A Quick Novel Method to Detect the Adulteration of Cow Milk in Goat Milk

Chi-Chei Lee[1], His-Shan Chang and Hua-Shan Sheen*

Department of Animal Science, National Chung Hsing University, 250 Kuo-Kuang Rd., Taichung 402, Taiwan, ROC

ABSTRACT : This study was to demonstrate a rapid novel method for detection of adulterated cow milk in goat milk using modified staining protocol after native polyacrylamide gel electrophoresis (PAGE). Samples of cow milk and goat milk containing 0, 0.5, 1.0 and 2.0% (v/v) of cow milk were analyzed. Low levels of cow milk mixed in goat milk were identified by the presence of higher mobility of β-lactoglobulin A (β-Lg A) in cow milk. By mini-gel electrophoresis, a distinguishable protein profile was visualized in 25 min using the modified Coomassie blue staining solution, in which methanol (50%) was replaced with ethanol (20%) and the concentrations of Coomassie blue and acetic acid were reduced from 2 to 0.13% and 10 to 5%, respectively. To visualize the milk proteins, gels in the staining solution were water-bathed in boiling water for 5 min and then cooled down immediately for 3-5 min. The sensitivity of this method is relatively high, allowing examination of 1% cow milk in goat milk. The procedure presented here is also very cost-effective due to less reagents needed. This simplified method would be useful and applicable to dairy industry for routine examination of goat milk. ***(Asian-Aust. J. Anim. Sci. 2004. Vol 17, No. 3 : 420-422)***

Key Words : PAGE, Cow, Goat, Milk

INTRODUCTION

Chinese people have believed that goat milk functions as a nutraceutical nourishment. As written in the Compendium of Materia Medica, a Chinese traditional medicine book, goat milk is not only nourishing the stomach and a tonic for the lungs, but also improving complexion. Therefore, the market price of goat milk is usually twice as high as that of cow milk. As a consequence, illegal adulteration of cow milk into goat milk can be found frequently in dairy market. For the ethical reasons as well as the benefits of the consumers, it is necessary to develop a technique for dairy factories to detect the adulterated cow milk in goat milk before further processing.

A number of researchers have proposed a variety of analytical methods based on ELSIA (Anguita et al., 1997), gas liquid chromatography (Iverson and Sheppard, 1989), HPLC (Romero et al., 1996), capillary zone electrophoresis (Cattaneo et al., 1996), PCR (Bania et al., 2001) and urea PAGE for α_{s1}-casein detection (Liang and Huang, 1999). Electrophoresis technique has been an essential tool for protein chemistry and biochemistry and has been used to characterize molecular architecture, determine homogeneity, and quantify proteins (Douglas and Zeece, 1988). Recently, this technique was successfully applied for the analysis of milk proteins (Sibel et al., 1996). In this study, we intended to demonstrate this technique, with some modifications in the gel staining procedures, to identify a low level of adulterated cow milk in goat milk using the native PAGE protocol.

* Corresponding Author: Hua-Shan Sheen. Tel: +886-4-2284-0365 (217), Fax: +886-4-2286-0265, E-mail: hssheen@dragon.nchu.edu.tw

[1] Ming-Dao High School, Taichung, Taiwan, ROC.

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MATERIALS AND METHODS

Preparation of skim milk and milk mixtures

Bulk milk samples of raw cow (Holstein) and goat milk from the dairy farm of National Chung Hsing University were used. Goat milk is collected from several breeds including Saanen, Alpine, Nubian, Lamancha and Toggenburg. Milk fat was removed from raw milk by centrifugation at 2,046×g for 5 min (microcentrifuge, Model UFO 2100, Pantech Co. Taiwan). Samples were prepared and kept at 4°C until use. Samples of goat milk containing 0.5, 1.0 and 2.0% of cow milk were prepared by mixing 0.5, 1.0 and 2.0 mL of cow milk with 99.5, 99.0 and 98.0 ml of goat milk, respectively. After completely mixed, milk samples were sampled for assay by native gel electrophoresis.

Native PAGE analysis

Mini vertical gel electrophoresis apparatus and power supply were used (ATTO, Model AE-7300, Japan). A native polyacrylamide gel was prepared as 12% separating gel (60×55×0.75 mm) at pH 8.8 and 3.5% stacking gel (60×5×0.75 mm) at pH 6.8. The electrophoretic chamber could hold one slab with a capacity of 12 samples.

Milk proteins were resolved at a constant current of 20.5 mA (output mode: protein H) in electrode buffer (3.03 g Tris and 14.4 g glycine in 1 L of distilled water) for a desired duration (15 or 30 min) according to the experimental design.

