

Protein Stability in Pasteurized Cow and Goat Milk using Protein Gel Electrophoresis

Mai Nguyen

Metuchen High School

ABSTRACT

Milk is a nutritious beverage that contains whey, casein, and milk fat globule membrane (MFGM) proteins. Pasteurization kills pathogenic microorganisms in milk, making it safe to drink. A high heat load during processing can affect the protein quality of milk. In this study, protein gel electrophoresis (Native and SDS-PAGE) was used to view the protein profiles of retail cow and goat milk samples pasteurized at different temperatures (145, 165, or 280 °F) compared to commercially spray dried milk (356–482 °F). SDS-PAGE provided better resolution of the milk proteins compared to Native-PAGE. There were clear species differences in the MFGM proteins. This could be used to spot adulteration of goat milk with cow milk, however none of the samples showed signs of adulteration. There were no overt patterns of protein degradation with increasing pasteurization temperature. However drying reduced band intensity, especially MFGM and whey proteins in the goat milk sample. Whereas most samples came from different herds where genetics and environmental conditions varied, two of the goat milk samples were from the same herd, one fresh and one spray dried. It was evident from these two samples that spray drying can alter proteins in goat milk. Even so, if fresh milk is unavailable or unaffordable, dry milk remains a valuable protein source.

Background

Milk is a popular, nutritious beverage across the world. It contains lactose, proteins (caseins, whey proteins, and minor proteins), essential amino acids, fats, minerals, and vitamins. People consume many different types of milk including bovine (cow), buffalo, caprine (goat), ovine (sheep), and camel. According to the Food and Agriculture Organization's Statistical Database (FAO Stat 2023), cow and buffalo are the two most widely produced milk worldwide, followed by goat milk. India and the United States are the two largest producers of cow milk and India is the largest producer of goat milk (FAO Stat 2023). Goat milk is less popular in the US compared to cow's milk and can have a distinctly strong "goaty" taste. This might be due to the liberation of short-chain fatty acids during rough handling, which gives off a goaty smell (Jandal 1996). Sanitary conditions in the barns and during milking can help the milk taste sweeter and less goaty (Richardson CW 2004). Although the milks have very similar nutrient content (Table 1), goat milk can be easier to digest than cow milk because it has smaller and softer curds and smaller fat globules with a greater surface area than cow milk (Jandal 1996). Fat enzymes in the gut are supposed to break them down more rapidly in goat's milk.

The concept of milk quality is directly related to the amount and types of spoilage bacteria present in milk as well as chemical indicators (freezing point, fat and protein content, and absence of inhibitory substances) (Fusco et al. 2020). Fresh milk spoils during prolonged storage, even after pasteurization that kills the spoilage microbiota like proteolytic and lipolytic bacteria (Fusco et al. 2020). These bacteria can produce heat-stable proteases and lipases, which remain active after pasteurization to break down protein and fats, respectively. Raw milk and raw milk cheese consumption is increasing worldwide with the growing demand of minimally processed, sustainable, healthy, and local foods (Fusco et al. 2020; Witzling and Shaw 2019). This affinity for minimally processed local foods may be

due to the idea that overprocessed foods are unhealthy. Buying local gives the impression that food will be fresher and therefore retain higher nutritional properties.

Table 1 lists the nutrients present in goat and cow milk. It is a general guide, as the quality and nutrients in milk vary depending on the animal's diet, overall health, stage of lactation, age, and breed genetics (Deeth and Hartanto 2009; Dimpler et al. 2020). The two major types of milk proteins are whey and caseins. Caseins make up about 80% of milk proteins (Deeth and Hartanto 2009). There are 4 subtypes: α_{s1} , α_{s2} , β , and κ in a ratio of approximately 40:10:35:12 (Deeth and Hartanto 2009). The two major whey proteins are beta-lactoglobulin and alpha-lactalbumin. When caseins separate from the liquid part of milk, they form a gelatinous material called the curd. Casein can be removed from milk by using a centrifuge or filtration. They are heat stable over a wide range of temperatures, especially compared to the heat sensitive whey proteins (Early 2012; Lin et al. 2010). From a nutritional point of view, caseins are calcium-rich sources of protein (Rollema and Muir 2009). Quality of the raw milk and heat load during processing are two major factors determining casein quality (Rollema and Muir 2009; Lin et al. 2010).

