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Journal of Chromatography A, 772 (1997) 231–234

JOURNAL OF
CHROMATOGRAPHY A

Short communication

Assessment of heat-induced denaturation of whey proteins

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Abstract

Milk proteins are significant for their sensitivity to heat so that they can act as suitable indicators for monitoring the heat treatment of milk. In the present work, raw skimmed milk was heated under laboratory conditions to 60°C, 74°C, 85°C and 90°C for 5 min and the extent of denaturation of major whey proteins (α-lactalbumin and β-lactoglobulin) was determined by using polyacrylamide gel electrophoresis (PAGE), CE and indirect competitive enzyme-linked immunosorbent assay (ELISA). ELISA showed higher sensitivity especially for lower temperatures of heat treatment. PAGE enables semiquantitative evaluation of whey protein fractions and is rather time consuming, while CE is a very rapid and quantitative method. The results given for denaturation were also compared to those yielded from theoretical kinetic equations.

Keywords: Whey; Milk; Protein denaturation; Food analysis; Proteins; Lactalbumin; Lactoglobulin

1. Introduction

During processing, milk is heated to various temperatures to ensure it is safe for human consumption and also to improve the yield or properties of some dairy products. Whey proteins constitute approximately 20% of milk proteins and are significant for their variable sensitivity to heat so they can act as suitable indicators for monitoring the heat treatment of milk. The main components of whey proteins are β-lactoglobulin (β-Lg) and α-lactalbumin (α-La). The former is of particular interest, since this protein contains two intramolecular disulfide bonds and one –SH group which, during heat treatment, are responsible for changes in flavour, properties of casein (adsorption and binding to κ-casein) and thus colloidal instability of some dairy products. Relatively little attention is paid to heat denaturation of α-La.

Knowledge of the precise mechanism of denaturation and aggregation is still inadequate, because the selection of a suitable method giving rapid and reliable results is not simple. Two main groups of methods for heat induced denaturation of whey proteins were tested in preliminary experiments: (i) assessment of quantity or a property of the whole casein or whey proteins fractions (determination of casein nitrogen—ADMI method [1], WPNI method [2], nondenaturated whey proteins using Kjeldahl (see Ref. [3]) and Bradford methods (see Ref. [4]), UV derivative spectroscopy [5]); (ii) assessment of individual whey proteins. Methods of (ii) give more information about the denaturation process. Therefore, further work was concerned with the determination of heat-induced denaturation of main whey proteins (β-lactoglobulin and α-lactalbumin) by CE,

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PII S0021-9673(97)00100-3

sodium dodecyl sulfate–polyacrylamide electrophoresis (SDS–PAGE) and indirect competitive enzyme-linked immunosorbent assay (ELISA) methods.

2. Materials and methods

2.1. Sample preparation

Raw milk samples were obtained from the dairy plant Trojská mlékárna, Prague. Milk was skimmed (4°C, 4000 rpm, 15 min) and samples (80 ml) were heated under laboratory conditions in a water bath with a stirrer to 60, 74, 85 and 90°C for 5 min and then immediately cooled. Caseins were removed by precipitation (1 *M* HCl, pH 4.6) and centrifugation (4000 rpm, 10 min). Whey samples were kept frozen until the next analysis.

2.2. Capillary electrophoresis

Standards and whey samples for CE were diluted by phosphate buffer (pH 2.5) and analysed with SpectraPhoresis 500 (UV detection at 214 nm). The capillary was untreated fused-silica [70 cm (63 cm to detector)×75 μm I.D.]. Samples were introduced by hydrodynamic injection and separated at a constant potential difference of 20 kV. This method was evaluated and tested by Otte et al. [6].

2.3. Other methods

Discontinuous vertical slab SDS–PAGE was conducted in 4% stacking gel and 15% running gel (82×86×0.75 mm slabs) according to Parnell-Clunies et al. [7]. After staining with Amidoblack 10B, the gel was scanned and evaluated by SigmaGel (Jandel Scientific Software).

Indirect competitive ELISA was carried out with polyclonal antibodies against α-La, β-Lg A and β-Lg B (made to order by Sevac, Prague, Czech Republic), as the anti-antibody swine anti-rabbit conjugate with peroxidase was used. The procedure is described by Fukal and Hadinec [8].

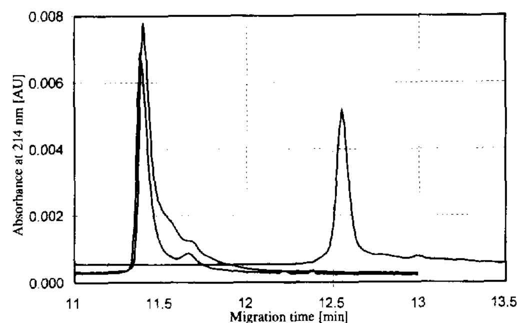

Fig. 1. CE of α-La and β-Lg. Conditions are described in the text. Migration times of β-Lg A, β-Lg B and α-La, were 11.41, 11.427 and 12.576 min, respectively.

