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Influence of lactation stage and fermentation environment on Kefir yield from cow's milk

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ABSTRACT

Kefir is a value-added fermented dairy product that can improve the profitability and sustainability of small-scale dairy farms by converting fresh milk into a nutritious product with an extended shelf life. However, limited information is available on how milk obtained at different lactation stages and simple fermentation environments influence kefir production under farm-level conditions. This study evaluated the effects of lactation stage and fermentation environment on the physicochemical properties of cow's milk and kefir yield. A randomized complete block design was employed using nine treatment combinations consisting of three lactation stages (early, mid, and late) and three fermentation environments (thick cloth cover, closed shelf, and warmed incubator), each replicated three times. Milk was analyzed for fat, solids-not-fat (SNF), protein, and lactose, while whey volume, kefir milk volume, and kefir grain weight were determined after fermentation. Milk composition differed significantly among treatments ($p<0.01$). The highest fat (6.67%), protein (3.01%), and lactose (4.74%) contents were obtained from mid-lactation milk fermented in a warmed incubator, whereas the highest SNF content (7.96%) was recorded in early-lactation milk. Whey volume was significantly affected ($p<0.05$), with the greatest yield (119.34 mL) obtained from early-lactation milk fermented in a warmed incubator and the lowest (95.82 mL) from late-lactation milk fermented under a thick cloth cover. In contrast, kefir milk volume (221.88–240.03 mL) and kefir grain weight (6.91–8.21 g) did not differ significantly among treatments. The findings demonstrate that milk composition varies with lactation stage, while a warmed fermentation environment enhances whey production without adversely affecting kefir yield or grain growth. These results support the use of mid-lactation milk and controlled warm fermentation to optimize kefir production in small-scale dairy systems.

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INTRODUCTION

Kefir is a practical, circular-economy solution for small dairy farms: a portion of surplus milk that would otherwise be wasted or used as low-value feed can be transformed into a higher-value, shelf-stable fermented product. Converting unsold or excess milk into kefir reduces waste, lowers on-farm disposal and hygiene risks, and creates an additional income stream through value-added sales. Producing kefir on-farm also closes material and nutrient loops-extending milk's useful life, improving resource efficiency, and enabling byproducts (e.g., whey) to be redirected into animal feed, crop fertilization, or other secondary uses. Evidence from farm-level studies indicates kefir processing is technically feasible and economically beneficial, making it a scalable, low-cost strategy to improve profitability and environmental performance within circular dairy systems.

Kefir can be produced by fermenting milk with commercial freeze-dried starter cultures, traditional kefir grains, and the product that remains after the removal of kefir grains (Bensmira *et al.*,2010). They are gelatinous, variable in size (0.3-3.5 cm in diameter), and composed of a symbiotic community of lactic acid bacteria, yeasts, and acetic acid bacteria at 10 CFU/g each, embedded in a polysaccharide matrix (Kurniawan *et al.*, 2026). After successive fermentations, kefir grains can break down into new-generation grains that have the same characteristics as the old ones (McGovern *et al.*, 2024). Kefir grains contain at least 3% of protein, 0.6% of lactic acid, and less than 10% of fat. It is a good source of Ca, Co, Cu, Fe, Mg, Mo, and Zn. During fermentation, proteins become easily digestible, and the amino acid profile of the kefir is similar to that of the milk used as a substrate, except for serine, lysine, alanine, threonine, tryptophan, valine, methionine, phenylalanine, and isoleucine, whose quantities are higher than those of unfermented milk. In addition, lactose is hydrolyzed by β-galactosidase, making it a good option for lactose-intolerant individuals (Rosa *et al.*, 2017).

Milk quality changes across lactation, and these changes may alter the composition and behavior of fermented products. Fat, protein, lactose, and solids-not-fat are especially important because they influence fermentation activity, whey separation, texture development, and product yield. In addition, the fermentation environment, whether milk is kept on a closed shelf, under a cloth cover, or in a warmed incubator, may affect microbial activity and the physicochemical behavior of the fermenting milk. These factors are particularly relevant in smallholder and semi-controlled dairy settings where processing conditions are often inconsistent.

