



# Differentiating Goat Breeds Based on Milk Physicochemical Properties and FTIR Spectral Profiles

YULIANTI FITRI KURNIA, HANIFAH NURYANI LIOE\*, ENDANG PRANGDIMURTI, NANCY DEWI YULIANA

*Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia.*

**Abstract** | This study aims to evaluate the quality of goat milks from Etawa, Etawa Crossbreed, Saanen, and Sapera breeds based on physicochemical characteristics and IR absorption profiles obtained from FTIR-ATR measurement. Physicochemical data were analyzed by ANOVA, meanwhile FTIR data were analyzed using multivariate analysis for the milk differentiation by OPLS-DA. The ANOVA results showed significant differences ( $p < 0.05$ ) of goat milk properties in protein, color ( $L^*$ ), and casein micelle sizes. FTIR profiles could distinguish milk samples according to goat breeds with fit values  $R^2Y$  0.89 and  $Q^2$  0.75 of OPLS-DA. This indicates the suitability and reliable predictive features of FTIR results in separating milk characteristics of different breeds. Distinct separating peak bands were observed at  $1553\text{--}1545\text{ cm}^{-1}$  (N-H, amide II) were markers for the first group (Etawa, Etawa Crossbreed and Sapera), and  $1675\text{--}1673\text{ cm}^{-1}$  (C=O, amide I) functional groups of protein as the primary marker for the second group (Saanen). These findings confirm that FTIR profiles combined with multivariate analysis has the potential yet reliable method for differentiating milk quality regarding different breeds (in the same farm).

**Keywords** | FTIR, Goats, Milk, Multivariate analysis, Physicochemical properties

**Received** | September 04, 2025; **Accepted** | October 29, 2025; **Published** | November 20, 2025

\***Correspondence** | Hanifah Nuryani Lioe, Department of Food Science and Technology, Faculty of Agricultural Engineering and Technology, IPB University, Bogor, Indonesia; **Email:** hanifahlioe@apps.ipb.ac.id

**Citation** | Kurnia YF, Lioe HN, Prangdimurti E, Yuliana ND (2025). Differentiating goat breeds based on milk physicochemical properties and FTIR spectral profiles. *Adv. Anim. Vet. Sci.*, 13(11):2535-2544.

**DOI** | <https://dx.doi.org/10.17582/journal.aavs/2025/13.11.2535.2544>

**ISSN (Online)** | 2307-8316



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## INTRODUCTION

The global demand for goat milk has shown an upward trend, including in Indonesia. According to ReportLinker (2025), the annual growth rate of dairy goat production in Indonesia is 1.33% (2018-2023) reflected a steady upward trend. Production of goat milk in globally is also expected to rise 2030 by 53% (Pulina *et al.*, 2018). Several factors contributing to this are that goat milks contain almost complete nutritional and bioactive components, thus they have potential health benefits, such as antidiabetic, antihypertensive and suitable for people with lactose intolerance (Du *et al.*, 2022; Ibrahim *et al.*, 2017; Getaneh *et al.*, 2016).

Some regions on the island of Java, such as Bogor, have become significant producers of goat milk. The substantial potential for goat milk production presents opportunities to optimize its benefits through milk quality studies. The quality of goat milk is affected by diverse biological and environmental factors, such as goat breed, type of feed, age, lactation stage, and environmental temperature (Fresno *et al.*, 2020; Kljajevic *et al.*, 2018). This study investigated the influence of the goat breed on milk composition. Etawa Senduro, Etawa Crossbreed, Saanen, and Sapera goats are goat breeds widely grown in Indonesia. According to Rahmatalla *et al.* (2021), these goat breeds can produce milk with relatively high protein, fat, vitamins, and minerals. Casein is the dominant protein ( $\pm 80\%$ ) of milk, which

plays a significant role in forming micelles (Chauhan *et al.*, 2021; Nayik *et al.*, 2022). Information on whether the different goat breed give different milk quality in Indonesia is still limited.

The description of Etawa Senduro, Etawa Crossbreed, Saanen, and Sapera goat breeds as the following sentences. Etawa Senduro goats are a cross between Jamnapari goats from the Etawah Region of India and Menggolo goats, which are local goats from the Lumajang Region of East Java. The characteristics of this goat are predominantly white fur, a convex face, ears that hang downward and curled, and udders shaped like a jar (Palayukan *et al.*, 2020). The Etawa Crossbreed are a cross between Jamnapari goats from India and indigenous Indonesian Kacang goats. It has udders shaped like bottles, hanging straight, parallel, and symmetrical, with fur combining black and white and brown and white (Budisatria *et al.*, 2018). The Saanen goat originates from the Saanen Valley in Switzerland. It has pale white (cream) fur and erect ears (Mohsin *et al.*, 2019). Meanwhile, according to Suranindyah *et al.* (2018), the Sapera goat is a cross between Saanen and Etawa Crossbreed. The distinctive features of this goat include a pure white coat, medium and upright ears, a triangular face, and small horns (Fatmawati *et al.*, 2022).

