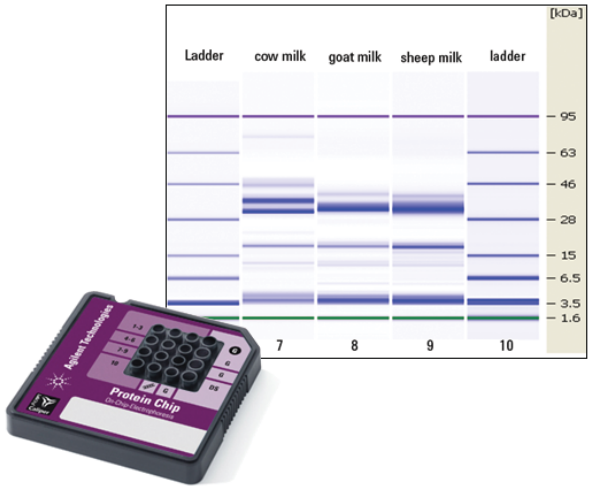
Milk protein analysis with the Agilent 2100 Bioanalyzer and the Agilent Protein 80 kit

Application Note

Food Analysis



Author

Rainer Nitsche

Agilent Technologies, Inc.

Waldbronn, Germany

Abstract

Protein content by type and amount is an important quality criterion in the analysis of milk from various animals. This Application Note describes the analysis of milk by on-chip electrophoresis with the Agilent Protein 80 kit and the Agilent 2100 Bioanalyzer. Advantages of this method for milk protein analysis in comparison to conventional SDS-PAGE include:

- Fast, reproducible and robust all in one method, replacing gel electrophoresis, staining and densitometric analysis
- Easy sample comparison by electropherogram overlay for protein assignment within a chip or in between chips
- Automated quantitative analysis for individual protein components

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Introduction

Cow milk contains approximately 33 g protein/L. The major milk protein fraction represents the caseins, which make up approximately 80% of the protein in cow milk (Table 1). The exact components of milk vary by species. Mammalian milk contains four different types of casein proteins αs1, αs2, β, and κ.

In addition to the caseins, milk contains a group of proteins known as the whey proteins; they make up the remaining 20% of the total proteins (Table 1). Whey proteins are typically a mixture of β-lactoglobulin, α-lactalbumin, serum albumin, immunoglobins and additional minor proteins and enzymes.

Cow milk protein	Amount [g/L]	% of total protein
Total protein	33	100
Total caseins	26	79.5
αs1	10	30.6
αs2	2.6	8.0
β	9.3	28.4
κ	3.3	10.1
Total whey proteins	6.3	19.3
α-lactalbumin	1.2	3.7
β-lactoglobulin	3.2	9.8
BSA	0.4	1.2
Immunoglobulins	0.7	2.1

Table 1
Average protein content in cow milk.[1]

Since the technological and nutritional characteristics of each protein are unique, it is of increasing interest to obtain qualitative and quantitative information about the main protein species in milk. Information on the occurrence and amount of a milk protein can provide useful information on technological treatments or fraudulent manipulations, for example, mixtures of milks of different species.

Various methods are currently used to analyze proteins in milk, dairy products and non-dairy products. This includes methods for the determination of the total protein, determination of milk protein fractions (for example, caseins, whey proteins, β-lactoglobulin), and various methods such as electrophoresis, liquid column chromatography and immunochemical tests, to detect and individually quantitate various milk proteins.[1] Electrophoresis is the most widely used procedure for phenotyping individual cows, distinguishing different species and for determining the main proteins in raw milk. Milk protein electrophoresis is commonly performed in the presence of urea, to dissociate protein aggregates, and a reducing agent, DTT or β-mercaptoethanol, to disrupt disulfide bridges.

