrophoresis.** The samples were separated with capillary zone electrophoresis (CZE) in a Type 2000 BioFocus Capillary Electrophoresis System (Bio-Rad, USA) with spectrophotometric detection at 200, 220, 260 and 280 nm directly on the capillary. The capillaries used, non-filled, were made of polyamide-coated glass (Bio-Rad); their inner and outer diameters were 50 and 375 $\mu$m, respectively; the capillary lengths used were 24, 36, and 45 cm; the effective lengths (up to the detection window) were 20, 32, and 41 cm, respectively. Prior to each assay, a capillary was rinsed for 5 min with phosphate buffer.

The assays were run using pressure injections within 1–l2 psi*s, usually 5 or 2 psi*s (1 psi*s = 6 894.76 $m^{-1} \cdot kg \cdot s^{-1}$); separation was carried out at a constant voltage of 5 to 20 kV (usually 20 kV), polarity + to -, and constant capillary temperature of 20 to 35°C (usually 35°C). The separation of a single sample took 15 to 40 min, depending on the capillary length and the voltage applied.

Separations involved 0.1 mol/L phosphate buffer of pH 2.5 (Bio-Rad), stored at 4°C; small amounts of the buffer were conditioned prior to each assay at room temperature.

The effect of buffer additives was analysed by using phosphate buffer with the addition of 10–30% of acetonitrile (ACN), 20–40% of methanol or 10–30 mmol/L of triethylamine (TEA) respectively.

The samples consisted of TCA extracts prepared as described above, stored at 4°C, conditioned – prior to the assay – at room temperature in small (0.5 mL) vials.

The data were collected and processed by the BioFocus 2000 system integrator.

**Purification of samples using a solid phase extraction (SPE).** TCA-deproteinated meat extracts (3 mL) were purified with a SPE method using a C18 column and an SPE kit (J.T. Baker).

Column was prepared by washing with 9 mL of 90% acetonitrile with addition of 0.1% trifluoroacetic acid (TFA), and then with the same amount of 2% acetonitrile and bi-distilled water. Contaminations were eliminated by washing out with 2% acetonitrile in bi-distilled water (3 mL). Compounds adsorbed on the column filling were eluted with 0.1% TFA in 90% acetonitrile (ACN), in two portions of 1 mL. The purified sample was subjected directly to CZE analysis.

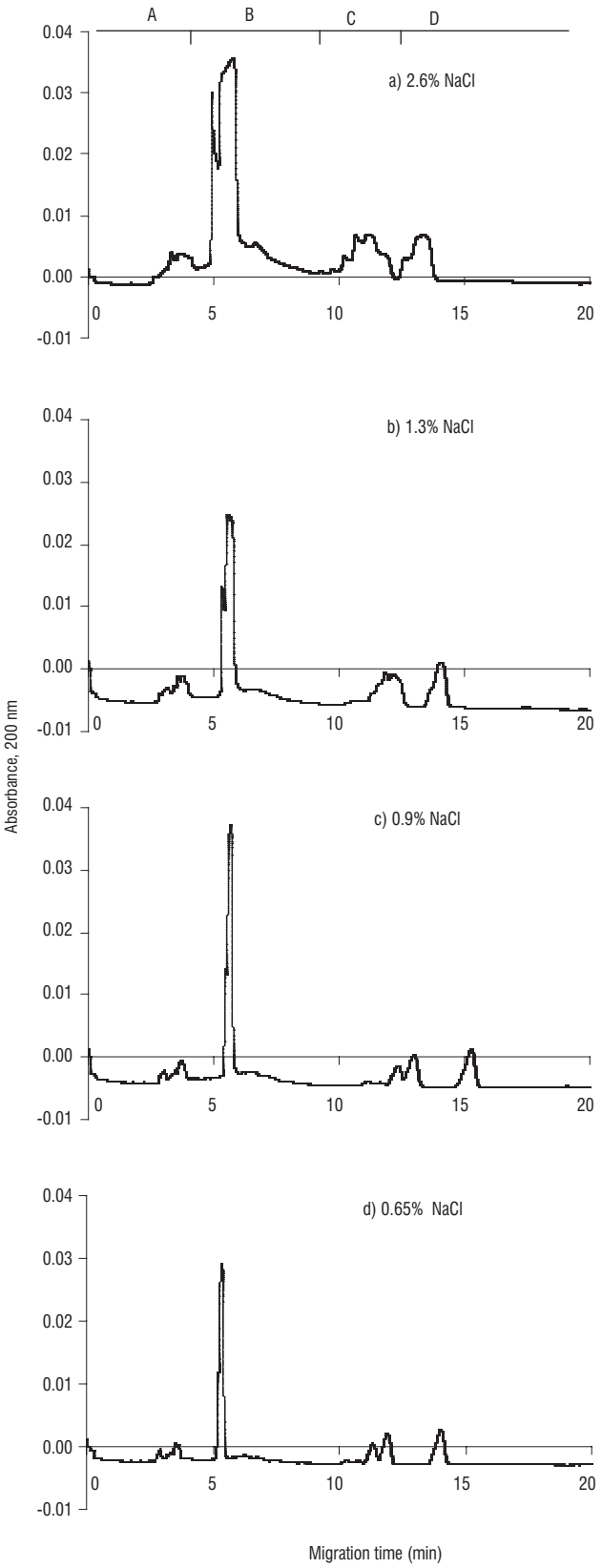


FIGURE 1. The influence of sodium chloride concentration in TCA extracts of salted Baltic herring meat on the separation quality.
Separation conditions: voltage 20 kV, injection pressure 5 psi*s, capillary temperature 35°C, capillary length 36 cm, detection 200 nm

**Sample purification by dialysing.** TCA-deproteinated meat extracts (3 mL) were purified by dialysing on a Spectra/Por®CE (Cellulose Ester) Float-A-Lyzer™ dialysis membrane (Roth, Germany) having the following parameters: MWCO 100, 100 mm diameter, 3 mL volume. A dialysing solution was bi-distilled water, changed after 3, 6 and 10 h of the process. Purified sample was subjected to CZE analysis directly after the process, without additional operations.

