JOURNAL OF TROPICAL LIFE SCIENCE
2023, Vol. 13, No. 2, 287 – 296
http://dx.doi.org/10.11594/jtls.13.02.06

Research Article

Proximate Composition and Anti-microbial Activity of Kefir Produced from Cow's and Almond Milk

Jing Xuan Hew [1], Siti Raihanah Shafie [1]*, Nurshahira Sulaiman [2]

[1] Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia
[2] Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

Article history:
Submission September 2022
Revised December 2022
Accepted December 2022

**Corresponding author:*
E-mail: sitiraihanah@upm.edu.my

ABSTRACT

Fermented foods are an important diet component of people around the world. Kefir, or fermented milk, is popular worldwide due to its high nutritional value, with cow's milk being the common substrate for traditional kefir fermentation. However, the scarcity of animal-based milk in some countries, plus cultural, religious, and health reasons, have seen non-dairy milk kefir from almond milk gaining popularity among consumers globally. This study aimed to evaluate and compare the proximate composition and anti-microbial activity of kefir produced from 100% cow or 100% almond or an equal (1:1) mixture of both kinds of milk. This study used the AOAC 2000 method for the proximate analysis, while the agar well diffusion method examined the anti-microbial activity of the milk samples against *Escherichia coli, Staphylococcus aureus, and Salmonella typhi*. Results revealed that the three kefir samples showed significantly different ($p<0.05$) moisture, total dietary fiber, and fat contents and were within the CODEX acceptable range for kefir. All samples exhibited varying degrees of inhibition between the different pathogens. The diameters of the inhibition zone of the tested kefir samples were significantly different toward *Salmonella typhi* ($p<0.05$), with the mixture of almond and cow milk notably producing better inhibition towards all tested bacteria. The above-said milk mixture also gave a better overall nutrient profile (lower fat and higher fibre). While almond milk might be a suitable substrate for kefir, it was not effectively inhibitory for all bacteria. The overall results thus conveyed the promising use of almond and cow milk mixture as an alternative substrate for kefir fermentation, further supporting its potential use as a probiotics source.

Keywords: Almond milk, Anti-microbial activity, Cow's milk, Fermentation, Kefir, Proximate composition

Introduction

People have been using fermentation to preserve foods for centuries before the invention of other technologies [1]. Evidence shows that fermented beverages appeared since 5000 B.C. in Babylon [2]. Because of the high sensory attributes and nutritional value, fermentation has a crucial function in the meals of individuals worldwide [3]. Fermented food is believed to improve gastrointestinal health, enhance systemic immunity, lower cholesterol and blood pressure, exert anti-hypertension, anti-inflammatory, and anti-carcinogenic effects, and maintain weight [4].

Milk kefir is fermented milk that originates from Tibet and Caucasus and has become an increasingly popular fermented beverage worldwide [5]. Kefir is said to be one of the "9 food trends to watch for in 2021" by the Institute of Food Technologists, with market size projected to increase by US$456 million from 2021 to 2025, with a compound annual growth rate of 4.37% in 2021 [6]. Hamida et al. (2021) [7] also described that the COVID-19 pandemic had boosted consumers' health consciousness, and they are seeking products that could enhance the immune system. The

How to cite:
Hew JX, Shafie SR, Sulaiman N (2023) Proximate Composition and Anti-microbial Activity of Kefir Produced from Cow's and Almond Milk. Journal of Tropical Life Science 13 (2): 287 – 296. doi: 10.11594/jtls.13.02.06.

term "kefir" remains commonplace despite multiple names in different regions [8]. Kefir is slightly acerbic (< pH 5), alcoholic, and easily digestible, effervescent creamy-textured fermented milk [9]. It is produced from the combination of acid and alcohol fermentation [9, 10], which yields ethanol, lactic acid, bioactive compounds, and carbon dioxide as products [11]. Kefir is generally consumed with meals or consumed alone as a probiotic drink [8]. Kefir has caught consumers' attention due to its health benefits, safety, affordability, and convenience, as it can be easily produced in home settings [5]. It is coined the 21st-century yogurt because of its substantial quantity and range of microorganisms, the bioactive metabolites from their metabolic processes, and the health benefits from kefir consumption [5]. Several studies have shown that kefir may have anti-microbial, anti-tumor, anti-carcinogenic, and immunomodulatory activity and improve lactose intolerance [11]. According to Rosa et al. (2017) [5], milk kefir production traditionally begins by preparing a fermentation substrate from a variety of sources, *viz.* whole, semi-skimmed, or skimmed pasteurized cow, goat, sheep, camel, or buffalo milk, with cows' milk being the most common. Then, the kefir grains are added to the fermentation substrate as the starter cultures and kept aside in a partially closed container for 24 hours at temperatures between 8 to 25˚C. After fermentation, the kefir grains are sieved, and milk kefir is the product of filtered fermented milk.

Although dairy milk is the traditionally common substrate for kefir fermentation, animal milk scarcity in some countries, high prices, dietary constraints, preferences, health concerns, or religious customs have seen consumers' preferences shifting toward non-dairy milk products [12]. Almond or *Prunus dulcis* (Mill.) D.A Webb is one of the most popular tree nuts produced and consumed worldwide due to its favourable fatty acids profile, vitamin E content, and polyphenols [13]. Futhermore, almonds' high fibre and polyphenols content could be a substrate for microbial fermentation in the gut [14]. In fact, due to the increased preference towards non-dairy milk, sales of such milk in the United States have increased by 61% in the last five years, in which almond milk remains a staple (64%) in this category [15]. Because of the increased demand for new non-dairy probiotic beverages [16], non-dairy milk matrices could be an emerging source to deliver probiotics with satisfactory viability levels. Thus, this study aims to evaluate and compare the nutrient composition and anti-microbial activity of kefir produced from cow and almond milk mixture with different ratios, for a better understanding of the nutrient profiles and potential health benefits.