Physicochemical evaluation is the most commonly used method in assessing milk quality. However, this method requires a relatively long processing time, high cost and chemical reagents that are not environmentally friendly (Hayes *et al.*, 2023). A spectroscopy-based analysis approach such as FTIR is a promising alternative. The ATR technique for sample measurement allows rapid, non-destructive analysis and provides simple sample preparation, making it very efficient for routine milk quality testing. Combined with multivariate analysis, it can analyze the complex data generated from FTIR measurements, making it a reliable analytical method for differentiating or classifying milk (Priyashantha *et al.*, 2021; Salleh *et al.*, 2019) as well as for milk authentication (Arifah *et al.*, 2022; Conceicao *et al.*, 2019; Julmohammad *et al.*, 2024; Windarsih *et al.*, 2022). Classifying goat milk from Malaysia has been revealed by evaluating FTIR profile data by partial least square (PLS) analysis (Salleh *et al.*, 2019). Classifying organic and conventional cow milks from multiple brands and regions in China by OPLS-DA has been reported (Bai *et al.*, 2022; Hou *et al.*, 2023). In addition, OPLS-DA has been widely used to assess milk quality, as demonstrated by Priyashantha *et al.* (2021) in assessing the effect of the feeding regimen on bovine milk quality in Northern Sweden, and by Sen *et al.* (2021), who combined PCA and OPLS-DA to differentiate the qualities of goat, buffalo and their mixtures.

To our knowledge, integrated approach of FTIR spectroscopy data as well as chemical proximate data, together with multivariate analysis by OPLS-DA to differentiate goat milk breeds grewed in Indonesia, such as Etawa, Etawa Crossbreed, Saanen and Sapera breeds, has never been reported. This study aims to evaluate the quality of goat milks from various breeds collected from different farm through a physicochemical and FTIR profile approach combined with multivariate analysis. However, this study has limitations due to the unavailability of a single farm in the country that raises all four goat breeds simultaneously, thus other aspects, likely enviromental condition (husbandry practices), microclimate, water, feed composition, and lactation can influence these milk samples. In this study, milks of Saanen and Sapera were obtained from the same farm (namely Farm A), meanwhile milk of Etawa from Farm B and Etawa Crossbreed from Farm C. The three farms are located at the same region and they applied the same feed sources, i.e. *odot* grass and tempeh dreg. All collected milks were of the same lactation period.

## MATERIALS AND METHODS

### MILK SAMPLES

Samples of fresh goat milk were collected from four different breeds, i.e., Etawa, Etawa Crossbreed, Saanen, and Sapera, at different farms located in Bogor Regency. All milk samples from different breeds were collected on the same week. There are three weeks for the milk sample collection, mentioning three replicates, all were within the end-period of their second lactation period across different farms (Farm A for Saanen and Sapera breeds, Farm B for Etawa breed and Farm C for Etawa Crossbreed). Feedstuffs for the goats in the three farms consisted of fresh *odot* grass (*Pennisetum purpureum* cv. Mott) and tempeh dregs as the main feeding from direct observation and interview. The composition of the tempeh dregs consisted of crude protein 14.53%, crude fiber 54.16% and crude fat 2.54% on wet basis (Handayanta, 2007), meanwhile the composition of *odot* grass consisted of crude fiber 78.32% and crude protein 13.90% on wet basis (Kamid *et al.*, 2024). Each goat milk sample, collected every week from five goats of the same breed, and pooled as a composite sample. The total composite samples from three replicates and four breeds were 12 samples. They were subjected to physicochemical analysis, including proximate analysis, pH, color and casein micelle sizes (12 data obtained). The same samples were analyzed using FTIR with two replicates in FTIR-ATR, resulting in a total of 24 samples for the analysis.

All goat milk samples are collected in the morning, transferred into plastic bottles (with screw cap) and stored at -20 °C prior to physicochemical quality analysis.

Meanwhile, for FTIR analysis, the milk samples were freeze-dried using a freeze dryer Alpha 2-4 LSC basic (Martin Christ GmbH, Germany) prior to the analysis.

### DETERMINATION OF PHYSICOCHEMICAL CHARACTERISTICS

Chemical characteristics include moisture, protein, fat, and ash contents, according to AOAC (2019). Moisture content was determined using the gravimetric method with a drying oven model UF110plus, Memmert GmbH, Germany (AOAC 926.08), ash content by high-temperature combustion in a muffle furnace model LE 6/11/R7, Nabertherm GmbH, Germany (AOAC 945.46), protein content using the Kjeldahl method (AOAC 991.20), and fat content by Soxhlet extraction method (AOAC 989.05). Total carbohydrates in milk were calculated by difference. Goat milk pH was determined using a pH meter (APERA 9500, APERA Instruments GmbH, Germany), with the procedure involving the use of standard buffers (pH 7.0 and 4.0).

