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**ASSESSMENT OF DAIRY COW MILK QUALITY BASED ON UTMT-1,
BOILING, AND ALCOHOL TESTS AT IJEN MAKMUR DAIRY FARM,
BANYUWANGI REGENCY, EAST JAVA**

Claudia Shinta Bella¹, I Gusti Ayu Agung Suartini², I Wayan Suardana^{3*}

¹Veterinary Education Student, Faculty of Veterinary Medicine, Udayana University;

²Biochemical Laboratory, Faculty of Veterinary Medicine, Udayana University;

³Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Udayana University;

*Corresponding author: wayan_suardana@unud.ac.id

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Abstract

Dairy milk is a highly nutritious animal-based food with significant economic value for local farmers. Milk quality is influenced by udder health and physicochemical stability, particularly in subclinical mastitis that often remains clinically undetected. This study aimed to evaluate the quality of Friesian Holstein (FH) milk using the Udayana Tokcer Mastitis Test (UTMT-1), boiling test, and alcohol test at Ijen Makmur Dairy Farm, Banyuwangi, East Java. A descriptive observational study was conducted using purposive sampling of 17 fresh milk samples from lactating FH cows. Most samples showed mild to moderate subclinical mastitis, with 64.7% testing positive based on UTMT-1. The boiling and alcohol tests, especially the 95% alcohol test, indicated decreased physicochemical stability with increasing UTMT-1 scores. Spearman correlation and Chi-square analyses showed significant and strong associations between UTMT-1 and both tests ($p < 0.05$). These findings indicate that the combined use of UTMT-1, boiling, and alcohol tests is an effective, practical, and economical screening method for milk quality assessment at smallholder dairy farms. This study was limited by the small sample size and single-farm location; future studies should include larger samples, wider areas, and further evaluation of these methods.

Keywords: dairy milk, milk quality, UTMT-1 test, boiling test, alcohol test.

INTRODUCTION

Milk is a nutritionally valuable animal-based product with important economic benefits for local dairy farmers (Zamrudi *et al.*, 2023). Banyuwangi Regency is one of the regions in East Java with considerable potential for dairy cattle development. However, milk production declined from 1,673,452 kg in 2018 to 1,397,635 kg, indicating challenges in maintaining productivity and milk quality at the farm level (Ningrum *et al.*, 2022). This decline underscores the need for effective quality monitoring strategies to support sustainable dairy production.

High-quality fresh milk is characterized by physical stability and reflects optimal udder health. In contrast, subclinical mastitis remains a major constraint on milk quality, as it increases somatic cell count and alters milk protein structure, leading to instability during heating or

alcohol testing (Harjanti *et al.*, 2020). Rapid screening methods such as the boiling and alcohol tests are therefore useful for early detection of physicochemical instability in fresh milk (Mukarromah, 2025). Subclinical mastitis is commonly detected using the California Mastitis Test (CMT), which estimates somatic cell levels through viscosity reactions between reagents and milk samples (Ndoy *et al.*, 2024). However, the high cost and limited availability of CMT reagents restrict their routine application in smallholder dairy systems.

To overcome these limitations, the Veterinary Public Health Laboratory, Faculty of Veterinary Medicine, Udayana University, developed the Udayana Tokcer Mastitis Test (UTMT-1), an economical and locally produced reagent that can be applied without complex equipment. Despite its potential as a mastitis screening tool, evidence linking UTMT-1 results with conventional milk quality tests remains limited. Therefore, this study aims to evaluate fresh milk quality by integrating UTMT-1, boiling test, and alcohol test results, providing a comprehensive physicochemical assessment of milk produced by the Ijen Makmur Dairy Farmer Group in Banyuwangi.

MATERIALS AND METHODS

Ethical Approval for Animal Subjects

Ethical approval was not required because this study did not involve animal experimentation or invasive procedures. Milk samples were collected through manual milking from lactating dairy cows during routine farm practices, with no interventions affecting animal welfare.

Research Subjects

The research subjects consisted of fresh milk samples collected from Friesian Holstein (FH) dairy cows raised at the Ijen Makmur Dairy Farmer Group, Banyuwangi Regency, East Java. Each cow represented one sampling unit, from which 15 mL of fresh milk was collected, resulting in a total of 17 samples from 17 lactating FH cows. The sample size was determined by the availability of healthy lactating cows at the study site. All selected cows had relatively similar age and lactation periods and were managed under uniform feeding, milking, and husbandry practices to minimize confounding factors.