Material and Methods

Sample preparation

Kefir grains, raw whole almonds, and fresh cow's milk were purchased from the local store in Malaysia. To prepare almond milk, the whole raw almond was soaked overnight, drained and dehulled, and blended with a 1:4.315 ratio of almond:water to standardize the total solid content with cow's milk. Finally, the almond slurry was filtered through a muslin cloth and collected as almond milk. The milk samples in this study were 100% cow's milk, a 1:1 ratio of cow's and almond milk mixture, and 100% almond milk [17].

Activation of the kefir grains involved incubating 20 g of kefir grains in 500 mL of fresh milk for 24 hours at room temperature, and the slurry was filtered. This process was repeated 3 times to obtain healthy and active kefir grains. According to the Codex Alimentarius Commission, (2011) [18], typical kefir produced from kefir grains should contain at least 2.7% of protein, 0.6% of lactic acid, and less than 10% of fat. Next, the activated kefir grains were added to the pasteurized milk kefir samples in 5% (w/v) and were fermented at 25 ˚C for 24 hours. The fermented milk samples were then filtered to yield the milk kefir samples for the subsequent experiments. The kefir sample preparation is summarized in Figure 1. The nutrient composition and anti-microbial activity tests were triplicated, and the results are presented as mean ± standard deviation.

Nutrient compositions analysis

The milk kefir samples were analyzed for moisture, ash, total available carbohydrate, protein, fats, and total dietary fiber contents, using the AOAC 2000 methods. The methods involved air-oven drying, dry ashing, Colorimetric Clegg-Anthrone method, Kjeldahl method, Gerber method, and enzyme gravitational method [15]. The chemical and reagents used in this study were of analytical grade.

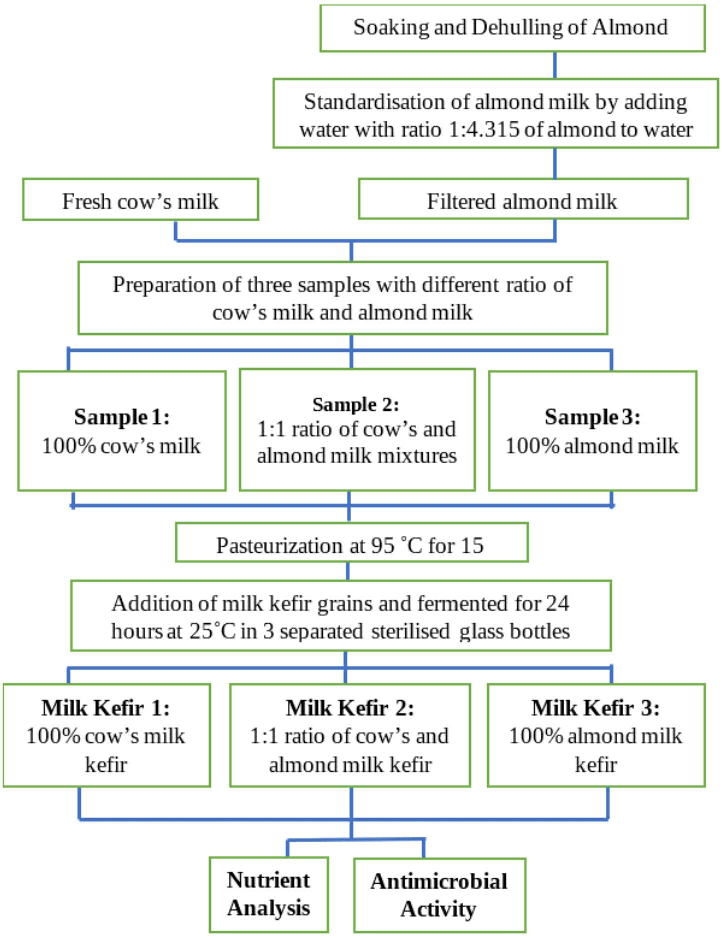

Figure 1. Steps for the preparation of kefir samples

Table 1. The culture media, microbial inoculum size and incubation conditions for anti-microbial susceptibility testing methods recommended by CLSI

	Micro-organism	Growth Medium	Final Inoculum Size	Incubation Temperature (˚C)	Incubation Time (h)	Ref.
Agar Well Diffusion	Bacteria	Mueller Hinton Agar (MHA)	0.5 McFarland	35±2	16-18	M02-A

Anti-microbial activity analysis

The *S. aureus, S. typhi, and E. coli* clinical isolates were provided by the Department of Biomedical Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. The anti-microbial assay used the Mueller-Hinton agar (MHA) powder, trypticase soy agar (TSA) powder, saline water, antibiotic gentamicin (10 mcg) disc, *Staphylococcus aureus 25923, Escherichia coli 25922 and Salmonella typhi* (wild type). The culture media, microbial inoculum size, and incubation conditions for anti-microbial susceptibility testing methods were prepared according to the Clinical and Laboratory Standards Institute (CLSI) standards, as shown in Table 1.