Physical characteristics include, color and particle size of casein. Milk color measurement was performed using a chromameter (Konika Minolta CR-410, Japan), with color data obtained based on the International Commission on Illumination (CIE) system  $L^*$  (lightness),  $a^*$  (redness > 0, greenness < 0), and  $b^*$  (yellowness > 0, blue < 0), while  $h^*$  (hue) using a formula presented by Milovanovic *et al.* (2020):  $^{\circ}\text{hue} = \tan^{-1}(a^*/b^*)$ . The determination of milk particle sizes (casein micelles) was measured using particle size analyzer (PSA) Litesizer<sup>TM</sup> 500 instrument (Anton Paar GmbH, Austria), based on dynamic light scattering (DLS), with the procedure involving goat milk diluted 100 times in redistilled water, then homogenized and measured at 25 °C following the procedure by Breunig *et al.* (2024).

### FTIR-ATR ANALYSIS

The analysis of the chemical profile of milk by FTIR was carried out according to the method described by Salleh *et al.* (2019). The FTIR used in this study was modified using a ZnSe ATR crystal cell (Bruker Optik GmbH, Germany). Approximately 10 mg of the sample (freeze-dried) was utilized. The spectra data were acquired over 24 seconds in the range of wavenumber 4000–400  $\text{cm}^{-1}$ , and a resolution of 4  $\text{cm}^{-1}$ . The spectra was processed using OPUS software version. 8.9.7. (developed by Bruker).

### STATISTICAL ANALYSIS

Physicochemical data are presented as mean  $\pm$  standard deviation (SD). IBM SPSS statistical software, version 22 (IBM Corp., USA) was used for statistical analysis. One-way ANOVA was used to assess differences in physicochemical properties. The effects of different goat breeds were evaluated based on differences between means

at a 95% confidence level using the Tukey HSD test. Additionally, an independent *t*-test was used to compare the physicochemical properties of milk in Farm A (Saanen and Sapera) under the same farm conditions. This test aimed to know the different properties of the milks from different breeds, in the same farm.

Spectral data obtained from the FTIR instrument were analyzed by multivariate analysis using SIMCA software, version 18 (Sartorius Umetric, Sweden), with data consisting of absorbance values at wavenumbers 4000–400  $\text{cm}^{-1}$ . The multivariate analyses used were PCA and OPLS-DA. PCA was used to observe the general clustering between goat milk from various breeds and became the basic stage to continue the differentiation analysis using OPLS-DA. The models were evaluated based on the  $R^2X$  and  $Q^2$  criteria, respectively, representing the models fit to the data and its predictive capability. Pranata *et al.* (2021) stated that a model with an  $R^2X$  and  $Q^2$  value of 0.97 is reliable. Sample grouping was performed using OPLS-DA to refine the classification pattern from PCA. According to Worley and Powers (2016), OPLS-DA is used to refine the PCA model and identify important variables contributing to group separation, thereby serving as candidate biomarkers for each observed group. Furthermore, the quality of the OPLS-DA model is considered a good-predictive model if the  $R^2Y$  and  $Q^2$  values are  $\geq 0.6$ , its CV ANOVA result is significant ( $p < 0.05$ ), and its validation result by permutation shows the original model exhibits  $R^2Y$  and  $Q^2$  values higher than the permuted model (Hou *et al.*, 2023).

## RESULTS AND DISCUSSION

### PHYSICOCHEMICAL CHARACTERISTICS

The chemical analysis results based on wet basis and physical analysis results are presented in Table 1.

The moisture contents of goat milk samples in this study (83.61–87.08%) were not significantly different among the goat breeds, indicating that it is not associated moisture content with the breeds. This result is in accordance to that reported by Jaafar *et al.* (2018) and Mohsin *et al.* (2019) (85.32–89.05%). These results align with the moisture content test in a controlled environment (Farm A), which showed a not significant difference ( $p = 0.204$  *t*-test result) between samples. This finding confirms that both in goats raised on the same farm and on different farms with similar environmental condition (husbandry practices), water, feed composition, and lactation stage, the influence of breed on moisture contents non significant difference.

