AJFAND — African Journal of Food, Agriculture, Nutrition and Development. Since 2001
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Afr. J. Food Agric. Nutr. Dev. 2026; 26(1): 28326-28353 https://doi.org/10.18697/ajfand.148.26575

Date	Submitted	Accepted	Published
	17th November 2025	19th December 2025	29th January 2026

INFLUENCE OF FERMENTATION VESSELS AND OLIVE (*Olea africana*) ASH ON PROXIMATE COMPOSITION, PHYTOCHEMICALS AND CONSUMER ACCEPTABILITY OF SPONTANEOUSLY FERMENTED MILK

Kimetu LM[1]*, Njoroge DM[1] and EE Owaga[1]

Lucy Munee Kimetu

*Corresponding author email: lucymunee2017@gmail.com
ORCID: https://orcid.org/0009-0009-7489-0601 - Kimetu LM
ORCID: https://orcid.org/0000-0002-3825-8651 - Njoroge DM
ORCID: https://orcid.org/0000-0003-2360-4563 - Owaga EE

[1]Institute of Food Bio-resources Technology, Dedan Kimathi University of Technology (DeKUT), Private Bag-10143, Dedan Kimathi, Nyeri, Kenya

ABSTRACT

In addition to its cultural significance, naturally flavored spontaneously fermented milk is linked to health and nutritional benefits. Traditionally, the milk is fermented in gourds, but considering their current scarcity, it is necessary to explore use of other fermentation vessels that could guarantee the continuation of these practices. This study investigated the effect of incorporation of *Olea africana* ash (OAA) in spontaneously fermented milk using different fermentation vessels, such as gourds (G) and polyethylene terephthalate (PET) plastic (P) vessels. Different concentrations of OAA were used; control 0.00% (GC0 and PC0), 0.05% (G05 and P05), 0.10% (G10 and P10), and 0.20% (G20 and P20). The proximate composition, mineral content, total phenolic and total flavonoid contents, antioxidant activity, physicochemical properties, microbial quality, and consumer acceptability of the spontaneously fermented milk were determined. The crude ash content and mineral composition increased significantly with addition of OAA in a dose-dependent manner. Specifically, macro elements calcium (106.14 – 130.04 mg/100g), potassium (201.93 – 250.26 mg/100g), sodium (36.83 – 42.55 mg/100g), magnesium (6.75 – 9.51 mg/100g), and phosphorus (123.61 – 138.86 mg/100g), and micro elements zinc (0.04 – 0.16 mg/100g), iron (0.08 – 0.60 mg/100g), and manganese (0.08 – 0.30 mg/100g). The OAA reduced the acidity of spontaneously fermented milk, but the pH and TTA were within the recommended standard range. Both gourds and OAA enhanced the viscosity, total phenolic, total flavonoid contents, and antioxidant activity. A strong positive relationship of antioxidants with both phenolics and flavonoids was observed from the Pearson's correlation. The addition of OAA significantly inhibited the growth of yeast and molds in a dose-dependent manner. The freshly fermented milk samples up to 0.10% OAA in both vessels were generally rated as acceptable by consumers. This study provides scientific knowledge, empowers small-scale producers, and promote the nutritional value of foods through home-based production of traditional fermented milk. In conclusion, based on the nutritional, functional, and consumer acceptability, spontaneously fermented milk in both gourds and PET vessels containing 0.10% OAA produced the best results. Therefore, PET vessels could be considered for milk fermentation after further validation.

Key words: *Olea africana*, milk, spontaneous fermentation, proximate, phytochemicals, consumer acceptability, fermentation vessel

Citation: **Kimetu LM, Njoroge DM and EE Owaga** Influence of Fermentation Vessels and Olive (*Olea africana*) Ash on Proximate Composition, Phytochemicals and Consumer Acceptability of Spontaneously Fermented Milk. *Afr. J. Food Agric. Nutr. Dev.* 2026; **26(1):** 28326-28353. https://doi.org/10.18697/ajfand.148.26575

Since 2001 ajfand AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

INTRODUCTION

Fermentation of foods and beverages has been used since ancient times as a traditional method of preserving food products, and leads to distinctive sensory characteristics. Spontaneously fermented milk in African communities has different characteristics depending on the geographical location, the cultural background, and the microbes present in the environment. Starter cultures are commercially utilized, and the complexity of the flavor of fermented milk is minimized due to the use of a selected species of fermentative microbes [1]. The fermentation process utilizes different kinds of vessels. Most of the ethnic communities in Kenya have traditionally used gourds in the fermentation of milk. Gourds present an interchange of phenolic compounds with the inner walls and hence affect the typical organoleptic properties of the resulting food products [2]. However, their decreasing supply as a result of environmental changes and limited cultivation puts a constraint on the continuation of the traditional practices. Therefore, there is a need to explore alternative fermentation vessels to ensure the sustainability of these traditional fermentation techniques. To this end, PETs could be used for fermentation process based on their availability. Although there are safety concerns pertaining to the presence of microplastics in thermally processed foods, PETs have been previously utilized in milk fermentation processes at ambient temperatures [3].

Different plants have been utilized amongst communities in Africa to enrich the flavor and extend the keeping quality of dairy products. African wild olive, also known as *Olea europaea spp. africana* is used by the Central people of Kenya. Locally, it bears different names depending on the tribe, such as *Mutamaiyu* (Kikuyu), *Muthata* (Meru), and *Oloirien* (Maasai). The plant is widely distributed in Africa, and it exists in different habitats like forest, open field, and farm. *Olea africana* ash (OAA) is likely to have high concentrations of minerals, which may assist in nutritional fortification. In addition, *Olea africana* contains bioactive compounds with high levels of antioxidants due to the presence of triterpenic acids and phenolic compounds [4].

Previous studies have been inclined towards investigating the characteristics of spontaneously fermented milk involving smoked fermentation vessels using local indigenous plants. In Kenya, the study of fermented milk has mainly focused on *Mursik* among the Kalenjin community and *Kule naoto* among the Maasai community. *Mursik* is prepared using *Acacia nilotica*, while *Kule naoto* is prepared by fermenting raw milk, with the addition of fresh raw blood in gourds smoked with *Olea africana*. Limited information exists on the fermented milk in the Central part of Kenya, where fermentation containers are smoked using *Olea africana* tree or charcoal powder is introduced to the milk before fermentation [2]. There is limited information on the use of OAA, which induces a distinctive flavor to the resulting fermented product. The combined effect of OAA and fermentation vessels on spontaneously fermented milk has also not been fully elucidated. This is because previous studies in Kenya have focused on the microbial safety of spontaneously fermented milk. With a combination of these aspects, this study will help to maintain the traditional knowledge and promote sustainable processing options that use locally available natural resources.

Therefore, this research aimed to investigate the effect of OAA and fermentation vessels (gourds and PET vessels) on the proximate composition, physicochemical properties, phytochemicals, antioxidant activity, microbial quality, and consumer acceptability of spontaneously fermented cow's milk. It is hypothesized that OAA and fermentation vessels have a significant effect on the mineral composition, phytochemicals, and consumer acceptability of spontaneously fermented milk.

MATERIALS AND METHODS

Raw materials

Raw cow's milk was purchased from Nyeri Hill Farm, Nyeri, Kenya. It was immediately transported to the laboratory, where platform tests were conducted, and confirmed that the milk was fit for use in the study. The tests included: organoleptic test (normal), clot-on-boiling (negative), alcohol test (negative), lactometer test (1.028), and inhibitory test. To ensure sample homogeneity, the milk was mixed well before sampling. *Olea africana* twigs of moderate size were harvested from mature trees at the Dedan Kimathi University field in Nyeri County. Mature gourds with a volume capacity of 1.5 liters were purchased from Nyeri Market, Kenya. Food-grade Polyethylene terephthalate (PET) plastic vessels with the same capacity were also purchased from a local supermarket, Nyeri, Kenya. They were thoroughly cleaned with water and detergent using a soft brush, dried, and then stored in a dry place.

Olea africana ash preparation

The moderately sized twigs of *Olea africana* were chopped into small pieces and dried in the solar dryer for two weeks, then burnt in open air to obtain the ash. Subsequently, it was sieved to remove unburnt residues, and the filtrate was powdered using a grinder. The OAA was kept in sealed glass containers.

Preparation of spontaneously fermented milk

Preliminary studies were conducted to determine the optimum concentration of OAA for flavoring fermented milk. Concentrations of 0.1%, 0.2%, and 0.4% were used in the initial trial. The pH, syneresis, and color of fermented milk samples were evaluated. It was noted that a 0.4% ash concentration led to excessive whey separation, undesirable dark color, and the desired final pH was not attained. In contrast, 0.2% ash concentration resulted in acceptable color and a pH of 4.6 was attained. The concentrations were then adjusted to 0.05%, 0.1%, and 0.2% (w/w)

https://doi.org/10.18697/ajfand.148.26575

for the experimental design used in this research (Table 1). The raw milk was divided into four batches of 2.4 liters. The ash was added to three batches in varying amounts (0.05%, 0.10%, and 0.20%), while the fourth batch was not treated with OAA (control). Each batch of milk was thermally treated at 80 – 85 ℃ for 30 minutes and then left to cool to 27 ℃. Each of the thermally treated batches were then divided into two parts, followed by fermentation of one lot in a gourd and the other in a PET vessel. To minimize contamination, both the gourds and plastic vessels were sanitized with 3% hydrogen peroxide before use. Wooden corks were used to close the gourds, while plastic vessels were closed loosely with fitting lids. The milk was left to ferment spontaneously in an incubator set at 27 ℃ for 48 hours. The incubation temperatures were below the temperature previously reported to facilitate PET microplastics leaching (50 ℃) [5]. After fermentation, the samples were stored under refrigeration (4 °C) and analyzed in triplicate.