The sale of raw, unpasteurized milk is restricted in many parts of the US for food safety reasons; it is prohibited in the state of New Jersey (N.J.S.A. 24:10-57.17). There are a few methods of pasteurization used in the US to kill milk pathogens (International Dairy Foods Association 2023; CFR§131.3). The original method is vat pasteurization where milk is heated to 145 °F for at least 30 minutes followed by rapid cooling and packaging under sanitary conditions. High Temperature Short Time (HTST) pasteurization is most commonly used today for large commercial operations. Milk is heated to a minimum of 161 °F for at least 15 seconds. Ultra-pasteurization is a method that heats milk to a minimum of 280 °F for at least two seconds. The packaging conditions are nearly sterile. Ultra High Temperature (UHT) milk is aseptically processed using commercially sterile equipment. It is filled under aseptic conditions into hermetically sealed packaging. UHT milk is less common in the US, especially compared to Europe (Kresova et al. 2022).

Fresh milk can be dried for a long shelf life. Many US consumers prefer the taste of fresh cow's milk to powdered milk, as the drying process can produce off-flavors (Karagül-Yüceer et al. 2001). Two types of dried milk are whole milk powder (WMP) and skimmed milk powder (SMP). According to the USDA, "Nonfat dry milk is the product resulting from the removal of fat and water from milk, and contains the lactose, milk proteins, and milk minerals in the same relative proportions as in the fresh milk from which made." It can only be made from pasteurized skim milk, not whey proteins or buttermilk. Dry milk is produced by spray drying or less commonly a roller process (Early 2012). It is well known that some milk proteins are denatured during the drying process, but the structural changes are subtle (Chen et al. 2004). Raw milk is first pasteurized and skimmed using a centrifugal cream separator. Milk is then heated to 75–120 °C and held at this temperature for several seconds to minutes. This process denatures whey proteins, causes whey protein gelation, as well as interactions between whey proteins and between whey proteins and caseins (Early 2012). The effects on proteins depend on treatment conditions, particularly heat load (Rollema and Muir 2009). Next, water is evaporated by boiling the milk under reduced pressure at low temperature (<72 °C). This concentrated milk is then sprayed in a fine mist into a chamber with hot air 180-250 °C (spray drying) to remove further moisture, forming a powder.

The primary goal of this study was to determine if commercial processing changes the protein profiles of milk using polyacrylamide gel electrophoresis (PAGE). This will test the consumer perception that "farm fresh" milk is more nutritious than highly processed milk, since proteins play a large role in milk nutrition. Protein gel electrophoresis was used to compare protein profiles of milk coming directly from local farms with traditional vat pasteurization with more highly processed milk from national dairies (HTST and ultra pasteurization) and commercially spray dried milk. Although there are studies using PAGE to demonstrate differences between goat and cow milk, we could not find many studies using PAGE to assess goat milk protein quality after pasteurization and no studies on commercial spray drying. This is an important distinction because these two species of milk show different technological-functional properties including heat stability (Pesic et al. 2012). Both cow and goat milk samples were tested, as they are readily available from farms and supermarkets in New Jersey. A secondary goal was to see if there were any signs of adulteration in the milk samples by evaluating the authenticity of milk proteins. For example, protein profiles could

be altered by goat's milk diluted with cow's milk, watering down, removal/remixing of protein fractions, or addition of "artificial" proteins such as collagen, plant proteins, or nitrogen-rich organic substances (Lin et al. 2010).

Table 1. Nutrition of goat milk vs. cow milk (Richardson CW 2004; Jandal 1996)

| Nutrient | Goat | Cow |
|-------------------------|--------|--------|
| Water (%) | 87.0 | 87.2 |
| Fat (%) | 4.25 | 3.70 |
| Protein (%) | 3.52 | 3.50 |
| Casein (%) | 2.47 | 2.63 |
| Whey (%) | 0.43 | 0.60 |
| Lactose g/100 g | 4.27 | 4.90 |
| Total solids | 13.0 | 12.8 |
| Vitamin A IU/liter | 2074 | 1560 |
| Vitamin D mg/liter | 23.7 | * |
| Vitamin B1 mg/liter | 0.4 | 0.44 |
| Vitamin B2 mg/liter | 1.84 | 1.75 |
| Nicotinic acid mg/liter | 1.87 | 1.75 |
| Vitamin B12 mg/liter | 0.0006 | 0.0043 |
| Vitamin C mg/liter | 15 | 21.1 |