Table 1 show significant differences ( $p < 0.05$ ) in protein content. The protein content range 2.43–3.60%. The protein content of goat milk from Etawa was significantly different

**Table 1:** Physicochemical characteristics of goat milks from four different breeds.

| Milk characteristic*     | Saanen (Farm A)           | Sapera (Farm A)            | Etawa (Farm B)             | Etawa Crossbreed (Farm C) |
|--------------------------|---------------------------|----------------------------|----------------------------|---------------------------|
| Moisture (% wb)          | 87.08 ± 0.59 <sup>a</sup> | 85.58± 1.61 <sup>a</sup>   | 83.94 ± 2.01 <sup>a</sup>  | 83.61 ± 1.52 <sup>a</sup> |
| Protein (% wb)           | 2.43 ± 0.17 <sup>a</sup>  | 3.43 ± 0.09 <sup>b</sup>   | 3.60 ± 0.41 <sup>b</sup>   | 3.14 ± 0.46 <sup>ab</sup> |
| Fat (% wb)               | 4.17 ± 0.79 <sup>a</sup>  | 3.48 ± 0.90 <sup>a</sup>   | 4.28 ± 1.67 <sup>a</sup>   | 3.91± 1.35 <sup>a</sup>   |
| Ash (% wb)               | 0.74± 0.12 <sup>a</sup>   | 0.73 ± 0.20 <sup>a</sup>   | 0.80 ± 0.13 <sup>a</sup>   | 0.74 ± 0.20 <sup>a</sup>  |
| Carbohydrate (% wb)      | 5.58 ± 1.10 <sup>a</sup>  | 6.79± 2.35 <sup>a</sup>    | 7.39 ± 2.78 <sup>a</sup>   | 8.60 ± 1.31 <sup>a</sup>  |
| pH                       | 6.45 ± 0.04 <sup>a</sup>  | 6.58 ± 0.12 <sup>a</sup>   | 6.40 ± 0.04 <sup>a</sup>   | 6.45 ± 0.04 <sup>a</sup>  |
| Color (°Hue)             | 117.83± 2.95 <sup>a</sup> | 114.34 ± 6.10 <sup>a</sup> | 115.64 ± 2.72 <sup>a</sup> | 118.38± 1.70 <sup>a</sup> |
| (L*)                     | 87.10 ± 0.10 <sup>a</sup> | 87.73 ± 0.08 <sup>ab</sup> | 88.53 ± 1.05 <sup>b</sup>  | 86.38 ± 0.24 <sup>a</sup> |
| (a*)                     | -3.24 ± 0.21 <sup>a</sup> | -3.20 ± 0.31 <sup>a</sup>  | -3.05 ± 0.22 <sup>a</sup>  | -3.19± 0.13 <sup>a</sup>  |
| (b*)                     | 6.18 ± 0.79 <sup>a</sup>  | 7.26 ± 1.23 <sup>a</sup>   | 6.39 ± 0.57 <sup>a</sup>   | 5.92 ± 0.44 <sup>a</sup>  |
| Casein micelle size (nm) | 271 ± 30.37 <sup>b</sup>  | 249 ± 22.95 <sup>ab</sup>  | 219 ± 18.49 <sup>ab</sup>  | 201 ± 9.51 <sup>a</sup>   |

Note: Different superscripts in the same row mention the significant different mean value ( $p < 0.05$ ), and wb is wet basis (calculated based on the weight of fresh milk sample).

from Saanen ( $p < 0.05$ ), but not significantly different from Etawa Crossbreed and Sapera. From the same farm, goat milk from Saanen was significantly different from Sapera. This finding indicates milk protein content could be different between breeds. The t-test results of the goat milk protein contents from the same farm (Farm A) also shows a significant difference ( $p$  value = 0.001) between the two breeds. This result indicates at uniform environmental condition, the genetic factor (breed) plays a significant role in determining milk quality, especially its protein content. This result is in accordance to that reported by Curro *et al.* (2019), who compared Saanen and local breed (from Italy) under uniform environmental condition, showing different milk protein content between the two breeds. Xiang *et al.* (2025) also reported differences in milk protein content between Saanen and Toggenburg goats in China under uniform environmental and feeding conditions.

The milk protein content of the Etawa goat (3.6%) was the highest compared to those of other samples, and Saanen goat milk has the lowest protein content (2.4%), this is due to the moisture content value or the lowest solid content of Saanen. The results of this study are lower than those reported by Mohsin *et al.* (2019) (5.80% for Etawa and 4.83% for Saanen goat milks) and by Curro *et al.* (2019) (3.42% for Saanen). In this case, Jamnapari goat milk reported by Mohsin *et al.* (2019) is considered as Etawa goat milk in Indonesia.

The protein content of Sapera goat milk in this study (3.4%) is also lower than that reported by Fatmawati *et al.* (2022) and Suranindyah *et al.* (2018) (3.71-4.63%). The standard quality of goat milk in this study, according to the Thai Agricultural Standard (TAS) 2008 shows that Etawa milk is of good grade (3.6%), meanwhile Etawa Crossbreed and Sapera milks are of standard grade (3.1-3.4% of protein content). Saanen milk is out of the standard quality. Many factors affect the protein content of goat milk, including

goat breed, feed, lactation period, and environmental factors (Park and Haenlein, 2017).

