

## Analysis of Different Types of Milk Proteins Using SDS-PAGE Electrophoresis Technique

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### Abstract

Electrophoresis is a method for separating particles or group of electrically charged particles. Protein properties to move in electric field are used to separate them from a mixture. The most powerful of the electrophoretic separation of proteins is that using this work polyacrylamide. In gels were analyzed proteins present in cow and goat milk, milk for human consumption, using SDS-PAGE electrophoresis technique.

**Keywords:** SDS-PAGE electrophoresis, milk proteins.

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### 1. Introduction

Electrophoresis is a method for separating particles or assemblies of charged particles under the action of a uniform electric field. The property sits on this method of electrically charged particles to move or migrate in electric field to the anode (+) or to the cathode (-). Electrophoresis can be used both analytical and preparative purposes [1].

Depending on the environment in which the electrophoresis separation can be of two types: the environment free and stable environment. Technique used for separating proteins present in milk in medium stabilized electrophoresis on polyacrylamide gel.

The electrophoresis methods, polyacrylamide gel electrophoresis (PAGE) plays a particularly important role. This method can be used to separate different types of membrane proteins, ribosomal protein, the histones, viral and bacterial proteins, etc [2].

Depending on working conditions, polyacrylamide gel electrophoresis can be: undistorted (PAGE) and electrophoresis distorted (SDS-PAGE).

Method undistorted (PAGE) separates proteins native to the ratio between electric charge and

their molecular mass. Proteins retain their integrity.

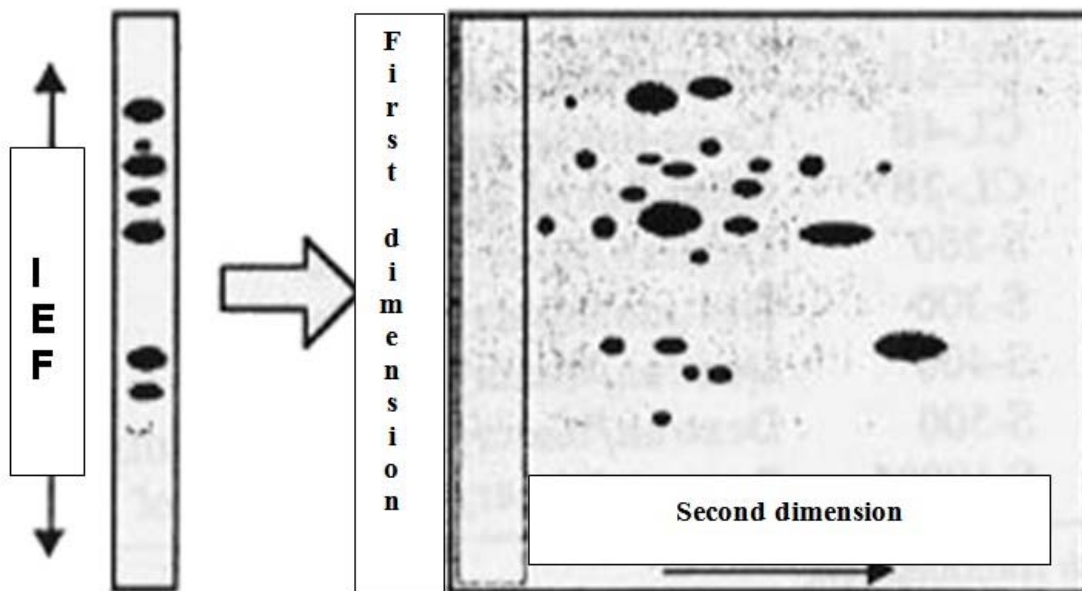
In the case of the denatured method (SDS-PAGE) in the gel is added an ionic detergent (negatively charged) SDS - sodium dodecyl sulphate. Samples for analysis are themselves treated, hot, with SDS and 2-mercaptoetanol. Detergent determines the break of the links between different proteins and lipid-protein interactions and binds to molecules of protein stoichiometry. Under the 2-mercaptoetanolului breakage occurs inter-and intermolecular disulfide linkages in protein structure, thereby separating oligoproteines subunits.

In the undistorted electrophoresis, protein bands can be recovered from the gel and subjected to further investigation (e.g. sequentialized, mass spectrometry, functional and immunological tests). Dimensional electrophoresis is a method mostly used in the analysis of protein mixtures in biological samples and was first introduced by P.H. O' Farrell in 1975 [3].

This is a technique similar to two-dimensional HPLC chromatography, separation is accomplished in two distinct phases, based on two different properties (Figure 1). The first dimension allows separation of proteins depending on pH and

is called each characteristic Isoelectric focusing (IEF). The second dimension is actually a classical electrophoresis / SDS-PAGE) allows

separation of proteins according to their molecular mass [4].



**Figure 1:** Separation of proteins by two dimensional electrophoresis

Initially, samples must be processed through various techniques that aim at different types of protein solubility. Processing of samples is performed in the presence of urea, various detergents and reducing agents that provide both protein solubility and distortion, phase strictly necessary to enable the separation of IEF. Urea is to help the solubilization and distort proteins. It is used in concentrations of about 8M. To improve solubility may be used in concentrations greater than or urea may be replaced with thiourea [5].

