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Effect of *Moringa oleifera* Leaf Powder Fortification on the Nutritional, Microbial, and Sensory Properties of Kefir

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ABSTRACT

Kefir is a probiotic-rich fermented milk product with numerous health benefits. This study investigates the impact of moringa leaf powder (MLP) fortification at different concentrations (0%, 3%, and 7%) on its proximate composition, viscosity, total plate count (TPC), pH, energy, dietary fibre, and sensory properties. Results showed that kefir fortified with 3% MLP had higher protein (7.42%) and ash (1.30%) than the control, while 7% MLP increased protein (7.73%) and ash (1.37%). Dietary fibre rose from 0.12% (control) to 0.36% (3% MLP) and 0.51% (7% MLP). Moisture decreased from 87.35% (control) to 85.67% (3% MLP) and 84.33% (7% MLP). Viscosity increased significantly, from 7.109 mPa (control) to 24.950 mPa (3% MLP) and 64.777 mPa (7% MLP). TPC of lactic acid bacteria increased from 9.13 Log_{10} CFU/ml (control) to 9.61 Log_{10} CFU/ml (7% MLP), indicating improved probiotic potential. The pH rose from 4.27 (control) to 4.43 (3% MLP) and 4.62 (7% MLP), while energy content increased to 61.53 kcal/100 g (3% MLP) and 64.78 kcal/100 g (7% MLP). Sensory evaluation showed that 3% MLP had the highest overall acceptability (7.2), with a smoother texture (6.8) and mild herbal flavour (6.5), while 7% MLP exhibited a coarser texture (5.4) and higher bitterness (5.1). A moderate MLP fortification (3%) improved kefir's nutritional value while maintaining sensory appeal. This study highlights MLP's potential as a functional ingredient in nutrient-dense dairy products.

INTRODUCTION

Moringa oleifera belongs to the family of *Moringaceae*. The common names such as drumstick tree, ben oil tree, and miracle tree [1]. *Moringa oleifera* in India and widely planted in Asia, Africa, and tropical and subtropical regions of Central America [2]. The leaves of *Moringa oleifera* are rich in essential nutrients, including proteins, vitamins (A, B-complex, C, and E), minerals such as calcium, potassium, iron, and magnesium, as well as bioactive compounds such as flavonoids, phenolic acids, glucosinolates, and isothiocyanates, which contribute to its potent antioxidant, anti-inflammatory, antimicrobial, and antihypertensive properties [3,4]. Kefir is a fermented milk produced from kefir grains that contain a specific and complex mixture of bacteria and yeast that live in symbiotic association [5]. Kefir contains a lot of health benefits because of its ability to improve digestion and tolerance to lactose, antioxidants, antimicrobials, and anti-inflammatory properties, control of plasma glucose, anti-hypertensive, anti-carcinogenic activity, anti-allergenic activity, and healing effect [5]. Kefir grains inoculated into milk will produce acidified fermented milk that is slightly carbonated and contains small amounts of alcohol. During the fermentation process, there will be the production of lactic acid, bioactive peptides, exopolysaccharides, antibiotics, and numerous bacteriocins [5]. According to *Codex Alimentarius* (Codex Stan 243-2003), the minimum milk protein for kefir is 2.7%, 0.6% lactic acid, and milk fat must be less than 10%.

Research on food fortification using *Moringa oleifera* leaf powder has gained increasing attention due to its potential to enhance the nutritional value of various food products.

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Fortification of bakery products, noodles, dairy products, and energy bars with *Moringa oleifera* leaf powder has been shown to increase protein, mineral, and antioxidant content [6,7]. Studies have demonstrated that fortification of cookies and biscuits with MLP improves their nutritional profile while maintaining acceptable sensory properties [8]. Similarly, wheat-based products fortified with MLP are beneficial in addressing protein-energy malnutrition and micronutrient deficiencies [9]. However, challenges such as altered sensory attributes, including changes in colour, texture, and taste, require optimization of formulation to balance nutrition and consumer acceptability [10].

MATERIALS AND METHODS

Materials

Moringa leaf powder was obtained from MitoMasa Sdn. Bhd., Ampang, Selangor. Kefir grains were obtained from MyKefirWorld, Cheras, Kuala Lumpur, and the milk used was Dutch Lady UHT milk.