Proximate and mineral composition

Proximate composition of spontaneously fermented milk

The moisture, ash, protein and fat content were determined following AOAC methods [6]. Specifically, moisture content of the spontaneously fermented milk was measured using the AOAC method 930.15, the ash content determination followed the AOAC method 923.03, the Kjeldahl method outlined by the AOAC method 991.23 to quantify the protein content, and the crude fat content was determined using the Soxhlet technique as described by the AOAC 992.23 method. The arithmetic difference approach was used to calculate the samples' carbohydrate content while the caloric value was calculated using Atwater's conversion factors [7, 8].

Mineral composition of OAA and spontaneously fermented milk

To determine the concentrations of calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), zinc (Zn), iron (Fe), manganese (Mn), and copper (Cu) in OAA and fermented milk samples, Atomic Absorption Spectroscopy (AAS) was used while UV-visible spectroscopy was used to determine phosphorus (P) content [9,10]. Standard curves were plotted using absorbance of standard solutions in the range of 0 to 10 ppm ($R^2 \geq 0.997$).

Physicochemical properties of spontaneously fermented milk

The pH of the spontaneously fermented milk was determined using the potentiometric method using pH meter (Hanna Instruments pHep). The titratable acidity was measured using the acid-base titration method [11]. In estimating the total solids (TS) content, the gravimetric method was used [12]. Rotational viscometry (NDJ-4 Rotational Viscometer, Nanjing, China) was used to determine the viscosity [13].

Phytochemicals and antioxidant activity of OAA and spontaneously fermented milk

Total phenolic content (TPC)

The Folin-Ciocalteu method was used to determine the TPC of OAA and spontaneously fermented milk, as directed by Tossou [14]. In a test tube, 1 mL of the sample extract was combined with 0.5 mL of 10% Folin-Ciocalteu reagent and 1.5 mL of a 7.5% sodium carbonate solution. Distilled water was added to the mixture's contents to make up 10 mL, and it was then thoroughly vortexed. The mixture was then incubated in darkness for a duration of 30 minutes. Subsequently, absorbance was measured using a Shimadzu UV-1800 UV spectrophotometer set at 765 nm. Gallic acid standards in the range of 0 to 100 ppm were used to plot the standard curve (R^2 = 0.9977). By comparing the absorbance of the samples to standard solutions of gallic acid, the TPC was calculated using the regression equation (y=0.0096x+0.0119) and presented as mg of gallic acid equivalents (GAE) /100 g of sample.

Total flavonoid content (TFC)

The TFC was quantified using the procedure described by Tossou [14]. In a test tube, 1 mL of the sample extract was mixed with 0.3 mL of 5% sodium nitrite and 4 mL of distilled water, and the mixture was left to stand for 5 minutes. After adding 0.3 mL of 10% aluminum chloride to the mixture, it was left to stand for another 6 minutes. Then, 2 mL of a 1 M sodium hydroxide solution was added to the mixture, followed by the addition of distilled water to bring the volume up to 10 mL The Shimadzu UV-1800 spectrophotometer was used to measure the absorbance at 415 nm. The standard curve was achieved using quercetin standard ranging from 0 to 1000 ppm (R^2 = 0.9949). The regression equation (y=0.001x-0.022) was used to calculate the TFC in the samples, and the results were expressed as milligrams of quercetin equivalents per 100 grams of sample (mg QE/ 100 g).

Antioxidant activity

The antioxidant activity was measured based on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging principle [15]. A DPPH solution of 0.002% (w/v) in 95% methanol was prepared. A volume of 2 mL of the DPPH in methanol was mixed with 2 mL of fermented milk extracts, vortexed thoroughly, and left to react for 30 minutes at room temperature in the dark. At 517 nm, the absorbance was measured with a Shimadzu UV-1800 spectrophotometer. Methanol served as the blank, while the control was a DPPH solution in methanol. The antioxidant activity was determined as a percentage of DPPH activity and expressed as follows:

$$\text{DPPH activity (\%)} = \frac{\text{Absorbance of DPPH solution} - \text{absorbance of sample}}{\text{Absorbance of DPPH solution}} \times 100\%$$

https://doi.org/10.18697/ajfand.148.26575

Microbial quality of spontaneously fermented milk

The Lactic acid bacteria (LAB) count was determined using de Man Rogosa and Sharpe (MRS) agar medium (ReadyMED, Chaitanya Agro Biotech PVT.LTD, India) by the pour plate method [16]. The inoculated plates were incubated anaerobically at 37 °C for 48 hours. The obtained colonies were counted and presented as colony-forming units per milliliter (CFU/mL). The plates considered were those having between 30 and 300 colonies per plate. The identification of LAB colonies was done morphologically since they appear as cream and round colonies on MRS agar.

Yeast and molds was determined using the spread plate method using Potato Dextrose Agar (PDA) (BIOCHEM Chemopharma, France) [17]. The inoculated plates were incubated aerobically at 25 °C for 5 days. The colonies were counted and recorded as CFU/mL Yeast colonies appeared round in shape and cream to white in color, while mold colonies were filamentous and dark in color.

Sensory evaluation of spontaneously fermented milk

Samples of spontaneously fermented milk were subjected to sensory evaluation using 50 semi-trained panelists. The panel consisted of 17 males (34%) and 33 females (66%), aged between 18 – 25 years (34%), 26 – 35 years (28%), and 36 – 47 years (38%). The participants were staff members and students of Dedan Kimathi University of Technology, Kenya, and they were recruited according to their willingness to participate. Those who were frequent consumers of fermented milk, and had previous experience with sensory evaluation of different food products were considered for the study. Individuals who were allergic to dairy products were excluded from the study. The test was conducted in a controlled sensory room with uniform white lighting, well ventilated, and controlled noise. Approximately 20 mL of fermented milk samples was served to each panelist. Each sample was coded with a random 3-digit code to avoid bias, and the order of presentation was randomized for each panelist. Clean water and spittoons were provided to rinse their palates in between sample tasting. The panelists were previously advised to fast for 1 – 2 hours and ensure they are not exposed to odors before the study. Specific instructions for the study, the attributes, and the hedonic rating scale were explained to each participant. The panelists signed a consent form before participating in the research. The panelists were provided with a scorecard bearing the respective codes of the samples to rate the observed attributes, which included: color, taste, aroma, mouth feel, consistency, and overall acceptability. The definitions of the attributes were indicated on the score cards. A hedonic rating system with 9 points was used to rate the consumers' liking, whereby 9 represented "like extremely" and 1 represented "dislike extremely" [18]. The intent to purchase was also recorded on the scorecards, using a 5-point hedonic scale, whereby 5 represented "Definitely would buy" and 1 represented "Definitely would not buy".

Statistical analysis

Minitab statistical software, Version 21 (Minitab, LLC, State College, PA, USA), was used to analyze the data using a two-way ANOVA. General linear models were used to determine the interactions between the fermentation vessels and the concentration of OAA. Pearson's correlation was used for correlation determination. The Fisher's pairwise Least Significant Difference (LSD) method was used to separate the means at 95% confidence interval. Means and standard deviations were used to present the results. Means and interactions having $p < 0.05$ were regarded as statistically significant. Equal variances were assumed for the analysis.

Ethical considerations

This research was approved by Dedan Kimathi University of Technology Scientific Ethics Review Committee (DeKUTSERC) after ethical considerations (Approval no: DeKUT/ISREC/03422/086). A research license (License no: NACOSTI/P/24/41402) was also obtained from the National Commission for Science, Technology and Innovation (NACOSTI). Consent of every panelist who took part in the sensory analysis was obtained. The products analyzed were safe for consumption.

RESULTS AND DISCUSSION

Mineral composition and phytochemicals of *Olea africana* ash

The results of OAA mineral composition analysis (Table 2) presents essential information for both macro and micro elements that suggests its potential use in food fortification. The main minerals in the wood and plant ashes are calcium and potassium, which commonly contain 20-25% of ash content, giving it the liming properties. The amounts of phytochemicals in OAA suggests its possible impact on the TPC, TFC, and antioxidant activity when added in spontaneously fermented (Table 3). They were in the range of other Olea species, including Olea europaea.

Proximate composition of spontaneously fermented milk

The proximate composition of spontaneously fermented milk is presented in Table 4. Significantly higher moisture contents in PET vessels-fermented milk were recorded as compared to that fermented in gourds. This may be due to plastic vessels retaining water during fermentation since their material is nonporous and doesn't allow escape of moisture to the external environment [19]. The internal walls of the gourds may also have absorbed some of the moisture from the milk during fermentation. Increasing OAA concentrations led to a lower moisture content compared to the control. A possible explanation is the enhanced gel strength since OAA minerals may bind some of the water molecules in the fermented milk. A similar reduction in moisture content in fermented soybean milk with the addition of corn germ meal was reported in a previous study [20]. The combined interaction between

https://doi.org/10.18697/ajfand.148.26575

the type of vessel and OAA concentration did not show a statistically significant effect on the moisture content.

Spontaneously fermented milk showed a significant increase in crude ash content with increased concentration of OAA. This may be attributed to OAA contributing to non-organic matter and minerals. A previous study on yoghurt supplemented with oat bran showed a significant increase in crude ash compared to the control sample [21]. The significant interaction between the type of vessel and OAA dosage may be explained by the non-porous interior surface of plastic vessels, which do not adsorb OAA particles. Gourds might have adsorbed some of the OAA particles on their walls, hence resulting in lower crude ash content as compared to their plastic counterparts.

