

Analysis of Casein and Whey Protein in Whole, 2%, and Skim Milk by Capillary Gel Electrophoresis

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Introduction

Milk is an important and popular source of nutrition that is widely consumed around the world. Milk for human consumption can be obtained from a number of domesticated animals including sheep, goat, buffalo and cow, whose milk is by far the most consumed. Fresh cow milk contains approximately 3.5% protein, 80% [casein](#), 15% whey protein, as well as vitamins, and lipids, all of which provide necessary ingredients for growth.

It is well documented the composition of milk changes between breeds, feed and of course species. Because they make up a significant proportion of the content, milk proteins have been a focus of research in order to better understand its makeup, quality, and health-related properties. Historically, these proteins have been analyzed using techniques like total nitrogen analysis, spectroscopy, and ELISA strategies. More recently, analytical technologies such as capillary electrophoresis (CE), liquid chromatography and immunochemistry have gained in popularity as methods by which to analyze these proteins. Capillary zone electrophoresis (CZE) has proven to be a very important analytical tool in the phenotyping of individual cows for characterization of proteins in raw milk as well as determining whether the milk has been adulterated¹. The principle of CZE is separation based on differences in mass to charge ratios of a protein and its isoforms thus providing heterogeneity information. We propose that CE-SDS can be used orthogonally as an automated analytical technique by which to obtain quantitative information about milk proteins.

In this bulletin, analysis of [casein](#) and whey protein in bovine milk (whole, 2%, and 1%) including protein fractionation by the Rowland Method and sample preparation for CE-SDS analysis on the PA 800 *plus* system is described.

Experimental

Protein standards:

Whey proteins α -lactalbumin, β -lactoglobulin, and bovine serum albumin in addition to α , β , and κ [casein](#) standards were purchased from Sigma Aldrich and used without further purification. A stock solution of whey proteins was prepared in distilled and deionized (ddi) water at a concentration of 10 mg/ml. The [casein](#) stock solutions were also prepared in 1 x PBS (phosphate buffered saline) at a concentration of 10 mg/ml. These solutions were stored at 4°C until used.

Charged
by possibilities.



Preparation of Whey proteins:

Bovine whole, 2%, and 1% milk was purchased from a local market. [Casein](#) was prepared by precipitation using HCl (Masci et al.²). A 10 ml volume of each milk sample was brought to room temperature and adjusted to pH 4.0 using 2.0 M HCl while subjected to constant shaking. The resulting curdled material corresponding to the [casein](#) fraction was separated from the whey fraction by centrifugation at 1800g for 20 min at room temperature. One ml of 0.2 M acetate buffer pH 4.0 was added to 2 ml of whey and stored at room temperature for 15 min then centrifuged for 15 minutes at 1800g also at room temperature. The supernatant was recovered and filtered through a 0.45 µm syringe filter. The resulting solution was diluted by mixing equal volume of the supernatant and ddi water and stored at 4°C until use.

Preparation of whey protein for CE SDS analysis:

The Beckman Coulter SDS MW Assay (Beckman Coulter PN 390953) was used in the preparation of samples for separation on the PA 800 *plus* instrument. Fifty microliters of sample were thoroughly mixed with 45 µl of CE SDS sample buffer, 2 µl of 10 kD internal marker, and 5 µl of beta-mercaptoethanol and then heated at 100°C for 3 minutes.

Preparation of Caseins:

The [casein](#) fraction was treated as described by Olieman et al.³. The precipitate was washed 3 times with 20 ml of acetate buffer (4% acetic acid and 0.4 M sodium acetate, pH 4.6). In the case of the whole milk sample, an extra wash with 10 ml of dichloromethane was performed to remove lipids, followed by another wash of 10 ml of acetate buffer pH 4.6. The resulting [casein](#) clean fraction was dried, collected, and frozen at -20°C until use.

[Casein](#) Sample Buffer (300 mM of Tris, 20 mM of EDTA, 0.1M urea and 10 mM DTT):

In a 50 ml flask containing 20 ml of ddi water were dispensed 1.82 g of Trizma base, 0.37 g of EDTA and 0.3 g of Urea, followed by the addition of 0.5 ml of 1 M DTT solution. ddi water was added to a final volume of 50 ml. It may take up to 15 minutes for the complete dissolution of all solids. This solution must be prepared fresh daily.