Kefir fortified with MLP

Kefir fortified with Moringa leaf powder (MLP) was prepared based on the method of Endah et al. [11] with slight modifications. Three formulations were prepared in triplicate: 0% (control), 3% and 7% MLP fortification. Specifically, 6 g and 14 g of MLP were added to 200 mL of fresh cow's milk, corresponding to 3% and 7% (w/v), respectively. These percentages are based on the volume of milk before fermentation. The MLP used in this study was not subjected to pasteurization prior to incorporation. This approach was done to preserve the bioactive compounds and natural microbial profile of the MLP, which may influence fermentation dynamics. Fresh cow's milk was mixed with kefir grains and incubated at 25 °C for 24 hours to produce the mother culture. After removing the grains, 3% of the resulting mother culture was inoculated into pasteurized milk containing MLP. The fortified milk was then incubated for another 10 to 16 hours at 25 °C until the pH reached 4.6–4.7. The final kefir samples were stored at 4–10 °C prior to analysis.

Proximate compositions

The proximate contents of the noodles and kefir were tested for moisture, protein, fat, ash, and fibre content by using the standard methods of analysis. Crude protein (N x 5.7) content was converted from the nitrogen measurement by the Kjeldahl method according to AOAC, 2000 [12]. Kefir crude fat was determined using the Gerber Method [13] by ash content assessed using a gravimetric method after incineration in a furnace at 550˚C for 24 hours [14]. The crude fibre contents were determined according to standard methods [14]. Carbohydrate content was estimated by difference [Carbohydrate (%) = 100% - % (moisture + crude protein + crude fat + ash)]. The calorie value of kefir was calculated as: Calorie value (kcal/100g) = (%crude protein x 4) + (% carbohydrate x 4) + (% fat x 9).

pH

The pH meter was prior calibrated using buffer solutions of pH 4.0 and 7.0. According to Firtrianingsih et al. [15], pH meter was dipped into 5 ml beaker glass containing 3 ml of kefir sample using pH meter 3505 Jenway (Altima Resources, Vancouver, Canada).

Colour

The colour of kefir was measured using portable calorimeter Chroma Meter CR-140 (Konica Minolta, Inc., Tokyo, Japan). The equipment was calibrated using white ceramic tiles (Konica Minolta, Inc., Tokyo, Japan). The kefir was analysed by placing them on a petri dish. The colour was defined numerically in terms of lightness or L^* value (0 = black, 100 = white), a^* value (greenness 0 to -100, redness 0 to +100), and b^* value (blueness 0 to -100, yellowness 0 to +100).

Viscosity

The viscosity of kefir was analysed based on Kök-Tas & Ozer [16], using a Modular Compact Rheometer (MCR72 Anton Paar GmbH, Austria). The rheometer with adapter CC27 was fixed to the rheometer. The shear rate, shear stress, and viscosity were measured at rotational speeds from 10-150 rpm. Triplicate analyses were carried out at 25 °C.

Total plate count (TPC)

The total lactic acid bacteria of kefir fortified with MLP was determined according to Hanum et al. [17] with slight modification. Three replicates were performed to determine the total lactic acid bacteria of kefir fortified with Moringa Leaf Powder at different concentrations (0%, 3%, and 7%). Serial dilutions were carried out by pipetting 10 µL of the kefir into 990 µL of peptone water in Eppendorf tube, creating 10^{-2}, 10^{-4}, and 10^{-6} dilutions. Then, 25 µL from each dilution was plated into de Man Rogosa Sharpe Agar (MRS-Agar, Merck) and incubated at 37 °C for 24 hours. This procedure was performed in triplicate to ensure data reliability. The calculation was determined by selecting colonies that grown from 30-300 colonies in each petri dish and counted as followed:

$$Colony/gram(CFU/mL) = \text{The number of colonies x (1/dilution factor)}$$

Sensory analysis

The kefir samples were prepared for sensory evaluation by using the 9-point hedonic scale (1-Dislike extremely, 5-Neither like or dislike, 9-Like extremely) [8]. For kefir, the samples were poured into a small glass and presented individually in a randomized order. The sensory panellists were 50 persons including students, staff, and lecturers from the Universiti Putra Malaysia. Drinking water was used to rinse the mouth between samples. The attributes of kefir were flavour, aroma, colour, texture and overall preference.

Statistical analysis

Statistical analysis was conducted using Minitab 2022 statistical software (Minitab LLC, USA). The results obtained from the present study were represented as the mean values of three individual replicates ± the standard deviation (S.D.) One-way analysis of variance was performed and significant differences between the mean values were determined using Duncan's multiple range tests at a significance level of $p < 0.05$.

RESULT AND DISCUSSION

Effect on Proximate Composition

Fig. 1 illustrates the moisture content (%) of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated no significant differences in moisture content between the control kefir (0%) and the kefir fortified with 3% MLP. However, a significant difference was observed between the 3% and 7% MLP-fortified kefir samples. The control kefir exhibited the highest moisture content (87.35%), followed by the 3% MLP-fortified kefir (86.81%), with the lowest moisture content recorded in the 7% MLP-fortified kefir (84.33%).

