International Journal of Agricultural Technology 2025 Vol. 21(5):1793-1804
DOI: https://doi.org/10.63369/ijat.2025.21.5.1793-1804
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Assessment of quality and safety at critical control points of cow's milk produced in Northern Mindanao

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Hebron, I. U., Cuizon, T. S., Furog, A. C., Gonzaga, J. M., Maglacion, L. N. A., Hallasgo, M. H. C. and Taculod, T. A. (2025). Assessment of quality and safety at critical control points of cow's milk produced in Northern Mindanao. International Journal of Agricultural Technology 21(5):1793-1804.

Abstract Results showed that 60% of raw milk samples tested positive in Alcohol Precipitation Test, but confirmatory tests with Clot on Boiling proved the samples otherwise. Methylene Blue Reduction Test at the different critical control points rated very good implying their acceptability for processing. Physico-chemical tests indicated butterfat at 4.58%, Solid Non-Fat at 8.92%, protein at 3.52%, Lactose at 4.72%, and Brix at 9.2%. Organoleptic quality tests showed acceptable appearance, color, and sedimentation. However, microbial culture shows *E. coli* and Total Coliform Counts are present beyond the acceptable limits in processed flavored milk, but none in plain pasteurized milk. The fat, SNF, and lactose contents were beyond minimum standards, while the protein was a little below the acceptable limits. These results may be attributed to the farms' compliance with the Risk Management Plan for milking required by the National Dairy Authority that ensured the production of safe and quality milk. Regular monitoring and evaluation with further tests for farm hygiene and sanitation with bacterial tests may be done in the future.

Keywords: Butterfat, *E. coli,* Milk handling, SNF

Introduction

Milk is a good source of nutrients for human health. However, if it is altered with other external materials by milk value chain actors or if it is contaminated with germs or contains residues of anti-microbial agents, among others, it may cause health hazards to consumers. Milk handling and hygiene during milking is one of the factors that affect milk quality. Tollossa *et al.* (2014) observed that the manner of storing milk, the extent of cleaning the equipment used in storage, and water quality determine the quality of milk that reaches the consumers.

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According to Schroder (2013), dairy farmers experienced a reduction in microbial contamination, which they attribute to the traditional methods that they no longer practice. Also, farmers no longer had inadequate numbers of milk containers, and transport distances were shortened, providing adequate cooling for milk at transport and training people about the importance of hygiene. The dairy farms in northern Mindanao are mostly small-scale, and they keep their milk for local processing and marketing. At the same time, the bulk of their production was brought to the processing plant owned by the Federation of Primary Cooperatives. This condition closely resembles the situation in Africa, where much of the national milk supply is sold without regulation. Thus, it is essential to test the milk supplied by formal and informal sectors to ensure the quality and safety of milk (Deka *et al*., 2020).

Milk is used throughout the world as human food in various forms. It is a sterile fluid when secreted from the alveoli of the udder of a healthy cow. However, once it is released from the healthy animal, its quality is subject to how the dairy farm workers handle the milk, microbial contamination might generally occur from different sources (Washaya, 2022). Conditions for contamination of raw milk at different critical points due to less hygienic practices in pre-milking udder preparation, sub-optimal hygiene of milk handlers, and poor sanitation practices associated with milking and storage equipment (Garedew *et al.,* 2012). The demand of consumers for safe and high-quality milk has placed a significant responsibility on dairy producers, retailers, and manufacturers to produce and market safe milk products (Mennane *et al.,* 2007).

Milk is a complete food containing the six basic nutrients, with water composing the bulk of it. With these nutrients, its high moisture content and neutral pH make it a perfect medium for bacterial growth and multiplication. These bacterial populations may include disease-causing agents. Paraffin *et al.* (2018) perceived that milk contamination could come from the milking environment, wind, milking equipment, feeds, soil, feces, farm personnel, and the barn. While it is believed that milk contamination can happen in various stages of the value chain, milk consumers usually emphasized to observe sound quality control measures at the farm level. Butler *et al.* (2008) linked the composition of milk to the stage of the pasture, grazing periods, diet composition, processing, and sensory and potential nutritional qualities of the milk.