Non-ionic detergents such as Triton X100 or CHAPS, in concentrations between 0.5% and 4% are used for a complete solubility and to prevent protein aggregation by hydrophobic interactions. Dithiothreitol is used as reducing agents (DTT) and Dithiothreitol (DTE), in concentrations of 20 to 100 mm and are designed to cleave disulfide bonds present in the protein [6].

Whatever type of electrophoresis used (mono or two-dimensional) is an important step to separate proteins viewing. There are several ways in which such views, the choice of optimal method taking into account the amount of protein present in the sample (sensitivity) and separate purposes

(whether subsequent analysis of proteins separated).

Two methods are commonly used in this view: method silver nitrate staining method with Coomassie blue.

The method of protein staining with silver nitrate is highly sensitive, allowing highlighting a number of separate proteins, 1000 to 2000 different spots [7]. Coomassie blue method is about 50 times less sensitive than silver nitrate method (detects a concentration of 100 ng / spot separately) but it is better to use the separated proteins must be analyzed by densitometry and mass spectrometry [8].

## 2. Materials and methods

Like all electrically charged molecules and proteins can move in an electric field, this property being used for separation of a mixture. The most efficient separation of proteins is electrophoresis, using polyacrylamide gels.

Clarified milk samples were dissolved in Laemmli buffer and heated for 5 minutes at 95° C and then deposited on the gel (15 µ l / sample).

Laemmli Swab contains a migration marker (blue bromphenol), an anionic detergent (sodium dodecylsulphate, 20%), an agent for reducing disulphide linkages (3% DTT) and glycerol 20%, all dissolved in a solution 125 mM Tris-HCl buffer pH 6.8.

Separation of proteins was performed using a Bio-Rad Mini Cell gel. Migration was performed in a first step to 80V, samples crossed gel concentration (Polyacrylamide gel 4.5%). After crossing the separation of proteins in the gel (Polyacrylamide gel more concentrated, 12.5%), voltage was increased to 120V.

Gel concentration (<<stacking gel>>) is to focus the various protein species in the form of narrow, uniform and thus increase the separation resolution. In this portion of the gel separates proteins not only migrate together to form more concentrated and uniform stripe. Gel separation (<<separating gel>> or <<running gel>>) is the gel in which the effective separation of protein species. In this area is more concentrated polyacrylamide and the proteins are separated based poets their different migration speed, speed is directly dependent on their molecular mass.

Migration was performed in a migration buffer prepared from glycine and SDS, in Tris buffer 0, 5M pH 8.3.

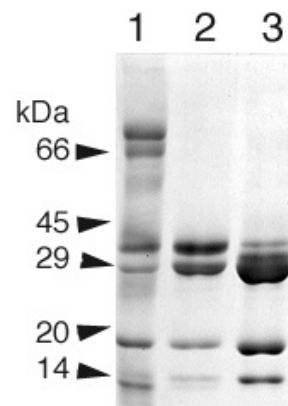
To identify the molecular weight of proteins separated on the same gel have been made and solutions (markers) of proteins with known molecular weights.

After migration gels were carefully removed from the glass plates and were washed with distilled water in three successive water baths for 5 minutes. To highlight the separate proteins, the gel was colored using a colloidal solution of Coomassie blue. This dye, in acid medium (necessary to prevent protein disseminate gel, so to fix them) and the presence of methanol has a high affinity for proteins. The fixing of the separated protein bands appear in the blue colored areas. After the gels were maintained for 30 minutes in color they were washed with water distilled (to remove excess coomassie blue), in successive water baths.

### 3. Results and discussion

Technique using denatured SDS-PAGE electrophoresis to examine the protein content of milk from two species - goats that cow, the gel obtained from migration is shown in Figure 2.

To identify protein electrophoresis separated using a protein marker (column 1) containing a mixture composed of lactoferrin (~ 80 kDa), serum albumin (~ 66 kDa), IgG (~ 55 kDa),  $\beta$ -casein and  $\kappa$ -casein (between 40 and 25 kDa), lactoglobuline (~ 18 kDa ) and  $\alpha$ -lactalbumin (~ 14 kDa).



**Figure 2:** Electrophoretic separation of proteins in cow milk (2) and goat milk (3)

Separated protein bands of cow's milk sample (column 2) correspond to the casein  $\beta$  and  $\kappa$  (32 and 25 kDa), lactoglobuline (~ 18 kDa) and  $\alpha$ -lactalbumin (~ 14 kDa). A profile also obtained in the case of goat milk (column 3) with the observation that casein is observed when an intense band at approximately 25kDa (band could correspond to  $\beta$ -casein).

### 4. Conclusions

1. Using SDS-PAGE electrophoresis technique was highlighted soluble protein lactoglobuline and  $\alpha$ -lactalbumin in cow's milk.
2. By this technique, in cow's milk was shown only  $\beta$  and  $\kappa$  caseins.
3. Using SDS-PAGE electrophoresis technique, in case of goat milk is obtain a profile similar to cow milk with the observation that when casein is noticed there is an intense band corresponding to  $\beta$ -casein.

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