3. Results and discussion

Separation of α-La and β-Lg by CE is shown in Fig. 1. Under used conditions α-La and β-Lg were well separated, but further development of this method is needed for better separation of genetic variants β-Lg A, β-Lg B and BSA. Comparison of the electropherograms of whey proteins isolated from raw skimmed milk and milk heated for 5 min indicated an influence of thermolabile components (e.g. immunoglobulins) on the peak shape (Fig. 2). This fact complicates the evaluation of the denaturation of the major whey proteins.

Used SDS–PAGE method gave good separation of the main components (Fig. 3). Some minor components near the start of gel were also changed by heating. To separate them sufficiently, the gel with

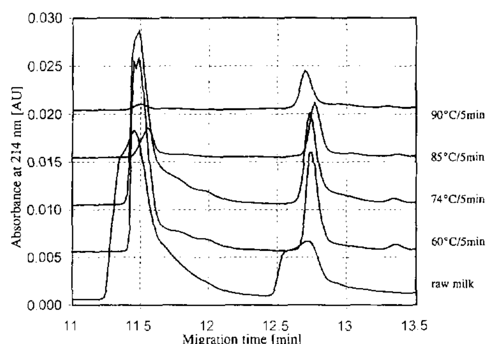

Fig. 2. CE of whey proteins. For better distinction, the electromigration profiles for 60, 74, 85 and 90°C, were shifted to absorbance units of 0.005, 0.010, 0.015 and 0.020, respectively.

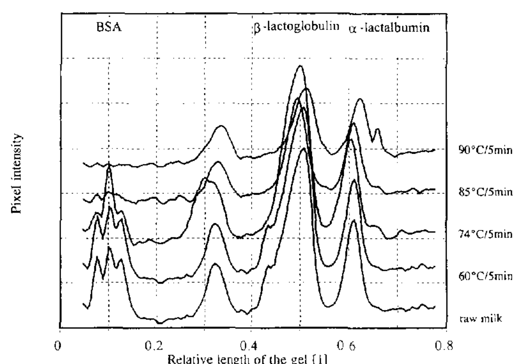

Fig. 3. SDS–PAGE of whey proteins. Conditions are described in the text. The peaks for α-La, β-Lg and BSA, belong to 0.6, 0.5 and 0.1, respectively, of the relative gel length.

lower acrylamide concentration is necessary. Whey proteins were quantified without an internal standard.

The higher denaturation detected by ELISA can be explained by the fact that the method is, due to more or less specific antigen–antibody interactions, sensitive to structural changes of proteins remaining in whey after heating. Interpretation of results can also be complicated by the fact that polyclonal antibodies were used. ELISA enables the estimation of genetic variants of β-Lg, but some cross reactions were observed.

The methods used for the determination of the extent of heat denaturation are compared in Fig. 4. The extent of denaturation is expressed in per cents. As for PAGE, owing to relatively high concentrations of the main whey proteins, the area of peaks given by integrated pixel intensity could be related to the content of whey proteins determined by the Kjeldahl method.

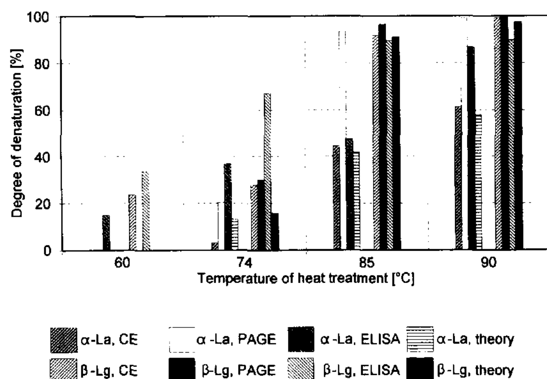

Fig. 4. Comparison of denaturation of α-La and β-Lg determined by CE, PAGE and ELISA, and obtained from theoretical equations [10].

Denaturation of α-La in milk heated at 60°C for 5 min is indicated only by ELISA; PAGE and CE showed no denaturation in this case. Relatively high difference occurred at 85°C when comparing to PAGE and other methods. Law et al. [9] used similar heat treatment conditions and observed lower denaturation (especially around 85°C) of α-La and β-Lg using the FPLC method.

Comparison with data obtained from kinetic equations derived by Dannenberg and Kessler [10] showed good consistency with the results given especially by CE. The authors used the method of isoelectric focusing to express the relations for whey protein denaturation.

4. Conclusion

It can be concluded that the results obtained by CE and PAGE showed the decrease of whey protein concentration after their partial binding on casein caused only by heat treatment. ELISA showed a higher sensitivity especially for lower temperatures of heat treatment when proteins could already have been partially denaturated but not yet reacted with casein. PAGE enables semiquantitative evaluation of whey protein fractions and it is rather time consuming. On the other hand, CE is a very rapid and quantitative method.

Acknowledgments

This project has been granted by the Grant Agency of the Czech Republic, project No. 525/96/0438.

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