**Eliminating $Cl^-$ ions with silver nitrate and silver sulphate.** Precipitation of $Cl^-$ ions with $AgNO_3$ or $Ag_2SO_4$ was carried out by mixing 0.4 mL of TCA-extracts with the proper amount of silver salt, adequate to bound $Cl^-$ ions in the extract, determined with the Mohr technique. Samples were mixed in 0.5 mL test tubes, left to stand 15 min and centrifuged for 10 min at 3200 × g. Then supernatant was transferred to a dry 0.5 mL vial and subjected to CZE analysis.

**Salt content determination.** Salt content in the brine obtained during salting of the Baltic herring and in meat and brine TCA extracts was determined with the Mohr technique according to the Polish Standard [PN 74/A-86739].

## RESULTS

Direct separation of the Baltic herring meat TCA extract resulted in four groups of fused peaks, termed areas (fields) A, B, C, and D; the peaks did not lend themselves to sufficiently precise identification, in areas A and C (Figure 1a). Dilution of the extract with distilled water resulted in a sharp, precise separation of peaks in the areas (Figure 1b, c), which may evidence a negative effect of substances present in the sample (most probably sodium chloride, TCA, or both) on separation quality. Therefore the present work focused primarily on elucidating effects of those substances on the quality of separation.

The area C was found to consist of four peaks which corresponded to free amino acids, in sequence tryptophan, methionine, phenylalanine and tyrosine. The last peak in the C field was Tyr (Figure 2).

Separation quality was quantified with an index describing the extent to which the Tyr peak, equal to the ratio (expressed as a percentage) between the height at which a Tyr peak is fused with the neighbouring peak and the Tyr peak itself ($W_{JR}$). An index equal to 100% is indicative of a complete separation of peaks; the lower the index, the more fused the peaks appeared. The Tyr peak was selected because it was easily identifiable in all the separations and its resolution was found to be correlated with separation quality. The voltage increase was found to be accompanied by an improved separation quality (Figure 3a); however, voltages higher than 20 kV resulted in an excessive increase in current, hence the difficulties in maintaining constant separation temperature, which resulted in premature termination of an assay. An increase in the injection pressure resulted in deteriorated

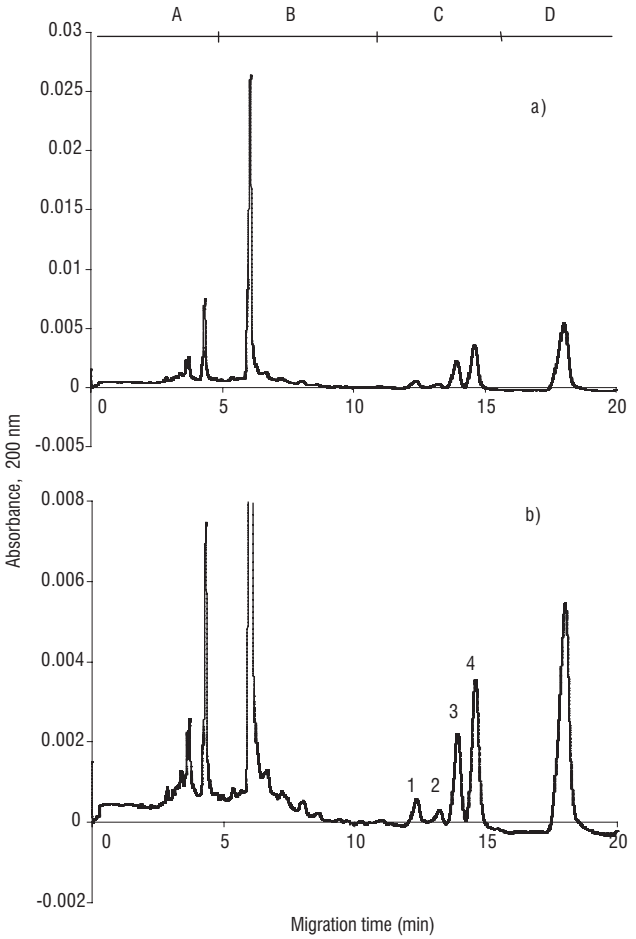


FIGURE 2. Electrophoregram of TCA extract of salted Baltic herring meat diluted three times with bi-distilled water.
a) in full scale, b) in reduced scale; 1 – Trp, 2 – Met, 3 – Phe, 4 – Tyr
Separation conditions: voltage 20 kV, injection pressure 2 psi*s, capillary temperature 35°C, capillary length 36 cm, detection 200 nm

separation quality (Figure 3b) – too small a sample resulted in the disappearance of small peaks. On the other hand, an excessively large sample produced fused peaks. The optimal injection pressure was found to be dependent on sample type, capillary length, and voltage. Longer capillaries required a higher injection pressure for a constant sample volume *vs.* capillary capacity to be maintained. The best resolution was obtained at injection pressure ranging within 2–5 psi*s. Elongation of the capillary improved separation quality, but the migration time became extended as well (Figure 3c). A temperature increase resulted in a slight reduction of the migration time and improved peak shape, but no particular effect on the separation quality was observed. Detection was carried out at 200 nm; most compounds showed absorbance at that wavelength and the signal was higher than that at 220 nm. Wavelengths beyond the 200–220 nm range made it possible to detect aromatic amino acids only.