Research Methods

Study Design

This study employed a descriptive observational design with purposive sampling. Lactating FH cows were selected as the sampling criteria. Milk quality was assessed using the Udayana Tokcer Mastitis Test (UTMT-1), boiling test, and alcohol test conducted at the Ijen Makmur dairy farm.

Materials and Equipment

The materials used included fresh FH milk samples (15 mL per cow; 17 samples in total), UTMT-1 reagent for subclinical mastitis detection, 70% and 95% alcohol solutions for milk stability testing, distilled water for cleaning equipment, and sample labels. The equipment consisted of a micropipette, mastitis paddle for UTMT-1 testing, test tubes and racks, Bunsen burner, spirit lamp and lighter, test tube holder, plastic sample bottles, and disposable gloves.

Sample Collection

Milk samples were collected once during the morning milking session. Approximately 15 mL of milk was aseptically collected into labeled plastic bottles using disposable gloves to prevent contamination. The samples were immediately subjected to testing.

Milk Quality Examination

Milk quality was evaluated using three methods: boiling test, alcohol test, and UTMT-1 test.

For the boiling test, 5 mL of milk was placed into a test tube and heated over a Bunsen flame until boiling. Milk that remained homogeneous was classified as good quality, whereas coagulation or curd formation indicated reduced quality.

For the alcohol test, 3 mL of milk was placed into two separate test tubes, followed by the addition of 70% and 95% alcohol, respectively. The mixtures were gently shaken and observed. The presence of clots indicated a positive result and reduced milk stability, while the absence of clots indicated good-quality milk.

For the UTMT-1 test, 2 mL of fresh milk was mixed with 2 mL of UTMT-1 reagent (1:1 ratio) in a mastitis paddle and gently agitated for 10–15 seconds until homogeneous. Results were interpreted based on gel formation and viscosity, with increased gel thickness indicating higher somatic cell counts and subclinical mastitis. Results were categorized from negative (–) to strongly positive (++++) based on gel formation intensity.

The results of the alcohol and boiling tests were categorized as negative (–), slightly positive (+), moderately positive (++), or strongly positive (+++), reflecting decreasing physicochemical stability of the milk.

Data Analysis

The data obtained from milk quality testing were analyzed using descriptive quantitative methods and presented in tabular form. The results of the UTMT-1 test, boiling test, and alcohol test were summarized to describe the distribution of milk quality parameters.

Spearman's rank correlation analysis was used to determine the relationships between UTMT-1 results, boiling test outcomes, and alcohol test outcomes in relation to the quality of Friesian Holstein dairy cow milk. In addition, the Chi-square test was applied to evaluate the associations between categorical variables. All statistical analyses were performed using IBM SPSS Statistics version 25.

RESULTS AND DISCUSSION

Results

The results comprise UTMT-1, boiling, and alcohol test outcomes obtained from 17 dairy cow milk samples at Ijen Makmur Dairy Farmer Group, Banyuwangi Regency.

UTMT-1 Test

The results of the Udayana Tokcer Mastitis Test-1 (UTMT-1) conducted on 17 Friesian Holstein milk samples are presented in Table 1. A total of 64.7% of the samples tested positive for subclinical mastitis, consisting of 11.8% strongly positive (+++), 35.3% moderately positive (++), and 17.6% mildly positive (+). Meanwhile, 35.3% of the samples showed negative results.

Table 1. UTMT-1 test results of Friesian Holstein dairy cows at KPSP Ijen Makmur

UTMT-1 Test Result	Frequency (n)	Percentage (%)
Negative	6	35.3
Positive 1 (+)	3	17.6
Positive 2 (++)	6	35.3
Positive 3 (+++)	2	11.8
Total	17	100.0

Overall, the findings indicate that the majority of FH dairy cows at KPSP Ijen Makmur were affected by mild to moderate subclinical mastitis, suggesting that subclinical mastitis remains a dominant issue with potential implications for milk quality.

Boiling Test

The boiling test results of the 17 milk samples are summarized in Table 2. The results showed that 41.2% of the samples were negative, 52.9% were mildly positive (+), and 5.9% were moderately positive (++). Most samples exhibited a mild positive reaction, indicating early physicochemical instability of the milk.

Table 2. Boiling test results of Friesian Holstein dairy cows at KPSP Ijen Makmur

Boiling Test Result	Frequency (n)	Percentage (%)
Negative	7	41.2
Positive 1 (+)	9	52.9
Positive 2 (++)	1	5.9
Total	17	100.0

Alcohol Test

Based on the alcohol 95% test results presented in Table 3, 35.3% of the samples were negative, while 23.5%, 29.4%, and 11.8% showed positive reactions at levels (+), (++), and (+++), respectively. These findings indicate that most milk samples exhibited positive reactions to the alcohol 95% test, reflecting reduced physicochemical stability.

