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Serbian Chemical Society



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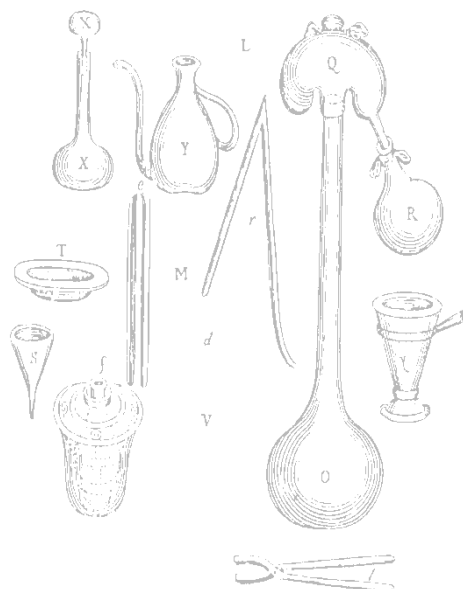
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Karakterizacija glavnih proteina mleka različitog porekla elektroforezom korišćenjem Tris pufera visokog molariteta

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Upotreba natrijum-dodecil-sulfat-poliakrilamidne gel elektroforeze (SDS-PAGE) za karakterizaciju proteina mleka različitog porekla je dobro poznata laboratorijska procedura. Najcitiranija SDS-PAGE procedura je po Laemmli-u. Korišćenjem ovog postupka ne uočavaju se razlike u proteinskim profilima različitih vrsta mleka. Primenom modifikovanog protokola, koju su predložili Fling i Gregerson (1984), razlike u proteinskim profilima kozjeg, kravljeg i ovčijeg mleka postaju uočljive. Dvostrukim povećanjem molariteta Tris-a u elektrodnom puferu i gelu za odvajanje, postignuta je bolja definisanost proteinskih traka, njihova rezolucija i detekcija glavnih proteina mleka.

U ovom radu primenili smo modifikovanu tehniku i pokazali značajne razlike u elektroforetskom ponašanju κ -kazeina i parak-kazeina analiziranih mleka. Redosled κ -kazeina prema rastućoj pokretljivosti u gelu bio je: kravljji κ -kazein, kozji κ -kazein i ovčiji κ -kazein. U slučaju para κ -kazeina redosled je bio: kozji parak-kazein, ovčiji parak-kazein i kravljji parak-kazein. Poznato je da je parak-kazein protein koji ne pokazuje proteolitičke promene tokom zrenja sireva. Zato upotreba ove procedure može biti vrlo efikasna za utvrđivanje autentičnosti mleka upotrebljenog za njihovu proizvodnju.

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-polyacrilamide 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 parak-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 parak-caseins, the order was: caprine parak-casein, ovine parak-casein and bovine parak-casein. It is known that parak-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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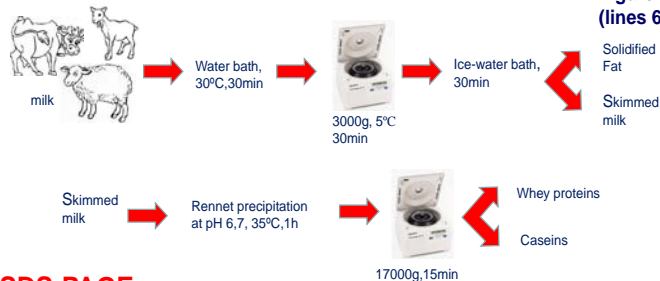
Results

Introduction

The use of sodium dodecyl sulfate-polyacrilamide 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 of 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 caprine and ovine caseins) with the sample buffer [0.055 M Tris-HCl, pH 6.8, 2% (wt/vol) SDS, 7% (vol/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 0.0025% (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 destained gels were scanned and then analyzed by SigmaGel software version 1.1 (Jandel Scientific, San Rafael, CA).



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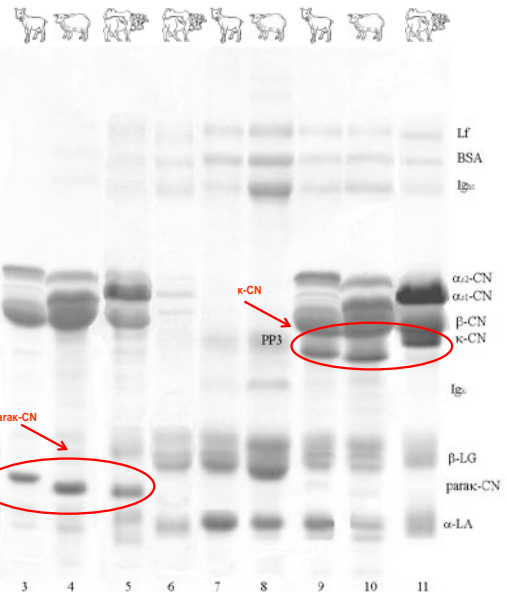


Figure 1. SDS electrophoregrams of bovine, caprine and ovine milk (lines 9-11), whey proteins (lines 6-8) and caseins (lines 3-5). Line 1 - LMW standards, line 2 -bovine milk protein standards

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 α₂-CN and low quantity of α₁-CN could be observed for caprine milk. The electrophoretic pattern of ovine milk revealed high quantity in both α₂-CN and α₁-CN, whereas significantly lower quantity of α₂-CN than for α₁-CN were established for bovine milk. Significant differences among electrophoretic properties of κ-caseins and parak-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, yielding artifactually high molecular weight estimates. In the case of parak-caseins, the order was: caprine parak-casein, ovine parak-casein and bovine parak-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 of the differences among protein profiles of bovine, caprine and ovine milk. Due to the fact that parak-casein of bovine milk has 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 parak-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 of the authenticity of milk species used for their production.