Skimmed goat milk containing 0, 0.5, 1.0 and 2.0% (v/v) of skimmed cow milk was assayed. Pure cow milk and

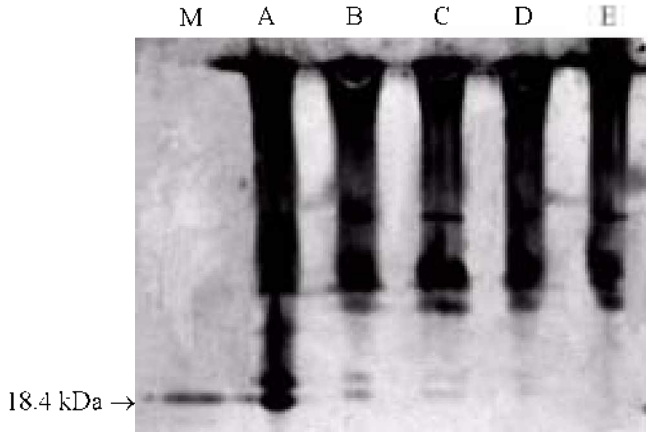

Figure 1. The protein profiles of cow and goat milk are identified by modified native PAGE. After 30 min of electrophoresis, the 12% native polyacryamide gel was soaked in modified Coomassie blue alcohol solution in boiling water for 5 min and subsequently cooled down in cold water for 3 to 5 min. β-Lactoglobulin A (18.4 kDa, arrow) is visible in Lanes A, B and C. Lane M, β-Lactoglobulin A; Lane A, 100% skimmed cow milk; Lane B, 2% skimmed cow milk in goat milk; Lane C, 1% skimmed cow milk in goat milk; Lane D, 0.5% skimmed cow milk in goat milk, Lane E, 100% skimmed goat milk.

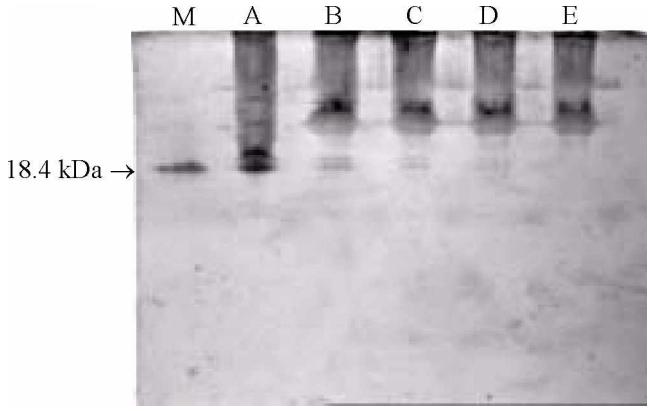

Figure 2. The protein profiles of cow and goat milk are identified by modified native PAGE. After 15 min of electrophoresis, the 12% native polyacryamide gel was soaked in modified Coomassie blue alcohol solution in boiling water for 5 min and subsequently cooled down in cold water for 3 to 5 min. Note that the β-Lactoglobulin A (18.4 kDa, arrow) is visible in Lanes A, B and C. Lane M, β-Lactoglobulin A; Lane A, 100% skimmed cow milk; Lane B, 2% skimmed cow milk in goat milk; Lane C, 1% skimmed cow milk in goat milk; Lane D, 0.5% skimmed cow milk in goat milk; Lane E, 100% skimmed goat milk.

pure goat milk were also analyzed as comparisons. Before applying samples to the electrophoretic wells, each sample was mixed with sample buffer (2:1) containing 20% glycerol, 0.04% bromophenol blue, and 0.25 M Tris-HCl (pH 6.8). Five μl of each diluted samples and marker (β-lactoglobulin A, β-Lg A) was loaded to each well of the gels. The marker β-Lg A was purchased from Sigma Chemical Co. was used as the protein marker.

Coomassie blue staining

After electrophoresis, some gels were stained in regular Coomassie blue solution containing 2% Coomassie blue (w/v), 50% methanol (v/v) and 10% acetic acid (v/v) at room temperature (RT) for 30 min. The gels were then transferred to destaining solution containing 50% methanol (v/v) and 10% acetic acid (v/v) for 30 min at RT. To simplify the procedures, other gels were soaked in the modified Coomassie blue alcohol solution, containing 0.13% Coomassie blue, 20% ethanol and 5% acetic acid and were water-bathed in boiling water for 5 min. After boiling, the gels were then cooled down in cold water for 3 to 5 min for examination.