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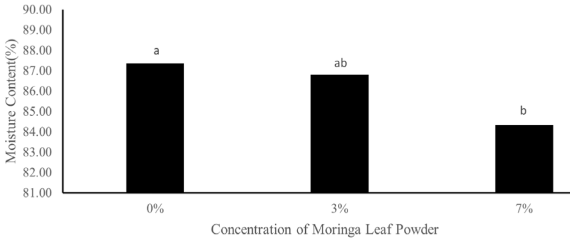

Fig. 1. Moisture content (%) of kefir fortified with MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The significant reduction in moisture content between the 3% and 7% MLP fortification levels was attributed to the water-binding properties of MLP. Moringa Leaf Powder possesses high water absorption and binding capacities due to its fibrous nature. The addition of MLP to food products introduced a competition for free water, thereby reducing the available moisture content. This trend was consistent with findings by Mukumbo et al. [19], who reported that MLP's fibrous composition contributes to its water-absorbing characteristics, particularly at higher concentrations.

Similarly, Dooshima et al. [20] observed a significant decrease in the moisture content of moringa-supplemented food products at higher concentrations of MLP. This reduction is linked to MLP's hygroscopic and fibrous properties, which absorbed and retained water during processing. Saeed et al. [21] further corroborated these findings, demonstrating that the addition of MLP to kefir reduced free water due to interactions between MLP and water molecules. These interactions occurred within the fibrous matrix of MLP, effectively binding water and reducing its availability as free moisture in the kefir. In conclusion, higher concentrations of MLP in milk-based products such as kefir resulted in reduced moisture content. This effect was driven by the water-binding properties and moisture adsorption capacity of MLP, as well as the reduction of free water due to its interaction with water molecules.

Fig. 2 shows the fat content of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated no significant differences in fat content as the concentration of MLP increased. A study by Endah et al. [11] confirmed that the addition of MLP into kefir did not significantly alter fat content. This was attributed to the primary influence of MLP on the moisture and protein content of food products, rather than on fat levels. MLP primarily contributed to increasing fibre, minerals, and antioxidant properties, while having a negligible effect on fat content.

This limited effect on fat content is not exclusive to dairy products such as kefir or yoghurt but has also been observed in other food products, including chicken sausages and beef products. Teye et al. [22] reported that incorporating MLP into these products did not significantly change their fat content, even at higher concentrations, due to the inherently low-fat content of MLP. Instead, MLP enhanced protein and micronutrient content.

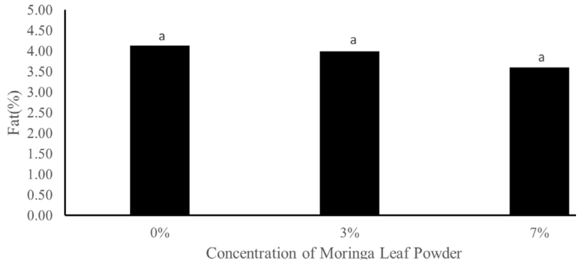

Fig. 2. Fat (%) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

Furthermore, numerous studies have supported the finding that MLP has a very low fat content, typically ranging between 1.1% and 9.51%. For instance, Sultana [23] and Gopalakrishnan et al. [24] both reported minimal fat content in MLP, which has explained its negligible impact on the fat content of fortified kefir, even at higher concentrations. In conclusion, the incorporation of MLP into kefir, even at higher concentrations, has minimal impact on the fat content of dairy products. This was due to the dominance of the inherent milk fat structure and the low-fat composition of MLP, which primarily contributed to other nutritional attributes such as fibre, minerals, and antioxidants.

Fig. 3 illustrates the protein content of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated no significant difference in protein content between the control kefir (0%) and the kefir fortified with 3% MLP. However, a significant difference was observed between the 3% and 7% MLP-fortified kefir. The control kefir exhibited the lowest protein content (4.83%), followed by the 3% MLP fortification (5.81%), with the highest protein content recorded in the 7% MLP fortification (7.73%). These findings suggested that increasing the concentration of MLP has increased the protein content of kefir.

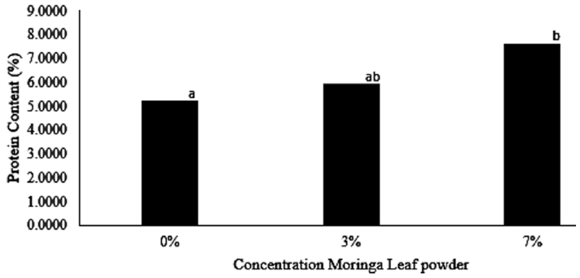

Fig. 3. Protein (%) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p* value <0.05.