As a farmer in a country with a developing dairy industry, there is limited information on kefir production. Abadl *et al.* (2023) investigated the effects of fermentation temperature and found that fermentation at 20°C resulted in kefir with a milder flavor and lower acidity compared to fermentation at 30°C and 40°C. They also noted that fermentation at 20°C requires a longer fermentation period and a lower microbial population than at higher temperatures.

Despite the growing literature on kefir microbiology and functionality, most recent studies have focused on its chemical profile, sensory quality, storage stability, and functional properties under controlled or household conditions. Fewer studies have examined how milk from different lactation stages and simple fermentation environments jointly affect kefir production under farm-level conditions. This is an important gap because milk composition changes throughout lactation, and fermentation temperature or storage environment can affect the development of acidity, whey separation, and product stability. In smallholder dairy systems, where processing conditions are often non-standardized, these factors may strongly shape the consistency and yield of kefir.

The study aimed to evaluate the effect of lactation stage, physicochemical composition of milk and fermentation environment on kefir production traits, specifically whey volume, kefir milk volume, and kefir grain weight. It sought to determine whether milk from early, mid, or late lactation, when fermented under different simple fermentation environments, would produce measurable differences in kefir quality and yield. The findings are expected to provide practical guidance for optimizing kefir production in small-scale and semi-controlled dairy systems. Its composition and quality may depend on the characteristics of the substrate, the microbiota in the grains, and the conditions used during fermentation and storage.

MATERIALS AND METHODS

Study site

The experiment was conducted at the Animal Science Unit, College of Agriculture, University of Science and Technology of Southern Philippines (USTP), Claveria Campus, Misamis Oriental, Philippines, from December 31, 2022 to February 28, 2023. The study site is located at 8.62°N, 124.87°E and has an average annual temperature of 23.2°C, relative humidity of 74.4%, and annual rainfall of approximately 2,351 mm.

Experimental design

The experiment was arranged in a randomized complete block design (RCBD), with lactation stage serving as the blocking factor. Nine treatment combinations were generated from three lactation stages (early, mid, and late) and three fermentation environments: thick cloth cover, closed shelf, and warmed incubator. Each treatment was replicated three times. Experimental cows were selected from the same herd and were comparable in breed type and parity to minimize biological variation.

Milk collection and physicochemical analysis

Fresh cow's milk was collected daily in 250-mL portions from cows representing the three lactation stages throughout the 60-day experimental period. Immediately after collection, milk samples were analyzed for fat, solids-not-fat (SNF), protein, and lactose using a portable Milkoscan milk analyzer (Germany).

Kefir preparation and fermentation

A total of 12 L of fresh milk was used for kefir production. Milk was inoculated with Irish kefir grains at a rate of one tablespoon of grains per cup of milk and fermented at 30°C for 24 h under one of three fermentation environments: (i) covered with a thick cloth, (ii) stored on a closed shelf, or (iii) incubated in a warmed incubator. Kefir grains were refreshed daily using milk obtained from cows at the corresponding lactation stage.

Following fermentation, the cultured milk was aseptically filtered to separate kefir grains from the fermented milk. The volumes of whey and kefir milk were measured, and kefir grains were weighed using a digital balance. These measurements were recorded daily throughout the experiment.

Statistical analysis

Data on milk composition (fat, SNF, protein, and lactose), whey volume (measured as whey separation), kefir milk volume, and kefir grain weight were subjected to analysis of variance (ANOVA) appropriate for a randomized complete block design. Treatment means were compared using Tukey's honestly significant difference (HSD) test at a 5% level of significance.

RESULTS AND DISCUSSION

Milk fat

Milk fat content differed significantly ($p<0.01$) among treatments, indicating that lactation stage influenced the lipid composition of the milk used for kefir production (Table 1). The highest fat content (6.67%) was recorded in milk obtained from cows at mid-lactation fermented in a warmed incubator (T8), whereas the lowest value (3.75%) was observed in late-lactation milk under the cloth-covered treatment (T3). Although variation occurred across all treatment combinations, mid-lactation generally produced milk with a more favorable fat concentration than late lactation.