Milk testing and quality control are critical components of any milk processing industry, large or small. To produce a high-quality dairy product, it is essential to ensure hygienic production and maintain a stable storage temperature. The study determined the quality of fresh, stored, and transported milk using the Clot on Boiling Test, Alcohol Precipitation Test, Methylene Blue

Dye Reduction Test, total coliform, and *E. Coli* colony forming units, determined the organoleptic test in terms of appearance, presence of off-flavor/odor and sediment, determined the percent fats, solid nonfat, protein, and lactose contents of milk at different critical control points and evaluated the milking and milk handling practices in dairy farms in accordance with Risk Management Plan for Dairy.

Materials and methods

The study was conducted in six dairy farms in northern Mindanao from January 2022 to September 2024. Pre-implementation protocol was done where the researcher sought permission from the farm manager and the owner of dairy farms. The purpose of the request was to test the milk quality in terms of microbial load, physicochemical contents, and milk handling practices. Backyard and semi-commercial dairy farms in northern Mindanao were the scope of the study. The data gathered were the responses of the milk collected at different critical control points on Clot on Boiling (COB), Alcohol Precipitation Test (APT), Methylene Blue Reduction Test (MBRT), *Escherichia coli*, and Total Coliform tests. The same milk was analyzed for the fats, proteins, lactose, solid non-fat, and Total Soluble Solids (% Brix) contents.

Microbial test for milk quality

Collection of milk and testing were done twice a day, at 3:00 am and 3:00 pm, for 60 days. Each bucket of milk that was produced was tested for COB, APT, and MBRT. The same buckets were tested when they were stored and transported. Equal amounts of milk sample and 72% alcohol solution were mixed in a test tube, and the formation of precipitates was observed. To confirm APT, COB was done using the same sample preparation, held above the flame of an alcohol lamp until it boiled, and showed clotting. In MBRT, five ml of milk sample was mixed with 0.5 ml of methylene blue solution. The upper part of the test tube was tightened with a rubber stopper and gently inverted to ensure proper mixing of the solution. The mixture was incubated at 37 °C in a water bath. Then, the time (in hours) for the blue coloration to disappear was measured, and the equivalent descriptive ratings, as defined by Canadian Public Health (Table 1).

Table 1. Rubrics for evaluating MBRT

Time Duration to change Blue to White Color	Quality
5 hours above	Very Good
3 to 4 hours	Good
1 to 2 hours	Fair
Less than ½ hours	Poor

Using Compact Dry EC and Compact Dry TC (NISSUI Pharmaceutical Co., Ltd. Japan), colony-forming units for *Escherichia coli* and total coliforms were tested from the bulk milk of the dairy farms randomly sampled throughout the study.

Organoleptic quality test of milk

This was determined based on its appearance, presence of off-flavor, and sediments. The appearance of milk was determined by swirling the cup and observed for signs of clotting and film formation. Rating was done using rubrics in the Likert scale in Table 2.

Table 2. Likert Scale for the appearance of milk

Appearance	Description	Excellent 8-10	Good 5-7	Fair 0-4
Color	Fat distributed throughout the milk determined in color	Creamy yellow	Whitish	Pale white
Separation of particles	Consistency of fat particles	Emulsion-like	Fat separates	Watery

Physico-chemical quality test of milk

Tests for fats, proteins, lactose, and solid non-fat were performed in a milk analyzer (Boeco, Germany). Total Soluble Solids were determined using a digital Brix Refractometer (Atago Co. Ltd. Japan). Data were obtained from the milk samples upon milking after they were stored prior to their delivery to the milk plant and after they were transported a certain distance and reached the processing site. A mixed design using percentages and averages triangulated with interviews and a farm survey was used to evaluate the extent of milk quality and safety at the different critical control points.

Results

Profile of different dairy farms

The study on ensuring cow's milk quality tested the microbial attributes in different critical control points of milk and was conducted in small and medium-scale dairy farms in Northern Mindanao, Philippines. They raised Holstein-Sahiwal and Holstein-Jersey crosses of dairy cattle and were technically supported by the National Dairy Authority. They raised three to 50 milking cows, yielding six to 10 liters per cow per day. The majority used bucket-type milking machines, while 17% did hand milking.

This practice ensured high-quality milk production in the region, which is characterized by a diverse range of dairy farming practices. The NDA plays a crucial role in promoting sustainable dairy farming through training and resources which aimed to enhance productivity and safety standards within the industry. This profile served as a foundation for understanding the operational dynamics of dairy farms in Northern Mindanao and their contributions to local milk production.