Here we demonstrate milk protein analysis using on-chip electrophoresis. The Agilent 2100 Bioanalyzer employs microfluidic capillary gel electrophoresis with laser-induced fluorescence (LIF) detection for protein separation and detection, where fluorescence intensities of proteins are measured as a function of their migration times. Ten protein samples are analyzed in less than 40 minutes on their protein size distribution and protein quantities. Data analysis is performed with the Agilent 2100 Expert software, which automatically determines molecular weight, concentration, and % of total of the individual proteins in the sample.[2]

Experimental

Materials and equipment

Individual milk proteins were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA). Cow, goat and sheep milk was obtained from a local grocery store. The 2100 Bioanalyzer and Agilent Protein 80 kit were obtained from Agilent Technologies GmbH (Waldbronn, Germany).

On-chip electrophoresis

The on-chip protein analysis was performed on the 2100 Bioanalyzer using the Protein 80 kit. The milk or milk protein samples were denatured as specified in the Protein 80 Reagent Kit Guide[3] in the presence of the reducing agent DTT. Chips were prepared according to the Reagent Kit Guide and analyzed directly on the 2100 Bioanalyzer. Commercially available milk samples were diluted 1:10 with deionized water before sample preparation.

Results and discussion

On-chip electrophoresis of milk proteins

The four bovine caseins and α-lactalbumin and β-lactoglobulin can be separated by SDS-PAGE in the presence of a reducing agent. Listed in the order of increasing mobility: α-lactalbumin < β-lactoglobulin < κ-casein < β-casein < αs1-casein < αs2-casein.[1,4]

For comparison, the five major milk proteins were analyzed individually on the 2100 Bioanalyzer using the Protein 80 kit . Figure 1 shows the overlay of the five different electropherogram traces and the corresponding gel-like images.

The migration behavior observed with the 2100 Bioanalyzer is comparable (Figure 1) to what is expected from SDS-PAGE. α-lactalbumin and β-lactoglobulin migrate according to their molecular weight (Table 2). All caseins migrate at higher molecular weights than expected (Table 2). Usually such migration time shifts as observed for the caseins derive from their tendency to form aggregates, or other chemical properties, such as glycosylation, phosphorylation pattern and overall hydrophobicity, that influence protein structure and interaction with the gel matrix during separation. Especially for the κ-casein, this effect

is very prominent, shifting it from an expected 19 kDa to approximately 46 kDa (Figure 1 and Table 2). However, this is a known effect using on-chip protein electrophoresis[5] and it is reproducible. In addition, this shift in apparent moleculer weight greatly helps with resolution and subsequent detection and quantitation of the individual milk proteins. For example, if κ-casein was migrating at its expected molecular weight of 19 kDa, it would not be resolved from β-lactoglobulin migrating at 18 kDa. The data thus demonstrate that the Protein 80 assay kit for the 2100 Bioanalyzer is suitable for the analysis of the five major proteins present in milk. It provides a fast and standardized tool for milk protein analysis.

Milk protein	Expected MW [kDa]	2100 Bioanalyzer [kDa]
α-casein	23/25	37
β-casein	24	33
κ-casein	19	46
β-lactoglobulin	18	18
α-lactalbumin	14	12

Table 2
Expected molecular weight of the five analyzed milk proteins compared to the apparent molecular weight as determined by the 2100 Bioanalyzer.

