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Karakterizacija glavnih proteina mleka različitog porekla elektroforezom korišćenjem Tris pufera visokog molariteta

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Characterisation of major milk proteins from different species by electrophoresis using a high-molarity Tris buffer

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The use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the characterisation of milk proteins has become a firmly established laboratory procedure. The most widely cited SDS-PAGE procedure is Laemmli procedure. Using this procedure no differences among protein profiles of different milk species was observed. Using the modified protocol, proposed by Fling and Gregerson (1984), the differences among protein profiles of bovine, caprine and ovine milk, could be detected. Increasing the molarity of Tris in the resolving gel and running buffer twofold over the Laemmli concentrations, yielded superior banding, resolution and detection of major milk proteins.

In this study we applied the modified technique and approved significant differences among electrophoretic properties of κ-caseins and paraκ-caseins of different species. The order of κ-caseins in electrophoretic gel according to increasing mobility was: bovine κ-casein, caprine κ-casein and ovine κ-casein. In the case of paraκ-caseins, the order was: caprine paraκ-casein, ovine paraκ-casein and bovine paraκ-casein. It is known that paraκ-casein is protein which does not show proteolytic changes during the cheeses ripening. Thus, the application of this procedure could be very effective in estimating the authenticity of milk species used for their production.
Characterisation of major milk proteins from different species by electrophoresis using a high-molarity Tris buffer

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Introduction

The use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for the characterisation of milk proteins has become a firmly established laboratory procedure. The most widely cited SDS-PAGE procedure is the Laemmli (1) modification of the discontinuous buffer system of Orstein and Davis (2). Using this procedure no differences between protein profiles of different milk species was observed. Fling and Gregerson (3) proposed the modified protocol. Sharp bands and high resolution were achieved in the wide range of protein molecular weights by increasing the molarity of Tris in the resolving gel and running buffer twofold over the Laemmli concentrations.

In this study we applied the modified technique to evaluate whether it is suitable for estimation the differences among electrophoretic patterns of proteins of bovine, caprine and ovine milk.

Sample preparation

SDS-PAGE

Samples of milk, whey proteins and caseins for SDS-PAGE were diluted to different extents (1.2 for bovine and caprine milk; 1.6 for ovine milk; 1.2 for bovine, caprine and ovine whey proteins; 1.3 for bovine casein and ovine caseins) with the sample buffer (0.055 M Tris-HCl, pH 6.8, 2% (wt/vol) SDS, 7% (vol/vol) glycerin, 5% (vol/vol) 2-mercaptoethanol, 0.002% (wt/vol) bromophenol blue).

The electrophoresis was carried out in 1.5-mm thick gels with 12.5% (wt/vol) separating gels and 5% (wt/vol) stacking gels. After boiling for 2 min, 20 μL of the cooled solution was loaded onto each well. The gels were run in a buffer solution [0.05 M tris(hydroxymethyl) aminomethane, 0.19 M glycine, 0.1% (wt/vol) SDS, pH 8.5] for 5 h to completion. Gels were fixed and stained with 0.23% (wt/vol) Coomassie brilliant blue R250 (dissolved in 3.9% (wt/vol) TCA, 6% (vol/vol) acetic acid, and 17% (vol/vol) methanol) for 1 h followed by two destaining steps with continuous agitation [18% (vol/vol) ethanol and 8% (vol/vol) acetic acid]. The stained gels were scanned and then analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA).

Results

Using the modified protocol, which proposed Fling and Gregerson, the differences between protein profiles of bovine, caprine and ovine milk could be detected. High quantity of κ-CN and low quantity of αs-CN could be observed for caprine milk. The electrophoretic pattern of ovine milk revealed high quantity in both αs-CN and αs2-CN, whereas significantly lower quantity of αs-CN than for κ-CN were established for bovine milk. Significant differences among electrophoretic properties of κ-caseins and para-k-caseins among different species could be noticed. The order of κ-caseins in electrophoretic gel according to increasing mobility was: bovine κ-casein, caprine κ-casein and ovine κ-casein. This could be due to small differences in molecular weights, different degree of phosphorylation and glycosylation of these proteins. It is known that many glycoproteins behave anomalously even when SDS and reducing agents are in excess, probably because they bind SDS only to the protein part of the molecule (4). The reduced net charge, resulting from reduced SDS binding, lowers the polypeptide mobility during electrophoresis, yealding artifically high molecular weight estimates. In the case of para-caseins, the order was: caprine para-casein, ovine para-casein and bovine para-casein. Possible explanation could be differences in amino acid sequences of these proteins, which implied to different extent of SDS binding (5). The other milk proteins showed no differences in electrophoretic mobility.

Discussion

Using the modified protocol, which proposed Fling and Gregerson, the differences between protein profiles of bovine, caprine and ovine milk could be detected. High quantity of κ-CN and low quantity of αs-CN could be observed for caprine milk. The electrophoretic pattern of ovine milk revealed high quantity in both αs-CN and αs2-CN, whereas significantly lower quantity of αs-CN than for κ-CN were established for bovine milk. Significant differences among electrophoretic properties of κ-caseins and para-k-caseins among different species could be noticed. The order of κ-caseins in electrophoretic gel according to increasing mobility was: bovine κ-casein, caprine κ-casein and ovine κ-casein. This could be due to small differences in molecular weights, different degree of phosphorylation and glycosylation of these proteins. It is known that many glycoproteins behave anomalously even when SDS and reducing agents are in excess, probably because they bind SDS only to the protein part of the molecule (4). The reduced net charge, resulting from reduced SDS binding, lowers the polypeptide mobility during electrophoresis, yealding artifically high molecular weight estimates. In the case of para-caseins, the order was: caprine para-casein, ovine para-casein and bovine para-casein. Possible explanation could be differences in amino acid sequences of these proteins, which implied to different extent of SDS binding (5). The other milk proteins showed no differences in electrophoretic mobility.

Conclusions

SDS-PAGE, according to the method of Fling and Gregerson, is suitable for determination the differences among protein profiles of bovine, caprine and ovine milk. Due to the fact that para-casein of bovine milk have the highest electrophoretic mobility than two other ones from caprine and ovine milk, the presented method could be useful analytical tool in the quantification of bovine milk adulteration. It is known that para-casein is protein which doesn't show proteolytic changes during the cheeses ripening. Thus, the application of this procedure could be very effective for estimation the authenticity of milk species used for their production.

References


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