Microbial test

Results showed that about 27% of the raw milk samples from selected farms tested positive for APT, where precipitates were visible in the sample. These were subjected for confirmation using COB. All of the milk samples from the selected dairy farms for processing at the milk plant passed the COB test, including those that tested positive in APT. The milk sampled from the buckets as they appear in the three critical control points showed no clotting at the boiling test. No changes in MBRT-Milk mixture color occurred before five hours.

The APT, COB, and MBRT qualitative tests showed that the milk sampled from the dairy farms in the region and tested at source, after storage, and after being transported to the processing plant indicated no significant risks.

These tested negatives (0 to 1 *Escherichia coli* colony-forming unit per milliliter) when the fresh, pasteurized milk was tested using Compact Dry EC (Japan) and Compact Dry TC (Japan), as indicated in Table 3. However, for the flavored milk, the number of colonies for both *E coli* and Total Coliforms found to be the minimum implying a certain degree of contamination.

Organoleptic test

Milk at different control points was creamy white to yellowish and without any unacceptable smell. Barny aromas were detected on the farm, but these totally disappeared after the milk was stored and transported. Very few to negligible sediments were collected in the sediment test.

Physico-chemical test

Physico-chemical parameters (fat, solid non-fat, lactose, and protein) were measured by an ultrasonic milk analyzer. The researcher noted that the analysis for all the test parameters did not change regardless of the time the milk was stored and handled, as shown in Table 3.

Table 3. Analysis of milk samples from dairy farms in Northern Mindanao

Milk components	Mean	Acceptable levels for Philippine milk processing plants	Remarks
Fat	4.57 %	3.5 - 4.00 % (NDA NLT3%)	Higher
Solid NonFat, SNF	8.92 %	More than 6% (NDA 8.25%)	Higher
Lactose	4.72 %	Not below 4.0	Within limits
Protein	3.16 %	3.0 - 3.3 %	Little lower
Total Soluble Solids (%Brix)	9.78 %	Not defined except for colostrum	
E. coli	0 cfu/ml	0	Fresh Milk
E. coli	16 cfu/ml	0	Flavored Milk
Total coliform	338 cfu/ml	10 cfu/ml	Flavored Milk

Farm's compliance to risk management plan for dairy

Re-milking process

The dairy farm milkers were all aware of the necessary hygiene and sanitary practices prior to the milking process. The farm personnel in dairy farms practice proper hygiene and wear Personal Protective Equipment (PPE) on the farm. When hand milking, the farm personnel keep their hands clean and their fingernails short. The farms are provided sanitary products and practices, such as hand washing with soap and a footbath. Milking machines and milk containers were cleaned and dried after milking. The milkers checked everything to be clean before using the containers and equipment. In cleaning the utensils, the dairy farms used tap water and liquid detergent. Sanitizers are seldom utilized such that cleanliness monitoring is essential. Dairy farms separated sick and treated cows from healthy ones. They isolate sick animals directly once they observed that the cows are found to be not in good condition. After milking, the dairy farms released their cattle from their milking stalls. Cows responded thirstily after milking, and farm workers water them soon after milking.

Milking proper

Before attaching the milking unit, the milkers guaranteed that the cows were mastitis-free. Foremilk was stripped to ensure the normal consistency of the milk. All of the dairy farms practiced forestripping prior to milking. However, forestripped milk was directly drained to the floor, providing greater chances of contamination. The milkers observed washing the teats and udders of cows with detergent. Teats were pre-dipped in iodine solution and wiped dry before milking clusters were attached. These practices were observed; however, the 30 seconds for the antiseptic to act on the teats were not religiously observed prior to wiping. Only 17% performed pre-dipping. All of the dairy farms washed the udders with water, it is making impossible to dry them properly before the cups were on.

Post milking process

All of the farms practiced to clean` the milking parlor after milking. The entire area was washed with soap and water and kept dry. They practiced post-dipping and were aware of the benefits it would provide to their cows. With uncertainty in this practice was the concentration of the iodine solution. Also, it was observed that no farm practiced adding the teat dips with emolients and humectants like glycerin that will keep the teats from getting dry and chappy. The cows were brought to the pasture after milking so that they remain standing and grazing and will not keep the opened teat canals touching the dirty floor if they lie down. The milk harvested was stored in food-grade containers, including a limited number of stainless-steel cans and plastic buckets. The majority of dairy farms usedfreezers to store milk prior to delivery to the processing plant, and the milkers ascertained that milk was stored in sanitary conditions.