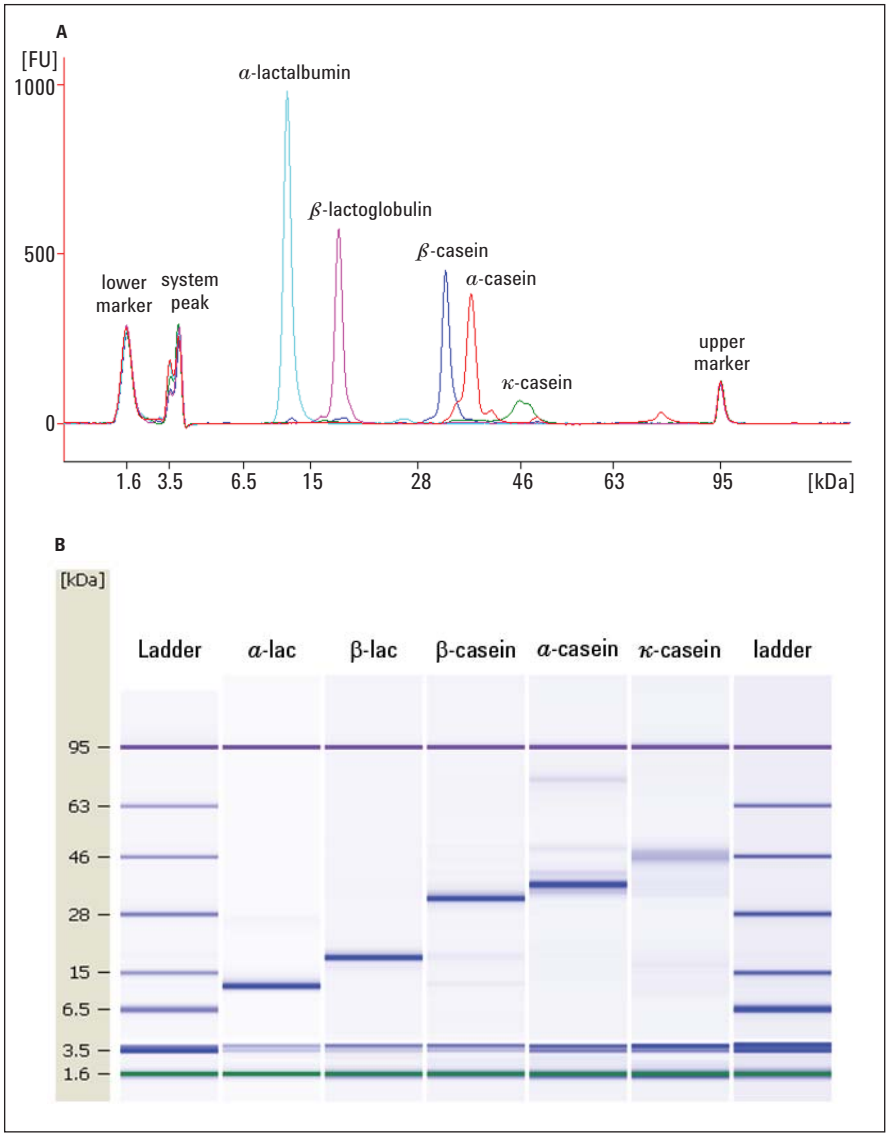

Figure 1
The milk proteins α-lactalbumin, β-lactoglobulin, κ-casein, β-casein, α-casein were analyzed with the Protein 80 kit. (A) shows the overlay of the five individual electropherogram traces, (B) shows the corresponding gel-like images.

Analysis of milk proteins in cow milk

Next, cow milk and the purified five major milk proteins were analyzed together in one data set to investigate if they can be readily identified in the original milk sample. Figure 2A shows the typical pattern obtained when analyzing commercially available cow milk with the Protein 80 kit. The main milk proteins are readily identified when overlaying the cow milk electropherogram with the electropherograms of the individual milk proteins (Figure 2B). The automated peak detection and analysis of the Agilent 2100 Expert software make the densitometric analysis of gels obsolete. The depiction as electropherogram by the 2100 Expert software allows an easy and reliable comparison of different samples, even when analyzed on different chips.

