J. Food Sci. Gastron. (January - June 2026) 4(1): 1-9
https://doi.org/10.5281/zenodo.18294016
ISSN 3073-1283

JFSG
JOURNAL OF FOOD SCIENCE AND GASTRONOMY

ORIGINAL ARTICLE

Artisanal fermentation practices and nutritional profiles of Yosha and Philu, traditional fermented yak milk products of Bhutan

Prácticas de fermentación artesanal y perfiles nutricionales de Yosha y Philu, productos tradicionales de leche de yak fermentada de Bután

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Received: 15 October 2025 / Accepted: 09 December 2025 / Published online: 23 January 2026

Abstract Yosha and Philu are traditional fermented yak milk products produced by communities in the highlands of Bhutan. The study aimed to document their traditional production methods and to characterize their nutritional profile using qualitative and quantitative approaches, including field observations, interviews with local producers, photographic documentation of the processing stages, and compositional analysis. Two production methods were identified for Yosha: one based on natural fermentation using a back-slopping technique, and another that uses whey from the previous batch for coagulation during heating, followed by ripening of the curd in animal skin. Philu, which has a creamy texture, is traditionally produced using birch or willow branches. During six months of ripening, Yosha showed significant changes ($p \leq 0.05$) in total solids, protein, ash, pH, and titratable acidity, while the increase in fat content was not statistically significant. Philu exhibited a significantly higher moisture content and moderate levels of protein, fat, ash, and pH. Statistical differences between Philu and Yosha, both fresh and ripened, were significant for all parameters. These findings demonstrate the influence of fermentation and ripening on the nutritional profile and highlight the cultural and artisanal value of these products, while underscoring the need for further studies on their microbial and sensory characteristics.

Keywords cheese, fermentation, traditional product, yak milk, nutrition, ethnic dairy products.

Resumen Yosha y Philu son productos tradicionales de leche de yak fermentada elaborados por comunidades de las tierras altas de Bután. El estudio tuvo como objetivo documentar sus métodos tradicionales de producción y caracterizar su perfil nutricional mediante enfoques cualitativos y cuantitativos, que incluyeron observaciones de campo, entrevistas con productores locales, registro fotográfico del proceso y análisis de composición. Se identificaron dos métodos de elaboración de Yosha: uno basado en fermentación natural con técnica de back-slopping y otro que emplea suero de la tanda previa para la coagulación durante el calentamiento, seguido de la maduración de la cuajada en piel animal. Philu, de textura cremosa, se produce tradicionalmente utilizando ramas de abedul o sauce. Durante seis meses de maduración, Yosha presentó cambios significativos ($p \leq 0.05$) en sólidos totales, proteína, cenizas, pH y acidez titulable, mientras que el aumento de grasa no fue estadísticamente significativo. Philu mostró mayor contenido de humedad y valores moderados de proteína, grasa, cenizas y pH. Las diferencias entre Philu y Yosha, tanto fresco como madurado, fueron estadísticamente significativas para todos los parámetros. Los resultados evidencian la influencia de la fermentación y la maduración en el perfil nutricional, así como el valor cultural y artesanal de estos productos, y señalan la necesidad de estudios adicionales sobre su microbiota y perfil sensorial.

Palabras clave queso, fermentación, producto tradicional, leche de yak, nutrición, productos lácteos étnicos.

How to cite
Nair, A., Gyelmo, P., Lhamo, P., & Raika, V. (2026). Artisanal fermentation practices and nutritional profiles of Yosha and Philu, traditional fermented Yak milk products of Bhutan. *Journal of Food Science and Gastronomy*, *4*(1), 1-9. https://doi.org/10.5281/zenodo.18294016

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PUBLIS EDITORIAL

Introduction

One of the earliest and most energy-efficient food preservation techniques used by humans is fermentation. For thousands of years, humans have included fermented foods in their diets, even before written accounts of their production and use appeared (Campbell-Platt, 1994). It is estimated that billions of people worldwide consume over 5000 different types of fermented foods and alcoholic beverages as staples and other culinary ingredients (Tamang & Kailasapathy, 2010). Fermented dairy products are widely consumed globally and represent a rapidly expanding market. The global fermented milk market, valued at USD 307.41 billion in 2024, is projected to reach approximately USD 457.66 billion by 2032. Yogurt remains the most dominant product, with the fastest anticipated growth between 2025 and 2032. This growth is driven by evolving dietary preferences and increasing consumer awareness of the health benefits associated with fermented dairy products (Gusain, 2024).