Discussion

Microbial Tests on milk done with APT, COB and MBRT all ensured good quality of milk evaluated at the different critical control points. APT tests whether milk can withstand thermal processing. When milk proteins become unstable due to mineral imbalance, they form precipitates (Pinto da Rosa *et al.,* 2020). Atasever (2012) emphasized that the precipitations observed in APT is not the lone method to check milk quality. Thus, many processors confirm APT results with COB to decide whether milk will be accepted for processing or not.

Further tests could be done on titratable acidity and bacterial cultures. MBRT is determined by observing the color that appears in the milk after the addition of Methylene Blue dye. It is a redox indicator, which loses its color when it comes under the effect of a lack of oxygen. Bacterial metabolism causes a decrease in oxygen in the milk due to the formation of reducing substances. The time taken by the dye to get reduced determines the number of microbes in the milk and the metabolic reactions in microorganisms. Similar to Silva *et al.* (2016), MBRT was occasionally used in Northern Mindanao as an alternative method for an effective, efficient, yet cheaper microbiological test in milk. When the milk was not decolorized within five hours, the Likert indicated that the milk quality was Very Good. The result implies that the milk sampled from the three critical control points in the milk value chain was acceptable for processing.

Organoleptic tests showed that the milk obtained from cows in Northern Mindanao was creamy white to yellowish, which is the typical milk color rated as excellent on the Likert scale. The odor did not reveal unacceptable smells. Off-flavor test helps to determine the aroma of milk. The results showed that the raw milk has a noticeable barny aroma. There were some minor off-flavors of the milk when it was at the farm, but it totally disappeared once it was stored and transported to the processing plant. Hence, it did not affect the quality of milk. The result implied that during the test, the evaluator smelled the unclear flavor of milk, which is probably masked by the odor of the barn. Raw milk sediment indicates negligence in milk handling and sanitation. There were no visible or extraneous matters in the filtrate, which conforms to the standards set by Dairy Safety Regulations per DA Administrative Circular number O4 series of 2019 (DA-NDA, 2019). The results indicate that milk from the selected dairy farms in northern Mindanao met the color, appearance, flavor, odor, and sedimentation tests for good-quality milk. Physico-chemical Tests on the butterfat, SNF, and lactose contents of milk exceeded the minimum set by the National Dairy Authority and the Northern Mindanao Federation of Dairy Cooperative Inc.'s Highland Fresh Processing Plant.

Cow's milk usually contains 8.8% SNF. Milk's solid non-fat contains lactose, casein, whey proteins, and minerals. Milk with a high solid non-fat content is valuable to consumers for its flavor and nutritional value. Milk processing plants require a minimum of 6%, while selected dairy farms in the study produced 8.92% (range 8.39 % - 9.20%). These contents imply that the milk is nutritious and delicious. The higher SNF content could be attributed to the high forage content in the diet, as most of the farms in the study practiced grazing, cutting, and carrying, and a little concentrate feed was given twice a day. Usually, cow's milk has about 4.6% lactose. Lactose, being the major carbohydrate source, its primary role is to draw water into the milk as it is formed in the udder epithelium (Sun, 2016). Lactose produced in the farms under study was greater than the standards, implying that the cows were fed adequately and produced the desired energy levels.

Milk contains approximately 3.25% protein of which approximately 80% is casein and 20% are whey proteins. According to science-based rating scales, both proteins are of good quality and include all essential amino acids at sufficient levels to sustain the numerous functions of the protein. The protein levels in the milk obtained from dairy farms in Northern Mindanao range from

3.0 to 3.35%. Its average (3.16%) is lower than the standard, which may imply that the protein in the diet may be inadequate. Considering the diet provided, there needs to be more protein feeds given. During the sampling, in-depth interviews were conducted and triangulated with farm observations. It was noted that all of the farms feed their cows with forage in a cut-and-carry scheme. They were fed as silage added with concentrate feeds provided during milking. This feeding practice has influenced the greater fat contents in the milk produced, concurring with the findings of Jaakamo (2019) that a greater portion of forages in the diet increases milk fat content. The incorporation of concentrate feeds among dairy farms in the region is intended to keep the cows in the milking parlor during milking. Several food processing by-products were supplemented to cut down costs and augment the limited corn and forage supplies. These include Brewer's Spent Grains, pineapple pulp, cassava pulp and banana peelings.