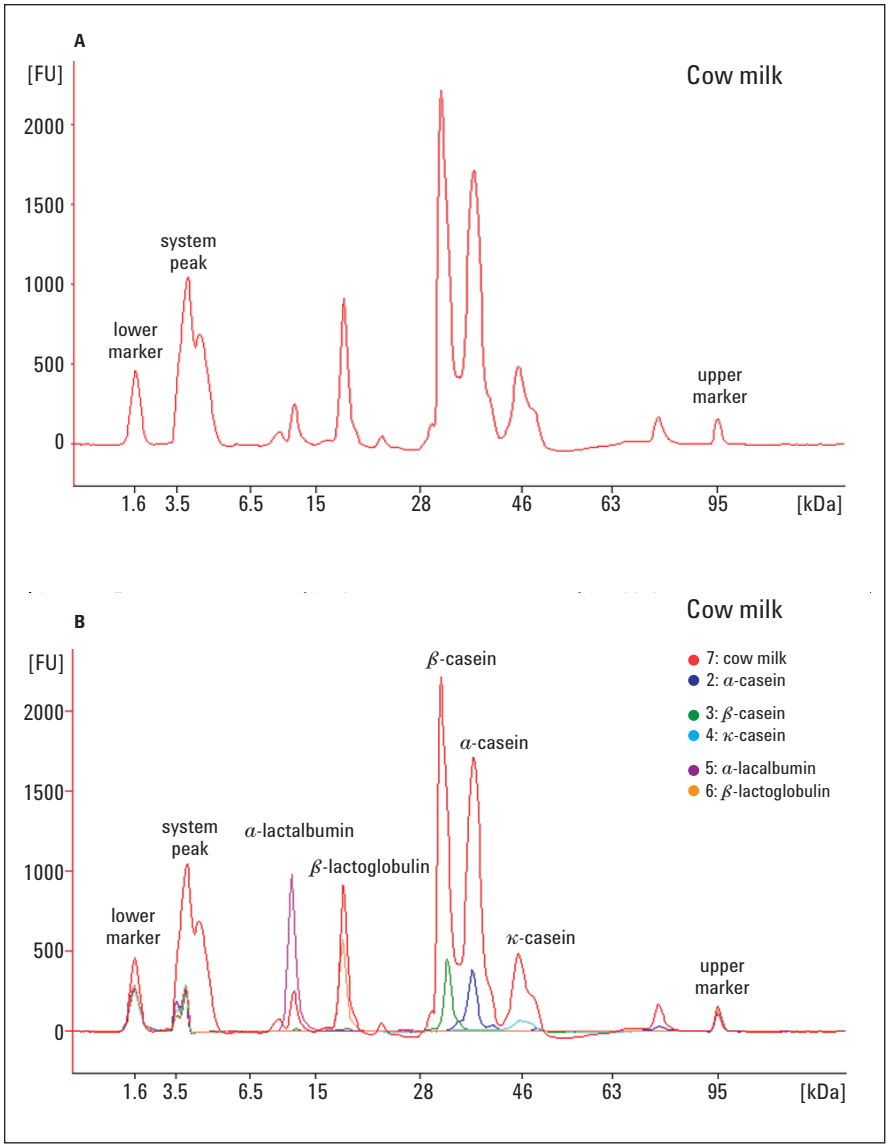

Figure 2
Analysis of milk proteins in cow milk. (A) shows the electropherogram trace of commercially available cow milk when analyzed on the Agilent 2100 Bioanalyzer. (B) shows an overlay of electropherogram traces of cow milk of the individual five most abundant milk proteins.

Analysis of milk from different species

The analysis of commercially available milk from cow, goat and sheep with the Protein 80 kit on the 2100 Bioanalyzer is shown in Figures 3 and 4. The electrophoretic pattern derived from goat (B) and sheep (C) milk is fairly similar, but the electropherogram of cow milk (A) shows a significantly different protein pattern. Two additional prominent peaks are observed in cow milk at 38 kDa and 45 kDa, which only represent minor components for the other two species. When overlaying the cow milk electropherogram with the individual milk proteins (Figure 2B), the 38 kDa peak can be attributed to α-casein and the 45 kDa peak to κ-casein. This indicates that this cow milk sample contains a higher percentage of α- and κ-casein as compared to sheep and goat milk.