The crude protein decreased significantly ($p<0.05$) as the percentage of OAA increased. This could probably be explained by proteins-minerals interactions, thus affecting protein availability and detection. Minerals bind to the negatively charged regions of the protein molecules, acting as crosslinking agents. In addition, the increased minerals may have favored microbial proteolysis, leading to the significant reduction in crude protein [22]. However, the protein content was higher in gourd fermented milk than in plastic fermented milk at equivalent concentrations of OAA. These results were similar to those obtained in *kindirmou* and *pendidam* fermented in calabash, which could be due to proteins of the inner walls of the vessel being released into the milk [3].

The crude fat of the controls did not differ significantly ($p<0.05$) from the other treatments. The type of the vessel had no significant impact on the crude fat content of the fermented milk. No significant interaction was observed between the type of vessel and OAA concentration. Depending on the fermentation vessel, significantly higher carbohydrates were recorded in gourd-fermented milk than that produced in plastic vessels. This could be explained by the hydrolytic enzymes secreted by fermentative microbes, which degrade macromolecular structures of the inner lining of the gourd, thereby elevating the content of carbohydrates in the fermented milk. Gourds have been previously reported to contain 18.61% of carbohydrates [23]. There was no significant difference in the caloric energy at different OAA dosages. However, significantly higher energy values were recorded for milk fermented in gourds than that fermented in plastic vessels. This trend was similar to that of carbohydrate and protein contents, since the caloric value is derived from carbohydrates, fats, and proteins.

Mineral composition in spontaneously fermented milk

The mineral contents of spontaneously fermented milk flavored with OAA at different concentrations are shown in Table 5. The OAA significantly increased mineral content in a dose-dependent manner, as represented in Figure 1. A possible

ajfand Since 2001 AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

explanation is the increased crude ash, which is directly linked to the mineral composition [24]. The characterization of OAA confirmed a high concentration of these minerals. Therefore, its addition to fermented milk at varying concentrations could explain the possible increase of the mineral content. Both the type of the vessel and OAA concentration had a significant effect on the mineral content of fermented milk, and a statistically significant interaction existed between the two variables. The significantly lower mineral content in milk fermented in gourds compared to that in PET vessels may be due to mineral retention, caused by the non-porous nature of the vessel surfaces. On the other hand, gourds are naturally porous materials, and therefore, milk components together with OAA may physically bind to the surfaces, thus causing a reduction in the minerals detected in milk [19].

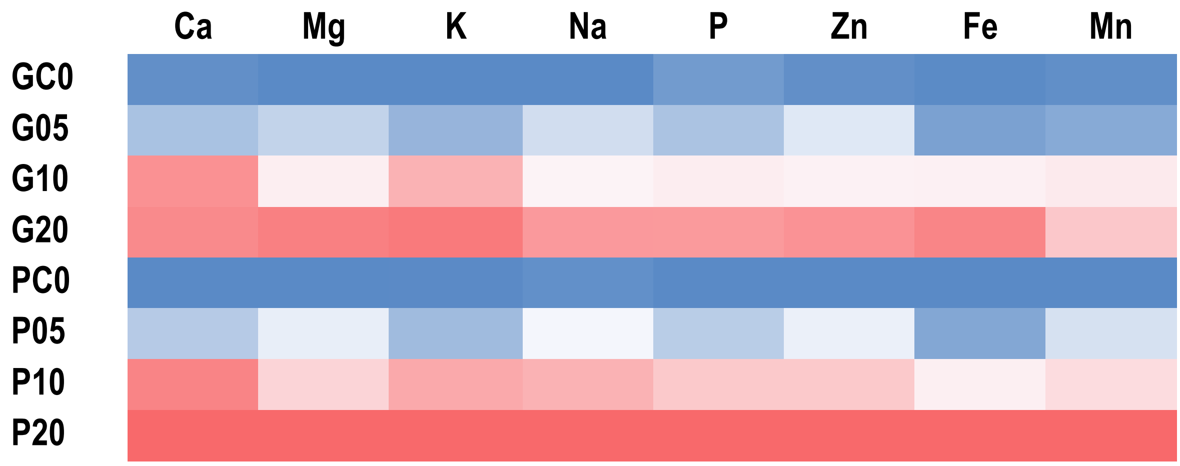

Figure 1: Heatmap showing the effect of *Olea africana* ash concentration on the mineral composition of spontaneously fermented milk. The minerals increased from blue – white – red color scales. G – gourd, P – PET, C0 – 0%, 05 – 0.05%, 10 – 0.10%, 20 – 0.20%

Total solids and viscosity of spontaneously fermented milk

It was observed that addition of OAA resulted in a significant increase in total solids for PET fermented milk, but the increase was not significant in gourds (Figure 2a). These results were consistent with those of moisture content. The increased OAA concentration raised the viscosity significantly in a dose dependent manner (Figure 2b). A possible explanation is that OAA may form a strong gel network and enhance interaction between milk proteins. Specifically, bioactive compounds and minerals act as cross-linkers and increase the electrostatic interactions between casein micelles during acidification. The increased network decreases the size of the pores and entraps less free water, which increases viscosity [25]. Fermentation in gourds resulted in more viscous fermented milk, probably due to absorption of excess whey by the internal surface of the gourds [19].

ajfand Since 2001
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

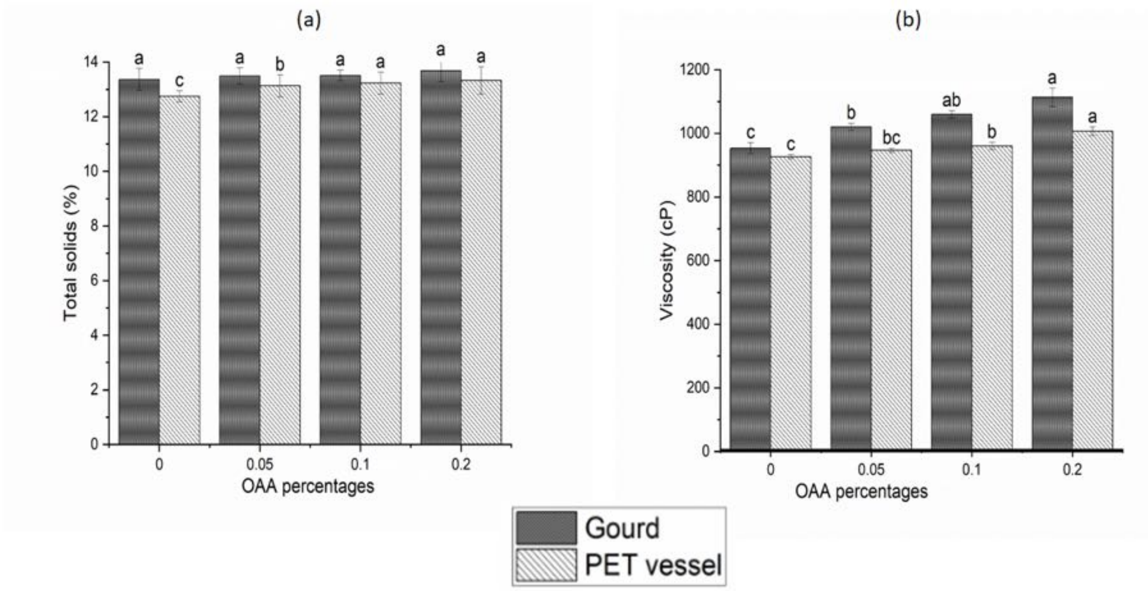

Figure 2: **(a) Total solids and (b) viscosity of spontaneously fermented milk. Data: Means ± standard deviation (n = 3). Different superscript letters indicate a significant difference (p<0.05) between vessels. OAA – *Olea africana* ash, 0% (GC0 and PC0), 0.05% (G05 and P05), 0.1% (G10 and P10), 0.2% (G20 and P20)**

pH and TTA of spontaneously fermented milk

pH values between 4.43 and 4.57 (Table 6) were attained for spontaneously fermented milk, confirming that the fermentation process was effectively completed by microbial activity. The ideal pH range for fermented milk has been reported to be between 4.0 and 4.6 [26]. Significant differences in pH only existed between the control and 0.2% OAA, suggesting that the buffering effect dominated at high concentrations of OAA. This may be due to OAA possibly neutralizing the lactic acid produced by LABs. The TTA varied from 0.77 to 0.85 (Table 6) for all fermented milk samples, which was within the recommended minimum value of 0.7%, according to the Kenya Bureau of Standards. This confirms that the fermentation process was successfully completed. In both fermentation vessels, OAA decreased the TTA in a dose-dependent manner and an inverse relationship with the pH. Milk fermented in PET vessels had significantly higher TTA values than that produced in gourds. This suggests that PET vessels may offer a fermentation environment that leads to increased acidity, as opposed to gourds which have a cooling effect hence minimize the possibility of over-fermentation [11]. A previous study on fermented cow's milk published a TTA of 0.96% [27]. This difference in TTA may be due to the differences in fermentation time. The production of lactic acid through bacterial metabolism also depends on the LAB species involved, which influence the balance of organic acids in fermented milk [28].

Phytochemicals and antioxidant activity in spontaneously fermented milk

The phytochemicals of spontaneously fermented milk are presented in Figure 3. The TPC (3a) and TFC (3b) of milk fermented in both gourds and PET vessels increased significantly with addition of OAA in a dose-dependent manner. The baseline analysis of OAA reported residual TPC and TFC, and hence its addition in fermented milk may directly contribute to the observed significant increase. *Olea africana* has previously been reported to contain phytochemicals such as hydroxytyrosol and oleuropein [29]. These findings were also in agreement with a previous study, which reported that adding herbal extracts (lemon grass and cloves) to natural fermented cow's milk increased its TPC and TFC [30]. The TPC and TFC were significantly higher in gourd-fermented milk than that produced in plastic vessels. A previous study reported that bioactive compounds may be transferred from the inner walls of the gourd via diffusion and migration processes during fermentation [3].