Preparation of Caseins for CE SDS analysis:

300 mg of [casein](#) derived from the three varieties of milk was each mixed with 700 µl of the [casein](#) sample buffer. The casein fraction generally does not dissolve completely and was therefore left to incubate for 30 min at room temperature. The mixture was centrifuged at 14,000 g for 10 min. The solid was discarded and the supernatant filtered through a 0.45 µm syringe filter. This solution was used for CE SDS analysis.

Preparation of Caseins for CE SDS analysis:

50 µl of each filtered sample was thoroughly mixed with 45 µl of CE SDS sample buffer, 2 µl of 10 kD internal standard, and 5 µl of β-mercaptoethanol. This mixture was heated at 100°C for 3 minutes.

Workflow:

The sample preparation workflow for both whey and casein species is illustrated in Chart 1.

Instrumentation:

All centrifugation steps were carried out using a Beckman Coulter Allegra X-22 Centrifuge equipped with the SX4250 rotor with tube inserts for 50 ml or 15 ml Falcon® tubes.

The CE SDS experiments were carried out on a PA 800 *plus* Pharmaceutical Analysis System equipped with a PDA detector. The methods used for CE SDS separations including capillary conditioning, CE SDS separations, and shutdown method were performed as described elsewhere⁴.

Results and Discussion

CE SDS of whey proteins:

Figure 1 illustrates CE SDS separation for whey proteins following HCl extraction from whole, 2% and 1% milk and the molecular weight size ladder used to calculate their respective MW. The molecular weights for these proteins were estimated based on interpolation of the observed migration time for each of the protein peaks into a quadratic fit of molecular weight size standard. Peak assignments were performed based on the migration time observed for the individual whey protein standards run separately (data not shown). The molecular weight values found were 13 kD, 18 kD and 59 kD for α -lactalbumin, β -lactoglobulin, and BSA respectively. These values are in very good agreement with those reported in literature⁵.