The carbohydrate contents of goat milk samples in this study (5.58-8.60%) were not significantly different among the goat breeds. The Saanen milk in this study are higher than those reported by Curro *et al.* (2019), Scano and Caboni (2022), Jaafar *et al.* (2018), and Salleh *et al.* (2019) which revealed that the carbohydrate contents at 4.01-4.54%.

The fat contents of goat milk samples in this study (3.5-4.3%) were not significantly different among the goat breeds, indicating that it is not associated with breeds. These results align with the report by Mohsin *et al.* (2019), the fat content (4.20% for Jamnapari and 3.93% for Saanen goat milks). However, the fat contents of Etawa Crossbreed (3.9%) and Sapera goat milk in this study (3.5%) are approximately 1.5 and 1.2 times lower, respectively, than those reported by Suranindyah *et al.* (2018) (5.80% for Etawa Crossbreed and 4.30% for Sapera goat milks). Based on the fat content, Etawa and Saanen goat milks are of premium grade ( $> 4\%$ ), meanwhile Etawa Crossbreed milk is of good grade ( $> 3.5$  to 4), and Sapera milk is of standard grade (3.25-3.5% of fat content) (TAS, 2008).

Ash content indicates the presence of minerals in milk. The ash content of goat milks in this study (0.7-0.8%) shows no significant differences among breeds. The findings align with previous studies by Jaafar *et al.* (2018), Mohsin *et al.* (2019), and Singh and Sharma (2016) the ash content of Jamnapari (0.81-0.85%) and Saanen goat milks (0.67-0.70%).

The mapping of milk samples based on their chemical composition (protein, fat, and carbohydrates) in dry basis, can be seen in Figure 1. PCA result shows, milk from

four goat breed show a tendency for partial clustering, especially between the milk groups of goats from the same farm (Farm A= SA and SP), which began to appear separate in PC2. This separation was clearly observed with SP being at the upper end (positive value) of PC2 and SA being at the lower end (negative value) of PC2. This indicates that the separation of the two breeds is primarily explained by variables in the PC2, which is likely relate to minor differences in milk composition such as protein. Meanwhile, the ET and PE groups still showed a significant overlap. This pattern indicates slight chemical differences between the breeds, but not yet fully separate clearly in the unsupervised PCA analysis. This result is in accordance to that conducted by Jaafar *et al.* (2018), which found that protein content can describe the difference of Jamnapari (corresponding to Etawa) and Saanen milks.



**Figure 1:** PCA biplot ( $R^2X$  0.998 and  $Q^2$  0.876) for protein, fat and carbohydrate contents of goat milks from Etawa (ET), Etawa Crossbreed (PE), Saanen (SA), and Sapera (SP) breeds.

The pH values of goat milks in this study (6.4-6.6) are considered at neutral pH. The pH values of goat milks are within the range reported by other studies, 6.35-6.78 (Alyaqoubi *et al.*, 2015; Atika *et al.*, 2023; Garcia-Olarte *et al.*, 2024).

Color  $L^*$  values among different milks were also significantly different. The  $L^*$  value (89) of Etawa goat milk is significantly higher than that of Etawa Crossbreed (86) and Saanen (87). The  $L^*$  values of goat milks in this study are within the range reported by Garzon *et al.* (2024), Milovanovic *et al.* (2020), and Tarchi *et al.* (2025) (83.47-88.22).

The  $b^*$  value of goat milk samples in this study (5.9-7.3%) were not significantly different among the goat breeds. The positive result of these values indicates the color of goat milk is yellow, the higher  $b^*$  value give the closer color of milk to yellow. According to Cheng *et al.* (2019), it can be caused by carotenoids in the milk fat portion. In addition, Laurent *et al.* (2023) reported that, apart from carotenoid content, the yellow color of goat milk depends on vitamin B2 (riboflavin) content. In this study, the  $b^*$  value of goat milks was slightly higher than the values reported by

Garzon *et al.* (2024), Laurent *et al.* (2023) and Milovanovic *et al.* (2020) (3.29-5.8).

The  $a^*$  values (-3.2 to -3.1) in this study show no significant differences among breeds. The negative result of  $a^*$  value indicates a greenish milk color. The more negative  $a^*$  give the closer color to green. The results are lower than those reported by Garzon *et al.* (2024), Laurent *et al.* (2023) and Milovanovic *et al.* (2020) (-2.1 to -1.12). The overall color results indicate that differences in goat breeds are particularly in lightness ( $L^*$ ) and yellowness ( $b^*$ ). However, the combine of  $L^*$ ,  $a^*$ , and  $b^*$  values could provide a more accurate prediction of consumer visual perception (Cheng *et al.*, 2019).

Hue values of goat milk samples in this study (114.3-118.4) were not significantly different among the goat breeds. According to Milovanovic *et al.* (2020), hue is a color property based on 360° spectrum of red, yellow, green and blue. The results of this study were slightly higher than those reported by Garzon *et al.* (2024), Laurent *et al.* (2023) and Milovanovic *et al.* (2020) (108.8-110.9).