The exact composition of milk varies significantly by species. It is known that α-casein is the major protein in cow milk, whereas β-casein is the major protein found in goat or sheep milk. The percentage of α-casein is higher in sheep's milk than in goat's milk, but significantly lower than in cow's milk. β-casein represents 1/3 of the total casein in cow's milk, 2/3 in goat's milk and 50% in sheep's milk.[1] Such variations in casein composition explain the observed differences in micelle structure, coagulation properties, taste and cheese making properties.

The whey protein composition also varies from species to species and during lactation. In the example shown here, the cow milk sample contains approximately 3% α-lactalbumin, 13% β-lactoglobulin, 32% β-casein, 36% α-casein and 11% κ-casein as automatically determined by the 2100 Expert software (Table 3).

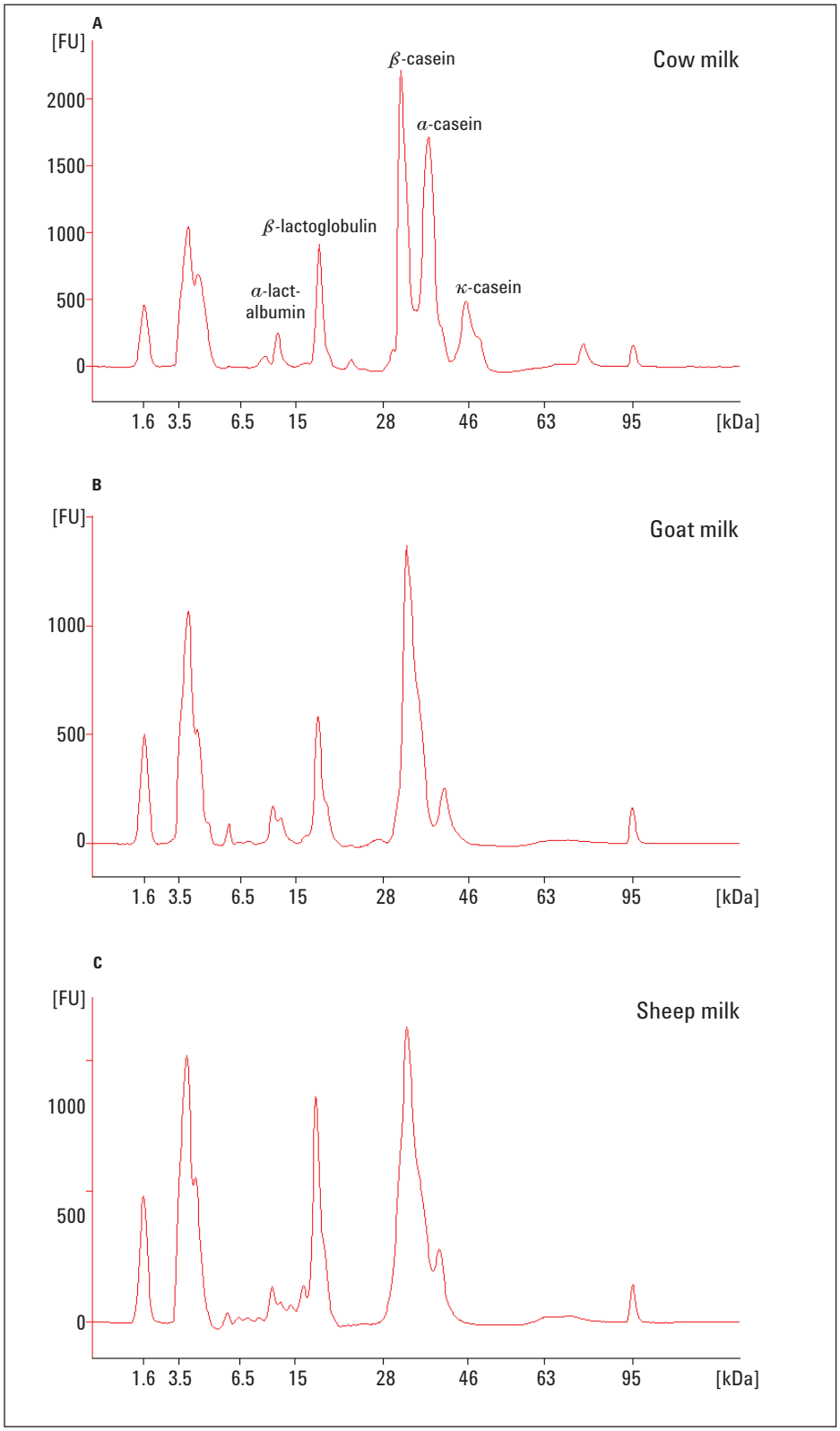

Figure 3
Analysis of milk from different species. Electropherogram traces obtained from an Agilent 2100 Bioanalyzer analysis are shown: (A) cow, (B) goat, and (C) sheep milk.

Conclusion