The Food and Agriculture Organization and International Dairy Federation (FAO and IDF, 2011) require that the milker wear suitable and clean working clothes, keep hands and arms clean, especially during milking, cover any cuts or wounds, and be free from infections or diseases that can be transmitted to the milk. However, some practices were often compromised due to the cold weather, such as taking a daily shower and the use of clean, unripped, non-latex gloves, and some facilities and containers need to be dedicated for use in the milking parlor. They complied with FAO's guidelines stipulated in Good Dairy Farming Practices that animals whose milk is unfit for human consumption were milked last or in a separate bucket system. Cows have continuous access to potable water and peak at feeding and milking periods (Cardot *et al*, 2008). Waterers were positioned near feeders and milking parlors as cows may consume 30% to 50% of its daily water intake within one hour after milking (Becker, 2024).

Milk is ensured to be handled properly after milking. It must be cooled on time, in a clean and tidy area with adequate temperature to hold the milk without spoiling. (FAO and IDF 2011). The Risk Management Plan (RMP) for dairy added the need for segregating the specialty milk (colostrum) from milk for human consumption. Risk Management Plan for Dairy (DA-NDA, 2019) emphasized that only detergents and sanitizers recommended will be used for cleaning and sanitizing the milking parlor, and they must be appropriate for the dairy farm water. The milking parlor is required to be washed with warm alkaline detergent at least once a week or when the microbial population increases to prevent carryover and contamination in milk. In this aspect, all of the farms in the study applied alkaline detergents in washing their milking machines and with warm alkaline wash at least weekly. They thoroughly cleaned the milking area, milk collection area, storage area, and yard. But most farms do not have adequate Personal Protective Equipment, and if there is, these are not kept clean.

In the Dairy Safety Regulations imposed by the DA-NDA (2019), post-dipping was required since it takes about an hour after miking for the streak canal to re-close. A stronger solution of iodine (1.5%) with 10% glycerin was recommended for dipping or spraying. Iodine will seal the teats that were opened during milking and prevent the entrance of pathogens into the udder, causing infections.

Food-grade plastic used as milk containers is quite challenging to clean. Also, the length of time it can store raw milk, preserving it in good condition, is not yet known and may be an area for research. This practice complied with the standards of the FAO and IDF (2011) that require milk storage equipment to be cleaned and sanitized before use. Milk contact surfaces were sanitized.

The study found that dairy farms in Northern Mindanao are producing milk that passed the minimum standards for processing, and they complied with most of the standards set in the Risk Management Plan for milking of the National Dairy Authority and the Good Dairy Practices of the Food and Agriculture Organization. However, there are more avenues that the farms can improve to further their productivity. The milking and milk handling practices they do in their daily routines were responsible for producing quality milk and ensuring them upon harvest, storage, and delivery to the milk processing plant. Routine tests on APT, COB, MBRT, *E. coli*, and Total Coliform colony counts, appearance, color, odor, and sediment tests indicated that the farm produced acceptable attributes within the critical control points of the value chain. The Fat, SNF, and Lactose contents were beyond the standards, while its protein and density were a little below the minimum. It is recommended that regular monitoring and evaluation be done on the milk produced at the farm level and the flavored milk processed in the plants to ensure the safety of the consumers. It is also recommended to conduct hygiene and sanitation tests for the equipment in contact with the milk to provide information necessary for decision-making and strategic actions for the dairy farmers.

Acknowledgements

The authors are thankful to the participating dairy farms and processing plants for their consent in conducting interviews and sampling milk. They also express sincere thanks for the fund support of the DOST-PCAARRD Livestock Research Division. Deep appreciation is extended to USTP Research Centers and Innovations, the Center for Dairy Research and Innovations and the College of Agriculture of USTP-Claveria.

Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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(Received: 2 October 2024, Revised: 12 June 2025, Accepted: 26 August 2025)