The anti-microbial activity was analyzed using the agar well diffusion test according to the methods described by Azizkhani, Saris, and Baniasadi (2021) [19] with some modifications. First, pure cultured bacteria were swabbed uniformly on the surface of the Mueller Hinton agar plates using sterile cotton swabs and gel punctures to create holes (depth x diameter: 6 mm x 9 mm). Next, 100 µL of filtered kefir supernatants were pipetted into the holes, and the study used 100 µL of distilled water and 10 µg discs of gentamicin as the negative- and positive controls, respectively. The agar plates were incubated at 35 ± 2 ˚C for 16-18 hours, followed by the zone of inhibition analysis.

Statistical analysis

IBM SPSS Statistics 25 was applied for the statical analysis. The study opted for the one-way ANOVA to determine the differences between the means ± standard deviations of proximate composition and anti-microbial activity between 3 samples (100% cow's milk kefir, 1:1 ratio of cow's and almond milk kefir, and 100% almond milk kefir), with an individual sample of 3 (n = 3) each group. Statistical comparisons were considered significant at $p<0.05$.

Results and Discussion

Chemical and nutrient composition

Moisture content

Table 2 shows that the moisture content of the kefir samples made from 100% almond milk (88.80±0.35%) was higher than the 1:1 ratio of cow's and almond milk mixture (88.20±0.08%) and 100% cow's milk (86.26±0.07%). Pertinently, the result demonstrated significant differences in the moisture content between 100% almond milk kefir, a 1:1 ratio of cow's and almond milk kefir, and 100% cow's milk kefir ($p<0.05$). The present study concurred with previously reported moisture content for kefir by Wszolek et al. (2001) [20], in which the moisture content of kefirs ranged between 85.1% to 89.4%. Likewise, Otles (2003) [21] revealed that their cow's milk kefir moisture content was 87.5%. However, minor differences in moisture contents were noted between the present study with Arslan's (2015) [22], where the latter showed 89-90% moisture content.

According to the present finding, the moisture content of all the kefir samples was within range compared to previous studies due to the standardized total solid content for the cow's milk and almond milk samples before fermentation. Milk blends containing higher total solids progressively displayed lower moisture content [17]. This study adopted the method described by Gul et al. (2015) [23] for standardizing the total solid content of samples for kefir production to ensure that the microbiological and chemical differences in kefir samples were associated with protein and minor compounds of milk.

It has been documented that moisture-related microbial growth is one of the major factors that cause food spoilage in developing countries [24]. All the kefir samples studied had moisture content higher than 80%, rendering them perishable. Thus, readily consumed kefir beverages should be kept at 4 °C [11, 23, 25]. While kefir shelf life tends to vary, storage at 4°C for 7 days is the most common method [25].

Ash content

According to Table 2, the 100% cow's milk kefir had the highest amount of ash content (0.73±0.11%), followed by a 1:1 ratio of cow's and almond milk kefir (0.71±0.10%) and the 100% almond milk kefir (0.60±0.00%). The ash contents for the three types of kefir milk produced from cow's milk and almond milk mixtures at different ratios were not statistically different (p=0.230).

This result tied well with previous studies wherein the ash content of cow's milk kefir is 0.75 % [26]. Compared to results by Wszolek et al. (2001) [20], the study's ash content for cow's milk

Table 2. Results of the proximate composition of kefir produced from cow's milk and almond milk mixtures with different ratios

	100 % almond milk kefir	1:1 ratio of cow's and almond milk kefir	100% cow's milk kefir
Moisture (%)	88.80±0.35^{a}	88.20±0.08^{b}	86.26±0.07^{c}
Ash content (%)	0.60±0.00^{a}	0.71±0.10^{a}	0.73±0.11^{a}
Protein (%)	5.69±0.45^{a}	5.87±0.11^{a}	5.80±0.10^{a}
Total Dietary Fibre per dried mass (%)	2.67±0.64^{a}	5.96±1.21^{b}	6.97±0.21^{bc}
Fat (%)	4.70±0.50^{a}	3.73±0.21^{b}	2.15±0.05^{c}
Total Available Carbohydrate (%)	5.24±0.58^{a}	5.02±0.33^{a}	6.18±0.49^{a}

Mean ± standard deviation values in the same row with different superscripts are significantly different from each other at $p<0.05$.

kefir (0.73%) agreed well with their findings, despite the different milk sources from mammals used to make the kefir produced in Poland and Scotland different species of mammals ranges (ash content 0.7-1.11%).

However, the ash content of almond milk kefir was highly dependent on the amount of added water during almond milk preparation. The total solids content represents the quantity of organic material remaining after all the moisture has been evaporated. Thus, more added water when blending almond milk leads to a lower ash content, following a more diluted kefir. This trend was clearly shown in the study by Kundu et al. (2018) [17], where the ash content decreased from 3.02% for 1:1 almond: water to 1.63% for 1:3 almond: water ratio of almond milk. Another finding by Gamba et al. (2015) [26] showed that unfermented and fermented cow's milk yielded similar ash content (0.75%), while unfermented soy milk (0.44%) showed comparable ash content as fermented soy milk (0.40%). This may suggest that fermentation in either kefir sample did not significantly change the ash content.

It is pertinent to indicate here that the total mineral content in ash includes the essential micronutrients, suggesting that the 100% cow's milk kefir contained a higher amount of total minerals than the other kefir samples in the present study. However, the difference is insignificant due to the prior standardization of all the samples' total solid content.

Protein

According to Table 2, the 100% almond milk kefir had the lowest amount of protein content (5.69±0.45%), followed by 100% cow's milk (5.80±0.10%) and the 1:1 ratio of cow's and almond milk kefir (5.87±0.11%). However, the protein content across the different kefir milk with different ratios was not statistically different ($p=0.714$).