*very low unless fed vitamin D supplements

Methods

Samples

Table 2 lists the characteristics of the milk samples. All samples were purchased in New Jersey, USA directly from farms (samples 1 and 5) or from commercial grocers. Raw, unpasteurized milk samples were included in the initial experiments (not shown in table). But since they are not legal for purchase in NJ, they were excluded in the final run. Both farm fresh samples (1 and 5) were around 48 hours old (milking to purchase). One problem with commercial cow milk samples is the opaque nature of the industry. Milk can be sourced regionally or from thousands of miles away; there is no way to verify its freshness, origin, or the breeds in the herd. The New Jersey dairy farm (sample 5) had a mixed herd of Holsteins and Jerseys. For the goat samples, only sample 1 came from a purebred herd (Alpine);

the other farms had mixed breeds. Samples 3 and 4 are from the same company. The producers of sample two stated they optimized their herd genetics for high protein content. This could be true for other farms as well, but it was not explicitly stated. Pasteurization temperature and homogenization was obtained from product labels, websites, or direct communication with farmers. Fat content was not independently verified; it was taken from the product's label or from personal communication with the farmer.

Table 2. Description of milk samples tested in this study

| Sample | Species | Type | Origin ¹ | Pasteurization ² | Homogenization | Fat / 240mL |
|--------|---------|-------|---------------------|-----------------------------|----------------|-------------|
| 1 | Goat | Fresh | NJ farm | 145 °F | No | 8g |
| 2 | Goat | Fresh | VT | 165 °F | Yes | 9g |
| 3 | Goat | Fresh | CA | 280 °F | Yes | 7g |
| 4 | Goat | Dry | CA | Yes | Yes | 8g |
| 5 | Cow | Fresh | NJ farm | 145 °F | No | 8g |
| 6 | Cow | Fresh | US | 165 °F | Yes | 2.5g |
| 7 | Cow | Fresh | US | 280 °F | Yes | 13g |
| 8 | Cow | Dry | US | Yes | No | 0g |

¹Samples 6–8 did not list a specific regional origin within the US. All were purchased in New Jersey, US.

²Dry milk samples were pasteurized but temperature and time is unknown

Milk aliquots were put in 15 mL sterile, labeled tubes and frozen at -20 °C for no more than one week until ready for assays. At the time of assay, samples were thawed to room temperature. For the powdered dry milks, they were reconstituted as per label instructions to match the protein content of fresh milk and vortexed to mix. All samples were spun briefly in a centrifuge at low speed. After spinning, milkfat rises to the top and was removed with a large gauge syringe. The tubes were kept at 4 °C until ready to use.

Protein Gel Electrophoresis

Polyacrylamide gels (PAGs) are commonly used to separate proteins into bands using electrophoresis. In PAGE, proteins move in an electric field based on net charge or charge to mass ratio. The gels are normally treated with protein binding dyes to visualize the proteins. Size and color intensity of the separated bands are directly related to protein concentration. There is a lot of published information about separating milk proteins on PAGE using different protocols, each with their own pros and cons. Examples include native, urea, SDS (reducing and non-reducing), tricine, and 2DE (Sharma et al. 2021).

Native PAGE

In Native-PAGE, proteins are separated in their native form based on their net charge, size, and shape under non-denaturing conditions (Sharma et al. 2021). Some of the advantages are low cost of reagents and less preparation steps compared to SDS-PAGE (Lee et al. 2004). It was reported to easily detect differences in the protein profiles between goat and cow milk (Lee et al. 2004). One milliliter of each milk sample was mixed with 0.5 mL of sample buffer containing 20% glycerol, 0.04% bromophenol blue, and 0.25 M Tris-HCl (Lee CC, 2004). A running buffer was made by mixing 1 L distilled water with two packages of instant Tris-Glycine buffer (Lee, CC 2004). Next, a NuSep pre-cast polyacrylamide gel cassette with 12% Tris-Glycine, 4% stacking gel, 1mm thickness, was removed from its refrigerated package and placed into the electrophoresis chamber (Bio-Rad Mini-Protean II) with the Tris-Glycine buffer. Using a syringe, 20 μ L of each prepared milk sample was carefully injected into each well. The gel was run until the dye front was close to the lower part of the gel. Carefully, the cassette was popped open and the gel was released into a pre-mixed protein stain solution containing one part Coomassie blue (45% 2-propanol, 45% water, 9.75% acetic acid, 0.25% Coomassie blue) and one part vinegar (Lee, CC 2004). It was stained in the Coomassie stain solution for 30 minutes. Then the gel was put into a de-stain solution containing one part ethanol and one part vinegar for 30 minutes.