With the growing trend in the consumption of fermented milk products, traditional fermented products represent a niche growth opportunity due to their unique flavor and traditional method of production. This aligns with global demand for natural, clean-label, and functional food (Gusain, 2024). Traditional products like cheese represent cultural heritage shaped by centuries of practical knowledge and experiences, and therefore play a pivotal role in the rural region's food culture (Alichanidis & Polychroniadou, 2008). Additionally, the production methods are deeply rooted in local and indigenous knowledge, unique to their origin, shaped by the social, environmental, and traditional practices (Alichanidis & Polychroniadou, 2008).

Yosha and Philu are traditional fermented yak milk products produced by yak-rearing communities living in the northern part of Bhutan. Nomads or semi-nomads in the Northern part of Bhutan (90% of them) depend on yak rearing as they cannot practice subsistence farming due to harsh weather conditions, and they not only depend on the yak for food, shelter, and clothing, but it is also one of the major sources of income (Wangchuk et al., 2013). The consumption of fresh milk is not common in Bhutan; therefore, the excess milk is either processed into butter, chugo (hard cheese), or Yosha (ripened cheese) and Philu (cream-like product) (Wangdi, 2015).

Yosha, a fermented yak cheese, is one of the special items produced by the nomads (Joshi & Gurung, 2009; Wangchuk et al., 2013). It is a naturally fermented product (NFP) traditionally produced using animal skin for fermentation and aging (Rai et al., 2016). For the nomadic people who traditionally produce it, Yosha not only serves as a valued food item, but it also plays a prominent role in income generation. Local producers highlighted that Yosha aged for a longer duration develops a unique flavor and texture, making it a highly sought-after product with premium prices (Personal communication, 2023). Similarly, Philu is a cream-like fermented yak milk product (Rai et al., 2016) produced mostly by ethnic people living in the western part of Bhutan. These products are produced using ancestral knowledge, which has been passed down from generation to generation, and they play a significant role in food culture, nutrition, and the livelihood of nomadic and semi-nomadic highland communities of Bhutan.

Despite its cultural and economic importance, there is limited information on the nutrition profile, functional and probiotic potentials, biochemical composition, or safety aspects in Bhutan. Additionally, the method of production is heterogeneous and varies among the producers, and is also restricted to the highland regions only. There are no standardized protocols for the productions that ensure consistency in quality, flavor, or safety. Such restrictions limit the potential for commercial potential and market recognition, where demand for clean-label fermented products is rapidly growing globally.

Thus, the primary aim of this study was to document the traditional method of production practiced by the communities, so that traditional knowledge and practices are preserved for future generations. Moreover, the study analyzed the nutritional composition of these products to provide information on their value. This study served as groundwork for developing standardized production protocols and understanding the nutrition profile to enhance the understanding of health benefits associated with the products.

The objectives of this study were to: (i) systematically document and describe the traditional production methods of Yosha and Philu among semi-nomadic communities, and (ii) compare the nutritional composition of freshly prepared Yosha, Yosha aged for six months, the minimum aging period, and Philu, to assess the effects of fermentation and aging on their quality attributes.

Methodology

Yosha is traditionally produced by semi-nomads living in Merak and Sakteng gewogs under Trashigang Dzongkhag in eastern Bhutan. For our study, Sakteng Gewog was purposely selected as the study site because both Gewogs share similar processing methods and herding practices. In the case of Philu, Haa Dzongkhag in western Bhutan was chosen as the study site because this product is specifically produced in this region. The households located near the village were selected randomly for the sample collection as well as to document the product and the production method. This was mainly done because of the inaccessibility of roads and the distant location.

The traditional processing method was documented through direct demonstrations by the nomads, using structured questionnaires, photographs, and video recordings to capture the details of Yosha and Philu production.

