Monitoring of Oligopeptides from Blue-veined Cheese during Ripening

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Abstract: The aim of this study is an isolation of oligopeptides from blue-veined cheese during different stages of ripening and sugests a method which allows their determination, especially electrophoresis. Extraction by water was used to isolate nitrogen compounds and the obtained extract was further fractionated and recleaned. First, high-molecular peptides and proteins were precipitated by methanol, second, the methanol-soluble fraction was further fractionated by gel permeation chromatography. The fractions obtained by this procedures were then analysed using capillary electrophoresis. The obtained results indicate that this procedure, may be applicable for isolation of oligopeptides from cheese allowing also determination of the individual peptides. This is necessary in particular for monitoring of formation and origin of bitter peptides in cheese, which cannegatively influence the final flavour of cheese.

Keywords: peptides; cheese; electrophoresis

INTRODUCTION

Cheese, milk and a variety of other products containing milk proteins are an integral part of the diet for much of the world's population. The overwhelming majority of these products currently are derived from bovine milk. The fresh cow's milk typically contains 3–3.5% protein, of which approximately 80% is casein, 15% whey proteins, and the remainder a variety of small molecules collectively termed nonprotein nitrogen. The casein fraction exists almost exclusively in spherical particles termed micelles, and is comprised of four major proteins, a_{S1}-, a_{S2}-, β- and κ-casein.

Peptides in cheeses arise from the proteolysis of milk casein and its fractions in the process of ripening of cheeses. The ability to assess the relative composition and integrity of proteins and peptides in cheeses and other dairy foods is important because these molecules have a profound effect on product functionality and quality [1].

Most methods for qualitative and quantitative determination of proteins and peptides require prior separation. An exception is capillary electrophoresis, which can provide rapid, high-resolution separation and good quantification of many compounds found in milk products.

An example is the application of capillary zone electrophoresis for the determination of small casein-derived peptides in mould cheese during different stages of the ripening [2, 3].

EXPERIMENTAL

Materials and methods. The CZE separation were performed using a PrinCE 460 capillary electrophoresis system (Prince Technologies B.V., the Netherlands), equipped with a fused-silica capillary (inner diameter 50 mm, total length 75 cm and effective length from injection to detector 50 cm). The backgroung electrolyte was phosphate buffer (0.1 mol/l, pH 2.5). This system was coupling with UV detector (wavelength 214 nm).The other condition were the follows: sample injection 30 s by pressure 50 mBar, voltage 25 kV, temperature 25°C.

Sample preparation. It was used a Czech blue-veined cheese Niva for analysis. It was prepared

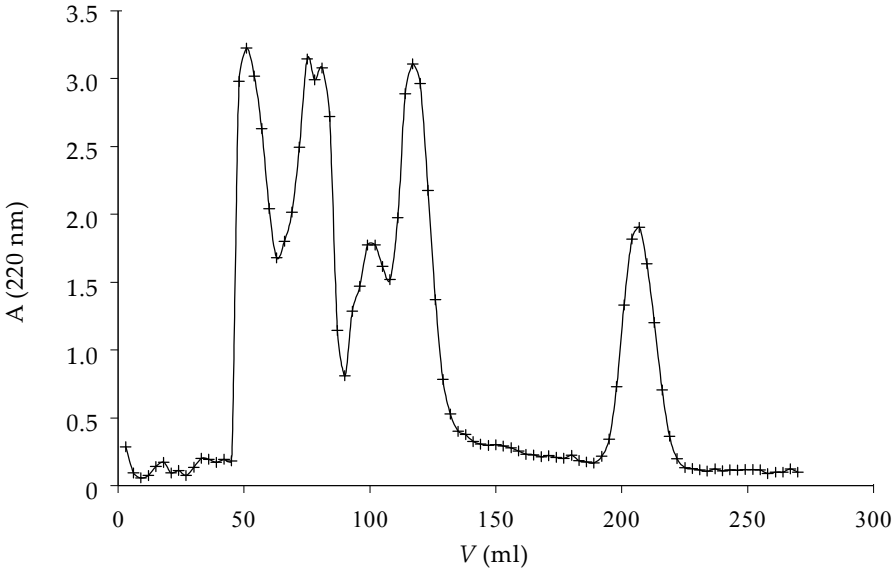

Figure 1. Gel permeation chromatography of blue-veined cheese Niva (30 days ripening)

an aqueous extraction by Kuchroo and Fox to isolate nitrogen compounds. Grated cheese was homogenized in a stomacher at 20°C for 10 min with twice its weight of water. The slurry was held at 40°C for 1 h, centrifuged and the supernatant filtered through glass wool and filter paper. The obtained extract was further fractionated and recleaned. First, high molecular peptides and proteins were precipitated by methanol, second, the methanol-soluble fraction was further fractionated by gel permeation chromatography on Sephadex G-15 (Amicon 16 × 500 mm, distilled water as mobile phase, flow 24 ml per an hour). The fractions obtained by this procedure were then analyzed using capillary zone electrophoresis [4–6].

RESULTS AND DISCUSSION

The obtained results indicate that this procedure, described above, is applicable for the isolation and the detection of small molecules and peptides that occur in aqueous cheese extracts. It would be suited for the monitoring of primary and secondary casein-breakdown in ripening cheese too. But it is necessary to use an other preconcentration step (e. g. solid phase extraction) for high dilution of samples by method of gel permeation chromatography.

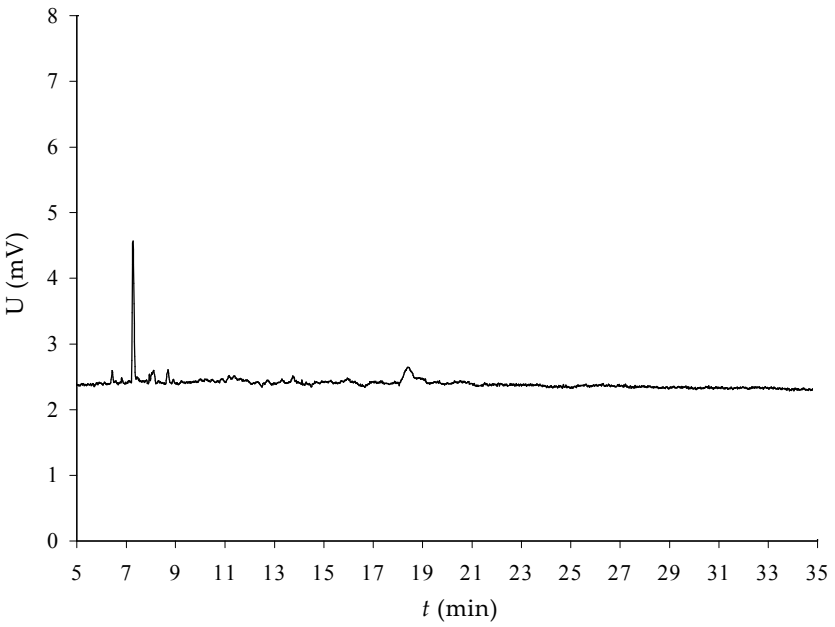

Figure 2. Electroforeogram of blue-veined cheese Niva (30 days ripening, fraction G15-f2)

CONCLUSIONS

Capillary zone electrophoresis (CZE) is a powerful microanalytical technique based on electrophoretic separation in narrow capillaries. The fast speed of analysis, high resolution and sensitivity make CZE an attractive method to separate a wide range of charged and uncharged compounds, including substances of food interest.

This analysis is necessary in particular for monitoring of formation and origin of bitter peptides in cheese, which can negativelly influence the final flavour of cheese.

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