A study by Endah et al. [11] corroborated these results, highlighting that MLP contains a high protein content. However, at low concentrations (0% to 3%), the overall contribution of MLP to protein content is minimal, and the addition of MLP did not significantly affect protein levels until higher concentrations were introduced.

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The significant increase in protein content at 7% MLP fortification was attributed to the direct contribution of MLP-derived protein at higher levels. This pattern was consistent with findings in other fortified products, such as maize-based complementary foods and taro porridge, where significant increases in protein content were observed only at high levels of MLP fortification [25]. At lower concentrations (0% to 3%), a protein dilution effect was observed, as the protein concentration is primarily determined by the matrix of the kefir. This effect was consistent with the findings of Sengev et al. [26], who reported similar patterns in fortified bread and maize-bread products, where low concentrations of MLP had minimal impact on the protein content of food products. In conclusion, the addition of MLP at lower concentrations did not significantly affect the protein content of food products. A threshold level of MLP fortification must be exceeded to achieve a meaningful increase in protein content, as demonstrated by the significant changes observed at higher concentrations.

Fig. 4 depicts the ash content (%) in kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results demonstrated a significant increase in ash content as the concentration of MLP increases, which can be attributed to the mineral-rich composition of MLP. The control kefir (0% MLP) exhibited the lowest ash content (0.61%), followed by kefir fortified with 3% MLP (0.87%), with the highest ash content recorded in kefir fortified with 7% MLP (1.37%).

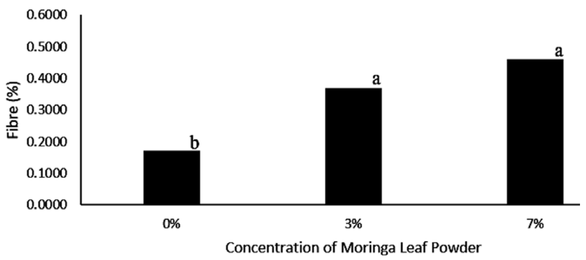

Fig. 4. Fibre (%) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The increase in fibre content in the kefir was attributed to the high dietary fibre content of MLP, which primarily consists of insoluble fibres such as cellulose and hemicellulose. A significant increase in fibre content was observed at the 3% fortification level, reflecting the addition of dietary fibre from MLP even at low concentrations [26]. This observation aligned with findings by El-Gammal et al. [27], who reported a similar linear increase in the fibre content in wheat bread fortified with MLP up to 3%, highlighting the inherent fibre density of MLP. The lack of significant difference between the 3% and 7% MLP fortification levels is likely due to fibre saturation within the kefir matrix at higher concentrations. At this saturation point, the liquid matrix of kefir becomes less effective at incorporating additional insoluble fibres. Similar findings have been reported by Herlina et al. [28] in other food products such as cookies and noodles fortified with MLP. Alhassan [29] further noted that while MLP contains both soluble and insoluble fibres, the liquid matrix of kefir has limited the incorporation efficiency of insoluble fibres, leading to stabilisation of fibre content at higher fortification levels.

In conclusion, the incorporation of MLP into kefir proportionally increased fibre content due to the fibre-rich composition of MLP. However, the absence of significant differences between the 3% and 7% fortification levels is likely a result of fibre saturation within the kefir matrix and reduced incorporation efficiency at higher concentrations. **Fig. 5** shows the carbohydrate content (%) of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated that the control kefir (0% MLP) exhibited the lowest carbohydrate content (2.67%), followed by 3% fortification (5.96%) and 7% fortification (6.34%). A significant difference in carbohydrate content was observed between the control kefir and the 3% and 7% MLP-fortified kefir, although no significant difference was noted between the 3% and 7% fortification levels.