The protein composition of kefir varies as it depends on the source of milk, the components of the grains or cultures, and the process of kefir fermentation [27]. Otles (2003) [21] and Gamba et al. (2020) [33] reported lower protein contents for traditional kefir samples made from kefir grains (3.22% and 4.54%) compared to kefir samples prepared by the present study at 5.69% to 5.87%. There may be an overestimation of the protein content, as there have been several studies showed that the nitrogen determination with subsequent conversion using 6.25 nitrogen-to-protein conversion factor may cause an overestimation of protein content in most diets, particularly plant foods [28].

In addition, the highest protein content in the 1:1 ratio of cow's and almond milk kefir may be due to the blending of different mixed matrices that increased the protein content of kefir. This was demonstrated by Barbosa et al. (2020) [29] who reported that beverages from hydrosoluble extracts comprising a fermented mixture of 75% soybean and 25% brazil nut gave the highest protein content (4.26%) than those prepared from fermented 100% soybean hydrosoluble extract (3.75%) and 100% brazil nut hydrosoluble extract (3.20%). This further showed that the mixture of different milk could increase the protein content of kefir.

Total dietary fibre

According to Table 2, the total dietary fiber per dried mass of kefir showed an increasing trend from 100% almond milk kefir, 1:1 ratio of cow's and almond milk kefir to 100% cow's milk kefir at 2.67±0.64%, 5.96±1.21%, and 6.97±0.21%, respectively. There was a statistical difference in the total dietary fiber content across kefir produced from cow's milk and almond milk mixtures at different ratios ($p<0.005$).

It is important to highlight that kefiran polymers [30, 31], comprising water-soluble glucogalactan polysaccharide matrix surrounding the microorganisms naturally present in the kefir grains [32]. Malaysian Food Composition Database, 1997 [32] showed that cow's milk did not contain any dietary fibre; thus, the 6.97% of dietary fiber found in the cow's milk kefir was attributable to the kefir grains inoculated into the milk during fermentation.

As shown in Table 2, the 100% cow's milk kefir contained the highest amount of total dietary fiber (6.97%), while the lowest was the 100% almond milk kefir (2.67%). The outcome implied that the 100% cow's milk kefir contained the highest amount of kefiran. According to Magalhães et al. (2011) [30], *Lactobacillus kefiri*, a commonplace probiotic cell found in kefir, is essential for producing kefiran. As kefiran is formed during microbiota cell growth under aerobic conditions [30], this suggests that 100% cow's milk was the better substrate for microbiota cell growth, contributing to the higher amount of kefiran produced.

Fat

Table 2 shows the fat content in the 100% almond milk kefir being the highest (4.70±0.50%), followed by a 1:1 ratio of cow's and almond milk kefir (3.73±0.21%) and 100% cow's milk kefir (2.15±0.05%), and differed significantly across kefirs produced from cow's milk and almond milk mixtures at different ratios ($p<0.005$). Notably, the fat content of cow's milk kefir in this study corroborated earlier reports showing cow's milk kefir having fat contents between 1.34 % to 3.5% [26, 27, 32]. According to Dinkçi et al. (2015) [3], 3% (w/v) inoculation of kefir grains in 100% cow's milk produced milk kefir with 2.83% of fat. Likewise, a 10% (w/v) inoculum used by Gamba et al. (2020) [33] yielded 1.34% fat content of fermented cow's milk [34], while Otles (2003) [21] and Shen et al. (2018) [27] showed that a 100 g cow's milk kefir samples contained 3.5g of fat.

As can be seen, the amount of fat in kefir varied greatly based on the milk used in the fermentation [5]. Abdolmaleki et al. (2015) [35] reached a similar conclusion, where the fat content of kefir beverages highly depended on the substrate. For example, soy milk (2.30%) contains a lower amount of fat than cow's milk (3.71%); thus explaining the lower fat content in soymilk kefir than cow's milk kefir.

Another fact to consider is that almond contains a high amount of fat, especially monounsaturated fat. Karimi et al (2021) [36] indicated that 66 % of these fats are monounsaturated, 26% polyunsaturated, and 8% are saturated fats. Hence, the high-fat content in the almond explains the almond milk kefir having the highest fat content among the prepared kefirs in this study. However, the value was still in line with the total fat content (less than 10%) of typical kefir [18].

Total available carbohydrate

According to Table 2, the 100% cow's milk kefir had the highest total available carbohydrate content (6.18±0.49%), while the 1:1 ratio of cow's and almond milk kefir was the lowest (5.02±0.33%). The total available carbohydrate content in the 100% almond milk kefir was 5.24±0.58% although the above-said total available carbohydrate contents were not statistically different across kefirs produced from cow's milk and almond milk mixtures at different ratios ($p=0.053$).

Farag et al. (2020) [37] reported that typical kefir consisted of 6.0% of total available carbohydrates, which agreed with the study's outcome of between 5.02% to 6.18% in the fermented milk kefir samples. However, Azizkhani et al. (2021) [19] reported a 3.95% carbohydrate content in cow's milk kefir, lower than the present study (6.18%). This is because the final percentage of the total sugar content of kefir highly depends on the fermented substrate [37], hence the slight difference in the final total available carbohydrate content of the three distinct kefir samples.