SDS-PAGE

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS PAGE) is a type of protein gel electrophoresis used to separate proteins by molecular weight. This method allows for visualization of the individual milk proteins along with relative quantity. Ten microliters of each milk sample was combined with 40 μ L of phosphate-buffered saline (PBS) and 50 μ L 2X loading buffer (Laemmli sample buffer: 4% SDS, 20% glycerol, 120 mM Tris pH 6.8, 0.01% bromophenol blue) with 50 mM dithiothreitol (DTT) reducing agent, for a final protein concentration of 3 μ g/ μ L. The samples were heated at 95 °C for 5 minutes before cooling to room temperature and loading onto a 10% NuPage Novex Bis-Tris 10% 1.5mm gel (Thermofisher). The first lane received commercial molecular weight size markers to estimate the size of the proteins. The next lanes each received 15 μ L sample (45 μ g protein/lane). The gel was placed in the electrophoresis chamber (Xcell SureLock mini cell) with Nupage MES SDS Running buffer (Thermofisher) and run until the dye front nearly reached the bottom of the gel. After running, the gel was immediately washed with deionized water for 30 sec twice to get rid of excess gel running buffer and SDS. The gel was stained with \leq 0.1% Coomassie Blue R250 in 10% acetic acid, 40% ethanol for at least 30 min at room temp with gentle shaking. Afterwards, the gel was de-stained with 7% acetic acid, 30% ethanol until desired effect.

Gel Image Analysis

ImageJ version 1.53t available through the National Institutes of Health (NIH) was used to analyze the density of the protein bands for selected lanes in the same gel. This was accomplished using the gel analyzer feature for 1D gels. The software generates a plot where higher peaks represent greater color density. In PAGE, greater color density is directly related to protein content. The software calculates areas under the curves for each peak. This can be used to quantify differences in protein content between samples.

Results

The gel shown in Figure 1(A) was the best of three runs. Native gel electrophoresis (non-denaturing PAGE) was run with a 12% Tris-Glycine gel with hopes to see separation based on the proteins' native charge, size, and shape without denaturation. Even related proteins have different acidic and basic amino acid compositions with different net charges

at the same pH. Therefore, they will move differently in the electric field causing protein separation. This was a suggested method to detect interspecies milk adulteration (Pesic et al. 2011). Indeed, the difference between cow and goat milk is evident in Figure 1. Since caseins have isoelectric points very close to each other, native-PAGE did not result in optimal separation. Even after several runs to optimize the method, large aggregates remained at the top of the gel (Figure 1A). According to the literature, these may have been κ -casein complexes of various lengths and net charge (Sharma et al. 2021). Raw milk samples were used at this stage of method development (Figure 1A Lane 1–3). The gel somewhat resembled published Native-PAGE gels for goat and cow’s milk (Figure 1B, Lee et al. 2004), but our resolution was too poor to detect small differences in protein profiles.