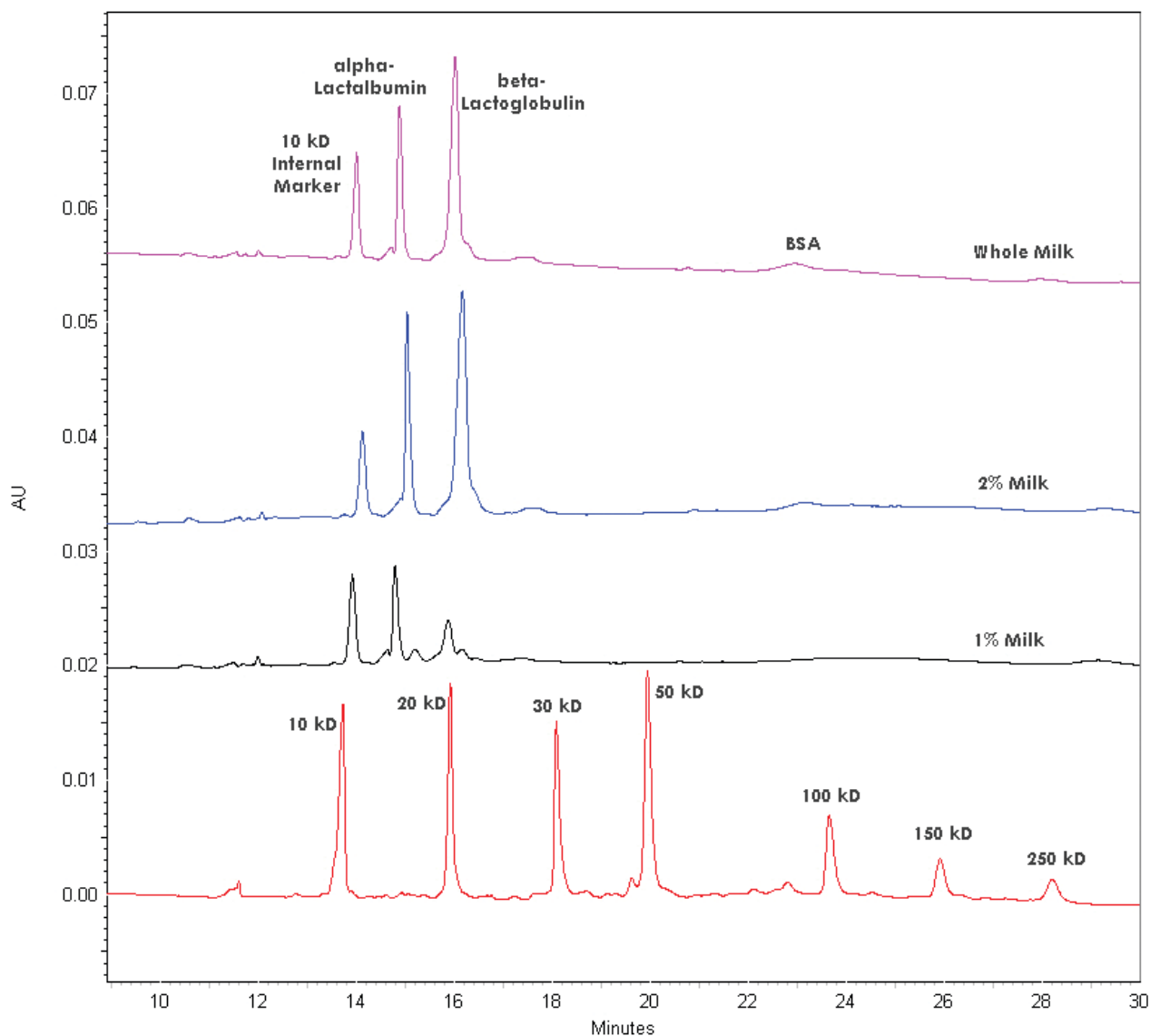


Figure 1. CE-SDS separations of milk whey proteins extracted with HCl.

Using CE-SDS MW it is also possible to estimate the quantity of whey proteins in milk. For this purpose, a calibration curve using purified commercial protein standards at various concentrations was constructed. Figure 2 illustrates the dilution curve obtained for separation of these standards.

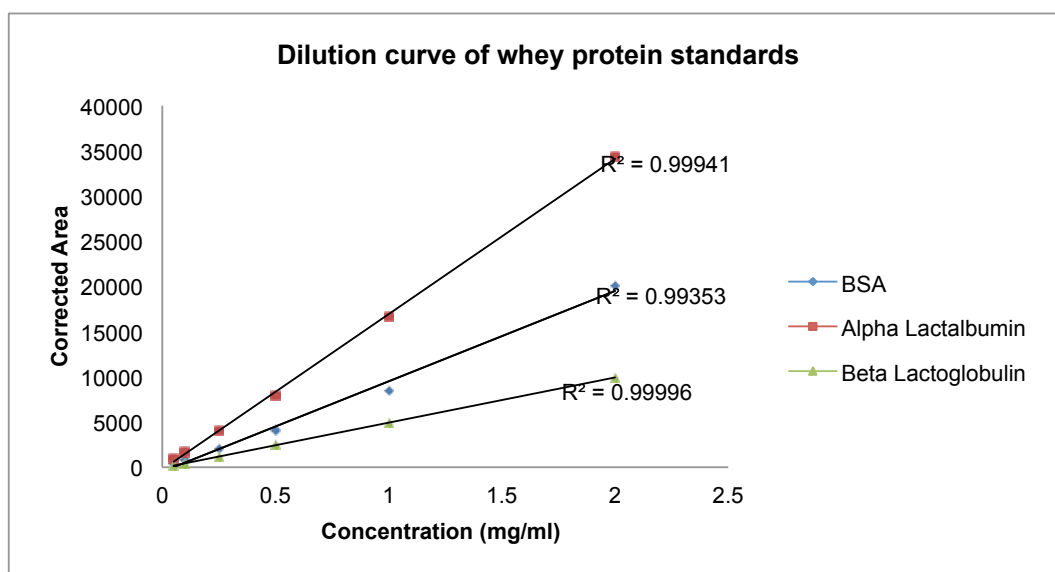


Figure 2. Calibration curve of milk whey protein commercial standards using CE-SDS.

By interpolating the corrected area of each milk protein from the calibration curve in fig. 2, the concentrations projected are in good agreement with values found in the literature for cow milk⁴. Table 1 shows the estimated amount of whey proteins present in the different types of milk studied compared to the average amount of whey protein found in cow milk⁵⁻⁷. Since the variations in the composition of milk are dependent on factors such as breed and feed, this can account for the slight difference found between average values in cow milk versus the amount found using CE-SDS. The difference between whole, 2% and 1% milk is related to their overall fat content and these 3 types of milk must contain no less than 8.25% of solid-not-fat (SNF)⁶. SNF consists of proteins, including whey, caseins and others, lactose, carbohydrates, water soluble minerals, and vitamins⁷. Our results indicate that both whole and 2% milk contain a very similar protein composition making up the whey fraction. Alternatively, whey in 1% milk was present at consistently lower concentrations for each of the major whey species identified. Although it is not clear why, the difference may be due to losses during milk filtration steps or possibly during sample preparation.