Adding substances modifying the electroosmotic flow to the buffer failed to produce expected results. Effects of acetonitrile added at the concentrations of 10–30% and methanol at the concentrations of 20–40% were examined. It was only 20% acetonitrile or 40% methanol that managed to slightly improve the separation quality, but extended the migration time. The optimal dose of triethylamine (20 mmol/L) produced improved resolution of peaks (Figure 4), but resulted also in the appearance of an additional TEA peak with migration time of about 3 min (within area A).

Chloride concentration effects on the quality of separation of standard amino acid mixtures and peptides were investigated. In both cases, resolution was markedly poorer, particularly with respect of peaks with shorter migration times, at 1% sodium chloride concentration (Figure 5). An increase in the concentration resulted in poorer resolution of peaks with longer time of migration, and even (at 5%) rendered their separation virtually impossible. The resolution was adversely affected by the presence of both $Na^+$ and $Cl^-$ as well as the presence of TCA at concentrations exceeding 1%. The meat TCA extract of herring salted for 7 days was found to contain 2.6% NaCl. Reduction of the NaCl concentration to 0.9% by three dilutions with distilled water resulted in a considerable improvement of peak resolution in all the areas, particularly in area C (Figure 1c). The peaks in areas A and B remained fused at the base.

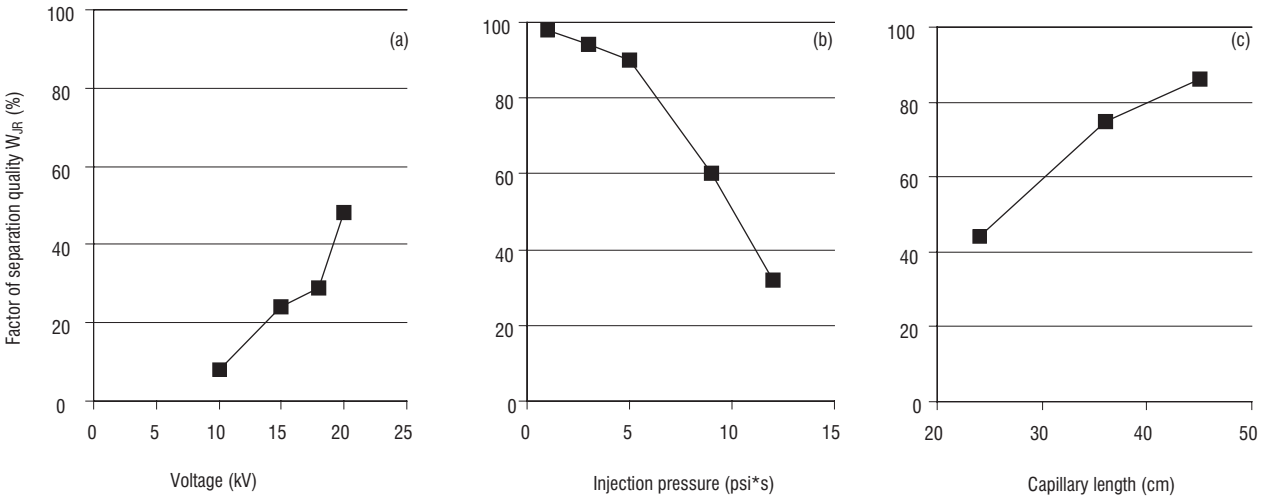


FIGURE 3. The influence of voltage (a), pressure injection (b) and capillary length (c) on the separations quality of TCA extracts of salted Baltic herring meat.
$W_{JR}$ – factor of separation quality, describing the ratio of peak Tyr separation