Changes in milk fat throughout lactation are consistent with the physiological adaptations of dairy cows during milk production. NRC (1988) identified both genetic and environmental factors as major determinants of milk fat concentration. Physiologically, milk fat is initially high in colostrum, declines during early lactation as milk yield rapidly increases, and subsequently rises as lactation progresses. Similarly, Wilms *et al.* (2022) reported that milk fat composition varies considerably during lactation, with concentrations generally increasing after the initial post-calving decline. In addition, feeding management influences milk fat synthesis, as demonstrated by Lock *et al.* (2013), who showed that dietary fat supplementation significantly alters milk fat yield and composition.

The present findings suggest that milk produced during mid-lactation provides a more favorable lipid profile for kefir manufacture. Adequate milk fat contributes to the texture, mouthfeel, and overall quality of fermented dairy products, making this stage particularly suitable for value-added processing.

Solid non-fat

Solid non-fat (SNF) content also differed significantly (p<0.01) among treatments (Table 1). The highest SNF value (7.96%) was observed in early-lactation milk (T1), whereas the lowest value (7.59%) occurred in mid-lactation milk (T2). Although the numerical differences were relatively small, the statistical significance indicates that lactation stage affected the concentration of non-fat milk solids.

Table 1. Nutrient composition of milk obtained at different stages of lactation, (%)

Treatments	Fat (%)	SNF (%)	Protein (%)	Lactose (%)
T_1 Early lactation	5.61^{ab}	7.96^{ab}	2.93^{ab}	4.34^{ab}
T_2 Mid lactation	4.44^{bc}	7.59^{b}	2.79^{b}	4.29^{b}
T_3 Late lactation	3.75^{c}	7.63^{b}	2.76^{b}	4.23^{b}
T_4 Early lactation	4.47^{bc}	7.69^{ab}	2.77^{b}	4.28^{b}
T_5 Mid lactation	5.52^{ab}	7.82^{ab}	2.92^{ab}	4.48^{ab}
T_6 Late lactation	4.94^{bc}	7.68^{ab}	2.78^{b}	4.28^{b}
T_7 Early lactation	5.64^{ab}	7.65^{b}	2.79^{b}	4.22^{b}
T_8 Mid lactation	6.67^{a}	7.72^{a}	3.01^{a}	4.74^{a}
T_9 Late lactation	4.44^{bc}	7.70^{ab}	2.80^{b}	4.35^{ab}
F-test	**	**	**	**
Coefficient of variation, %	9.47	1.70	2.13	2.59

Variation in SNF across lactation reflects changes in the proportions of proteins, lactose, and minerals that naturally occur during milk synthesis. Lammers et al. (2000) reported that milk solids, including SNF, are important indicators of milk quality and processing performance because they influence the nutritional and functional properties of dairy products. Likewise, changes in milk composition throughout lactation have been associated with shifts in protein and solid fractions, with early-lactation milk often containing relatively higher concentrations of total solids. Zilstra *et al.* (2016) further reported that early-lactation milk contains a higher proportion of casein, an important contributor to total solids and an essential component influencing the coagulation properties of fermented milk products.

The higher SNF observed during early lactation may therefore enhance the functional characteristics of milk during fermentation by providing a richer substrate for microbial activity and gel formation.

Protein

Protein concentration varied significantly among treatments (p<0.01) (Table 1). The highest protein content (3.01%) was obtained from mid-lactation milk fermented in a warmed incubator (T8), whereas the lowest value (2.76%) was recorded in late-lactation milk (T3). Overall, milk obtained during mid-lactation exhibited consistently higher protein concentrations than milk from the other lactation stages.

These findings agree with Hayes *et al.* (2023) and Ostersen and Foldager (2019), who reported that the proportion of casein relative to total milk nitrogen reaches its maximum during mid-lactation, while the concentrations of individual milk proteins change progressively throughout the lactation cycle. The protein concentrations observed in the present study (2.76–3.01%) were slightly lower than the average value of approximately 3.2% reported by PCC (2026) for dairy milk in the Philippines. However, they remain comparable to values reported by Gellrich et al. (2014), who documented protein concentrations of 3.03% in low-protein cows and 3.49% in high-protein cows during the first 155 days of lactation.