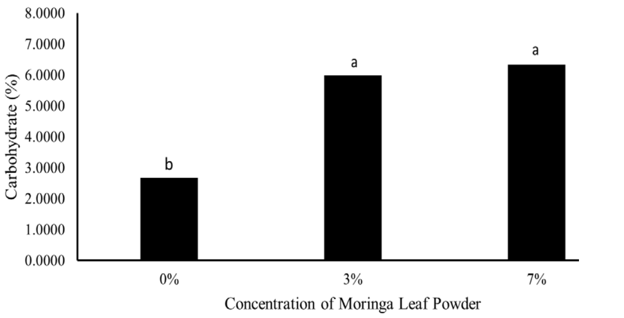

Fig. 5. Carbohydrate (%) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The observed increase in carbohydrate content has been attributed to the compositional contribution of MLP, which is rich in carbohydrates, including polysaccharides. At low concentrations (3% MLP), MLP introduced additional carbohydrates into the kefir, resulting in a substantial increase. This finding was consistent with a study by Endah et al. [11], which reported a significant increase of carbohydrate at low MLP concentrations in kefir fortified with goat milk. At higher concentrations (7% MLP), the carbohydrate content stabilised due to the saturation effect within the kefir matrix, which has limited its ability to incorporate additional carbohydrate components. This saturation effect has diminished the relative contribution of carbohydrates as the kefir matrix reached its incorporation threshold. Moreover, the increase in other macronutrients, such as proteins and fibres, with higher MLP concentrations may dilute the proportional contribution of carbohydrates, resulting in no significant differences between 3% and 7% MLP fortification [30]. In conclusion, the significant increase in carbohydrate content between 0% and 3% MLP fortification in kefir has been attributed to the carbohydrate-rich composition of MLP. However, the absence of significant differences between 3% and 7% fortification has reflected the saturation point of the kefir matrix, limiting its capacity to incorporate additional carbohydrates at higher concentrations.

Fig. 6 depicts the energy content (kcal/100 g) of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results have indicated significant differences in energy content between the control kefir (0% MLP) and kefir fortified with 3% and 7% MLP.

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However, no significant difference was observed between the 3% and 7% fortification levels. The energy content of kefir has increased as the concentration of MLP increased, which was attributed to the nutrient composition of MLP and its energy contribution at higher concentrations.

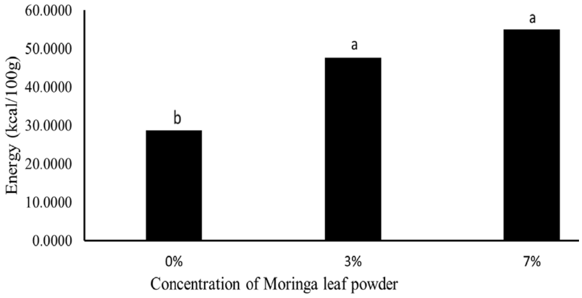

Fig. 6. Energy (kcal/100 g) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

Moringa Leaf Powder is rich in macronutrients, including proteins, carbohydrates, and fats, which significantly have contributed to the increased energy content of fortified products. The addition of 3% MLP to kefir resulted in a notable increase in energy value due to the incorporation of these macronutrients [11]. Mamy et al. [25] reported similar findings in fortified maize and taro products, where energy content increased significantly at moderate levels of MLP addition. At higher concentrations (7% MLP), the energy contribution was stabilised. This was because the additional nutrients provided by MLP beyond 3% do not proportionally increase the energy content. This trend aligned with findings by Sengev et al. [26], who observed stabilisation in energy levels in fortified food products at higher MLP concentrations. The saturation effect of MLP within the kefir matrix may also limit its capacity to contribute additional energy beyond a certain threshold.

In conclusion, the significant increase in energy content from 0% to 3% MLP fortification was due to the addition of macronutrients from MLP. However, the lack of significant differences between 3% and 7% MLP fortification has reflected the saturation point in the kefir matrix, limiting the contribution of MLP to energy content at higher concentrations.

Physical properties

Effect on Colour

Fig. 7 illustrates the colour parameters (*L**, *a**, and *b**) of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated a significant decrease in lightness (*L**) as the concentration of MLP increased. The control kefir (0% MLP) exhibited the highest *L** value (brightest), while kefir fortified with 7% MLP showed the lowest L* value (darkest). Significant differences were observed in the red-green component (*a**) between the control and kefir fortified with 3% and 7% MLP, although no significant differences were noted between the 3% and 7% fortification levels. Similarly, significant differences in the yellow-blue component (*b**) were observed between the control kefir and kefir fortified with 3% and 7% MLP, but no significant differences were detected between the 3% and 7% MLP fortifications.

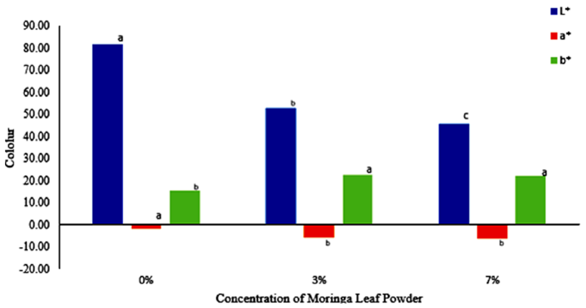

Fig. 7. Colour (*L**, *a**, *b**) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The decrease in *L** values is attributed to the green pigmentation of MLP, which contains chlorophyll that darkens the product as its concentration increases, thereby reducing the lightness. This observation aligned with findings by Mariyam et al. [31], who reported that fortified dairy and food products enriched with MLP exhibited lower *L** values due to the chlorophyll content. Additionally, the negative *a** values correspond to the green hue of MLP, which becomes more pronounced at higher concentrations. A study by Sengev et al. [26] reported similar trends in fortified products, including noodles and beverages, where the intensity of the green hue increased with higher MLP levels. The increase in the yellow component (*b**) is likely due to the presence of carotenoids in MLP, which has contributed to its yellow-greenish pigmentation. However, as the concentration of MLP increases and stabilises, the additional carotenoids do not significantly influence the overall *b** values [32]. In conclusion, fortifying kefir with MLP resulted in significant changes to its colour parameters. The reduction in lightness and shifts in the red-green and yellow-blue components are primarily attributed to the presence of chlorophyll and carotenoids in MLP, with the most pronounced effects occurring at higher fortification levels.