Table 3. Alcohol 95% test results of Friesian Holstein dairy cows at KPSP Ijen Makmur

Alcohol 95% Test Result	Frequency (n)	Percentage (%)
Negative	6	35.3
Positive 1 (+)	4	23.5
Positive 2 (++)	5	29.4
Positive 3 (+++)	2	11.8
Total	17	100.0

In contrast, the alcohol 70% test results shown in Table 4 revealed that 76.5% of samples were negative, while 11.8%, 5.9%, and 5.9% showed positive reactions at levels (+), (++), and (+++), respectively. These results indicate that most milk samples remained stable under the alcohol 70% test.

Table 4. Alcohol 70% test results of Friesian Holstein dairy cows at KPSP Ijen Makmur

Alcohol 70% Test Result	Frequency (n)	Percentage (%)
Negative	13	76.5
Positive 1 (+)	2	11.8
Positive 2 (++)	1	5.9
Positive 3 (+++)	1	5.9
Total	17	100.0

The differences between alcohol 95% and 70% test results suggest that higher alcohol concentrations provide greater sensitivity in detecting reduced milk stability, particularly in milk from cows affected by subclinical mastitis.

Correlation between UTMT-1, Boiling Test, and Alcohol Test Results

To evaluate the relationship between subclinical mastitis severity and milk physicochemical stability, correlation analyses were performed between UTMT-1 scores and the results of the boiling and alcohol tests.

Correlation between UTMT-1 and Boiling Test

As shown in Table 5, the Chi-square test revealed a statistically significant association between UTMT-1 results and boiling test outcomes (Asymptotic Significance (2-sided) = 0.001; $p < 0.05$). The Cramer's V value of 0.798 indicated a strong association between the two variables.

Table 5. Correlation between UTMT-1 and Boiling Test Outcomes

Independent Variable	Dependent Variable	N	Statistical Test	p-value	Correlation Value	Strength of Association
Boiling Test Results	UTMT-1 Results	17	Chi-square	0.001 ($p < 0.05$)	Cramer's V = 0.798	Strong
			Spearman's rho	0.001	r = 0.899	Very Strong

This finding was supported by Spearman's rank correlation analysis, which yielded a very strong positive correlation ($r = 0.899$; $p = 0.001$), indicating that increasing UTMT-1 scores were associated with stronger positive reactions in the boiling test.

Correlation between UTMT-1 and Alcohol 70% Test

The Chi-square test results presented in Table 6 showed a significant association between UTMT-1 results and alcohol 70% test outcomes (Asymptotic Significance (2-sided) = 0.013; $p < 0.05$; $N = 17$). The Cramer's V value of 0.641 indicated a strong relationship between the two variables.

Table 6. Correlation between UTMT-1 and Alcohol 70% Test Outcomes

Independent Variable	Dependent Variable	N	Statistical Test	p-value	Correlation Value	Strength of Association
Alcohol 70% Results	UTMT-1 Results	17	Chi-square	0.013 ($p < 0.05$)	Cramer's V = 0.641	Strong
			Spearman's rho	0.003	r = 0.675	Strong

Spearman's correlation analysis further confirmed a positive and significant correlation ($r = 0.675$; $p = 0.003$), demonstrating that higher UTMT-1 scores tended to correspond with increased positive reactions in the alcohol 70% test.

Correlation between UTMT-1 and Alcohol 95% Test

As presented in Table 7, the Chi-square test indicated a highly significant association between UTMT-1 results and alcohol 95% test outcomes (Asymptotic Significance (2-sided) = 0.001; $p < 0.05$). The Cramer's V value of 0.935 reflected a very strong association between the two variables.

Table 7. Correlation between UTMT-1 and Alcohol 95% Test Outcomes

Independent Variable	Dependent Variable	N	Statistical Test	p-value	Correlation Value	Strength of Association
Alcohol 95% Results	UTMT-1 Results	17	<i>Chi-square</i>	0.001 ($p < 0.05$)	<i>Cramer's V</i> = 0.935	Very Strong
			<i>Spearman's rho</i>	0.001	r = 0.976	Very Strong

This result was strongly supported by Spearman's rank correlation analysis, which showed a very strong positive correlation ($r = 0.976$; $p = 0.001$), indicating a high level of agreement between UTMT-1 scores and alcohol 95% test responses.