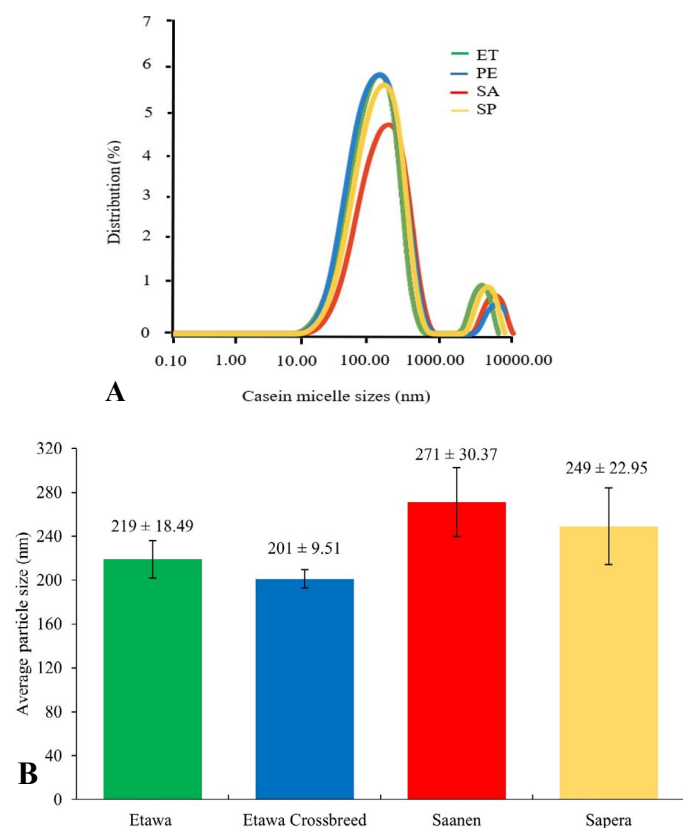
#### CASEIN MICELLE SIZES OF MILKS FROM FOUR BREEDS

Table 1 also shows that casein micelle sizes of the milks were significantly different among the breeds. The Figure 2A shows that goat milk particle size distributions (casein micelle sizes), averaged ranges from 201 to 271 nm, with the order of the smallest to the highest averaged milk particles (Figure 2B): Etawa Crossbreed, Etawa, Sapera, and Saanen milks. These casein micelle size differences reflect casein protein composition variations among different breeds.

The results of goat milk casein micelle sizes measurements (Figure 2A) show that the milk particle distribution has an irregular bimodal pattern, mainly localized around 200 nm. The average distribution of goat milk micelle size in this study is similar to that reported by Inglingstad *et al.* (2014), Pierre *et al.* (1999) and Zhao *et al.* (2021), with the casein micelle sizes of goat milk ranging from 200 to 280 nm.

In this study (Figure 2B), Etawa Crossbreed milk has a smallest casein micelle sizes (averaged, 201 nm), followed by Etawa (219 nm), Sapera (249 nm), and Saanen with the largest casein micelle size (271 nm), this indicates that variations in casein micelle sizes could be influenced by genetic differences that regulate protein composition, particularly casein, which is known to be highly polymorphic (Breunig *et al.*, 2024). Additionally Breunig *et al.* (2024) have found a negative correlation between  $\alpha_{s1}$ -casein level in milk and its casein micelle size, where higher  $\alpha_{s1}$ -casein levels result in smaller micelles. In this study, milk with smaller casein micelle sizes, such as those

of Etawa, Etawa Crossbreed and Sapera, exhibits higher protein contents than that of milk with larger casein micelle sizes, as found in Saanen goat milk. This fact could contribute to the future investigation for exploring the relationship between casein micelle sizes and milk protein contents as well as goat breed diversity.