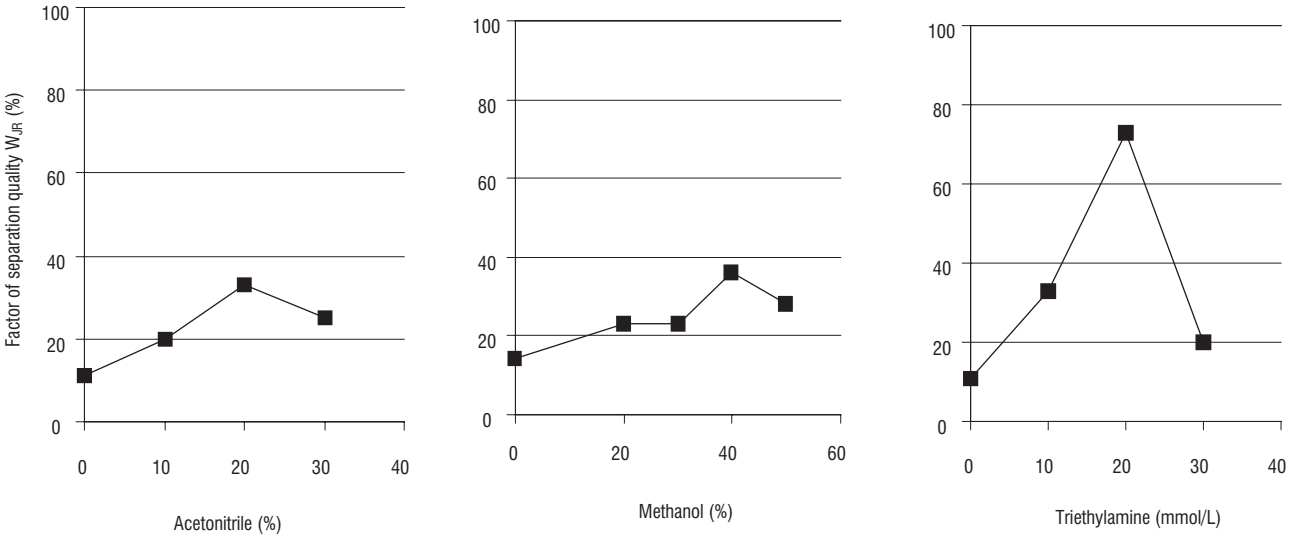


FIGURE 4. The influence of modifiers in buffer on the separations quality of TCA extracts of salted Baltic herring meat.
$W_{JR}$ – factor of separation quality, describing the ratio of peak Tyr separation

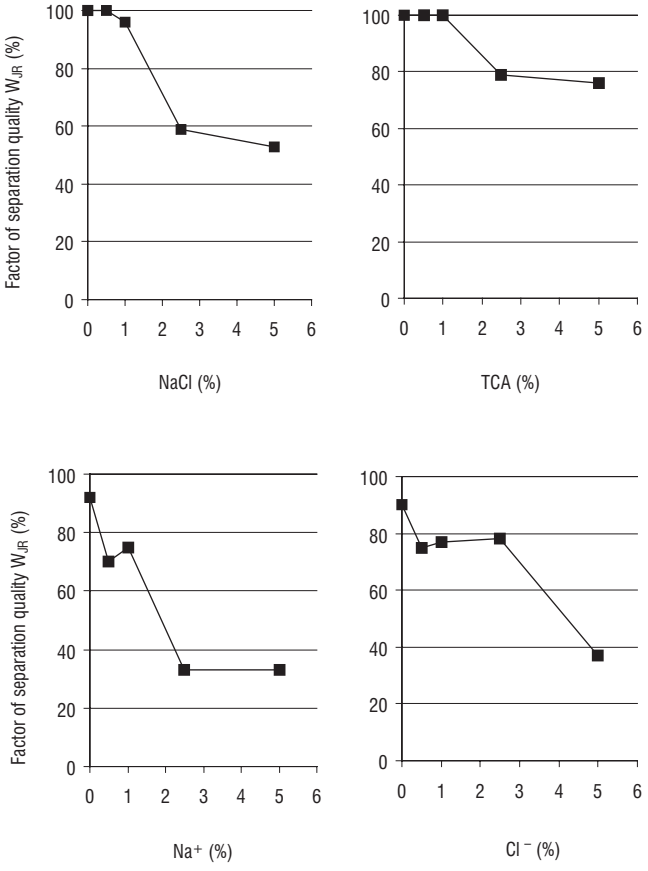


FIGURE 5. The influence of sodium chloride, trichloroacetic acid and ions $Na^+$ and $Cl^-$ in amino acids mixture on the quality of separation by CE.
$W_{JR}$ – factor of separation quality, describing the ratio of peak Tyr separation

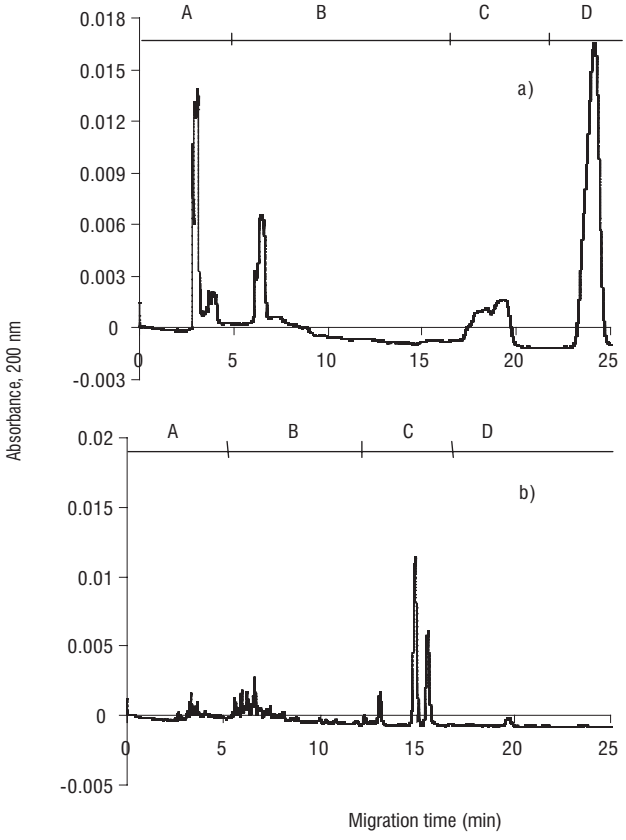


FIGURE 6. Electrophoregram of TCA extract of salted Baltic herring meat, before and after purifying by SPE.
a) TCA extract, b) purified sample.
Separation conditions: voltage 20 kV, injection pressure 2 psi*s, capillary temperature 35°C, capillary length 36 cm, detection 200 nm.