The Protein 80 kit and the 2100 Bioanalyzer are ideally suited to replace SDS-PAGE and densitometry for the analysis of dairy products such as milk. The main protein fractions can be identified by running the individual purified milk proteins for comparison. Furthermore, milk from different species could be distinguished based on their protein pattern which facilitates a fast incoming inspection in routine labs. In addition the 2100 Expert software provides tools for quantitative analysis of individual protein components[6].

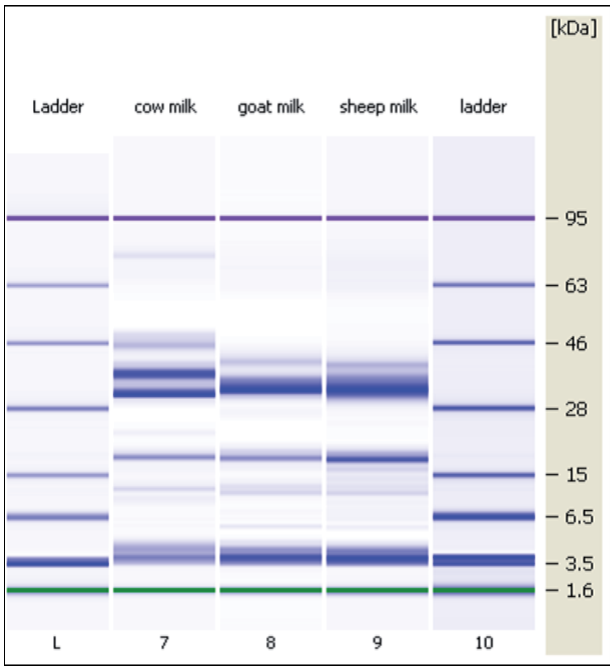

Figure 4
Electrophoretic patterns in different types of milk. Gel-like image derived from an Agilent 2100 Bioanalyzer analysis of cow, goat, and sheep milk.

Peak Size [kDa]	Rel. conc. [ng/µL]	% total	Observations
1.6	0.0	0.0	Lower marker
4.0	0.0	0.0	System peak
4.5	0.0	0.0	System peak
10.3	80.9	1.3	
12.3	207.0	3.4	α-lactalbumin
16.6	27.1	0.4	
18.4	768.5	12.7	β-lactoglobulin
23.3	41.5	0.7	
30.1	79.3	1.3	
31.7	1,968.6	32.4	β-casein
37.5	2,152.9	35.5	α-casein
45.5	657.1	10.8	κ-casein
77.1	89.8	1.5	
95.0	60.0	0.0	Upper marker

Table 3
Protein composition of cow milk as analyzed by the Agilent 2100 Bioanalyzer. Detected protein peaks are automatically assigned their molecular weights, their concentrations in the sample, and their overall contributions to the total protein composition in the sample.

References

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