Milk Type	Whey Proteins (mg/ml)		
	alpha-Lactalbumin	beta-Lactoglobulin	BSA
1% milk	0.44	0.98	0.05
2% milk	0.65	4.4	0.25
whole milk	0.62	4.2	0.2
Average protein content in cow milk (ref. 4)	1.2	3.2	0.4

Table 1. Amount of whey protein found in whole, 1% and 2% milk using CE-SDS assay.

Analysis of the [casein](#) fraction extracted from whole, 2% and 1% milk by CE-SDS is illustrated in Figure 3

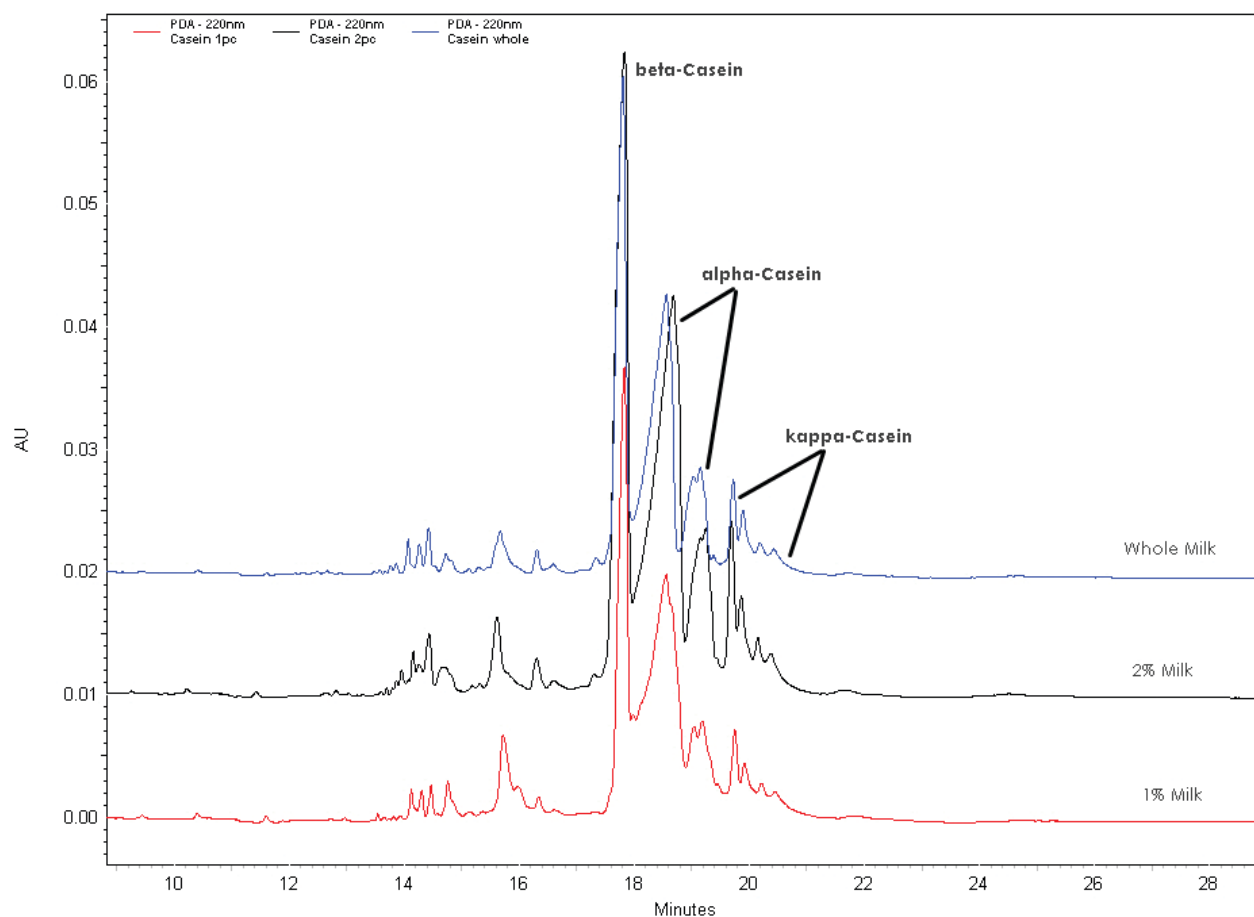


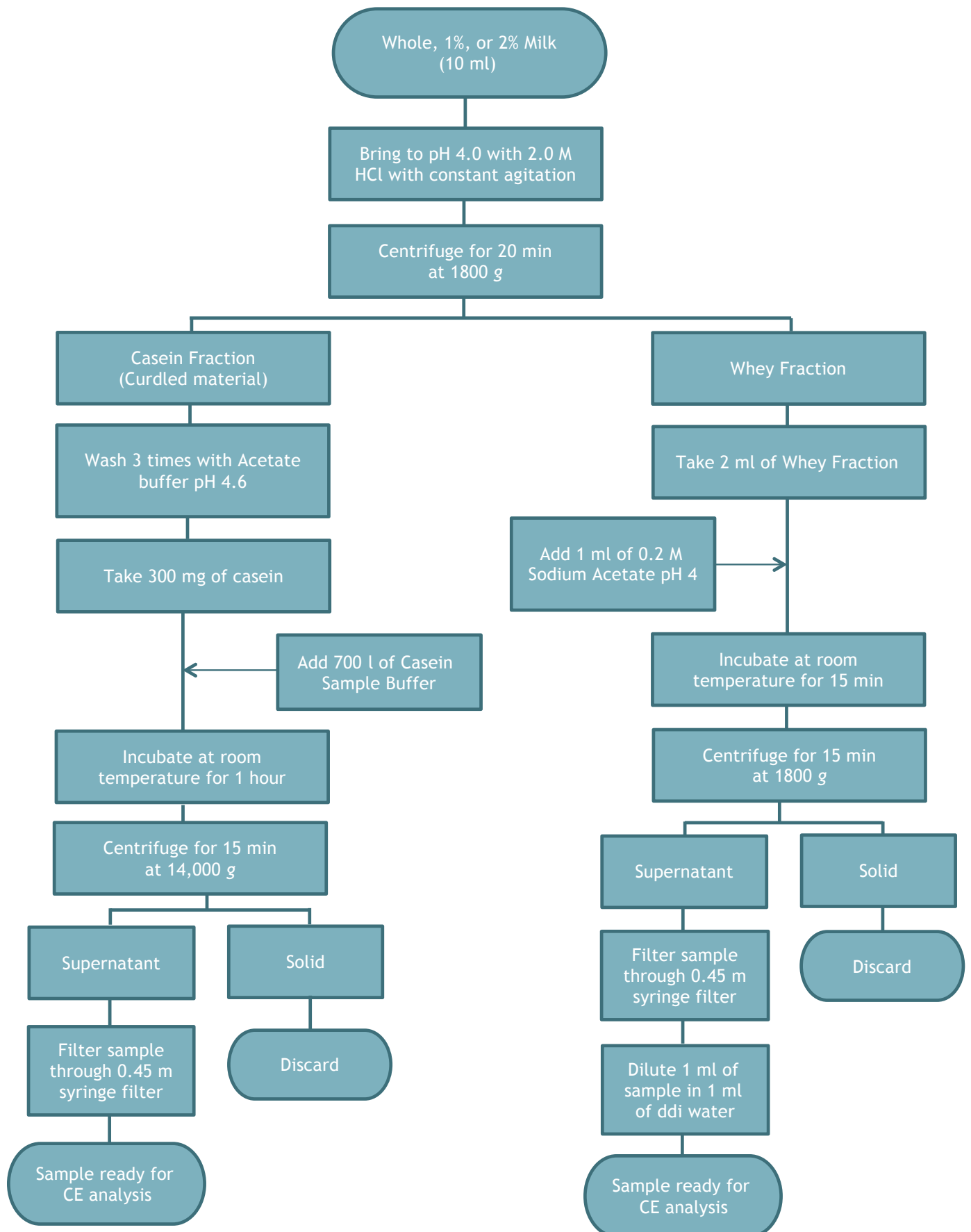
Figure 3. CE-SDS separations of caseins derived from whole, 2% and 1% milk.

Compared to whey proteins, [casein](#) content is expected to be similar between whole, 1% and 2% milk. Although we have not yet performed quantitative work on [casein](#), visual analysis of this fraction separated using CE-SDS revealed the different milk types were reasonably similar to one another (Fig. 3).

Conclusion

The data presented here illustrates that capillary electrophoresis can be used to obtain protein separation information for complex matrices like milk. This type of analysis is important as it provides a means by which to assess protein quality and quantity in the area of food safety and quality control. Although we may have experienced losses during sample preparation, the highly resolving separation conditions provided using the Beckman Coulter CE SDS gel in combination with the PA 800 *plus* instrumentation offers a robust platform by which to analyze samples like milk.

Chart 1. Milk Sample Preparation Workflow



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