Effect on Viscosity

Fig. 8 shows the viscosity (mPa) of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results showed a significant increase in viscosity at higher concentrations of MLP. The lowest viscosity was observed in the control kefir (0% MLP) at 7.109 mPa, followed by kefir fortified with 3% MLP (24.950 mPa), and the highest viscosity recorded in kefir fortified with 7% MLP (64.777 mPa).

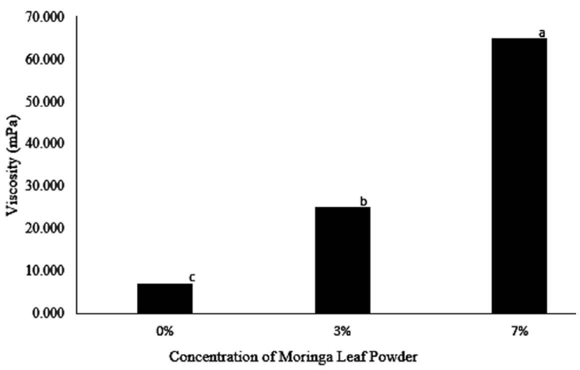

Fig. 8. Viscosity (mPa) kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

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The absence of MLP in the control kefir indicated that its viscosity was primarily determined by the fermentation process, which produced insufficient structural components to create a dense matrix. Without additional stabilisers or thickeners, the kefir structure relies solely on the casein gel formed during fermentation. This resulted in a limited ability to trap water and form a cohesive gel network [33]. Similarly, Ghanimah et al. [34] have observed that unfortified dairy products exhibited naturally lower viscosity due to the absence of external polysaccharides, fibres, or stabilisers. The addition of MLP significantly increased viscosity at 3% and 7% fortification levels. MLP is rich in dietary fibres and polysaccharides, which enhanced the water-holding capacity of the kefir matrix. Moreover, the fermentation of MLP by bacteria and yeast produced exopolysaccharides, which further contribute to increased viscosity [35].

Studies by Jakopović et al. [36] on yoghurt fortified with MLP reported similar findings, which increased in viscosity by polysaccharides and fibres improved water-binding capacity. The highest viscosity observed at 7% MLP fortification was due to the cumulative effects of dietary fibres and insoluble polysaccharides, which enhanced water retention and the structural integrity of the kefir. These fibres has interacted with milk proteins to form a robust network, resulting in a thicker texture. Alhassan [37] found that MLP-fortified yoghurt and kefir exhibited a stronger gel network at higher MLP concentrations, as plant fibres and polysaccharides stabilised the protein structure. In conclusion, the viscosity of kefir increased significantly at the higher concentrations of MLP, driven by the incorporation of plant fibres, polysaccharides, and enhanced water-holding capacity. These components contributed to a denser gel structure, improving the viscosity and overall texture of the kefir.

Effect on pH

Fig. 9 portrays the pH of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated no significant differences in pH values as the concentration of MLP increased. However, the pH of kefir decreased progressively with increasing MLP concentrations, suggesting an increase in acidity. The control kefir (0% MLP) exhibited the highest pH, followed by kefir fortified with 3% MLP, and the lowest pH was observed in kefir fortified with 7% MLP.

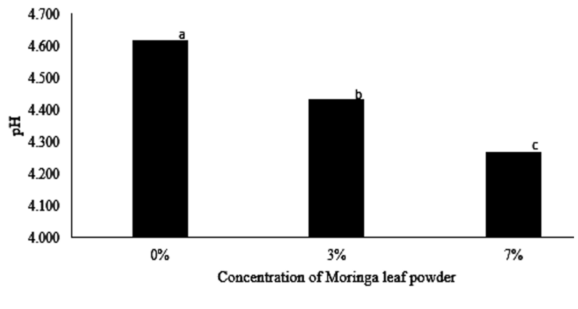

Fig. 9. pH kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The control kefir (0% MLP) contained no external acidic components from MLP, and its pH was primarily influenced by natural fermentation.