United States Department of Agriculture's (USDA) database of nutritional information revealed that the total sugar content of whole milk is 12.3% [38], while whole milk kefir had almost half of the total sugar content (6%) after fermentation. The possible reason is that in fermentation by lactic acid bacteria, lactose is hydrolyzed to glucose and galactose, decreasing carbohydrate content. The galactosidase enzyme hydrolyzes about 30% milk lactose into glucose and galactose [14]. Kefir's microorganisms also convert glucose to lactic acid, making it a good alternative for lactose-intolerant people in this circumstance [5]. The outcome reported in this study agreed with a report by Gamba et al. (2020) [33] which showed lactose as the main sugar used by microbes in cow's milk kefir, which decreased from 4703 to 3314 mg per 100 mL during fermentation. They also found that the sugar content in fermented cow's milk decreased by 1.06 % and 1.36 % by proximate and High-Performance Anion Exchange (HPAE) chromatography analysis, respectively [33]. However, there is yet any research on the main sugars used in the production of almond milk kefir.

The present result shows that the fermented 1:1 ratio of cow's and almond milk kefir consisted of the lowest amount of total available carbohydrates among the three kefir samples. This might be due to the mixture of cow's milk and almond milk having more matrices for delivery of probiotics, resulting in more active microorganisms that consumed more sugar, compared to 100% cow's milk kefir and 100% almond milk kefir. More active probiotics tend to consume higher amounts of sugar in kefir, lowering the total available carbohydrate content in the kefir samples. Thus, the total available carbohydrate may indirectly reflect the activity of the probiotics in kefir.

Anti-microbial activity

Table 3 summarizes the antibacterial activity of the kefir samples toward the pathogens. All the tested pathogens were sensitive to the positive control gentamicin by showing more than 15 mm of the diameter of the zone of inhibition according to the Clinical Laboratory Standards Institute (CLSI) [39] while resistant to the negative control distilled water.

According to Table 3, cow's milk kefir exhibited antibacterial activity against all three types of bacteria, corresponding to mean zone of inhibitions of 3.67±6.35 mm, 11.33±0.58 mm, and 3.67±6.35 mm for *E. coli, S. aureus, and S. typhi*, respectively. Next, the 1:1 ratio of cow's and almond milk kefir also exhibited antibacterial activity against all three types of bacteria, showing inhibition zones of 8.00±7.00 mm, 12.33±1.53 mm, and 12.67±1.15 mm for *E. coli, S. aureus, and S. typhi*, respectively. Almond milk kefir yielded an inhibition zone of 7.33±6.43 mm and 10.67±0.58 mm for *E. coli* and *S. aureus*, respectively, but did not inhibit *S. typhi*. Among all the kefir samples, the 1:1 ratio of cow's and almond milk kefir showed the highest anti-microbial activity against all three bacterial isolates. The One-way ANOVA showed no significant difference between the tested kefir samples for *E. coli* and *S. aureus* (*p*-value> 0.05). In contrast, a significant difference was observed for *S. typhi* (p<0.05). Comparatively, the positive control gave the highest zone of inhibition than the tested samples (p< 0.05).

Several studies looked at how different varieties of kefir affect the suppression of bacterial activity by using agar well diffusion, disc diffusion, and spot-on lawn methods [16, 27, 38, 39], and demonstrated that kefir, kefiran, kefir suspensions, kefir cell-free supernatant or bacterial isolates from kefir could inhibit the growth of *Salmonellae typhimurium, Salmonella spp, S. typhi, S. enteritidis, S. aureus, S. epidermidis, E. coli, Bacillus cereus, Clostridium tyrobutyricum, Yersinia enterocolitica, Listeria monocytogenes, Pseudomonas aeruginosa, Aspergillus flavus, Candida albicans, Shigella sonnei, and Klebsiella pneumoniae* [16, 25, 40, 41].

The highest anti-bacterial activity of the kefir samples was against *S. aureus* compared to *E. coli* and *S. typhi*, which supported a similar outcome reported by Ulusoy et al. (2007) [42]. However, the study result contradicted a report by Gamba et al. (2020) [33], which exhibited that cow's milk and soymilk kefirs exhibited anti-bacterial activity against *S. aureus*, *E. coli* and *S.*

Table 3. The inhibition zone of kefir produced from cow's milk and almond milk mixtures with different ratios against *Escherichia coli*, *Staphylococcus aureus*, and *Salmonella typhi*, together with gentamicin and distilled water as the positive and negative controls, respectively

Test pathogens	Sample	Diameter of zone of inhibition in mm (mean ± SD)	P-value (between 3 kefir samples)
Escherichia coli	100 % almond milk kefir	7.33±6.43[a]	P=0.702
	1:1 ratio of cow's and almond milk kefir	8.00±7.00[a]	
	100% cow's milk kefir	3.67±6.35[a]	
	Distilled water	0.00±0.00[ab]	
	Gentamicin	16.67±0.58[ac]	
Staphylococcus aureus	100 % almond milk kefir	10.67±0.58[a]	P=0.202
	1:1 ratio of cow's and almond milk kefir	12.33±1.53[a]	
	100% cow's milk kefir	11.33±0.58[a]	
	Distilled water	0.00±0.00[b]	
	Gentamicin	21.00±0.00[c]	
Salmonella typhi	100 % almond milk kefir	0.00±0.00[a]	P=0.015
	1:1 ratio of cow's and almond milk kefir	12.67±1.15[b]	
	100% cow's milk kefir	3.67±6.35[ac]	
	Distilled water	0.00±0.00[acd]	
	Gentamicin	27.00±1.73[e]	

Mean ± standard deviations values in the same column with different superscripts are significantly different from each other at p<0.05

typhi. The study also showed that *S. aureus* was least sensitive towards 25% cow's milk kefir solution and 75% soymilk kefir solution. Üstün-Aytekin et al. (2020) [43] postulated that kefir exhibited better anti-microbial activity against Gram-negative bacteria such as *E.coli* and *S. typhi* than Gram-positive bacteria such as *S. aureus*. The difference in the result might be due to the different compositions of the kefir grains and fermentation conditions that produced the different anti-microbial activity [19].