For the compositional analysis for Yosha, a total of thirteen samples (n=13) were collected from the 13 households that have migrated to low lowlands in winter. The samples were collected in two phases; for the fresh Yosha, approximately 500 g of freshly prepared Yosha were collected from every thirteen households aseptically into a sterile zip-lock bag. The samples were immediately stored in the iceboxes and transported to the laboratory at the College of Natural Resources for compositional analysis. Further, from the same batch of freshly prepared Yosha, the remaining portions were sewn into an animal skin bag and left with nomadic producers to undergo the traditional aging process. After six months of aging, the samples (n = 13) were collected similarly to fresh Yosha and taken to the laboratory for further analysis. The six-month time duration for aging was selected based on the observation reported (Joshi & Gurung, 2009), who reported that six months is a minimum time for Yosha to develop its sensory properties. For the Philu, the samples were collected similarly to fresh Yosha, where 13 samples (n=13) were collected from the semi-nomads and transported to the laboratory for further analysis.

For the compositional analysis, samples of fresh Yosha (n = 13), aged Yosha (n = 13), and Philu (n = 13) were thoroughly mixed separately to obtain a three-homogenized composite sample for each product type. From each composite sample, 20 analytical sub-samples were prepared to obtain representative measurements while reducing analytical variability and resource constraints. Composite sampling was employed to generate a representative average profile while minimizing analytical variability and resource constraints. However, it is acknowledged that this approach may mask natural variability among individual samples, and this limitation is considered when interpreting the results. All analyses for the following parameters were performed in triplicate. The triplicates refer to analytical replicates, generated from each composite sample type (fresh Yosha, aged Yosha, and Philu). Analytical replicates were used to assess measurement precision. All instruments were calibrated before analysis, following manufacturer protocols.

The moisture contents of fresh Yosha, aged Yosha, and Philu were determined by drying three grams of the samples in a dry forced draft oven (Stainless Steel Memmert Type Hot Air Oven, Germany) as per (Nielsen, 2010), and the moisture percentage was calculated. For the total mineral content, the dry ash method was used, in which the samples were heated at a muffle furnace (model EF3, Vecsar, UK), and the ash percentage was calculated (Seifu, 2013).

The Soxhlet extraction method was used for the determination of fat percentage using the Soxhlet apparatus (SCS-6, Hanna, India) (Nielsen, 2010). The total nitrogen content of the samples was determined by the Kjeldahl method using the Kjeldahl apparatus (mod. Classic Dx, Pelican Company, India), used for the total protein content, and a conversion factor of 6.38 was used for the calculation of crude protein (Rouch et al., 2008; Nielsen, 2010).

The pH of the samples was analyzed using a benchtop pH meter (mod. 211 microprocessor, HANNA, USA) according to the procedure described by Panda et al. (2016). Titratable acidity was determined by adapting the method mentioned before. The percentage of titratable acidity was calculated as a percentage of lactic acid.

The composition of both fresh and ripened Yosha was analyzed in triplicate, and the values were presented as percentages, means, and standard deviations. A paired T-test was performed to compare the proximate changes before and after the fermentation period using IBM SPSS Statistics 25. The level of significance of the differences was determined at $p \leq 0.05$. For Philu, values were represented as mean ± standard deviation.

Results and discussion

The preparation of animal skin is one of the fundamental procedures in the traditional production of Yosha (Figure 1). To prepare the skin, firstly, the skin is soaked in water for approximately one week to soften the texture and facilitate the easy removal of animal hair. After cleaning, the skin is cut into the desired size and sewn into a bag.

Once the bags for aging were ready, the nomads commenced the Yosha production, which generally follows two methods. The choice of method of production largely depends on the nomad's preference for either immediate production or allowing the milk to undergo natural fermentation. In both methods, the whole yak milk is heated for some time until it reaches 40–42 °C, and the cream is separated. After the cream separation, the skim milk is gently heated over a medium fire with continuous stirring. The whey from the previous batch is added gently to initiate the coagulation of the casein proteins, continuing until firm, solid casein precipitation is formed. The fully precipitated casein is then transferred to a bamboo sieve to remove the whey. The casein precipitates are then left undisturbed under their own weight overnight to drain out the excess whey. Once the casein is completely drained out, it is transferred into the prepared animal skin bag and compressed tightly to eliminate any air pockets, and then stitched closed. The bag is then subsequently placed near the hearth, where it is regularly turned over a period ranging from weeks to months to ensure uniform fermentation.