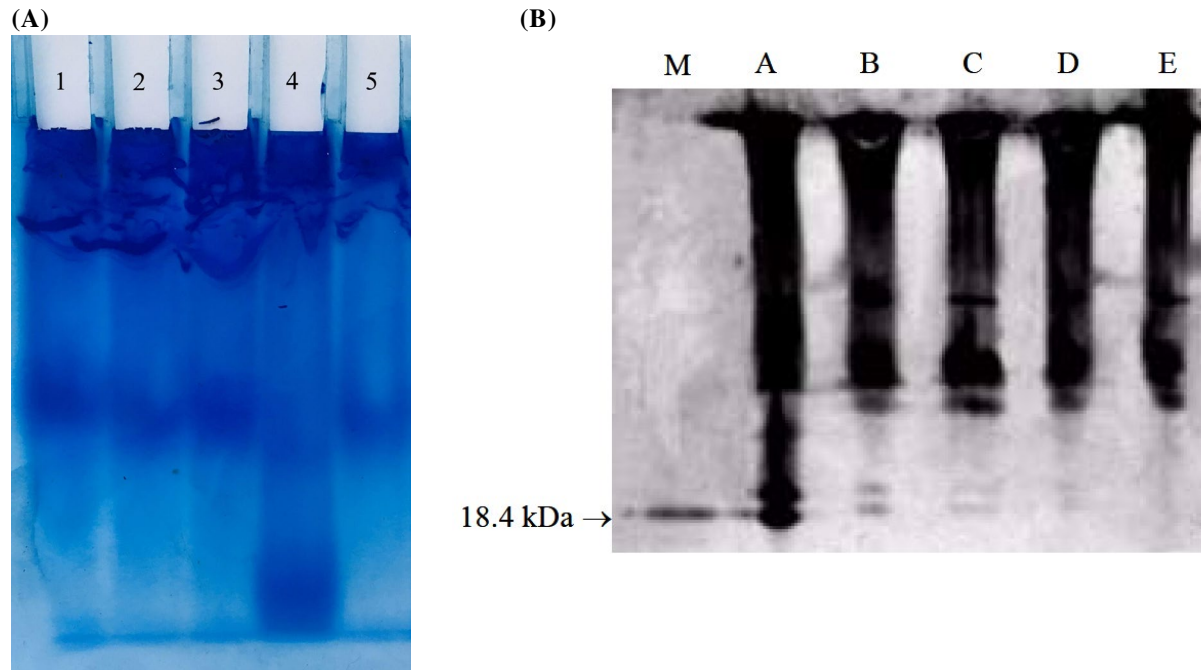


Figure 1. (A) Native-PAGE with Tris-Glycine (12%) gel showing stained protein from different milk samples. Lane 1–3, skimmed goat milk, unpasteurized; Lane 4, skimmed cow milk, pasteurized; Lane 5, skimmed goat milk, pasteurized (B) Native-PAGE with Tris-Glycine (12%) gel from Lee et al. 2004 showing 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. (Reproduced under creative commons license 4.0)

In order to reduce the aggregation of proteins and get better separation of the caseins, SDS-PAGE was used. SDS binds to proteins, gives them all a net negative charge, and unfolds them, so they are separated based primarily on molecular weight. The disulphide reducing agent DTT was added prior to electrophoretic separation, since SDS alone cannot cleave disulphide bonds and completely unfold proteins. The first run was performed at twice the concentration (30 μ L milk sample; 90 μ g protein/lane). But this somewhat overloaded the gel leading to poor resolution of the casein bands (data not shown). The following run with 15 μ L sample resulted in better casein resolution without sacrificing visualization of whey or large protein bands.

On the SDS gel (Figure 2), the first striking differences are the large molecular weight protein bands when comparing cow to goat milk samples. The pattern is quite different, especially between 60–90 kDa. This could be used as a way to spot adulteration of goat milk with cow milk; none of the samples tested showed signs of adulteration.

According to the literature, these proteins are likely serum albumin and milk fat globule membrane (MFGM) proteins such as fatty acid synthase, perilipin, mucin 1, xanthine oxidase, butyrophilin, and lactadherin (Sharma et al. 2021; Widodo et al. 2021; Saadaoui et al. 2013, Ma et al. 2019). Others have found goat milk has different milk fat globule (MFG) size and MFGM proteome compared to cow milk (Ma et al. 2019). Heat treatment such as pasteurization may cause aggregation with either MFGM proteins or skim milk proteins through disulphide bonds or transfer of whey proteins to the MFGM (Ma et al. 2019).