RESULTS AND DISCUSSION

Gel electrophoresis technique was developed more than 30 years. Its reliability and convenience have made it an indispensable laboratory tool. In this study, we modified this tool to detect cow milk adulterated in goat milk. Due to differences in the protein profiles between goat and cow milk, it is possible to identify these differences by electrophoresis. For example, α_{s1}-casein exits in cow milk but undetectable in goat milk. Theoretically, components of cow milk could be distinguished from goat milk by SDS-PAGE analysis (El Ghannam, 1994). Liang and Huang (1999) reported that 0.5% cow milk could be detected using urea-PAGE. However, the tedious pretreatment procedures of samples including adding water (30 ml for 2 ml milk), stirring (30 min), adjustment of pH (4.6), and centrifugation (15 min), and the high cost of reagents (El Ghannam, 1994; Sibel et al., 1996) made the examination for adulteration unfavorable. How to simplify the protocol and reduce the cost of reagents used for electrophoresis are among the main issues of dairy industry.

Other proteins such as β-Lg A and β-lactoglobulin B (β-Lg B) could be used as indicator to distinguish cow milk from goat milk. Goat β-Lg has less negatively charged and one more positively charged group than bovine β-Lg at pH of 5.0 to 9.0. This difference in ionized groups could explain the slower electrophoretic mobility of goat β-Lg in alkaline gels (Jenness, 1980; Amigo et al., 1991; Sibel et al., 1996).

Native PAGE had also been used for this purpose with a relatively low sensitivity of detecting 25% of adulterated cow milk in the goat milk (Sibel et al., 1996). In the present study, mini gel electrophoresis apparatus was used based on the same techniques with some modifications. The volume of the separating gel in our protocol is much less than that in the previous report (2.5 ml vs. 26.5 ml). The time

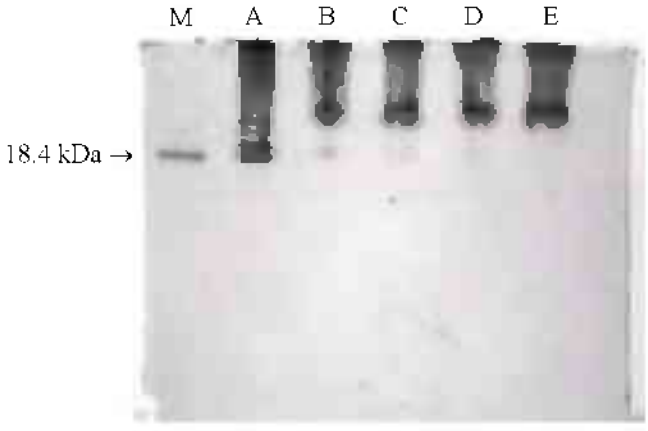

Figure 3. The protein profiles of cow and goat milk after electrophoresis for 15 min. The 12% native polyacryamide gel was stained with regular Coomassie blue staining solution at room temperature for 30 min and subsequently destained for 30 min. ß-Lactoglobulin A (18.4 kDa, arrow) is visible in Lane A and B, and barely visible in Lane C. Lane M, ß-Lactoglobulin A; Lane A, 100% skimmed cow milk; Lane B, 2% skimmed cow milk in goat milk; Lane C, 1 % skimmed cow milk in goat milk; Lane D, 0.5% skimmed cow milk in goat milk; Lane E, 100% skimmed goat milk.

required for running gel is also greatly shortened (30 min vs. 6-8 h). In the standard procedures of Coomassie blue staining and destaining, it usually takes about 1 h to be accomplished. In the present protocol, after the modified Coomassie blue staining, we eliminated the destaining step, which cut off the experimental duration and reagent cost. Figure 1 shows the result of native PAGE after a 30 min electrophoresis when the tracking dye front ended at 0.5 cm away from the bottom of the gel. It only took 40 min to complete this assay, including modified Coomassie blue staining and destaining procedures. The intensity or amount of β-Lg A from cow milk increases with the increase of cow milk in the goat milk samples. When as little as 1% of cow milk was mixed, it was still detectable by the higher mobility of β-Lg A after a 30 min of electrophoresis (Figure 1). In the reduced duration (15 min) of electrophoresis, the band of β-Lg A was also distinguishable although the time for running gel is shortened (Figure 2). Figure 3 shows the result after regular Coomassie blue staining which has no differences in the resolution of β-Lg A and time of running gel from that in Figure 2. However its time required for the whole procedure is greatly increased due to regular staining and destaining procedures.

In conclusion, routine examination of the adulteration of cow milk into goat milk has been performed in some countries (Szijarto and Voort, 1983), including Taiwan. The procedures demonstrated in this study (native PAGE, 15 min) not only reduced the cost of reagents but also the time required for the whole process. This novel technique would be beneficial for routine examination of adulteration in dairy industry.

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