Attempts to precipitate $Cl^-$ ions from the extracts with silver nitrate or silver sulphate proved unsuccessful.

Purification of extracts by solid phase extraction (SPE) produced well-resolved, sharp peaks (Figure 6). A particularly precise separation of peaks was obtained in area C. However, due to column selectivity, it was impossible to retain the full composition of a sample; particularly marked losses were observed among amino acids.

Dialysis resulted in a distinct improvement in the quality of separation, but the desired effect was obtained as late as after 3 h for peaks with short migration time and after 6–12 h for peaks with long migration time (Figure 7).

The study allowed concluding that the conditions optimal for CZE separation of salted Baltic herring meat TCA extracts involve: capillary length 36 cm; voltage 20 kV; injection pressure 2 psi*s; separation (capillary) temperature

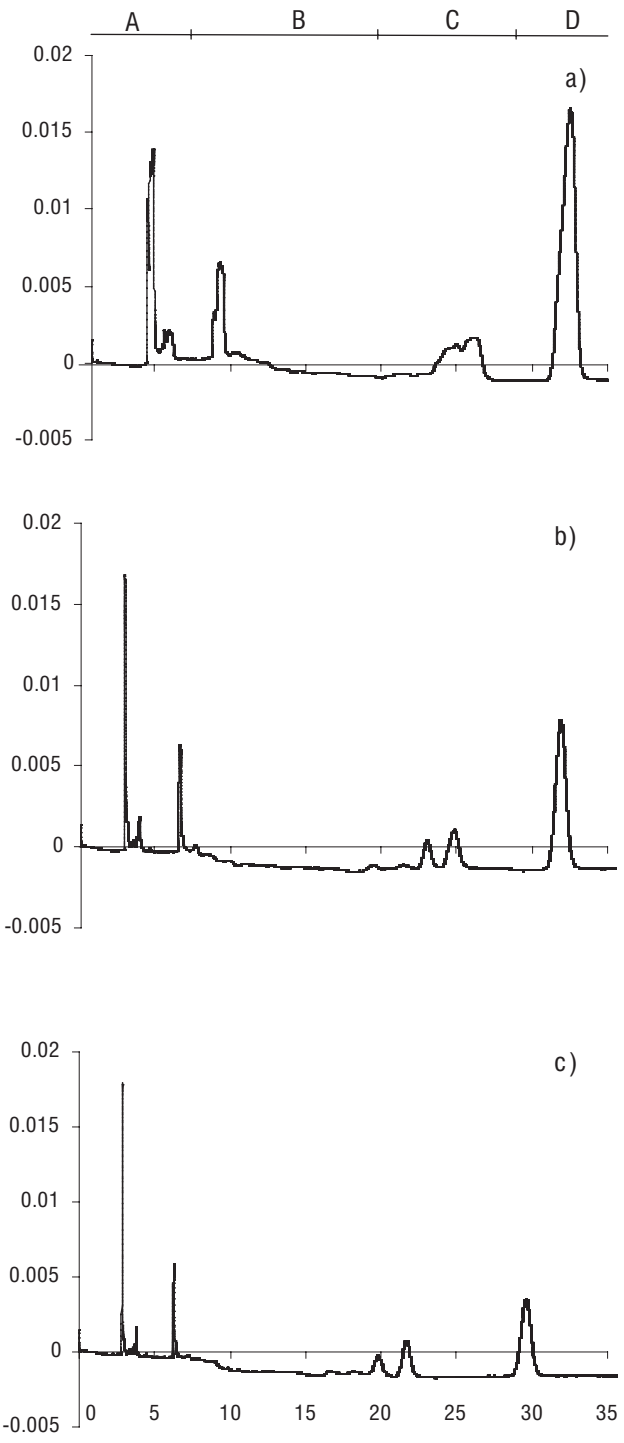


FIGURE 7. The influence of dialysis of TCA extract from salted Baltic herring meat on the separation quality.
a) sample before dialysis, b) sample after 6 h of dialysis, c) sample after 12 h of dialysis
Separation conditions: voltage 20 kV, injection pressure 2 psi*s, capillary temperature 35°C, capillary length 36 cm, detection 200 nm.

35°C; detection wavelength 200 nm; assay duration 20 min; and preliminary dilution 1:2.

## DISCUSSION

Numerous authors have generally recommended that conditions of analyses be determined separately for each problem under study [Dolník, 1996; Messana *et al.,* 1997]. The separation quality is affected by the following parameters: capillary length, voltage, injection pressure, capillary temperature, and detection wavelength.

Capillary length affects peak resolution and shape. Too long a capillary will diffuse the peaks due to the too long time an analyte would be staying in the detection window; too short a capillary will cause excessive accumulation of analytes and fusion of peaks [Oda & Landers, 1997]. A capillary 36 cm in length allows obtaining an optimal peak shape and optimal separation of components at a sufficient time. In addition, that length allowed optimal utilisation of the available 40 cm-long Bio-Rad capillary. Those results are in agreement with data reported by other authors who, when analysing peptides, used capillaries ranging in length from 24 cm [Yang *et al*., 1996] to 60 cm [Madsen & Qvist, 1997], *ca*. 40 cm being the most frequent length [Shihabi, 1996; Molina *et al*., 1998].