Protein is one of the principal determinants of kefir quality because it directly influences gel formation, viscosity, water-holding capacity, and texture during fermentation. Consequently, the relatively higher protein concentration observed in mid-lactation milk may contribute to improved structural characteristics of the fermented product.

Lactose

Lactose concentration differed significantly among treatments ($p<0.01$), with the highest value (4.74%) recorded in mid-lactation milk fermented in a warmed incubator (T8) and the lowest value (4.22%) in early-lactation milk under the warmed incubator treatment (T7) (Table 1). These findings indicate that both lactation stage and fermentation environment influenced lactose availability in milk used for kefir production.

The observed variation is consistent with the physiological regulation of lactose synthesis throughout lactation. Costa *et al.* (2019) explained that milk lactose concentration is primarily influenced by energy balance, metabolic status, and mammary gland health. Early lactation is commonly characterized by negative energy balance, which may reduce lactose synthesis, whereas lactose concentration generally reaches its maximum during mid-lactation before gradually declining toward the end of the lactation cycle. Because lactose functions as the principal osmotic regulator of milk, fluctuations in its concentration also reflect changes in water secretion within the mammary gland.

Lactose serves as the primary carbohydrate substrate for lactic acid bacteria and yeasts during kefir fermentation. Therefore, the relatively higher lactose concentration observed in mid-lactation milk may provide more favorable conditions for microbial metabolism, acid production, and fermentation efficiency, thereby contributing to improved processing performance.

Whey volume

The effects of lactation stage and fermentation environment on whey volume, kefir milk volume, and kefir grain weight are presented in Table 2. Among these production traits, only whey volume differed significantly among treatments ($p<0.05$), whereas kefir milk volume and kefir grain weight were not significantly affected.

The highest whey volume (119.34 mL) was obtained from early-lactation milk fermented in a warmed incubator (T7), followed closely by early-lactation milk stored on a closed shelf (116.65 mL) and mid-lactation milk fermented in a warmed incubator (115.82 mL). In contrast, the lowest whey volume (95.82 mL) was recorded in late-lactation milk fermented under a thick cloth cover (T3). These results indicate that both milk composition and fermentation environment influenced whey separation during kefir production.

The greater whey volume observed under warmed incubation may be attributed to enhanced microbial fermentation and acid development, which accelerate protein coagulation and gel contraction, thereby promoting whey expulsion. Recent studies have demonstrated that kefir syneresis, viscosity, and water-holding capacity are strongly influenced by milk characteristics and incubation temperature, indicating that fermentation conditions modify the structural properties of fermented milk gels. Guan et al. (2025) similarly reported that the interaction between substrate composition, microbial metabolism, and processing temperature plays an important role in determining the physicochemical characteristics of fermented dairy products.

Although increased whey separation is often associated with greater syneresis, the present production system differs from conventional yogurt manufacture because the separated whey was reincorporated into the fermented kefir after the removal of kefir grains. Consequently, greater whey production under warmed incubation contributes to a larger recoverable volume of kefir rather than representing product loss. This finding has practical importance for small-scale dairy processors because maintaining a warm fermentation environment may improve overall product recovery while preserving desirable fermentation characteristics.

Kefir milk

Kefir milk volume did not differ significantly among treatments (p>0.05), although numerical differences were observed (Table 2). Mean kefir milk volume ranged from 221.88 to 240.03 mL, with the highest value recorded in late-lactation milk fermented in a warmed incubator (T9) and the lowest in early-lactation milk fermented under a thick cloth cover (T1). The absence of significant treatment effects indicates that neither lactation stage nor fermentation environment substantially influenced the amount of fermented milk recovered after processing.