The bacteria in kefir grains have been documented to produce peptides, organic acids, hydrogen peroxide, carbon dioxide, diacetyl, ethanol, and other biologically active components, which hinder the growth of harmful bacteria [27]. Likewise, the antioxidant and antibacterial activity of kefir is due to lactic acid produced from the fermentation process and bioactive peptides created by protein breakdown [10]. Since kefir supernatant contains various bioactive components and inhibitory substances, these molecules may interfere with one another to strengthen or impair their anti-microbial effects, hence the various anti-microbial activity against bacteria [44].

Many parameters could affect the final anti-microbial activity of kefir. First, the type of milk substrate could affect the antibacterial activity of kefir samples [45]. The fatty acid composition and lactose content could affect the product's final pH and acid content, in addition to the kinds of peptides and other substances that make up the bioactive substances. Also, the observed anti-microbial activity in kefir milk hinges on microorganism type of population, and various enzymes in the grains [19]. Moreover, kefir produced from low-fat and plant-based milk, such as rice and soy milk, generally exhibited lower anti-microbial activity than kefir produced using full-fat and animal-based milk, such as cow's milk [27, 43, 44]. According to the study by Triwibowo et al. (2020) [46], the added 10%, 20%, and 30% almond milk in the cow's milk fermentation substrate could increase the total lactic acid bacteria of kefir from 1.67×10^7, 13.37×10^7 to 18.27×10^7, respectively. However, the study showed that the 1:1 ratio of cow's and almond milk kefir gave the highest anti-microbial activity against all tested bacterial isolates. The outcome seen here conveys the promising use of this kefir milk mixture as a better alternative substrate for kefir production, with higher anti-microbial activity.

Furthermore, fermentation time may be another factor that could affect the final anti-microbial activity of the kefir. Previous studies reported that the anti-bacterial activity of kefir samples against *S. aureus* and *E. coli* began after a certain time [40, 47]. Also, kefir samples fermented for 48 hours began to inhibit the growth of *S. aureus,* but this was not observed for kefir fermented for more than 72 hours [44]. Hence, it can be construed that the anti-bacterial action of kefir is influenced by distinct components produced at fermentation at different stages, resulting in a varied anti-microbial pattern as a function of time [44]. In another study, 48 hours and 72 hours of fermented kefir samples showed higher anti-bacterial activity against *E. coli* and *S. aureus,* respectively [45]. Thus, the shorter fermentation time (24 hours) in this study, when compared to earlier studies, might be the reason for the partial inhibition of bacteria (smaller zone of inhibition).

Next, acidity or pH has been known to affect the anti-microbial activity of kefir, in which a higher initial inoculum proportion (grain/milk proportion) yields a higher total acid content. This yields a lower pH value in the fermentation medium comprising lactic acid- and other bacterial carbohydrate metabolism end-products [25, 47]. Al-Mohammadi et al. (2021) [48] observed that anti-microbial activity is higher in kefir beverage than neutralized ones, however the study stated that this was not only due to pH but also due to many metabolites detected in kefir beverage. Meanwhile, Kim et al. (2016) [44] reported that almost all the bacteria strains were resistant to organic acid solutions. In this study, the pH values of the supernatant of kefirs ranged from 3.89,4.18, and 4.29 for the 1:1 ratio of cow's and almond milk kefir, 100% almond milk kefir, and 100% cow's milk kefir, respectively. The 100% almond milk kefir, with the second-lowest pH, gave no or lowest zone of inhibition towards *S. typhi* and *S. aureus*. This outcome further supported that pH is not the only factor that determines the anti-microbial activity of kefir, as it is also related to its specific compositions. Overall, fermentation conditions notably impacted the quantity and type of microorganisms and intermediate chemicals generated in the tested kefir samples. It is recommended that future works consider using different fermentation times for certain pathogens, such as 48, 72, 120, and 120 hours, for *E. coli, S. aureus, B. cereus, and S. dysenteriae,* respectively [45].

Conclusion

The mixture of cow's milk and almond milk could be a better substrate for kefir fermentation as the produced 1:1 cow's and almond milk kefir demonstrated better nutrient profile and higher anti-microbial activity towards *E. coli*, *S. aureus*, *and S. typhi* compared to 100% cow's milk- and 100% almond milk kefir. Research on kefir and a mixture of kefir with other plant-based milk should be further explored, considering the well-reported qualities as a healthy food or beverage based on their nutritional contents and anti-microbial activity toward various pathogens.

Acknowledgment

The authors acknowledge the Department of Nutrition, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for providing the facilities and technical assistance rendered.