A higher voltage reduces the assay duration, hence the time the analyte stays in the detection window; therefore narrow peaks are obtained. However, an excessively increased voltage adversely affects the resolution. The voltage of 20 kV is within the range most often used in the assays [Oda & Landers, 1997]. When separating peptides formed during enzymatic casein hydrolysis and testing a voltage range of 25–35 kV, Macedo *et al*. [1999], arrived at the best resolution at 25 kV.

Injection pressure which controls the amount of analyte taken up by the capillary affects the resolution as well [Oda & Landers, 1997]. A too low a pressure (1 psi*s) makes it only possible to detect compounds that are present in the sample at high concentrations or that show a strong absorbance at the wavelength used. On the other hand, a too high pressure leads to fusion of peaks due to an excessively high concentration of the sample. A low injection pressure is recommended to be applied to samples containing acetonitrile due to the necessity of restricting adverse effects of organic solvents on current rate [Sarmini & Kenndler, 1997]. Generally, the injection pressure used by various authors in 50 $\mu$m capillaries ranged from 1 [Molina *et al*., 1998] to 35 psi*s [Shihabi, 1998].

Of the separation temperature range tested (20–40°C), 35°C was regarded as optimal: the peaks were sharper than at the other temperatures and the assay took shorter. Identical effects were reported by other authors [Oda & Landers, 1997]. Improvement in peak shape and reduction of migration time resulted most probably from a temperature-induced reduction in separation buffer viscosity [Yang *et al.* 1996].

Detection was carried out at 200 nm, an absorbance wavelength of most compounds; in addition, the signal was higher than that at 220 nm. The wavelengths of 260 and 280 nm allowed detecting aromatic amino acids only. Other authors applied detection both at 200 nm [Hamrníková *et al*., 1998] and 214 nm [Harmníková *et al*., 1999], and seldom at 220 nm [Idei *et al*., 1992].

Attempts to reduce the electroosmotic flow by applying buffer modifiers (organic solvents and fourth-order amines) did not improve resolution, either. Addition of 30% methanol to the buffer slightly improved the resolution of Baltic herring meat TCA extract separation. This was related to methanol-induced reduction of electroosmotic flow [van de Goor *et al*., 1997]. According to Hamrníkova *et al*. [1999], addition of 10 or 20% methanol to a low pH buffer brought no positive effect during separation of a dipeptide mixture. Adding aceto-

nitrile (ACN) to the separation buffer produced the best effect at 20% ACN. Lower concentrations resulted in no significant change, while at 30% the migration time of the last component was excessively extended. The results obtained confirm the findings described in the literature [Shihabi, 1997] which show acetonitrile to reduce the extent of dissociation of silanol groups on the capillary surface and to decrease the current, which in turn increases the efficiency of the analysis. However, a too high acetonitrile concentration reduces the degree of dissociation of the compounds in the sample, which leads to widening of the peaks to the detriment of separation efficiency. Li *et al.* [1999] found 15% acetonitrile to be an optimal addition to the pH 3 phosphate buffer. It was thus concluded that application of organic solvent does produce an effect by slightly improving the resolution, but – at the same time – the migration time of anionic components in significantly extended and changes in the peak order may ensue. Moreover, fused peaks in areas A, B, and C could not be separated, so the method was deemed useless for separation of salted Baltic herring meat TCA extracts.

Of the remaining modifiers, triethylamine proved less efficient than acetonitrile. The best results were obtained at 20 mmol/L TEA, an additional amine peak being visible on electrophoregrams in all the cases. An increase in TEA concentrations resulted in extension of migration times, particularly those of anionic sample components. This concurs with results reported by Lee & Desiderio [1997] who used TEA at concentrations of 10–40 mmol/L in nitrogen formalate at pH 2.5 and concluded that 20 mmol/L was an optimal concentration. Thus, it does not seem purposeful to modify the electroosmotic flow with fourth-order amines, either. The modification brought about no improvement in resolution of fused peak groups; it made it only possible to better separate individual groups one from another.

The sample's NaCl concentration was found to be important, because the resolution was noticeably impaired already at a concentration as low as 0.5%. Shihabi [1999] demonstrated that peak height became significantly depressed at NaCl concentrations exceeding 1%, the peak disappearing completely at 3%. This is related to an impaired loading of a sample and increased ionic strength, and hence to an increase in Joule heat generated. This in turn leads to increased electroosmotic flow and reduced resolution of the components [Shihabi, 1997].

To eliminate adverse effects of NaCl on resolution of the peptide separation, samples are most often purified by solid phase extraction (SPE), mainly on a hydrophobic C18 column [Herraiz & Casal, 1995]. Using a C18 column to purify TCA extracts of salted herring meat enabled obtaining legible electrophoregrams; however, only hydrophobic components (mainly amino acids in area C) could be followed. Similar results were reported by Molina *et al*. [1998] who studied proteolysis products in a ripening cheese extract fractionated on C18.

Dialysis, applied mainly to purify polypeptides and proteins, proved successful also in purifying TCA extracts of salted herring meat or brine. However, the long time necessary for the analysis was definitely a drawback. For that reason, dialysis does not belong to methods used commonly for preparing samples for CE separation [Messana *at al.*, 1997].