In the natural fermentation method, the skim milk is stored in a container, and a small amount of Yosha from the previous batch is added to initiate the fermentation process (back-

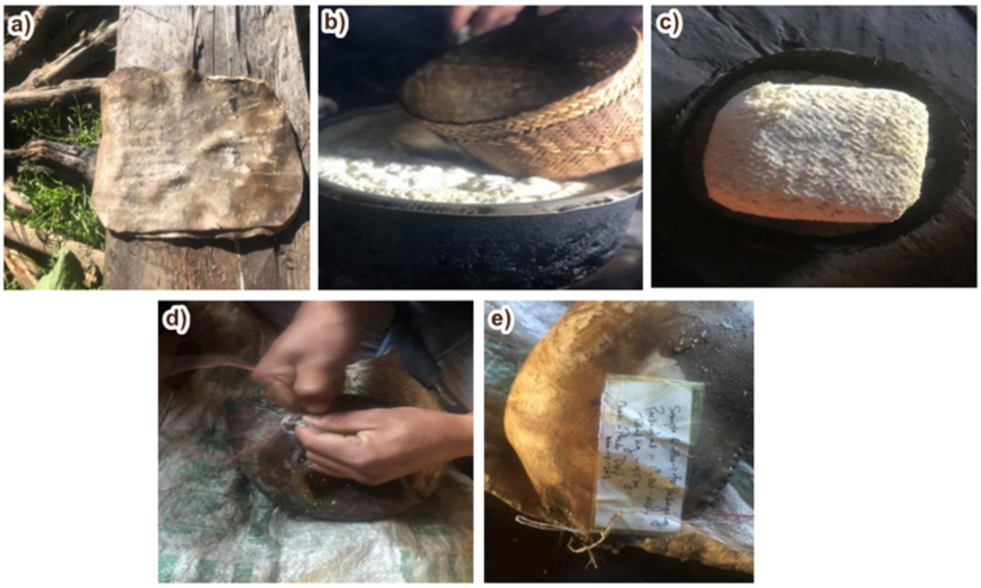

Figure 1. Flow graph illustrating the sequential steps of Yosha production: a) animal skin bag, b) straining of excess whey in a bamboo strainer, c) drained casein ready for packing, d) casein packed in animal skin bag, e) casein packed and ready for ripening.

slopping). The milk is then allowed to ferment naturally for approximately two to five days. The fermented milk is then heated gently over a medium fire with gentle stirring until complete coagulation is achieved. After the coagulation, the procedure mentioned above is followed. The procedures for both methods are reflected in Figure 2.

In the case of Philu (Figures 3 and 4), the first step involves the preparation of Zoa, the starter culture. Zoa is prepared by placing the boiled milk in a container and covering it with a blanket to maintain a stable ambient temperature, and keeping it for approximately 12 hours for fermentation. In the absence of Zoa, nomads also use bangchang, a local fermented alcoholic beverage, as zoa to initiate the fermentation process. After the zoa is prepared, the container for the fermentation is made ready. In earlier times, wooden container was commonly used; however, they are now replaced with plastic containers. The containers are filled with twigs of birch or willow trees. Birch tree twigs are generally used in the summer because the nomads believed that they help to regulate temperature during the summer, and willow twigs in winter, as it is thought to retain heat and create warmer conditions for the fermentation process.

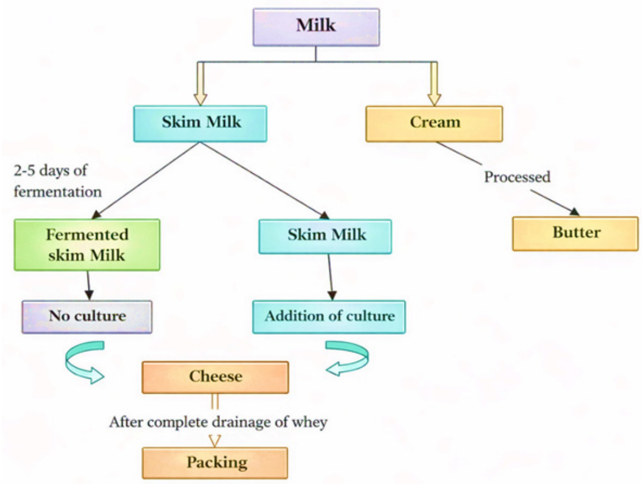

Figure 2. Flow chart of Yosha production.