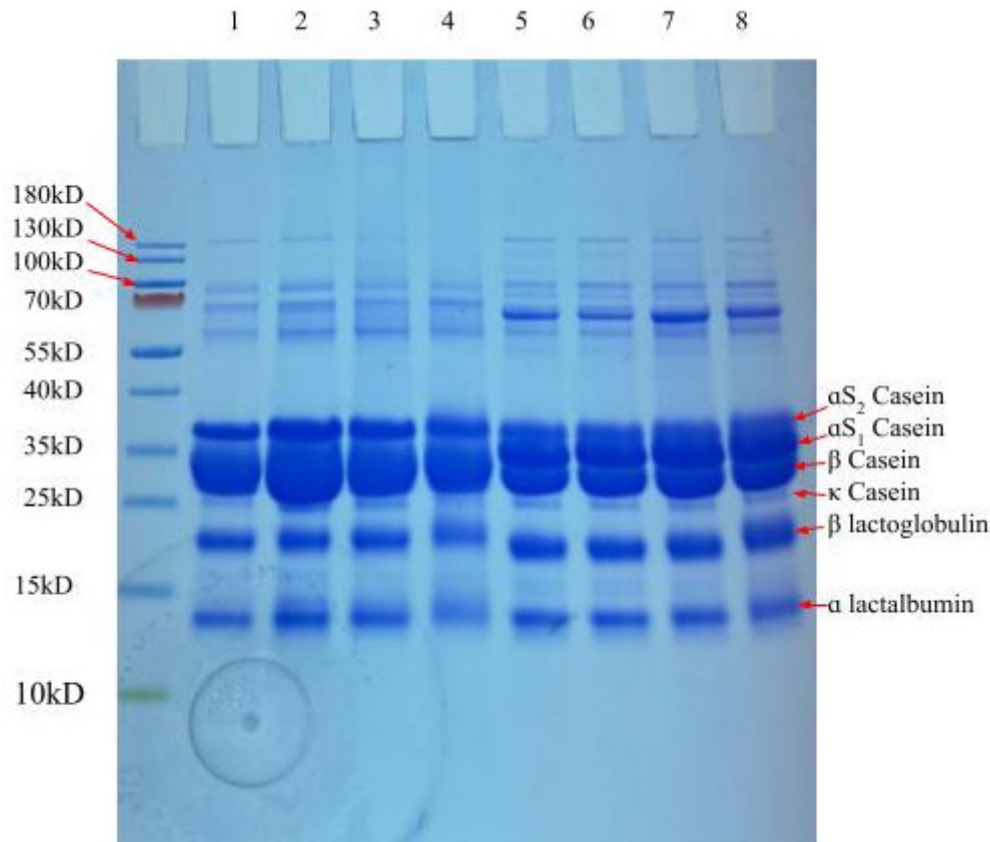


Figure 2. SDS-PAGE with Tris-Glycine (10%) gel showing stained protein from different milk samples. First lane (unlabeled) contains molecular weight markers for reference; Lane 1–4, goat milk samples; Lanes 5–8, cow milk samples (see Table 2 for sample descriptions). Casein and whey proteins were identified based on published electrophoresis data (Sharma et al. 2017; Sharma et al. 2021; Widodo et al. 2021; AlGhsyar et al. 2018; Kumar et al. 2013).

A second difference is the relative abundance of the caseins between samples. The farm fresh samples with the lowest heat used for processing (lanes 1 and 5) did not appear to have more abundant caseins. In fact, all the cow samples (lanes 5–8) were very similar. For the goat milk samples, sample 2 had noticeably more caseins and was slightly overloaded with moderately poor resolution. This was not a result of human error, since sample 2 had the highest amount of casein in the first gel run as well. This sample was pasteurized at 165 °F and came from Vermont. The company stated they select a mix of different goat breeds for high protein milk production and this data would support that claim.

Looking at lanes 4 and 8, the reconstituted dry milks, there appears to be a slight reduction in dry goat milk protein (sample 4). The whey protein bands are noticeably lighter with possible changes in molecular weight. Some of the heat sensitive MFGM protein bands are reduced or missing as well. Sample 3 is a fresh milk sample from the same company, allowing for direct comparison of fresh vs dry milk (sample 3 vs 4) in the same species with the same

herd genetics. Figure 3 shows a quantitative analysis of the density of the protein bands expressed as area under the curve (AUC). According to this analysis, nearly every band in the fresh milk sample has a higher amount of proteins compared to the corresponding dry milk sample bands.

Compared to the dry goat milk sample, there were less changes in the dry cow milk. One major difference between these samples, aside from species, was fat content. The cow milk sample 8 is from skim milk (fat free) and the goat milk sample 4 is from whole milk. It is interesting, even though sample 8 is from skim milk, MFGM proteins are still present in about the same amount as whole milks (samples 5 and 7).