An attempt to remove NaCl with $Cl^-$ ions-precipitating silver nitrate brought no satisfactory results. Although an insoluble silver chloride precipitate was produced, the ions remaining in the sample most likely prevented resolution from improving. Most probably, the remaining sodium ions as well as the presence of trichloroacetic acid still acted in concert to prevent any improvement of resolution.

Dilution with distilled water improves the separation resolution as a result of a reduction in the concentration of interfering substances. By decreasing sodium chloride and trichloroacetic acid concentrations below 1% and down to *ca*. 1.5%, respectively, legible separations could be obtained. Although dilution causes reduction in concentrations of the analytes studied, the reduction is uniform. A 1:2 dilution with distilled water is a successful way to restrict the adverse effect of sodium chloride and TCA on the resolution of separation.

## CONCLUSIONS

1. The presence of sodium chloride in deproteinated extracts of salted Baltic herring meat adversely affects legibility and precision of capillary electrophoresis (CE) separation.
2. Silver nitrate precipitation of $Cl^-$ ions from salted meat extracts causes no significant improvement in the resolution of separation.
3. Dialysis improves the resolution in all the electrophoregram fields (A, B, C and D), but takes from 3 to 12 h, depending on the migration time of individual peaks.
4. SPE purification of TCA extract allows obtaining legible separations, but – due to column selectivity – does not guarantee the full composition of the sample.
5. Addition of 20% acetonitrile and 40% methanol to separation buffer slightly improves the quality of separation, particularly in area A, but extends the duration of the assays.
6. Addition of 20 mmol/L triethylamine to separation buffer results in improved separation of peaks in areas A, C, and D, but induces an additional TEA peak to appear and extends the duration of the analysis.
7. The best CE separation of TCA extracts of salted Baltic herring meat was obtained under the following conditions: extract dilution to reduce NaCl concentration to at least 0.9%; 20 kV; injection pressure 2 psi*s; capillary temperature 35°C; capillary length 36 cm; and detection wavelength 200 nm.

## ACKNOWLEDGEMENTS

I gratefully thank Professor Edward Kołakowski DSc, Ph.D. (Head of Department of Food Science and Technology, Agricultural University of Szczecin) for his goodwill, help and valuable advice, which enabled making of this work.

The study was supported in 2004-2005 by the Polish State Committee for Scientific Research grant No. 2 P06T 002 26.

## REFERENCES

1. Cross R.F., Cao J., Salt effects in capillary electrophoresis. II. Mechanism of electrophoretic mobility modification due to Joule heating at high buffer concentrations. J. Chromatogr. A, 1998, 809, 159–171.
2. Cross R.F., Smairl A.M., Salt effects in capillary electrophoresis. V. Adsorption and retention of electrically neutral analytes. J. Chromatogr. A, 2001, 929, 113–121.
3. Dolník V., Selectivity, differential mobility and resolution as