The antioxidant activity showed a significant increase ($p<0.05$) from controls as the dose of OAA increased (3c). Previous study findings confirmed that increasing the amount of olive leaf extract in yogurt boosted its antioxidant activity [31]. The trend of antioxidant activity also followed that of TPC and TFC. This suggests their positive contribution to the enhanced antioxidant activity, and hence increased radical scavenging potential. Although lactic fermentation increases the antioxidant activity, a strong positive relationship of antioxidants with both TPC and TFC was noted from the Pearson's correlation analysis (Table 7). Phenolic compounds give up their hydrogen atoms or electrons to neutralize reactive oxygen species (ROS), disrupting radical chain reactions that result in the production of oxidative damage [32]. Milk fermented in gourds had significantly higher antioxidant activity than that in PET vessels, probably due to the potential leaching of bioactive compounds from the internal walls of the gourd [33].

Since 2001
ajfand
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

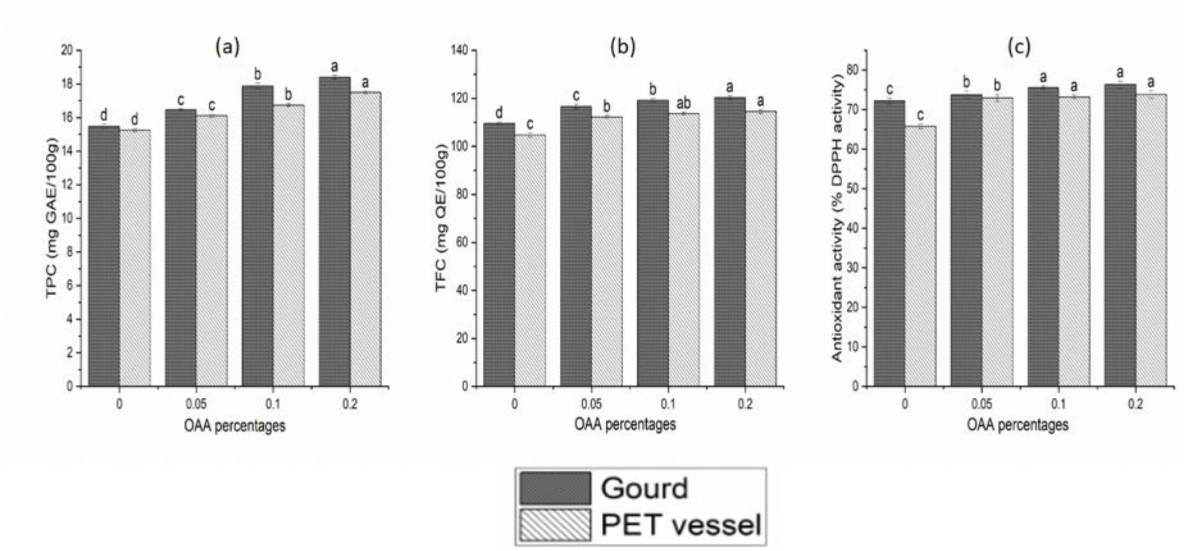

Figure 3: (a) TPC, (b) TFC, and (c) Antioxidant activity of spontaneously fermented milk. Data: Means ± standard deviation (n = 3). Different superscript letters indicate a significant difference (p<0.05) between vessels. TPC – Total phenolic content, TFC – Total flavonoid content, OAA – *Olea africana* ash., 0% (GC0 and PC0), 0.05% (G05 and P05), 0.1% (G10 and P10), 0.2% (G20 and P20)

Lactic acid bacteria (LAB) count

The LAB counts after fermentation (Figure 4) were within the Codex Alimentarius standard, which has been set at not less than 10^7 CFU/g sum of microorganisms for fermented milk. The dosage of OAA did not show a significant effect (p<0.05) on the LAB count. Milk fermented in gourds had significantly higher LAB counts than that fermented in PET vessels. The nature of the gourd's internal surface facilitates the formation of LAB biofilms, hence offering a back-slopping effect. This provides a starter culture to kick start the fermentation process, hence reducing the lag phase period [34]. Previous studies on spontaneously fermented milk from Western Kenya reported that the dominant bacterial groups were *Lactobacillus*, *Streptococcus*, and *Lactococcus* spp. In addition, *Leuconostocs* were reported to be few in numbers, due to their inability to compete with other LAB species when growing in a mixed population [35].

ajfand Since 2001
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

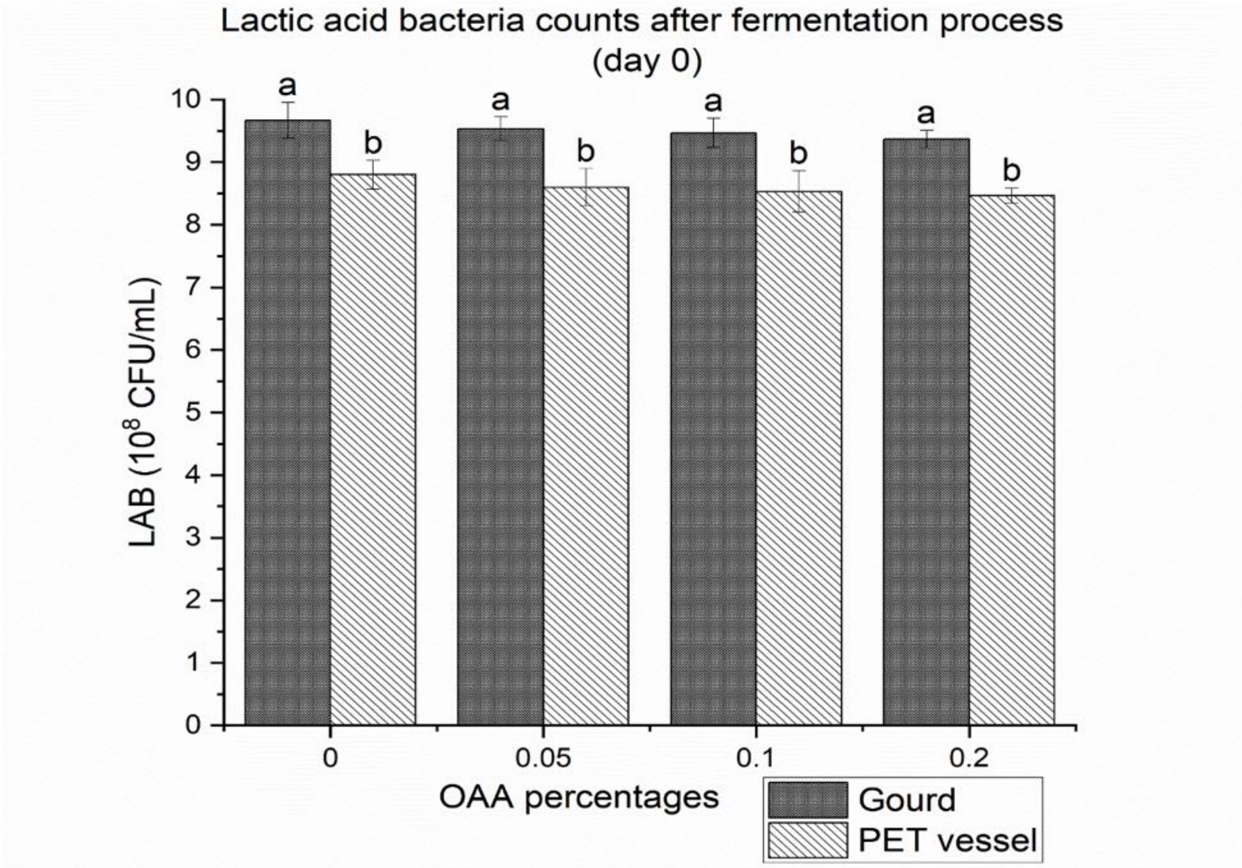

Figure 4: Lactic Acid Bacteria counts (CFU/mL) in spontaneously fermented milk after fermentation process. The data is presented as means (n = 3). Different superscript letters indicate a significant difference (p<0.05) between vessels. LAB – Lactic acid bacteria, CFU/mL – colony forming units per millilitre, OAA – *Olea africana* ash, 0% (GC0 and PC0), 0.05% (G05 and P05), 0.1% (G10 and P10), 0.2% (G20 and P20)

Yeast and molds count

Yeast and molds counts declined with increased concentration of OAA, as presented in Figure 5. This may be as a result of antifungal properties of OAA arising from bioactive compounds that are present in *Olea africana*, such as phenolics, flavonoids, and oleuropein [36]. The findings of the present study are consistent with the conclusions drawn from earlier research, which provided evidence that plant extracts exhibited potent antimicrobial activity against pathogenic fungi and bacteria [37]. Significantly higher counts of yeast and molds were found in GC0 than in PC0. The physical nature of the inner walls of gourds facilitates easier adhesion of residual microorganisms, colonization, and biofilm formation [38].