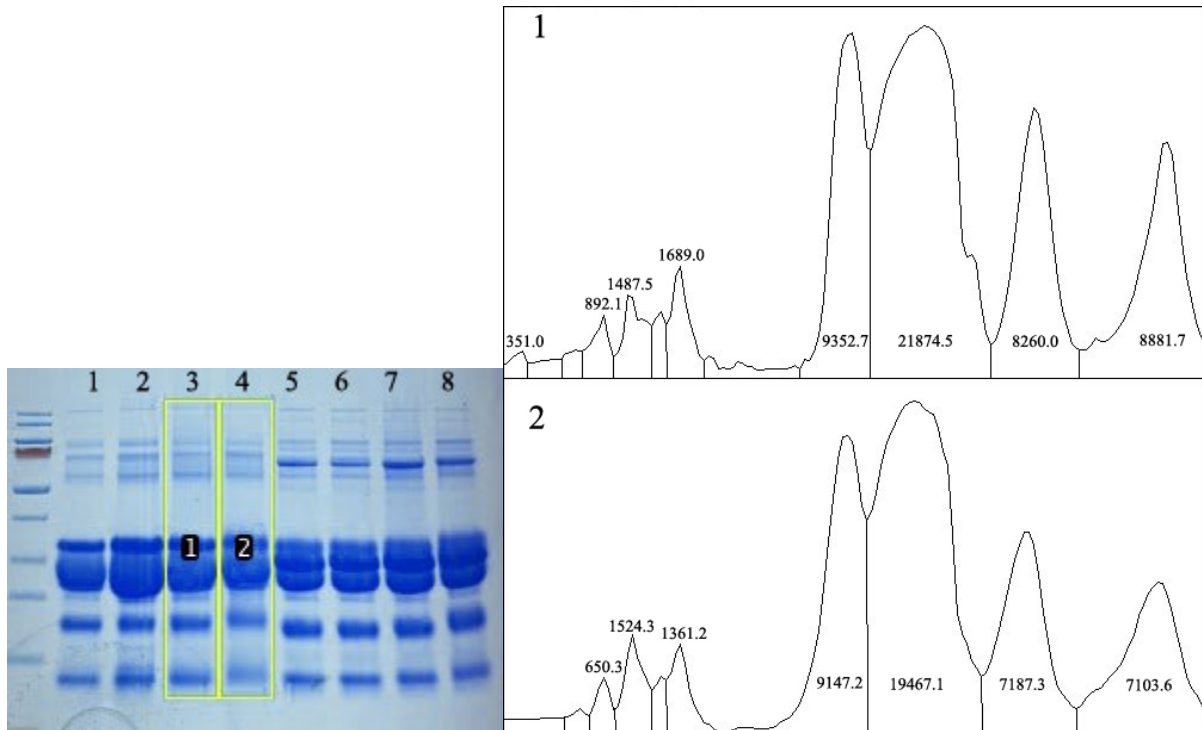


Figure 3. Plot of protein band density expressed as area under the curve (AUC) for lanes 3 & 4, fresh and dry goat milk from the same producer. Plot one = sample 3, fresh ultra pasteurized whole goat milk; Plot two = sample 4, spray dried whole goat milk. AUC is lower for nearly every peak in the dried milk sample.

Discussion

There are a number of ways to analyze milk proteins using gel electrophoresis (Sharma et al. 2021), each with their own advantages and disadvantages. Native-PAGE uses less expensive reagents and requires fewer preparation steps, making it an easier method for students and dairy workers. It did not work well in our case, with too much protein agglomeration, even in unheated raw milk samples. Possibly with more method development, we could have achieved better resolution similar to Lee et al. (2004). But studies show it is not the best method for resolving the caseins (Sharma et al. 2021). With advice from experts at the Rutgers University Biological Mass Spectrometry Facility, SDS-PAGE was chosen as the better method for overall protein separation. Using this method, there was excellent resolution of the MFGM proteins and good separation of the whey proteins. The caseins were somewhat distinguishable from each other, with overlapping bands.

Raw and pasteurized milk samples were used for method development (see Native gel Figure 1 Lanes 1–3), with the original hypothesis that any heat processing will reduce protein quality/quantity. Preliminary results looked

similar for unpasteurized and pasteurized samples from the farm (Lanes 1–3 vs Lane 5). As noted, the Native-PAGE protocol was not providing good resolution, so it is possible there were differences not detectable by this method. It was decided to only include milks that could be legally purchased in NJ for the final gel, to test the hypothesis that “farm fresh” pasteurized milk is more nutritious in terms of protein than highly processed fresh milk or dry milk. According to the NJ Department of Health “Raw milk, improperly pasteurized milk and raw milk fresh cheeses have been implicated in foodborne illness outbreaks of *Salmonella*, *Campylobacter*, *Listeria*, *E. coli* 0157:H7, and *Brucellosis* in recent years.” (New Jersey Department of Health 2023). Even if raw milk turned out to have the highest amount of protein, it is still unsafe to drink from a food safety perspective. Raw milk contains bacteria coming from the cow’s udder and in the environment (e.g. fecal contamination of udder or dirty milking equipment). Bacteria counts can be low if the cows are in good health and the facilities are sanitary (Skanderby et al. 2009) but this is impossible for consumers to know. While some consumers feel the bacteria in raw milk may be beneficial for the gut microbiome, it comes at the risk of infection with serious pathogens including Shiga toxin-producing *Escherichia coli* (STEC), *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter* spp., *Salmonella* spp., and coagulase-positive *Staphylococcus* spp. (Skanderby et al. 2009; Fusco et al. 2013).