References

1. Chaves-López C, Serio A, Grande-Tovar CD et al. (2014) Traditional Fermented Foods and Beverages from a Microbiological and Nutritional Perspective: The Colombian Heritage. Comprehensive Reviews in Food Science and Food Safety 13 (5): 1031–1048. doi: 10.1111/1541-4337.12098.
2. Saranraj P, Sivasakthivelan P, Naveen M (2017) Fermentation of fruit wine and its quality analysis: A review. Australian Journal of Science and Technology 1 (2): 85–97.
3. Kabak B, Dobson ADW (2011) An introduction to the traditional fermented foods and beverages of Turkey. Critical Reviews in Food Science and Nutrition 51 (3): 248–260. doi: 10.1080/10408390903569640.
4. Marsh AJ, Hill C, Ross RP, Cotter PD (2014) Fermented beverages with health-promoting potential: Past and future perspectives. Trends in Food Science and Technology 113–124. doi: 10.1016/j.tifs.2014.05.002.
5. Rosa DD, Dias MMS, Grześkowiak ŁM et al. (2017) Milk kefir: Nutritional, microbiological and health benefits. Nutrition Research Reviews 30 (1): 82–96. doi: 10.1017/S0954422416000275.
6. González-Orozco BD, García-Cano I, Jiménez-Flores R, Alvárez VB (2022) Invited review: Milk kefir microbiota—Direct and indirect antimicrobial effects. Journal of Dairy Science 105 (5): 3703–3715. doi: 10.3168/jds.2021-21382.
7. Hamida RS, Shami A, Ali MA et al. (2021) Kefir: A protective dietary supplementation against viral infection. Biomedicine and Pharmacotherapy. doi: 10.1016/j.biopha.2020.110974.
8. Atalar I (2019) Functional kefir production from high pressure homogenized hazelnut milk. LWT 107 (March): 256–263. doi: 10.1016/j.lwt.2019.03.013.
9. Talib N, Mohamad NE, Yeap SK et al. (2019) Isolation and characterization of Lactobacillus spp. From kefir samples in Malaysia. Molecules 24 (14), 2606: 1-18. doi: 10.3390/molecules24142606.
10. Shiby VK, Mishra HN (2013) Fermented Milks and Milk Products as Functional Foods-A Review. Critical Reviews in Food Science and Nutrition 53 (5): 482–496. doi: 10.1080/10408398.2010.547398.
11. Leite AM de O, Miguel MAL, Peixoto RS et al. (2013) Microbiological, technological and therapeutic properties of kefir: A natural probiotic beverage. Brazilian Journal of Microbiology 44 (2): 341-349. doi: 10.1590/S1517-83822013000200001.
12. Vijaya Kumar B, Vijayendra SVN, Reddy OVS (2015) Trends in dairy and non-dairy probiotic products - a review. Journal of Food Science and Technology 52 (10): 6112–6124. doi: 10.1007/s13197-015-1795-2.
13. Bolling BW (2017) Almond Polyphenols: Methods of Analysis, Contribution to Food Quality, and Health Promotion. Comprehensive Reviews in Food Science and Food Safety 16 (3): 346–368. doi: 10.1111/1541-4337.12260.
14. Barreca D, Nabavi SM, Sureda A et al. (2020) Almonds (Prunus dulcis Mill. D. A. webb): A source of nutrients and health-promoting compounds. Nutrients 12 (3), 672: 1-24. doi: 10.3390/nu12030672. doi: 10.3390/nu12030672.
15. Mintel Press Team (2018) Us Non-Dairy Milk Sales Grow 61% Over the Last Five Years. (2018). Accessed date: March 2022.
16. Ranadheera CS, Vidanarachchi JK, Rocha RS et al. (2017) Probiotic delivery through fermentation: Dairy vs. non-dairy beverages. Fermentation. 3 (4), 67: 1-17. doi: 10.3390/fermentation3040067.
17. Kundu P, Dhankhar J, Sharma A (2018) Development of non dairy milk alternative using soymilk and almond milk. Current Research in Nutrition and Food Science 6 (1): 203–210. doi: 10.12944/CRNFSJ.6.1.23.
18 Codex Alimentarius Commission (2011) Milk and Milk Products (CODEX STAN 243-2003), vol. 2 edition, pp. 6–16. Rome, Italy: World Health Organization (WHO) and Food and Agriculture Organization of the United Nations (FAO).
19. Azizkhani M, Saris PEJ, Baniasadi M (2021) An in-vitro assessment of antifungal and antibacterial activity of cow, camel, ewe, and goat milk kefir and probiotic yogurt. Journal of Food Measurement and Characterization 15 (1): 406–415. doi: 10.1007/s11694-020-00645-4.
20. Wszolek M, Tamime AY, Muir DD, Barclay MNI (2001) Properties of Kefir made in Scotland and Poland using Bovine, Caprine and Ovine Milk with Different Starter Cultures. LWT - Food Science and Technology 34 (4): 251–261. doi: 10.1006/fstl.2001.0773.
21. Otles, Semih CO (2003) Kefir: A Probiotic Dairy-Composition, Nutritional and Therapeutic Aspects. Pakistan Journal of Nutrition 2 (2): 54–59. doi: 10.3923/pjn.2003.54.59.
22. Arslan S (2015) A review: Chemical, microbiological and nutritional characteristics of kefir. CYTA - Journal of Food 13 (3): 340–345. doi: 10.1080/19476337.2014.981588.
23. Gul O, Mortas M, Atalar I et al. (2015) Manufacture and characterization of kefir made from cow and buffalo milk, using kefir grain and starter culture. Journal of Dairy Science. doi: 10.3168/jds.2014-8755.
24. Vera Zambrano M, Dutta B, Mercer DG et al. (2019) Assessment of moisture content measurement methods of

dried food products in small-scale operations in developing countries: A review. Trends in Food Science and Technology 88 (2019): 484–496. doi: 10.1016/j.tifs.2019.04.006.