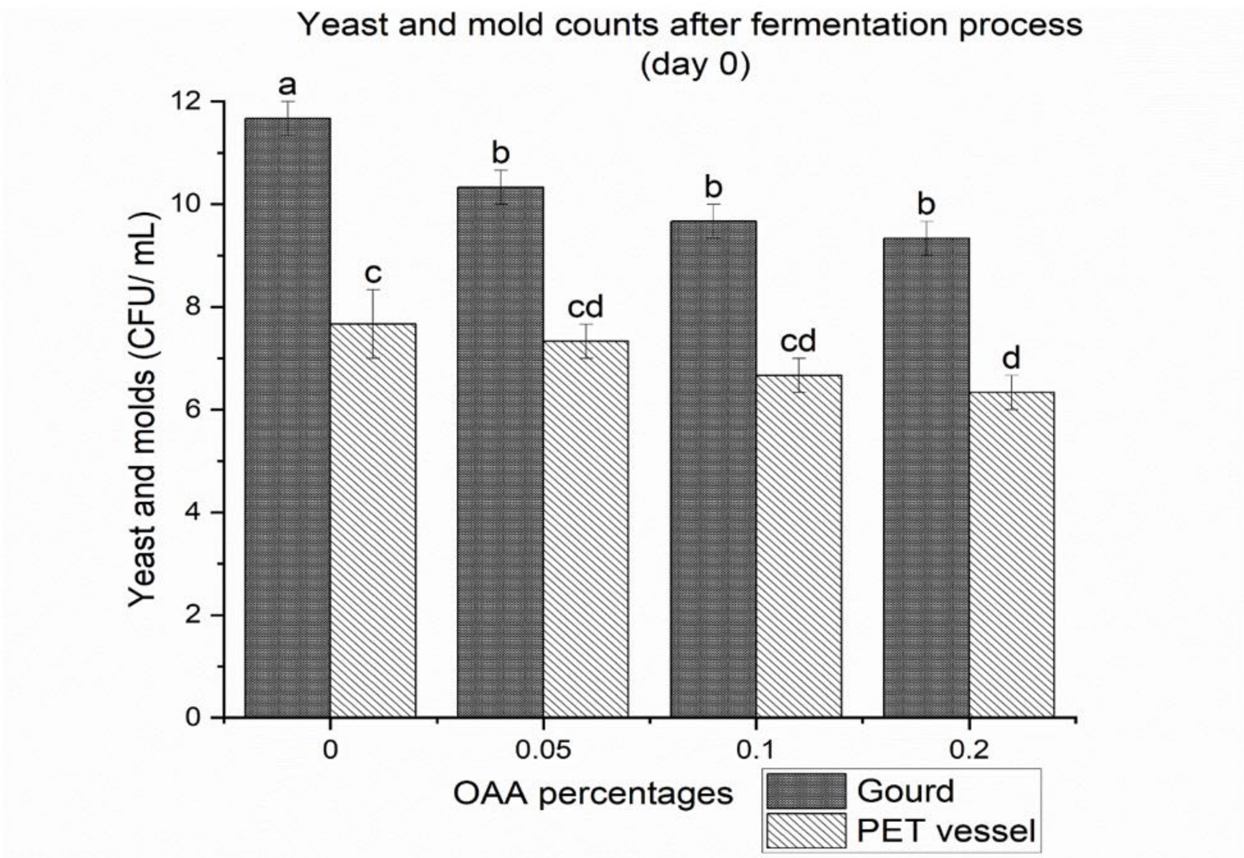

Figure 5: **Yeast and mould counts in spontaneously fermented milk after fermentation process. The data is presented as means (n = 3). Different superscript letters indicate a significant difference (p<0.05) between vessels. CFU/mL – colony forming units per millilitre, OAA – *Olea africana* ash, 0% (GC0 and PC0), 0.05% (G05 and P05), 0.1% (G10 and P10), 0.2% (G20 and P20)**

Sensory evaluation of spontaneously fermented milk

Consumer acceptability was tested by offering the different treatments of fermented milk to panelists, who rated them using a nine-point hedonic scale (Figure 6). The dosage of OAA significantly (p<0.05) affected the color ratings in a dose-dependent manner. The high scores on color for the controls are attributed to the absence of OAA. This may be due to the presence of ash pigmentations. Similar results on the effect of fortifying yogurt with *Moringa oleifera* were also reported in a previous study [39].

ajfand Since 2001
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

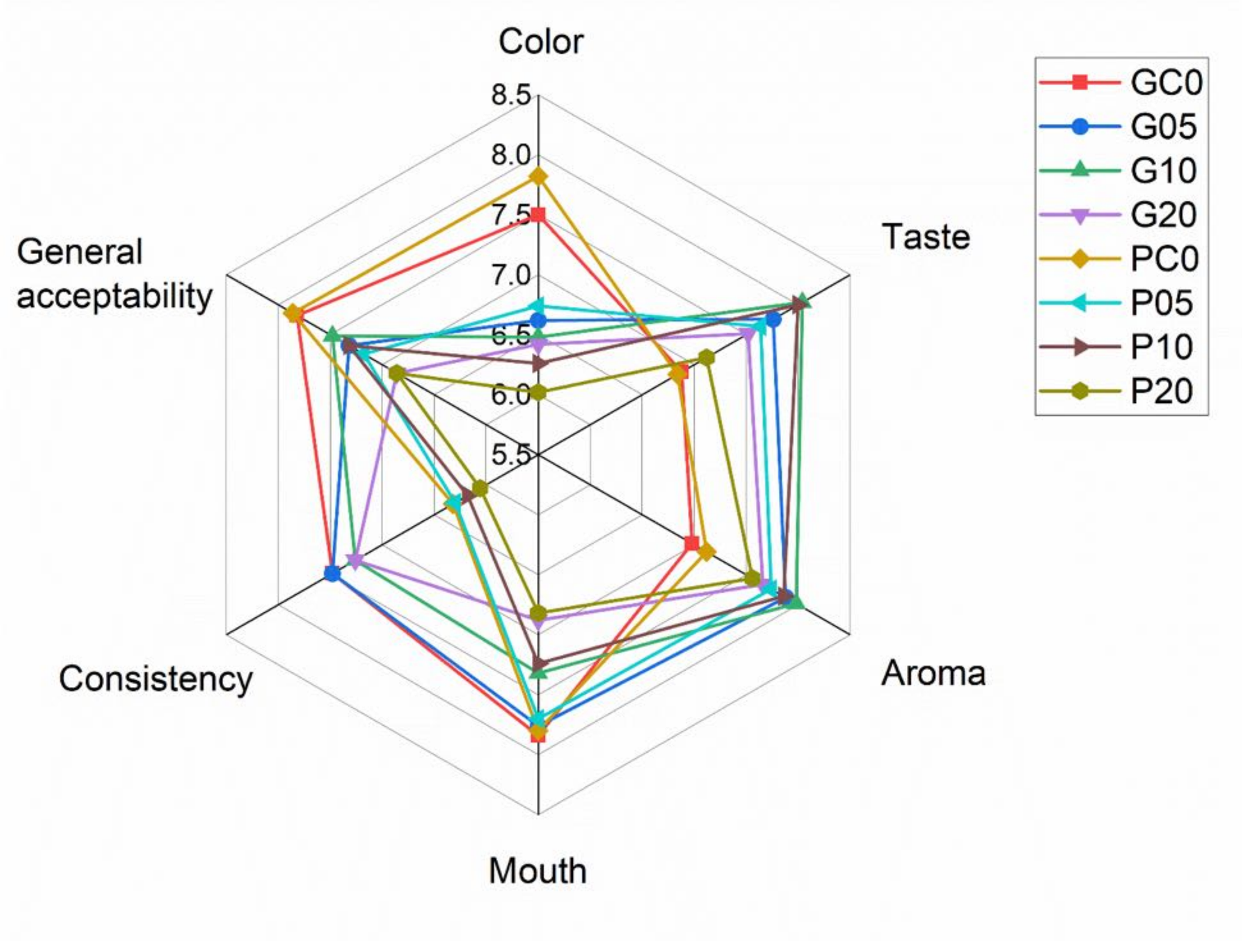

Figure 6: A spider chart representing the consumer acceptability of spontaneously fermented milk. The data is presented as means (n = 50). Hedonic rating scale ranges from 1 – 9 (1 – dislike extremely and 9 – like extremely). P – PET vessel, G – gourd, 0% (GC0 and PC0), 0.05% (G05 and P05), 0.1% (G10 and P10), 0.2% (G20 and P20)

ajfand Since 2001
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

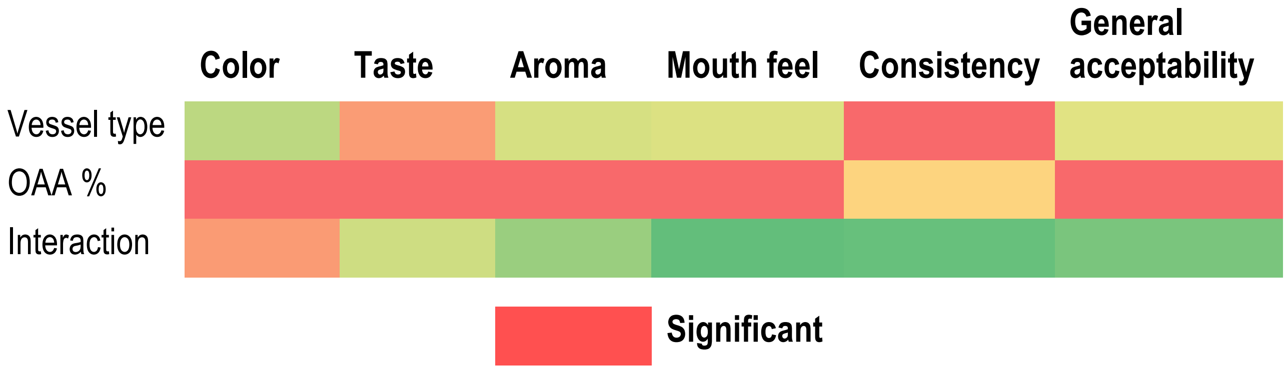

Figure 7: Heatmap showing the effect of vessel type, OAA concentration, and the interaction of the variables on the sensory evaluation attributes. OAA – *Olea africana* ash. Green – yellow – red color scale, with red showing a significant effect

The taste scores of the controls was significantly lower ($p<0.05$) than for OAA flavored fermented milk, suggesting a positive effect of OAA. Specifically, G10 was the most preferred, indicating that the combination of moderate concentration of OAA and polyphenols on the surface of the gourd enhanced the taste of fermented milk. The panelists detected a significant difference between the aroma of unflavored milk and that of OAA flavored milk. The difference may be attributed to presence of volatile organic compounds, such as terpenes which are released when wood is thermally treated as in the process of OAA preparation [40]. The mouth feel of GC0 was highly rated, whereas that of P20 was least liked. The panelists' liking for mouth feel significantly decreased with increased concentration of OAA. The observed phenomenon may be due to the presence of OAA particles, which probably were not soluble in the milk, thereby producing a gritty mouth feel.