The lactic acid bacteria (LAB) involved in the fermentation process resulted in a pH of approximately 4.6, consistent with findings reported by Endah et al. [11] and Farag et al. [38]. This pH value reflects the acid production by kefir grains in the absence of MLP. At 3% MLP fortification, the introduction of bioactive compounds such as polyphenols (tannins and phenolic acids), contributed to a slight decrease in pH. MLP is rich in fibres and micronutrient components that enhance the activity of LAB, leading to increased acid production and a reduction in pH [36]. The lowest pH was recorded at 7% MLP fortification, attributed to the higher concentration of MLP introducing additional acidic compounds such as tannins and phenolic acids.

These compounds further reduced the pH value. Additionally, the increase in the availability of substrates from MLP stimulates LAB activity, resulting in greater acid production. This dual effect has enhanced microbial activity, and the presence of acidic compounds leads to a more pronounced reduction in pH [21]. In conclusion, the fortification of kefir with MLP caused a reduction in pH due to the introduction of acidic compounds and the enhancement of microbial fermentation. The observed trend highlights the impact of MLP on the acidity and fermentation dynamics of fortified kefir.

Total plate count (TPC)

Fig. 10 depicts the total plate count (TPC) of lactic acid bacteria (LAB) in kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The results indicated an increasing trend in LAB count with significant differences across the fortification levels. The control kefir (0% MLP) exhibited the lowest LAB count (9.13_{Log10} CFU/mL), followed by kefir fortified with 3% MLP (9.286_{Log10} CFU/mL), and the highest LAB count was observed in kefir fortified with 7% MLP ($_{Log10}9.612$ CFU/mL).

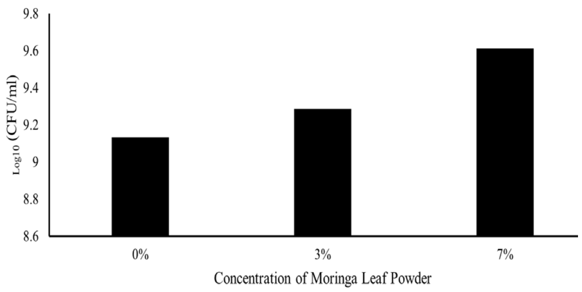

Fig. 10. Total plate count (TPC) of kefir fortified with different concentrations of MLP (0%, 3%, and 7%). Different letters denote significant differences at *p-value* <0.05.

The low TPC observed in the control kefir is attributed to its reliance on the inherent microbial ecosystem in milk and kefir. The absence of external nutrients, such as prebiotic fibres and bioactive compounds found in MLP, limits the ability of LAB to proliferate. Without these additional substrates, LAB growth depends solely on the limited availability of sugars (lactose) and proteins from milk, resulting in slower metabolism and reduced microbial activity [11]. The TPC increased slightly in kefir fortified with 3% MLP due to the introduction of prebiotic compounds. MLP contains dietary fibres that serve as fermentable substrates, enabling LAB to produce more lactic acid bacteria and proliferate. Additionally, bioactive compounds such as polyphenols and flavonoids in MLP stimulated LAB metabolism and protected the bacteria from oxidative stress.

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The moderate MLP concentration provides sufficient nutrients to enhance LAB activity without disrupting the kefir matrix, maintaining an optimal environment for microbial stability [39]. The highest TPC was observed in kefir fortified with 7% MLP. At this concentration, the high level of prebiotic fibres in MLP further enhanced LAB growth by serving as fermentable energy sources. Furthermore, MLP's bioactive compounds, including antioxidants and phenolics, reduce oxidative stress and create a favourable environment for exponential microbial growth.

The high concentration of MLP also provides additional vitamins and minerals, such as iron and potassium, which further support LAB metabolism. These cumulative effects lead to maximum microbial proliferation. However, this concentration also approaches the upper threshold of kefir's capacity to sustain additional microbial growth without compromising its structural integrity [40]. In conclusion, the total plate count of LAB in kefir fortified with MLP increased significantly due to the prebiotic and nutrient-enhancing properties of MLP. Higher MLP concentrations promoted exponential microbial growth, demonstrating that fortifying kefir with MLP not only enhances its nutritional value but also boosts its probiotic content.

Effect on sensory quality

Table 1 presents the sensory analysis of kefir fortified with Moringa leaf powder (MLP) at concentrations of 0%, 3%, and 7%. The sensory scores for attributes such as colour, aroma, acidity, smoothness, aftertaste, and overall acceptability are summarised. The results indicated that sensory scores decreased significantly with increasing MLP concentration.

Table 1. Sensory evaluation kefir fortified with different concentrations of MLP (0%, 3%, and 7%).