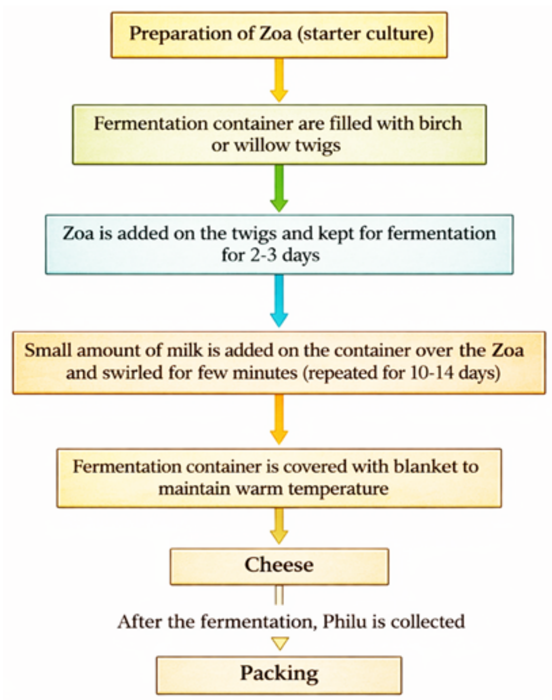

Figure 3. Flow chart of Philu production.

Once the container is filled with the preferred twigs, zoa is added gently over the twigs, and the zoa is swirled to ensure the uniform coating of the twigs. Then the container is covered with the blanket to maintain the warm temperature and kept undisturbed for two to three days. After the incubation of zoa, dew drops like droplets are formed on the twigs. Then, the small amount of milk is added gently over the twigs and swirled to ensure the even distribution of milk on the twigs. The container is then covered with the blanket to ensure a warm temperature. The process is repeated daily for 10-14 days. After the fermentation period, Philu is collected from each twig and used in the culinary preparations.

Figure 4. Flow graph illustrating the sequential steps of Philu production: a) wooden container, b) birch twigs, c) formation of Zoa, d) Philu.

The chemical composition of Yosha underwent significant changes during the six-month period of ripening (Table 1). The total solid content increased substantially from 32.6 ± 4.6 % to 75.9 ± 6.1 %. A similar trend was also observed with other parameters. The pH of of Yosha increased from 5.6 ± 0.2 to 7.5 ± 0.6, protein content rose from 28.2 ± 1.1% to 42.9 ± 8.5%, titratable acidity exhibited a sharp increase from 0.2 ± 0.1% to 6.8%, ash content increased from 3.4% in fresh Yosha to 13.8% after six months. The fat content also showed a moderate increase, rising from 2 ± 0.8% in the fresh sample to 3.5 ± 2.2% in the ripened Yosha. In the case of Philu, the moisture content was 80.05 ± 4.7%, ash 3.09 ± 1.3%, pH 6.41 ± 0.3, protein 11.12 ± 2.3%, fat 5.36 ± 1.4% (Table 2).

Table 1. Chemical composition of Fresh Yosha and ripened Yosha

Parameter	Storage period (day)	
	Fresh[a]	Six month
Total solids (%)	32.6 ± 4.6	75.9 ± 6.1
pH	5.6 ± 0.2	7.5 ± 0.6
Proteins (%)	28.2 ± 1.1	42.9 ± 8.5
Fats (%)	2.0 ± 0.8	3.5 ± 2.2
Ash (%)	3.4 ± 0.8	13.8 ± 5.2
Titrable acidity (lactic acid %)[b]	0.2 ± 0.1	6.8 ± 1.4

*Mean ± standard deviation.

[a] Fresh (1-3 days old cheese).

In Bhutan, generally ethnic groups known as Brokpas (eastern), Bjobs (western), and Lakhaps (west-central) regions rear yaks in a traditional migratory system and practice limited subsistence farming (Joshi et al., 2020). Yaks are the lifeline of these ethnic communities because they not only depend on yaks for food, shelter, and income, but they are also an indispensable part of their culture. The products derived from the yaks are the main source of income for these tribes, whereby they sell products like hard and soft cheeses, butter, yak meat, yak hair, and yak skins (Wangchuk, 2013).