Consistency of the fermented milk was not significantly affected by different OAA concentrations, but a significant difference existed between the two fermentation vessel types. Gourd fermented milk had significantly higher consistency compared to that fermented in PET vessels, confirming that gourds were crucial in the development of thicker textures of the fermented milk. The results on consistency followed a similar trend of viscosity, since high viscosity reflects a strong gel network and improved consistency. The results indicated that the panelists appreciated the improved taste and aroma at moderate OAA concentration (0.10%). The high concentration (0.20%) may mask other attributes of fermented milk, including acidity, which influences the overall rating of the final product. Overall, the controls were the most generally accepted samples, followed by 0.1% OAA, and also panelists showed a great intention to purchase (Figure 8). There was also no significant difference in general acceptability between 0.10% and the controls. The dosage of OAA mostly influenced the general acceptability of the fermented milk. This could be linked to the familiarity of most consumers with the unflavored and cream white color of fermented milk. The results for intent to purchase were in line with those of general acceptability.

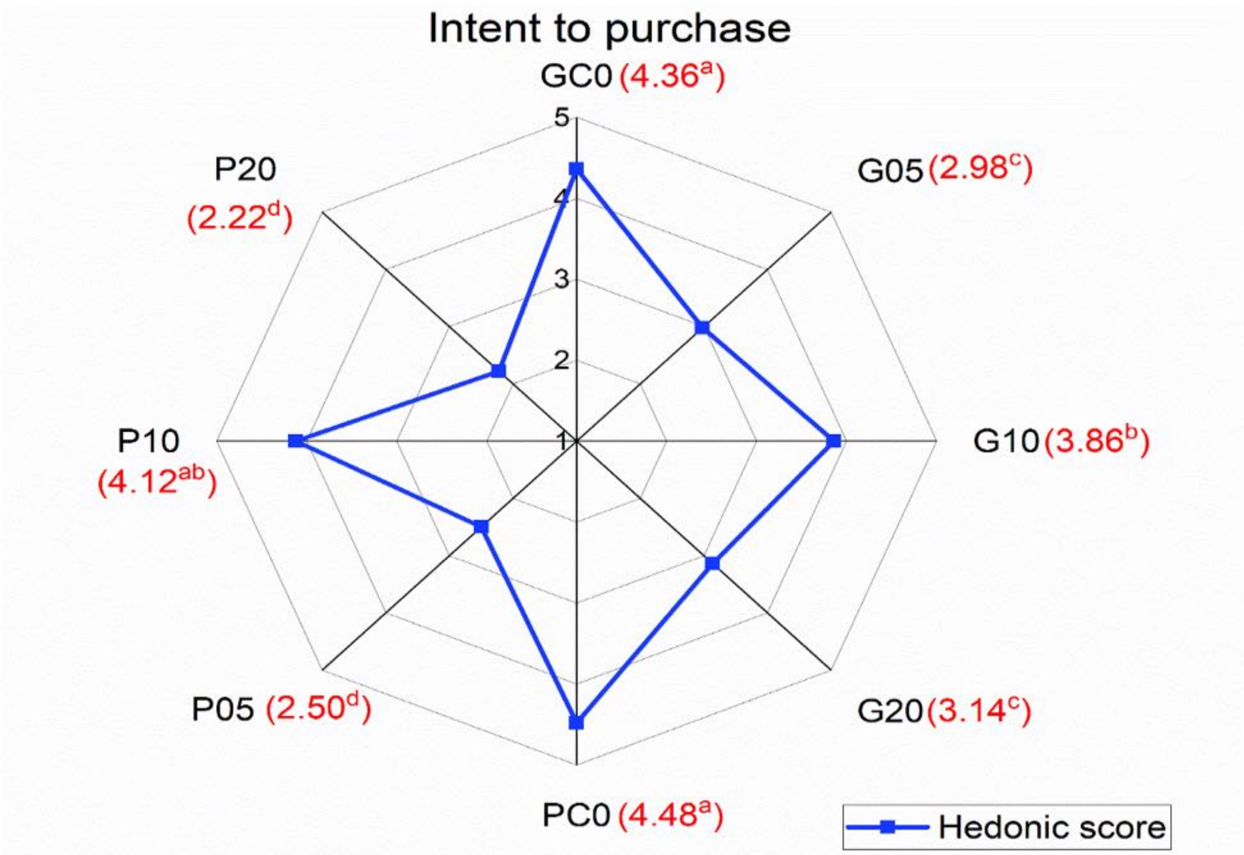

Figure 8: A spider chart representing the purchase intention of spontaneously fermented milk. The data is presented as means (n = 50). Significant differences ($p<0.05$) exist between values that do not share a superscript letter. Overall liking: 5=definitely would buy, to 1=definitely would not buy. G – gourd, P – PET vessel, C0 – 0%, 05 – 0.05%, 10 – 0.10%, 20 – 0.20%

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Sustainability of local production of spontaneously fermented milk may be achieved through the investigation of alternative fermentation vessels. Being a natural additive, OAA may improve the mineral composition, bioactive compounds, and microbial quality in spontaneously fermented milk. The characterization of OAA revealed its high mineral composition and phytochemicals. Its incorporation in spontaneously fermented milk enhanced the crude ash, mineral content, total solids, viscosity, TPC, TFC, and antioxidant activity. Gourds contributed more to crude protein, carbohydrates, and functional properties. The lactic acid bacteria, yeast and molds counts were within the recommended limits to guarantee safety. In addition, OAA also inhibited the growth of yeast and molds. The spontaneously fermented milk up to 0.10% OAA in both gourds and PETs was generally accepted. However, the controls were the most preferred due to familiarity of unflavored milk.

Further investigations on heavy metals and other appropriate safety parameters are needed as part of the validation of the study findings. The characterization of gourd internal surfaces is recommended to understand the possible leaching of bioactive compounds into the milk during fermentation. Additionally, for sensory evaluation, it is recommended to expand age group diversity, targeting community-based consumers who are familiar with similar fermented dairy products.

The nutritional, functional, and consumer acceptability findings indicate fermented milk in gourd with 0.1% OAA is the most preferred. However, PET vessels with 0.10% OAA demonstrated comparable parameters, suggesting potential practical application of PETs upon further validation. In general, food-grade PETs and 0.10% OAA are recommended as an alternative for spontaneous fermentation of milk.

ACKNOWLEDGEMENTS

The authors acknowledge all the staff at Dedan Kimathi University of Technology and Jomo Kenyatta University of Agriculture and Technology, Kenya, for their technical assistance in different areas during this research. All the sensory panelists are acknowledged for taking part in this study.

DECLARATION OF COMPETING INTERESTS

The authors declare no conflict of interest.

Table 1: Experimental design of the spontaneously fermented milk

% of OAA	Gourd	Plastic vessel
Treatment 1 (0.00)	GC0 (control)	PC0 (control)
Treatment 2 (0.05)	G05	P05
Treatment 3 (0.10)	G10	P10
Treatment 4 (0.20)	G20	P20

Table 2: Mineral composition of *Olea africana* ash

Category/ (units)	Mineral	Levels
Minerals	Calcium	670.76±0.13
(mg/100g)	Magnesium	26.74±0.21
	Potassium	1664.90±0.28
	Sodium	110.65±0.16
	Phosphorus	207.49±0.44
	Zinc	28.33±0.09
	Iron	45.24±0.08
	Manganese	25.34±0.09
	Copper	2.37±0.07

Data: Means ± standard deviation (n = 3)

Table 3: Phytochemicals and antioxidant activity of *Olea africana* ash

Category	Parameter	Result
Phytochemicals	TPC (mg/100g)	10.26±0.14
	TFC (mg/100g)	30.68±0.50
	% Antioxidant activity	47.60±0.34

Data: Means ± standard deviation (n = 3). TPC – Total phenolic compounds, TFC – Total flavonoid content

ajfand Since 2001 AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 4: Proximate composition of spontaneously fermented milk