parameters to optimize capillary electrophoresis. J. Chromatogr. A, 1996, 744, 115–121.
4. van de Goor, T., Apffel A., Chakel J., Hancock W., Capillary electrophoresis of peptides. 1997, *in*: Handbook of Capillary Electrophoresis (ed. J.P. Landers). CRC Press, Boca Raton, Boston, London, New York, Washington D.C., pp. 213–258.
5. Hamrníková I., Mikšík I., Uhrová M., Deyl Z., Ultraviolet detector response of glycine and alanine homopeptides: Some specific features in capillary electrophoresis. Analytica Chimica Acta, 1998, 372, 257–272.
6. Hamrníková I., Mikšík I., Deyl Z., Kašicka V., Binding of proline- and hydroxyproline-containing peptides and proteins to the capillary wall. J. Chromatogr. A, 1999, 838, 167–177.
7. Herraiz T., Casal V., Evaluation of solid-phase extraction procedures in peptide analysis. J. Chromatogr. A, 1995, 708, 209–221.
8. Idei M., Mezo I., Vadasz Z., Horvath A., Teplan I., Keri G., Capillary electrophoretic analysis of somatostatin analog peptides. Effect of organic solvents as buffer modifiers. J. Liq. Chromatogr., 1992, 15, 18, 3181.
9. Kołakowski E., Determination of fish meat peptides in technological processes. 1973, Wydawnictwo Akademii Rolniczej w Szczecinie, Rozprawy, No. 32 (in Polish; English abstract).
10. Kołakowski E., Analysis of proteins, peptides, and amino acids in food. 2005, *in*: Methods of Analysis of Food Components and Additives (ed. S. Ötleş). Taylor & Francis, Boca Raton, London, New York, Singapore, pp. 59–96.
11. Kołakowski E., Bednarczyk B., Effects of acetic acid addition on physical and sensory properties of salted Baltic herring. 1997, *in*: Materials of XXVIII Session of KTiChŻ PAN, 21–23 September 1997, Gdańsk, Poland, p. 193 (in Polish).
12. Lee H.G., Desiderio D.M., Optimization of the capillary zone electrophoresis loading limit and resolution of proteins, using triethylamine, ammonium formate and acidic pH. J. Chromatogr. B, 1997, 691, 67–75.
13. Li G., Zhang Z., Chen X., Hu Z., Zhao Z., Hooper M., Analysis of ephedrine in ephedra callus by acetonitrile modified capillary zone electrophoresis. Talanta, 1999, 48, 1023–1029.
14. Macedo Q., Jovilet P., Meunier J.-C., Capillary electrophoresis of caseino-peptides: influence of applied voltage and column temperature. Biotechnology Techniques, 1999, 13, 647–651.
15. Madsen J.S., Qvist K.B., Hydrolysis of milk protein by a *Bacillus licheniformis* protease specific for acidic amino acid residues. J. Food Sci., 1997, 62, 579–582.
16. Messana I., Rossetti D.V., Cassiano L., Misiti F., Giardina B., Castagnola M., Peptide analysis by capillary (zone) electrophoresis. J. Chromatogr. B, 1997, 699, 149–171.
17. Michaelsen S., Sørensen H., High performance capillary electrophoresis: principle, present possibilities and future potentialities in studies of low and high molecular weight charged and uncharged biomolecules. Pol. J. Food Nutr. Sci., 1994, 3/44, 1, 5–44.
18. Molina E., Ramos M., Cifuentes A., Lopez-Fandino R., Characterization of cheese proteolysis by capillary electrophoresis and reverse-phase HPLC analyses of peptides. Z. Lebensm. Unters. Forsch. A, 1998, 206, 259–263.
19. Mucha P., Rekowski P., Szyk A., Kupryszewski G., Barciszewski J., Capillary electrophoresis: a new tool for biomolecule analysis. Post. Biochem., 1997, 43, 208–216 (in Polish; English abstract).
20. Polish Standard PN-55/A-86761. Fresh fish. Chilling with ice during loading (in Polish).
21. Polish Standard PN 74/A-86739. Fish and fish products. Determination of table salt content (in Polish).
22. Oda R.P., Landers J.P., Introduction to capillary electrophoresis. 1997, *in*: Handbook of Capillary Electrophoresis (ed. J.P. Landers). CRC Press, Boca Raton, Boston, London, New York, Washington D.C., pp. 1–48.
23. Sarmini K., Kenndler E., Influence of organic solvents on the separation selectivity in capillary electrophoresis. J. Chromatogr. A, 1997, 792, 3–11.
24. Shihabi Z.K., Peptide stacking by acetonitrile-salt mixtures for capillary zone electrophoresis. J. Chromatogr. A, 1996, 744, 231–240.
25. Shihabi Z.K., Effects of sample matrix on capillary electrophoretic analysis. 1997, *in*: Handbook of Capillary Electrophoresis (ed. J.P. Landers), CRC Press, Boca Raton, Boston, London, New York, Washington D.C., pp. 457–478.
26. Shihabi Z.K., Stacking of weakly cationic compounds by acetonitrile for capillary electrophoresis. J. Chromatogr. A, 1998, 817, 25–30.
27. Song L., Ou Q., Yu W., Xu G., Effect of high concentrations of salts in samples on capillary electrophoresis of anions. J. Chromatogr. A, 1995, 696, 307–319.
28. Yang J., Bose S., Hage D.S., Improved reproducibility in capillary electrophoresis through the use of mobility and migration time ratios. J. Chromatogr. A, 1996, 735, 209–220.

Received April 2006. Revision received July and accepted August 2006.

## OPTYMALIZACJA WARUNKÓW ROZDZIAŁU TRICHLOROOCTOWYCH EKSTRAKTÓW MIĘSA SOLONEGO ŚLEDZIA BAŁTYCKIEGO METODĄ STREFOWEJ ELEKTROFOREZY KAPILARNEJ (CZE)

***Katarzyna Felisiak***

*Katedra Technologii Żywności i Przechowalnictwa, Akademia Rolnicza, Szczecin*

Zbadano wpływ parametrów CZE oraz substancji modyfikujących na jakość rozdziału trichlorooctowych (TCA) ekstraktów mięsa solonego śledzia bałtyckiego. Optymalne parametry rozdziału wynosiły: długość kapilary 36 cm, wielkości napięcia 20 kV, ciśnienie iniekcji 2 psi*s, temperatura kapilary 35°C, długość fali detekcji 200 nm. Dodatek do buforu acetonitrylu w stężeniu 20% oraz metanolu w stężeniu 40% powodowały nieznaczną poprawę jakości rozdziałów, zaś trietyloamina (TEA) w stężeniu 20 mmol/L prowadziła do pewnej poprawy, jednocześnie będąc źródłem dodatkowego piku w obszarze A (rys. 3). Wykazano, że stężenie soli ≥1% powoduje zlewanie się pików, zmniejszenie ich wysokości, aż do niemal całkowitego zaniku pików przy stężeniu 5% (rys. 4). Oczyszczanie próby metodami SPE (rys. 5) i dializy poprawiało rozdział, lecz było czasochłonne lub nie zapewniało zachowania pełnego składu próby. Strącanie jonów $Cl^-$ azotanem srebra nie przyniosło poprawy rozdziału. Obniżenie stężenia chlorku sodu poniżej 1% oraz kwasu trichlorooctowego do ok. 1,5% przez rozcieńczenie ekstraktu TCA wodą destylowaną dało najlepsze rezultaty (rys. 1). Pozwoliło to uzyskać dokładne rozdzielenie pików wszystkich obszarów, przy jednocześnie najmniejszym wkładzie pracy i przy zachowaniu pełnego składu próby.