The differences between proteins in cow and goat milk are well documented in the literature (Lee et al. 2004; Sharma et al. 2017) and this was evident in both the Native-PAGE and SDS-PAGE gels (Figures 1 & 2, respectively). The main difference seen in the SDS gel was the MFGM protein profile. Although a lot of attention has been paid to the casein and whey proteins because of their high commercial value in the food industry, the comparatively minor MFGM proteins have been studied for their health promoting properties such as anti-adhesive and antimicrobial functions (Ma et al. 2019; Cebo et al. 2010; Muñoz-Salinas et al. 2022). Others have found changes in MFGM proteins after pasteurization, for example protein transfer from whey to the MFGM (Ma et al. 2019). Our data suggest different pasteurization temperatures do not significantly affect MFGM proteins, but a more carefully controlled, quantitative experiment is needed to rule out differences coming from the genetics or environment of different herds. It did appear commercial drying affects MFGM proteins, especially in goat milk. Here we had a direct comparison of the same herd with two different types of processing (Figure 2, lanes 3 & 4; Figure 3).

From this study, pasteurization did not appear to negatively affect milk proteins. When comparing pasteurization at 145, 165, and 280 °F, there was no apparent reduction in any of the casein, whey, or MFGM proteins. The only process that seemed to reduce protein content was drying. This process puts a high heat load on the milk at many steps (Rollema and Muir 2009; Early 2012). The dry milk samples in this study were carefully reconstituted, running duplicate samples and replicate gels, to assure accurate results. With every run we saw the same lighter colored whey protein (β lactoglobulin and α lactalbumin) and large molecular weight MFGM protein bands in the dry goat milk. In the dry cow milk, there was only a slight reduction in intensity of the α lactalbumin band, with no obvious effects on β lactoglobulin or MFGM proteins. However, unlike goat milk, we did not have a sample from the same herd to directly compare fresh pasteurized and dried.

It was surprising to see the goat milk sample from Vermont had the most intense casein bands, as we had expected the local NJ fresh milk (goat and cow) to perform the best. This hypothesis was based on three factors: faster time from milking to purchase (less opportunity for microorganisms to degrade proteins); lower temperature pasteurization (less heat load); grass fed, free-range, healthy-looking herds. Details beyond our control and outside our knowledge could have played a major role in the outcome of this study. Number one is herd genetics. The VT goat herd may have been optimized for high casein production, as the farmers claimed. Many studies have shown that genetic polymorphisms determine milk composition, milk production traits, and technological properties of milk (Rahmatalla et al. 2022; Muñoz-Salinas et al. 2022; Gustavsson et al. 2010). Currently 20 protein variants have been reported in goats for α_{s1} -casein, eight for β -casein, 14 for α_{s2} -casein, and 24 for κ -casein (Rahmatalla et al. 2022). In addition, local and quick to market doesn’t necessarily mean the milk was more sanitary or held under strict conditions to reduce microorganism growth. Sometimes food illness outbreaks come from pasteurized milk because there was something off about the pasteurization equipment or method (Fusco et al. 2013). Thus, we can’t completely rule out inadequate pasteurization at the local farms, allowing microorganism growth and protein degradation.

Conclusions

Dry milk had less abundant proteins compared to fresh milk in this study. This was more pronounced in goat milk and could reflect a species difference in protein heat tolerance. Secondly, we speculate that factors such as herd genetics and/or environmental factors play a larger role in milk protein content than “freshness” and gentle processing of fresh milk. Overall, we observed no advantage of one form of pasteurization over another in the context of protein nutrition. If fresh milk is unavailable or unaffordable, dry milk is still a good source of milk proteins.

Limitations

This was a small study with only one sample to represent each processing condition. Since the milk samples were all point of purchase samples, we did not have control over any of the processing steps or data on animal or farm conditions. We did not visit any of the out of state farms to know how their conditions compared to the local NJ family farms or how herd genetics and health may have played a role in milk protein quality. We relied on information from farmers, product labels, or websites, which may not have been accurate. A more controlled pasteurization and drying study where milk with the same % fat from the same herd is subject to different heat loads would eliminate the uncontrolled factors of herd genetics, health, and environmental conditions. Secondly, microorganisms were not measured. If we were to do this study again, we would measure microorganism load as another way to assess milk quality and see if this correlates with protein content.

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