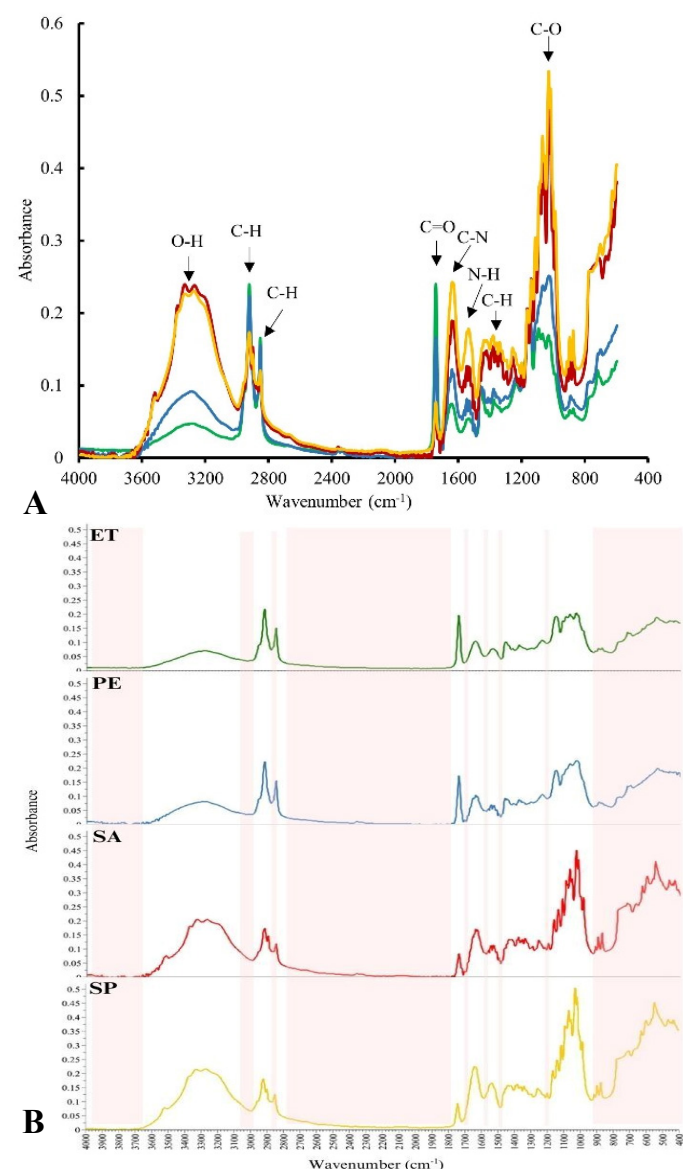
**Figure 2:** Particle size analysis results: (A) Casein micelle size distributions, (B) average casein micelle sizes (data  $\pm$  SD) of goat milks from different breeds (ET: Etawa, PE: Etawa Crossbreed, SA: Saanen, and SP: Sapera).

### FTIR SPECTRAL PROFILE

The FTIR spectral profiles of goat milks are shown in Figure 3. The results reveal relatively similar profiles in all goat milk samples (Figure 3A), i.e. similar in almost all peak bands observed from their FTIR spectra, however the intensities of certain bands are differed. The intensities were similar between Etawa and Etawa Crossbreed, and between Saanen and Sapera breeds (Figure 3B). The peak bands observed (Figure 3A, B) are in the region of N-H and O-H ( $3498\text{--}3000\text{ cm}^{-1}$ ), C-H ( $2990\text{--}2844\text{ cm}^{-1}$ ), C=O ( $1780\text{--}1640\text{ cm}^{-1}$ ), C-N ( $1630\text{--}1604\text{ cm}^{-1}$ ), N-H ( $1555\text{--}1500\text{ cm}^{-1}$ ), C-H ( $1465\text{--}1332\text{ cm}^{-1}$ ), and C-O ( $1186\text{--}980\text{ cm}^{-1}$ ) functional groups.

Some peak bands observed in the functional group region of  $3498\text{--}3000\text{ cm}^{-1}$  can be associated with N-H and O-H stretching vibrations, indicating protein and carbohydrate contents in milk (Sen et al., 2021; Dimitriou et al., 2025). The peak bands observed in the  $2990\text{--}2844\text{ cm}^{-1}$

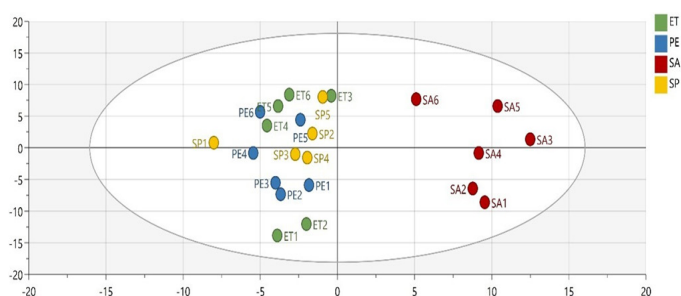
corresponds to C-H bound ( $\text{CH}_2$  stretching), regarding to the characteristics of milk fat (Balan et al., 2020; Pereira et al., 2020). Furthermore, the fat-related C=O stretching vibration of triglycerides and protein at peak bands around  $1750\text{--}1700\text{ cm}^{-1}$  are also observed, which is also found in other studies (Balan et al., 2020; Pereira et al., 2020). In addition, peak bands within  $1700\text{--}1500\text{ cm}^{-1}$  are related with amide I and amide II, indicating protein content, were also observed in milk of another study (Arifah et al., 2022). Meanwhile, the fingerprint region ( $1500\text{--}400\text{ cm}^{-1}$ ) showed several peaks bands that can be associated with C-O stretching (at around  $1000\text{ cm}^{-1}$ ) of fat and C-H stretching vibrations ( $1400\text{--}1200\text{ cm}^{-1}$ ) of carbohydrate or lactose. These results were also found in other study (Pereira et al., 2020).