Fermentation vessels	Sample treatments (% OAA)	Moisture Content (g/100 g)	Crude ash content (g/100 g)	Crude Protein (g/100 g)	Crude Fat (g/100 g)	CHO (g/100 g)	Energy Value (kcal/100g)
Gourd	GC0 (0.00)	86.64± 0.40^{a}	0.71±0.06^{b}	3.51±0.08^{a}	4.45±0.10^{a}	4.69±0.34^{b}	72.85±1.35^{a}
	G05 (0.05)	86.51±0.13^{a}	0.73±0.22^{b}	3.45±0.07^{b}	4.45±0.14^{a}	4.86±0.13^{ab}	73.25±0.45^{a}
	G10 (0.10)	86.49±0.01^{a}	0.76±0.13^{ab}	3.27±0.11^{c}	4.44±0.13^{a}	5.04±0.03^{ab}	73.19±0.06^{a}
	G20 (0.20)	86.32±0.47^{a}	0.81±0.10^{a}	3.11±0.14^{d}	4.44±0.13^{a}	5.33±0.47^{a}	73.66±1.86^{a}
PET plastic	PC0 (0.00)	87.25±0.05^{a}	0.71±0.11^{d}	3.39±0.10^{a}	4.45±0.10^{a}	4.20±0.05^{c}	70.40±0.25^{a}
	P05 (0.05)	86.87±0.21^{b}	0.74±0.20^{c}	3.37±0.19^{a}	4.45±0.09^{a}	4.58±0.06^{b}	71.80±0.32^{a}
	P10 (0.10)	86.77±0.2^{bc}	0.76±0.24^{b}	3.15±0.14^{b}	4.44±0.14^{a}	4.88±0.03^{a}	72.08±0.10^{a}
	P20 (0.20)	86.67±0.09^{c}	0.91±0.12^{a}	3.04±0.22^{c}	4.43±0.07^{a}	4.94±0.08^{a}	71.85±0.38^{b}
ANOVA	Vessel	0.000**	0.013**	0.000**	0.936	0.002**	0.000**
significance	OAA %	0.022**	0.000**	0.000**	0.053	0.000**	0.129
(P-values)	Interaction	0.601	0.003**	0.089	0.993	0.589	0.581

The data represent means ± standard deviation (n = 3). Significant differences ($p < 0.05$) exist between values in a column that do not share the same superscript letter. CHO – carbohydrates, OAA – *Olea africana* ash, G – gourd, P – PET vessel, C0 – 0%, 05 – 0.05%, 10 – 0.10%, 20 – 0.20%. ** indicates statistically significant differences in the comparison of all samples within the same column

ajfand Since 2001
AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 5: Mineral composition of spontaneously fermented milk, expressed in mg/100 g

Fermentation vessels	Sample treatments (% OAA)	Ca	Mg	K	Na	P	Zn	Fe	Mn
Gourd	GC0 (0.00)	106.91 ±0.09d	6.75± 0.02d	201.93± 0.20d	36.83± 0.10d	124.08± 0.28c	0.04± 0.01d	0.08± 0.11c	0.08± 0.01d
	G05 (0.05)	113.53± 0.07c	7.55± 0.02c	210.53± 0.37c	39.22± 0.327c	125.16± 0.80c	0.08± 0.01c	0.10± 0.13c	0.14± 0.01c
	G10 (0.10)	127.64± 0.12b	8.14± 0.04b	237.69± 0.53b	40.23± 0.13b	127.98± 0.42b	0.10± 0.01b	0.18± 0.14b	0.17± 0.01b
	G20 (0.20)	128.09± 0.32a	9.28± 0.05a	247.43± 0.31a	41.74± 0.40a	134.85± 0.88a	0.14± 0.01a	0.52± 0.09a	0.21± 0.01a
PET plastic	PC0 (0.00)	106.14± 0.41d	6.75± 0.12d	202.08± 0.41d	37.01± 0.18d	123.61± 1.16d	0.04± 0.01d	0.08± 0.13d	0.08± 0.01d
	P05 (0.05)	114.69± 0.30c	7.85± 0.04c	211.82± 0.32c	39.92± 0.17c	125.43± 0.26c	0.09± 0.01c	0.10± 0.08c	0.14± 0.01c
	P10 (0.10)	128.45± 0.29b	8.42± 0.08b	239.30± 0.45b	41.33± 0.26b	130.99± 0.48b	0.12± 0.01b	0.18± 0.12b	0.19± 0.01b
	P20 (0.20)	130.04± 0.30a	9.51± 0.01a	250.26± 0.53a	42.55± 0.41a	138.86± 0.16a	0.16± 0.01a	0.60± 0.10a	0.30± 0.01a
ANOVA	Vessel	0.000**	0.000**	0.000**	0.000**	0.000**	0.001**	0.000**	0.000**
Significance	OAA %	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**
(P-values)	Interaction	0.000**	0.002**	0.000**	0.049**	0.000**	0.009**	0.000**	0.000**

The data represent means ± standard deviation (n = 3). Significant differences ($p < 0.05$) exist between values in a column that do not share the same superscript letter. Ca - calcium, Mg - Magnesium, K - Potassium, Na - Sodium, P - Phosphorus, Zn - Zinc, Fe - Iron, Mn – Manganese, OAA – *Olea africana* ash, G – gourd, P – PET vessel, C0 – 0%, 05 – 0.05%, 10 – 0.10%, 20 – 0.20%. ** denotes statistically significant differences when comparing all samples in the same column

ajfand Since 2001 AFRICAN JOURNAL OF FOOD, AGRICULTURE, NUTRITION AND DEVELOPMENT
SCHOLARLY, PEER REVIEWED
Volume 26 No. 1
January 2026
PUBLISHED BY AFRICAN SCHOLARLY SCIENCE COMMUNICATIONS TRUST
ISSN 1684 5374

Table 6: The pH and TTA of spontaneously fermented milk after fermentation process

Fermentation vessels	Sample Treatments (% OAA)	pH	TTA (%)
Gourd	GC0 (0.00)	4.47±0.07^{b}	0.81±0.01^{a}
	G05 (0.05)	4.50±0.10^{ab}	0.80±0.01^{b}
	G10 (0.10)	4.53±0.06^{ab}	0.79±0.01^{bc}
	G20 (0.20)	4.60±0.01^{a}	0.78±0.01^{c}
PET plastic	PC0 (0.00)	4.43±0.06^{b}	0.85±0.01^{a}
	P05 (0.05)	4.47±0.06^{ab}	0.84±0.01^{a}
	P10 (0.10)	4.53±0.06^{ab}	0.82±0.01^{b}
	P20 (0.20)	4.57±0.07^{a}	0.77±0.02^{c}
ANOVA	Vessel	0.332	0.000**
Significance	OAA %	0.009**	0.000**
(P-Values)	Interaction	0.952	0.001**

Data: Means ± standard deviation (n = 3). Significant differences ($p < 0.05$) exist between values in a column that do not have the same superscript letter. TTA – total titratable acidity, OAA – *Olea africana* ash, G – gourd, P – PET vessel, C0 – 0%, 05 – 0.05%, 10 – 0.10%, 20 – 0.20%. ** represents statistically significant differences when comparing all samples in the same column

Table 7: Pearson correlation coefficients (r) and p-values between antioxidant activity, TPC, and flavonoids of spontaneously fermented milk

Variables	Correlation coefficient (r)	p-value
TPC and antioxidant activity	0.818	0.013
TFC and antioxidant activity	0.938	0.001

Significant correlation exists where $p < 0.05$. TPC – Total phenolic content, TFC – Total flavonoid content

REFERENCES

1. **Farag MA, El Hawary EA and MM Elmassry** Rediscovering acidophilus milk, its quality characteristics, manufacturing methods, flavor chemistry and nutritional value. *Critical Reviews in Food Science and Nutrition*, 2020; **60(18)**: 3024–3041. https://doi.org/10.1080/10408398.2019.1675584

2. **Nodem Sohanang FS, Coton M, Debaets S, Coton E, Ngoune Tatsadjieu L and BA Mohammadou** Bacterial diversity of traditional fermented milks from Cameroon and safety and antifungal activity assessment for selected lactic acid bacteria. LWT, 2021; **138:** 110635. https://doi.org/10.1016/j.lwt.2020.110635

3. **Tchamba NMM, Mohammadou BA, Pahane M, Maiwore J and LN Tatsadjieu** Physicochemical, Sensory, and Microbial Characteristics of Kindirmou and Pendidam, Two Traditional Fermented Milks of Adamawa - Cameroon, as Affected by the Fermentation Vessels. *Asian Journal of Biotechnology and Bioresource Technology*, 2021; 25–38. https://doi.org/10.9734/ajb2t/2021/v7i430107

4. **Masoko P and DM Makgapeetja** Antibacterial, antifungal and antioxidant activity of Olea africana against pathogenic yeast and nosocomial pathogens. *BMC Complementary Alternative Medicine*, 2015; **15(1):** 409. https://doi.org/10.1186/s12906-015-0941-8

5. **Allafi AR** The effect of temperature and storage time on the migration of antimony from polyethylene terephthalate (PET) into commercial bottled water in Kuwait. *Acta Bio Medica: Atenei Parmensis*, 2020; **91(4):** e2020105. https://doi.org/10.23750/abm.v91i4.8463

6. **AOAC.** Association of Official Analytical Chemists. Official Methods of Analysis, 18th Edition, Washington, DC. 2005. www.aoac.org *Accessed September 2025.*

7. **Ihemeje A, Nwachukwu CN and CC Ekwe** Production and quality evaluation of flavoured yoghurts using carrot, pineapple, and spiced yoghurts using ginger and pepper fruit. *African Journal of Food Science*, 2015; **9(3):** 163–169. https://doi.org/10.5897/AJFS2014.1244

8. **Mefleh M, Pasqualone A, Caponio F, De Angelis D, Natrella G, Summo C and M Faccia** Spreadable plant-based cheese analogue with dry-fractioned pea protein and inulin–olive oil emulsion-filled gel. *Journal of Science of Food and Agriculture*, 2022; **102(12):** 5478–5487. https://doi.org/10.1002/jsfa.11902

9. **Paszczyk B and E Tońska** Fatty Acid Content, Lipid Quality Indices, and Mineral Composition of Cow Milk and Yogurts Produced with Different Starter Cultures Enriched with *Bifidobacterium bifidum. Applied Sciences*, 2022; 12(13): 6558. https://doi.org/10.3390/app12136558

10. **Sun W, Chen X, Li C, Huang J, Wei M, Chen Z and J Liu** Spectrophotometric Determination of Total Phosphorus in Fresh Water Using Ammonium Molybdate. *Sensors and Materials*, 2024; **36(5):** 2113. https://doi.org/10.18494/SAM4876

11. **Matela KS, Pillai MK and T Thamae** Evaluation of pH, titratable acidity, syneresis and sensory profiles of some yoghurt samples from the Kingdom of Lesotho. *Food Research*, 2019; **3(6):** 693–697. https://doi.org/10.26656/fr.2017.3(6).177

12. **Arjuna VM** Development and Validation of Formulae for the Estimation of Solids Not Fat and Total Solids Content in Cow and Buffalo Milk [PhD Thesis]. (SRS) NDRI; 2017.