Overall, the correlation analyses demonstrated that increases in UTMT-1 scores were consistently associated with stronger positive reactions in the boiling and alcohol tests, particularly in the alcohol 95% test. These findings indicate a close relationship between subclinical mastitis severity and decreased physicochemical stability of Friesian Holstein dairy cow milk.

Discussion

The UTMT-1 results indicated that most Friesian Holstein (FH) milk samples from KPSP Ijen Makmur were classified as mild to moderate subclinical mastitis, as reflected by dominant + to ++ scores. UTMT-1 detects inflammatory responses in the mammary gland primarily through increased somatic cell levels. During the early stages of intramammary infection, alveolar epithelial cells activate local immune mechanisms that promote neutrophil migration into the alveolar lumen (Neves *et al.*, 2025). At this stage, inflammation is predominantly cellular, while the integrity of the alveolar epithelium is largely preserved by tight junctions, thereby limiting the leakage of blood-derived components into milk (Stocco *et al.*, 2023; Bisutti *et al.*, 2021).

Although epithelial damage remains minimal, early inflammation activates endogenous proteolytic systems, particularly plasmin (Tiantong *et al.*, 2023). Increased plasmin activity induces partial β -casein degradation, reducing calcium binding and micellar stability (Khan *et al.*, 2025). These biochemical changes precede visible physical defects, consistent with the characterization of early subclinical mastitis as a condition dominated by internal physiological alterations (Rochmah *et al.*, 2023).

Boiling test results were dominated by negative and weak positive (+) reactions, indicating mild heat instability. This condition is associated with plasmin-mediated casein degradation and partial weakening of κ -casein protection (Khan *et al.*, 2025). During heating, partial denaturation of whey proteins, particularly β -lactoglobulin, promotes limited aggregation with structurally compromised κ -casein (Sinthary & Arief, 2023). Because mastitis severity was predominantly mild to moderate, κ -casein degradation remained insufficient to cause extensive coagulation (Ćurlej *et al.*, 2022). Differences between UTMT-1 and boiling test results reflect their distinct biological targets, with UTMT-1 indicating inflammation and the boiling test reflecting downstream protein instability (Jadhav *et al.*, 2024).

Alcohol testing showed increased sensitivity at higher concentrations. Most samples were negative at 70% alcohol but showed positive reactions at 95%. Alcohol destabilizes milk by reducing κ -casein hydration and surface charge, thereby weakening micellar repulsion (Syahbanu & Kurnianto, 2023). In subclinical mastitis milk, early proteolysis and mineral imbalance increase susceptibility to this effect, while higher alcohol concentrations enhance

detection of early protein instability (Lewis *et al.*, 2022; Priyashantha *et al.*, 2019). This supports previous findings that alcohol tests primarily reflect changes in milk protein stability rather than inflammatory severity alone (Rochmah *et al.*, 2023).

Correlation analysis demonstrated significant associations among UTMT-1, boiling, and alcohol tests. Chi-square analysis indicated non-independence, while Cramer's V and Spearman's rho revealed strong to very strong relationships, particularly between UTMT-1 and the 95% alcohol test (Conover, 2020; Prayoga & Suliadi, 2024). Overall, the combined use of UTMT-1, boiling, and alcohol tests provides a complementary and cost-effective approach for farm-level milk quality assessment, integrating inflammatory detection with physicochemical evaluation in dairy systems such as KPSP Ijen Makmur.

CONCLUSIONS AND SUGGESTIONS

Conclusions

The UTMT-1 test identified 64.7% of samples as positive for mild to moderate subclinical mastitis, while 35.3% were classified as negative. The boiling test showed positive reactions in 58.8% of dairy milk samples obtained from FH cows at KPSP Ijen Makmur. In the alcohol stability test, positive reactions were observed in 64.7% of samples at 95% alcohol concentration and in 23.5% at 70%. A strong and positive correlation between UTMT-1 scores and both alcohol and boiling test outcomes suggests that UTMT-1 provides a consistent reflection of subclinical mastitis severity in dairy cow milk.

Suggestions

Routine application of UTMT-1 is recommended for early detection of subclinical mastitis, supported by improved milking hygiene and housing sanitation, with UTMT-1–positive milk handled separately to maintain overall milk quality. Milk processors should implement simple physical stability tests, such as boiling and alcohol tests, before processing to strengthen raw milk quality control, while consumers are advised to consume pasteurized milk and avoid raw milk, particularly from farms without routine mastitis monitoring. Future studies should expand sample size and study area to enhance representativeness and further validate the applicability of UTMT-1, boiling, and alcohol tests in smallholder dairy production systems.

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