The relatively consistent kefir milk volume across treatments suggests that the fermentation conditions evaluated in this study maintained stable fermentation performance despite differences in milk composition. While temperature affected whey separation, it did not significantly alter the total volume of recoverable kefir milk. This observation indicates that simple fermentation environments commonly used by smallholder dairy producers can produce comparable quantities of kefir provided that appropriate fermentation time and inoculum levels are maintained.

Table 2. Volumes of Whey (mL), Kefir Milk (mL) and kefir grains (g) grown from milk at early, mid and late lactation stages, stored in different warming conditions

Treatments	Whey	Kefir milk	Kefir grains
T_1 Early cloth-covered	109.19 ^{ab}	221.883	6.9067
T_2 Mid cloth-covered	103.99 ^{ab}	235.327	7.3657
T_3 Late cloth-covered	95.82 ^{b}	234.8	7.5207
T_4 Early closed Shelf	116.65 ^{a}	233.697	8.2074
T_5 Mid closed Shelf	99.29 ^{ab}	235.2	7.6273
T_6 Late closed Shelf	102.28 ^{ab}	236.39	7.8267
T_7 Early warmed incubator	119.34 ^{a}	236.62	7.8247
T_8 Mid warmed incubator	115.82 ^{ab}	238.897	7.7297
T_9 Late warmed incubator	114.93 ^{ab}	240.027	7.7800
F-test	*	ns	ns
CV (%)	7.09	10.69	3.0700

*significant (p>=.01), ns=non-significant (p>=0.05)

Kefir grains

Kefir grain weight was likewise not significantly affected by the different treatment combinations (p>0.05). Grain weight ranged from 6.91 to 8.21 g, with the highest mean value obtained from early-lactation milk fermented on a closed shelf (T4). Although numerical variation was observed among treatments, these differences were insufficient to demonstrate a significant effect of lactation stage or fermentation environment on grain biomass.

The stability of kefir grain weight suggests that the symbiotic microbial consortium within the grains remained metabolically active under all fermentation environments evaluated in the study. Grain growth depends on the balance among nutrient availability, microbial interactions, and fermentation conditions. Under the standardized fermentation period of 24 h and identical inoculum ratio used in the present experiment, these factors appeared sufficient to sustain grain viability without producing substantial differences in biomass accumulation. This finding is encouraging for small-scale producers because it indicates that kefir grains can be propagated successfully under relatively simple processing conditions without compromising grain integrity.

The absence of significant differences in kefir grain weight further demonstrates the resilience of kefir grains to moderate variations in fermentation environment. Consequently, producers may select fermentation conditions based on practicality and production objectives rather than concerns regarding grain survival or biomass development.

The present study demonstrates that lactation stage primarily influenced the physicochemical composition of milk, whereas the fermentation environment exerted a greater effect on whey separation during kefir production. Milk obtained during mid-lactation generally exhibited superior compositional characteristics, particularly with respect to protein and lactose concentrations, while fermentation in a warmed incubator enhanced whey production without adversely affecting kefir milk yield or grain growth. These findings indicate that combining high-quality milk from mid-lactation cows with controlled warm fermentation provides a practical approach for improving kefir production under farm conditions. The results also support the feasibility of on-farm kefir manufacture as a value-added strategy for increasing milk utilization, improving production efficiency, and promoting sustainable dairy processing.

CONCLUSION

The study demonstrated that lactation stage and fermentation environment influenced several quality attributes of milk used for kefir production. Fat, protein, and lactose are optimum in cows at the mid-lactation stage, while solid non-fat is highest at early lactation. Because whey is returned to the kefir milk during production, fermenting cow's milk at a warmer incubation temperature is preferable to increase kefir volume. Kefir grain weight did not differ significantly among treatments, indicating that any of the tested fermentation environments can be adopted without harming grain mass.

RECOMMENDATIONS

For kefir processors, mid-lactation milk and a controlled warming environment are recommended to improve compositional quality, particularly protein and lactose content, and to increase kefir volume. Further studies are recommended to include sensory evaluation, microbial profiling, and shelf-life assessment to verify whether the best physicochemical treatment also yields the best consumer acceptance and storage stability.

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