Table 2. Chemical composition of Philu

Parameter	Mean ± standard deviation
Total solids (%)	80.05 ± 4.7
pH	6.41 ± 0.3
Proteins (%)	11.12 ± 2.3
Fats (%)	5.36 ± 1.4
Ash (%)	3.09 ± 1.3
Titrable acidity (lactic acid %) [b]	0.95 ± 0.3

Among these traditional dairy products, Yosha and Philu are distinct regional specialties, Yosha being predominantly produced in eastern Bhutan, while Philu is in the western region. Each product represents a long-established knowledge system unique to its community, its environment, and local resources. Beyond their nutritional and economic importance, these products hold strong cultural significance, which highlights nomadic heritage, identity, and traditional knowledge. Their continued practice of the traditional production method not only supports the livelihood of the ethnic people, it also plays a pivotal role in the preservation of Bhutan's tangible cultural heritage and traditional food cultures.

One of the unique features of Yosha processing is the use of animal skin for ripening. The cheese ripened in animal skin is generally known as traditional cheese and has a deep-rooted connection to the cultural heritage and historical tradition of their origin (Tudor et al., 2020). Traditional cheeses in different regions are commonly ripened in the goat or whole lamb skin (Tudor et al., 2020), however, in Bhutan, Yak skin is generally used for the ripening.

Though the method of processing skin as well as the type of skin used for ripening vary among the traditional cheeses, proper preparation of the skin is crucial because it plays a significant role during the ripening period and influences the sensory characteristics of the cheese (Tudor et al., 2020). For Philu, the main feature is the use of tree twigs, which is very specific to this product. Similar ethnobotanical practices are seen across different cultures. For instance, fresh twigs of Artemisia sp. are traditionally used in the preparation of Phabs, a starter culture used in the production of traditional fermented alcoholic beverages in Lhadakh, India (Kunzes & Bhalla, 2014). Similarly, the tribal communities of Assam also incorporate various parts of plant for the preparation of starter culture for local rice beer (Bhuyan & Baishya, 2013). These practices highlight the widespread use of plants and tree materials in traditional food processing and fermentation. The practice of fermentation using animal skins and tree twigs highlights the integration of indigenous knowledge and resource sustainability, reflecting the sustainable livelihood and traditional food system.

In terms of the chemical composition of Yosha, the study revealed a significant increase ($p \leq 0.05$) in the total solid content, pH, protein, mineral, and titratable acidity throughout the ripening period. The increase in the total solids aligns with observations reported for other cheeses ripened in animal skins, such as Lebanese artisanal cheese (Serhan et al., 2010) and Crotian traditional cheese (Tudor et al., 2014), where substantial moisture loss has been documented, resulting in the increase in total solids. Ripening temperature and permeability of animal skin are reported to influence the moisture loss. Studies have shown that when the ripening temperature is high, there is greater loss of moisture, and similarly, enhanced permeability of animal skin bags accelerates increased moisture loss (Serhan et al., 2010; Tudor et al., 2014).

A comparative study on Tulum cheese showed that more moisture was lost in the cheese ripened in the animal skin in comparison to cheese ripened in the plastic barrier, indicating the roles of porosity of the ripening container (Hayaloglu et al., 2007). Furthermore, reduced pH during the fermentation process as a result of the formation of lactic acid reduces the protein's ability to retain water, resulting in the release of the moisture, which is lost through porous skin (Tudor et al., 2014). Enhanced total solid content at the end of the ripening period indicates that Yosha loses significant moisture during the process, which can influence the overall quality and safety of the product. Similarly, after the ripening period, the protein content of Yosha increases significantly, and this rise is also associated with the loss. The current study also showed increased protein content after 60 days of ripening.

A similar trend was also observed in the Crotian cheese, which showed a gradual rise in protein content with the progression of the ripening period (Tudor et al., 2014). The increase in the protein content can be attributed to both loss of moisture and enhanced proteolytic activities linked to the ripening process. Proteolytic activities, such as the breakdown and hydrolysis of casein, are associated with an increase in water-soluble nitrogen and trichloroacetic acid-soluble nitrogen, leading to a higher concentration of proteins (Tudor et al., 2014; Rako et al., 2019; Tudor et al., 2020). Although the pH was not monitored during the process, measurements of initial pH and final pH were recorded. An increase in pH was observed in Yosha at the end of six months of ripening. Sir iz misine, a Crotian cheese, Ahumado de A´ liva cheese, a Spanish cheese, similar trends while ripening in the animal skins (Akın et al., 2003; Franco et al., 2001). According to previous studies, initial phase of ripening is characterized by the decrease in pH due to the formation of lactic acid, however with the progression of ripening period, pH tends to rise due to the formation of ammonia and ammonia acids and consumption of lactic by yeast and molds (Franco et al., 2001; Akın et al., 2003; McSweeney, 2004).