Attribute	0% (MLP)	3% (MLP)	7% (MLP)
Colour	7.00±1.863[a]	6.06±2.478[ab]	5.20±2.204[b]
Aroma	6.00±2.339[a]	3.38±1.999[b]	3.08±1.614[b]
Acidity	4.90±2.234[a]	2.68±1.801[b]	2.42±1.853[b]
Smoothness	5.98±2.236[a]	3.34±1.745[b]	2.48±1.555[b]
Aftertaste	5.28±2.382[a]	2.00±1.370[b]	1.78±1.130[b]
Overall Acceptability	5.32±2.394[a]	2.24±1.364[b]	1.84±1.057[b]

The aroma scores declined significantly as the concentration of MLP increased. The control kefir (0% MLP) achieved the highest score for aroma, while 3% MLP and 7% MLP were rated as moderately disliked. The grass-like odour of MLP becomes more pronounced as its concentration increases, which may negatively impact consumer perception. The colour of the kefir was significantly affected by MLP fortification due to the introduction of dark green pigments. The control kefir (0% MLP) achieved the highest score, followed by 3% MLP and 7% MLP. Panellists rated the control kefir's colour as moderately liked, while kefir fortified with 3% MLP was rated as slightly liked, and 7% MLP was rated as neither liked nor disliked. Colour is a critical sensory attribute as it forms the first impression, and higher MLP concentrations introduce pigments that negatively influence this perception [41].

Acidity scores decreased significantly with increasing MLP concentration. The presence of bioactive compounds in MLP, such as polyphenols, can alter or mask the acidic taste, leading to a lower perception of acidity. Maintaining a balance of acidity is crucial for dairy-based beverages, as deviations from expected acidity levels often reduce sensory acceptability [11].

The smoothness of kefir also declined significantly with increasing MLP concentrations. This decline is attributed to the fibre content and particulate nature of MLP, which introduced a coarser texture that disrupts the smooth, creamy mouthfeel typically associated with traditional kefir. The presence of fibrous materials creates a gritty sensation, significantly impacting textural quality and reducing smoothness scores at higher concentrations [42].

The aftertaste scores decreased markedly with increased MLP concentrations. At higher levels, the bitter, herbal, and acidic aftertaste from phytochemicals such as tannins and saponins became more pronounced. These compounds linger on the palate, leaving an undesirable aftertaste. Traditional kefir, characterised by mild flavours, contrasts with the strong, lingering herbal notes of MLP-fortified kefir. Similar findings were reported by Ndabigengesere and Narasiah [43], where fortified cookies and yoghurts with higher MLP levels exhibited stronger bitter aftertaste. Lastly, the overall acceptability of kefir decreased significantly with increasing MLP concentrations. The combination of changes in colour, aroma, acidity, smoothness, and aftertaste resulted in lower overall scores.

Kefir fortified with 3% MLP maintained some consumer acceptability, whereas 7% MLP was rated as extremely disliked due to the pronounced negative sensory changes. The panellists scores reflect that the sensory drawbacks outweighed the perceived nutritional benefits of MLP fortification at higher levels [41]. Overall, the control kefir provides a baseline sensory profile with traditional attributes and is the most preferred by the panellists. Kefir fortified with 3% MLP represents a balance between sensory quality and nutritional enhancement and remains acceptable. However, fortification with 7% MLP leads to significant declines in sensory attributes due to intense herbal characteristics, textural changes, and bitter aftertaste. In sum, our findings are in line with the reported studies on the health benefits of the *Moringa oleifera* [44-46].

CONCLUSION

The fortification of kefir with Moringa leaf powder (MLP) enhanced its nutritional and probiotic potential by significantly increasing the total plate count (TPC) of lactic acid bacteria (LAB). The prebiotic properties of MLP, including dietary fibre and bioactive compounds, supported microbial growth, with the highest concentration (7%) demonstrating the most substantial improvement. However, higher MLP concentrations adversely affected the sensory attributes of kefir. The herbal aroma, earthy taste, and bitter aftertaste introduced by MLP compounds, such as flavonoids and saponins, became increasingly prominent, resulting in lower acceptability scores. Additionally, the fibrous content of MLP disrupted the smooth and creamy texture of kefir, adversely affecting mouthfeel. The acidity of kefir was also reduced due to the buffering effects of MLP, which altered its characteristic tangy flavour. Moderate fortification (3%) was identified as the optimal level, maintaining acceptable sensory qualities while enhancing the probiotic and nutritional benefits of kefir. This finding demonstrates that MLP can be effectively utilised to improve the functional properties of kefir, but careful control of its concentration is essential to meet consumer preferences and ensure product acceptability.

CONFLICT OF INTEREST

The authors have declared that no conflict of interest exists.

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ETHICS STATEMENT

Ethical approval for the involvement of human subjects in this study was granted by the Ethics Committee for Research Involving Human Subjects (JKEUPM), reference number: JKEUPM-2024-1305.

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