**Figure 3:** FTIR spectra of goat milks (freeze-dried), obtained from FTIR-ATR instrument: (A) overlay of representative FTIR spectra for each breed (ET: Etawa, PE: Etawa Crossbreed, SA: Saanen, and SP: Sapera) and (B) averaged FTIR spectra data.

## DIFFERENTIATING GOAT MILK SAMPLES BY MULTIVARIATE ANALYSIS OF FTIR PROFILES

Figure 4 shows multivariate analysis results of FTIR profiles at 1700-1500  $\text{cm}^{-1}$  wavenumbers with fit values  $R^2X$  0.997,  $R^2Y$  0.89 and  $Q^2$  0.751 for OPLS-DA. A randomization test involving 100 permutations was also employed to validate the OPLS-DA result. The validity of the result was confirmed by looking at the original data of  $Q^2$  and  $R^2Y$ . Further validation using CV ANOVA showed a significant result with p-value of  $8.45 \times 10^{-3}$ .



**Figure 4:** OPLS-DA score plot in the wavenumber region of 1700-1500  $\text{cm}^{-1}$  ( $R^2Y$  0.89 and  $Q^2$  0.75) of Etawa (ET), Etawa Crossbreed (PE), Saanen (SA), and Sapera (SP).

The score plots of OPLS-DA from FTIR profiles from the wavenumber region, they could divide the milk samples into two groups, according to goat breeds, i.e. Etawa, Etawa Crossbreed, Sapera (Group 1) and Saanen (Group 2). Milks from the different breeds (Saanen and Sapera breeds) but from the same farm (Farm A) could be separated obviously in different groups (Figure 4). Etawa, Etawa Crossbreed, and Sapera goat milk has important wavenumbers at region 1553-1545  $\text{cm}^{-1}$  (amide II, N-H) functional groups, indicating protein. According to Barth (2007) the amide II is associated N-H bending vibrations and C-N stretching vibrations. For Saanen goat milk, the important wavenumbers at region 1675-1673  $\text{cm}^{-1}$  were mentioned for protein (C=O, amide I). The amide I region, which are showing the chemical bound of protein backbone, is also observed in the studies of skim milks (Li *et al.*, 2021) and chicken meat sample (Molee *et al.*, 2022). The substantial contribution from the amide regions (amide I and II) indicates that differences in protein composition are one of the main features of FTIR profiles to differentiate milks. This is confirmed by some studies on thermal processing of goat milk (Chen *et al.*, 2025), dairy product (Saji *et al.*, 2024), and cow milk sample during lactation (Rachah *et al.*, 2021). Through the finding of this study by multivariate analysis, FTIR profiles could give a substantial benefit for the differentiation of goat milks. This study revealed that different breeds could affect different chemical properties of milks, described by FTIR profiles. This study confirms the thoughtful results of FTIR profiles used together with multivariate analysis for differentiating milks from different breeds.

## CONCLUSION AND RECOMMENDATION

Protein content and color value  $L^*$  were the main properties which could divide the milk qualities from different breeds. Milk properties of Sapera and Saanen breeds from the same farm prove this finding. Multivariate analysis result of the FTIR profiles also show the same result for the two breeds. This can lead to the future research using FTIR for differentiation of milks from different breeds (in the same farm) which is a fast method.

## ACKNOWLEDGEMENT

The authors would like to thank the Indonesian Education Scholarship, the Center for Higher Education Financing and Assessment, Ministry of Higher Education, Science, and Technology of Republic Indonesia and Endowment Fund for Education Agency, Ministry of Finance of Republic Indonesia for the scholarship support (ID Number: 202209091617) and funding for this research.

## NOVELTY STATEMENT

This study addresses the first report of FTIR profiles of goat milks from different breeds (in the same farm) could differentiate milk qualities based on multivariate analysis (OPLS-DA). Micelle sizes and size distribution profiles of the milks are also revealed. These findings also fill a research gap by presenting the physicochemical characterization, thereby this study could provide a new perspective on potential and reliable methods for assessing goat milk quality and differentiation.

## AUTHOR'S CONTRIBUTION

YFK: Data curation, formal analysis, investigation, methodology, visualization, funding acquisition, and drafting the manuscript and critical review. HNL: Conception and design of the study, methodology, supervision, visualization, analysis and interpretation of data, project administration, drafting the manuscript and critical review. EP: Supervision, critical review. NDY: analysis and interpretation of data, supervision and critical review.

## GENERATIVE AI AND AI-ASSISTED TECHNOLOGY STATEMENT

The authors of this manuscript declare that no generative AI and AI-assisted technology were used to create content and data as well as text languages

## CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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