Likewise, increased overall ash content was also observed at the end of the ripening period of Yosha, which corresponds to the significant rise in ash content in other ripened cheeses (Prieto et al., 2000; Milani et al., 2014). The rise in mineral content for many types of traditional cheeses is associated with the addition of salt, which contributes significantly to the overall ash content (Macedo & Malcata, 1997; Prieto et al., 2002; Milani et al., 2014). However, in the case of Yosha, no salts are added during the processing. The mineral content of cheese is influenced by several factors, such as the origin of milk, geographical areas, and processing techniques (Milani et al., 2014). For Yosha, the rise in ash content during the ripening process could be attributed to ripening temperature, yak milk composition, or use type of animal skin used for ripening. The increase in fat % may be attributed to the loss of moisture, increasing dry matter. The results correlate with the findings of Milani et al. (2014) and Ioannidou et al. (2022), which show that a slight increase in fat percentage was also observed in Graviera cheese produced from raw and pasteurized milk and Kurdish cheese.

In the case of Philu, it contains high moisture content, which is higher than yak cheese, which ranges from 20-30% (Wang et al., 2023). The high moisture content contributes to Philu's softer texture and shorter shelf-life in comparison to hard yak cheese. The fat and protein content of the Philu is similar to that of yak milk and fermented yak milk, such as Kurut (Zhang et al., 2008; Zhang et al., 2020). This suggests that the traditional method of Philu processing does not significantly alter the overall protein and fat percentage of the raw milk. However, when compared with the cheddar cheese produced from yak milk, Philu contains relatively lower fat and protein content (Zhang et al., 2020). These differences may be attributed to variation in the yak breed, processing method, and ripening period. Additionally, the total content of Philu is slightly lower than that of cheddar cheese but significantly higher than that of Kurut (Zhang et al., 2008; Zhang et al., 2020). This difference in the composition positions Philu as an intermediate product between soft fermented milk products and hard-ripened cheeses.

Conclusions

This study provided a first comprehensive documentation of the traditional processing method and nutrition profile of the two traditional fermented yak milk products, unique to the highland communities of Bhutan. The findings showed that the production methods are deeply rooted in the culture and tradition of the semi-nomads and nomads, reflecting the sustainable use of local resources and the indigenous knowledge system to adapt and thrive in a harsh mountainous environment. Since Yosha is consumed only after it has gone ripe, a minimum of six months of ripening is required before it is ready for consumption. During this ripening period, biochemical changes take place that influence the sensory characteristics and nutritional content. The composition analysis revealed that Yosha undergoes significant changes during the six-month ripening period, with enhanced total solids, protein, fat, ash, and titratable acidity. The use of animal skin as a vessel for ripening plays a significant role in influencing the texture and flavor of Yosha. In contrast, Philu is consumed directly without ripening, the composition varies wth Yosha. It has high moisture content, making it susceptible to spoilage. The composition of Philu varies among the yak cheese, fresh yak milk, and fermented yak milk, and its creaminess and soft texture distinguish it as an intermediate product between soft fermented milk and hard ripened cheese. The present study illustrated the economic potential, cultural relevance, and nutritional importance of Yosha and Philu for Bhutan's highland communities. The study helps to preserve indigenous knowledge by recording the traditional production method and proximate composition analysis. It also lays the groundwork for creating standardized manufacturing protocols that guarantee quality, safety, and competitiveness in the market. For future studies, microbial profiling, functional characteristics, and consumer acceptance should be explored, which would provide scientific evidence to support quality standardization and cultural preservation initiatives.

Acknowledgments

The authors would also like to thank laboratory technicians Mr. Sonam Moktan and Mr. Ugyen Tenzin of the College of Natural Resources, Royal University of Bhutan, for their assistance with guidance and sample analysis, as well as the Dzongkhag Livestock officer for their support in the field.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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Data availability statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Statement on the use of AI

The authors acknowledge the use of generative AI and AI-assisted technologies to improve the readability and clarity of the article.

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