13. **Debon J, Prudêncio ES and JCC Petrus** Rheological and physico-chemical characterization of prebiotic microfiltered fermented milk. *Journal of Food Engineering*, 2010; **99(2):** 128–135. http://dx.doi.org/10.1016/j.jfoodeng.2010.02.008

14. **Tossou LM** Nutritional quality and safety of processed Wagashi cheese using Calotropis procera found in Benin and Kenya [PhD Thesis]. JKUAT, 2018. http://ir.jkuat.ac.ke/handle/123456789/4695 *Accessed September 2025.*

15. **Soleymanzadeh N, Mirdamadi S and M Kianirad** Antioxidant activity of camel and bovine milk fermented by lactic acid bacteria isolated from traditional fermented camel milk (Chal). *Dairy Science & Technology*, 2016; **96(4):** 443–457. https://doi.org/10.1007/s13594-016-0278-1

16. **Venu K and I Pan** Isolation of low pH resistant lactose fermenter from curd by serial dilution and pour plate method to optimize growth. *AIP Conference Proceedings*, 2024; **2871(1):** 050014. https://doi.org/10.1063/5.0227863

17. **Acharya T and J Hare** Sabouraud Agar and Other Fungal Growth Media. In Laboratory Protocols in Fungal Biology: Current Methods in Fungal Biology. *Springer International Publishing*, 2022; 69–86. https://doi.org/10.1007/978-3-030-83749-5_2

18. **Conti-Silva AC and PK Souza-Borges** Sensory characteristics, brand and probiotic claim on the overall liking of commercial probiotic fermented milks: Which one is more relevant? *Food Research International*, 2019; **116:** 184–189. https://doi.org/10.1016/j.foodres.2018.08.011

19. **Nejeliski DM, L da Cunha Duarte L, JE de Araujo Mariath and FL Palombini** Characterization of the Gradient Cellular Structure of Bottle Gourd (*Lagenaria siceraria*) and Implications for Bioinspired Applications. *Bionics and Sustainable Design*. Singapore: Springer Nature, 2022; 125–140 https://doi.org/10.1007/978-981-19-1812-4_5

20. **Li J, Wang X, Zhao J, Liu C, Sun F, Qiao X, Li J, Wei G and Y Li** Effects of different additives on fermentation quality and aerobic stability of soybean milk type and soybean dregs type fermented feeds. CABI Digital Library, 2020. https://www.cabidigitallibrary.org/doi/full/10.5555/20203199971 *Accessed September 2025.*

21. **Paszczyk B and E Tońska** Influence of Plant Additives on Changes in the Composition of Fatty Acids, Lipid Quality Indices and Minerals of Fermented Dairy Products from Cow's Milk. *Molecules*, 2025; **30(2):** 235. https://doi.org/10.3390/molecules30020235

22. **Jaimez-Ordaz J, Martínez-Ramírez X, Cruz-Guerrero AE, Contreras-López E, Ayala-Niño A, Castro-Rosas J and LG Gonzalez-Olivares** Survival and proteolytic capacity of probiotics in a fermented milk enriched with agave juice and stored in refrigeration. *Food Science and Technology,* 2019; **39:** 188–94. https://doi.org/10.1590/fst.41117

23. **Nwogwugwu NU, Abu GO and O Akaranta** Bioethanol Production from an Underutilized Plant, Calabash (*Crescentia cujete*) Using Co-Culture of Saccharomyces Cerevisiae and Cronobacter Malonaticus. *Journal of Advances in Microbiology*, 2021; **21(4):** 17–33. https://doi.org/10.9734/jamb/2021/v21i430339

24. **Akubuenyi FC and OH Sylvanus** Proximate Composition and Microbiological Quality of "Kunu-Aya": A Locally Produced Non-Fermented Beverage in Nigeria. *European Journal of Nutrition & Food Safety*, 2022; **14(12):** 87–96. https://doi.org/10.9734/ejnfs/2022/v14i121292

25. **Chen K, Zhao L, Bao H and L Liang** Formation and characterization of cold-set whey protein gels induced by L-ascorbic acid/calcium for their encapsulation and release. *Food Bioscience*, 2024; **62:** 105191. https://doi.org/10.1016/j.fbio.2024.105191

26. **Okur HH, Yıldırım HK, Yousefvand A and PE Saris** Production of Fermented Soy and Soy/Cow Milk Products with Probiotic Lacticaseibacillus rhamnosus GG Strain. *Food Bioprocess Technology*, 2025; **18(6):** 5736–5748. https://doi.org/10.1007/s11947-025-03804-x

27. **Afiati F, Setiyoningrum F, Priadi G and V Qyasaty** Quantification of Lactic Acid as Secondary Metabolite of Lactic Acid Bacteria Isolated from Milk and Its Derivatived Products. *The Food Ingredient Asia Conference (FiAC)*, 2022; 132–136. https://org.doi=10.5220/0010546700003108

28. **Sungatullina A, Petrova T and E Nikitina** Investigation on Fermented Milk Quality after the Addition of Flaxseed Mucilage and the Use of Lactobacillus delbrueckii subsp. bulgaricus and Lactiplantibacillus plantarum AG9. *Frontiers in Bioscience-Elite*, 2024; **16(2):** 11. https://doi.org/10.31083/j.fbe1602011

29. **Kabbash EM, Abdel-Shakour ZT, El-Ahmady SH, Wink M and IM Ayoub** Comparative metabolic profiling of olive leaf extracts from twelve different cultivars collected in both fruiting and flowering seasons. *Science Reports,* 2023; **13(1):** 612. https://doi.org/10.1038/s41598-022-27119-5

30. **Ramos LR, Santos JS, Daguer H, Valese AC, Cruz AG and D Granato** Analytical optimization of a phenolic-rich herbal extract and supplementation in fermented milk containing sweet potato pulp. *Food Chemistry*, 2017; **221:** 950–958. https://doi.org/10.1016/j.foodchem.2016.11.069

31. **Peker H and S Arslan** Effect of Olive Leaf Extract on the Quality of Low Fat Apricot Yogurt. *Journal of Food Processing and Preservation*, 2017; **41(5):** e13107. https://doi.org/10.1111/jfpp.13107

32. **Zeb A** Concept, mechanism, and applications of phenolic antioxidants in foods. *Journal of Food Biochemistry*, 2020; 44(9): e13394. https://doi.org/10.1111/jfbc.13394

33. **Sarkar T and S Pati** Bioactive Extraction and Application in Food and Nutraceutical Industries. *Springer Nature*, 2024; 480. https://doi.org/10.1007/978-1-0716-3601-5

34. **Lyu H, Hernalsteens S, Cong H, Quek SY and XD Chen** Solid state fermentation of mung beans by Bacillus subtilis subsp. natto on static, shaking flask and soft elastic tubular reactors. *Food Science and Technology International,* 2024; **30(7):** 623–635. https://doi.org/10.1177/10820132231162167

35. **Ogwaro BA, Gibson H, Hill DJ and EA O'Gara** Microbiological Quality of Typical Traditional Fermented Milk from Northern Uganda and Western Kenya. *Dairy*, 2023; **4(3):** 445–461. https://doi.org/10.3390/dairy4030030

36. **Nirmala C, Shahar B, Dolma N and O Santosh** Promising underutilized wild plants of cold desert Ladakh, India for nutritional security and health benefits. *Applied Food Research*, 2022; **2(2):** 100145. https://doi.org/10.1016/j.afres.2022.100145

37. **Gadhoumi H, Hayouni ELA, Martinez-Rojas E, Yeddes W and MS Tounsi** Biochemical composition, antimicrobial and antifungal activities assessment of the fermented medicinal plants extract using lactic acid bacteria. *Archives of Microbiology*, 2022; **204(7):** 374. https://doi.org/10.1007/s00203-022-02985-9

38. **Owusu-Kwarteng J, Akabanda F, Agyei D and L Jespersen** Microbial Safety of Milk Production and Fermented Dairy Products in Africa. *Microorganisms*, 2020; **8(5):** 752. https://doi.org/10.3390/microorganisms8050752

39. **Lisak Jakopović K, Repajić M, Rumora Samarin I, Božanić R, Blažić M and I Barukčić Jurina** Fortification of Cow Milk with Moringa oleifera Extract: Influence on Physicochemical Characteristics, Antioxidant Capacity and Mineral Content of Yoghurt. *Fermentation*, 2022; **8(10):** 545. https://doi.org/10.3390/fermentation8100545

40. **Pohleven J, Burnard MD and A Kutnar** Volatile organic compounds emitted from untreated and thermally modified wood - a review. *Wood Fiber Science*, 2019; **51(3):** 231–254.