25. Akan SA (2020) Impact of Storage Time on the Content of Kefir. Eurasian Journal of Food Science and Technology 4 (2): 82–90.
26. Gamba RR, Colo CN, Correa M et al. (2015) Antifungal Activity against Aspergillus parasiticus of Supernatants from Whey Permeates Fermented with Kefir Grains. Advances in Microbiology 05 (06): 479–492. doi: 10.4236/aim.2015.56049.
27. Shen Y, Kim D-H, Chon J-W et al. (2018) Nutritional Effects and Antimicrobial Activity of Kefir (Grains). Journal of Milk Science and Biotechnology 36 (1): 1–13. doi: 10.22424/jmsb.2018.36.1.1.
28. Mæhre HK, Dalheim L, Edvinsen GK et al. (2018) Protein determination—method matters. Foods 7 (1), 5: 1-11 doi: 10.3390/foods7010005.
29. Barbosa MLS, de Alencar ER, Leandro E dos S et al. (2020) Characterization of fermented beverages made with soybean and Brazil nut hydrosoluble extracts. International Journal of Gastronomy and Food Science 21, 100228: 1-6. doi: 10.1016/j.ijgfs.2020.100228.
30. Magalhães KT, de Melo Pereira GV, Campos CR et al. (2011) Brazilian kefir: Structure, microbial communities and chemical composition. Brazilian Journal of Microbiology 42 (2): 693–702. doi: 10.1590/S1517-83822011000200034.
31. Rodrigues KL, Gaudino Caputo LR, Tavares Carvalho JC et al. (2005) Antimicrobial and healing activity of kefir and kefiran extract. International Journal of Antimicrobial Agents 25 (5): 404–408. doi: 10.1016/j.ijantimicag.2004.09.020.
32. MyFCD (1997). Welcome to Malaysian Food Composition Database (MyFCD). From https://myfcd.moh.gov.my/. Accessed date: October 2022.
33. Gamba RR, Yamamoto S, Abdel-Hamid M et al. (2020) Chemical, Microbiological, and Functional Characterization of Kefir Produced from Cow’s Milk and Soy Milk. International Journal of Microbiology 2020, 7019286: 1-11. doi: 10.1155/2020/7019286.
34. Dinkçi N, Kesenkaş H, Korel F, Kınık Ö (2015) An innovative approach: cow/oat milk based kefir. Mljekarstvo 65 (3): 177–186. doi: 10.15567/mljekarstvo.2015.0304.
35. Abdolmaleki F, Mazaheri Assadi M, Akbarirad H (2015) Assessment of beverages made from milk, soya milk and whey using Iranian kefir starter culture. International Journal of Dairy Technology 68 (3): 441–447. doi: 10.1111/1471-0307.12197.
36. Karimi Z, Firouzi M, Dadmehr M et al. (2021) Almond as a nutraceutical and therapeutic agent in Persian medicine and modern phytotherapy: A narrative review. Phytotherapy Research 35 (6): 2997–3012. doi: 10.1002/ptr.7006.
37. Farag MA, Jomaa SA, El-wahed AA, El-seedi HR (2020) The many faces of kefir fermented dairy products: Quality characteristics, flavour chemistry, nutritional value, health benefits, and safety. Nutrients 12 (2), 346: 1-21 . doi: 10.3390/nu12020346.
38. Collard KM, McCormick DP (2021) A Nutritional Comparison of Cow's Milk and Alternative Milk Products. Academic Pediatrics 21(6):1067-1069. doi: 10.1016/j.acap.2020.12.007.
39. Clinical and Laboratory Standards Institute (CLSI) (2018) Performance Standards for Antimicrobial Susceptibility Testing. CLSI Approved Standard M100-S15. Clinical and Laboratory Standards Institute, Wayne. doi: 10.1108/08876049410065598.
40. Suhartanti D, Septian R (2014) Comparison of the antibacterial activity of cow milk kefir and goat milk kefir against bacteria Bacillus cereus. Jurnal Kesehatan Masyarakat (Journal of Public Health) 8 (2): 71–76. doi: 10.12928/kesmas.v8i2.1030.
41. Kesenkaş H, Gürsoy O, Özbaş H (2017) Chapter 14 Kefir. Fermented Foods in Health and Disease Prevention. Academic Press 339–361. doi: 10.1016/B978-0-12-802309-9.00014-5.
42. Ulusoy, BH, Çolak, H, Hampikyan, H et al. (2007). An in vitro study on the antibacterial effect of kefir against some food-borne pathogens. *Turkish Microbiological Society*, 37, 103–107.
43. Üstün-Aytekin Ö, Şeker A, Arısoy S (2020) The effect of in vitro gastrointestinal simulation on bioactivities of kefir. International Journal of Food Science and Technology 55 (1): 283–292. doi: 10.1111/ijfs.14274.
44. Kim DH, Jeong D, Kim H et al. (2016) Anti-microbial activity of kefir against various food pathogens and spoilage bacteria. Korean Journal for Food Science of Animal Resources 36 (6): 787–790. doi: 10.5851/kosfa.2016.36.6.787.
45. Ajam F, Koohsari H (2020) Effect of some fermentation conditions on antibacterial activity of fermented milk by kefir grains. Journal of Food Processing and Preservation 44 (12), e14913: 1-30. doi: 10.1111/jfpp.14913.
46. Triwibowo B, Wicaksono R, Antika Y et al. (2020) The effect of kefir grain concentration and fermentation duration on characteristics of cow milk-based kefir. Journal of Physics: Conference Series. doi: 10.1088/1742-6596/1444/1/012001.
47. Sirirat D, Jelena P (2010) Bacterial inhibition and antioxidant activity of kefir produced from Thai jasmine rice milk. Biotechnology 9 (3): 332–337. doi: 10.3923/biotech.2010.332.337.
48. Al-Mohammadi AR, Ibrahim RA, Moustafa AH et al. (2021) Chemical constitution and anti-microbial activity of kefir fermented beverage. Molecules 26 (9), 2635: 1-19. doi: 10.3390/molecules26092635.