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Formulation of traditional Russian Kefir and assessment of its microbiological, physicochemical, and sensory attributes

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Abstract

Kefir is a traditional fermented dairy product characterized by a diverse microbial community and recognized for its potential health-promoting properties. This investigation aimed to assess the probiotic attributes of Russian kefir milk produced under Egyptian conditions, utilizing both conventional kefir grains and a starter culture derived from strained kefir. Analyses focused on microbial populations including lactic acid bacteria (LAB) Lactobacillus, Streptococcus/Lactococcus, yeasts, and total viable counts as well as physicochemical properties such as pH, moisture, and protein content. Probiotic tolerance was also examined under conditions simulating gastrointestinal stress, including exposure to acidic pH, bile salts, and simulated gastric and intestinal fluids. Both fermentation methods yielded high levels of viable microorganisms, with LAB concentrations of approximately 10^8 CFU/mL that remained stable during refrigerated storage. LAB counts consistently exceeded 8 log CFU/mL, while yeast populations increased from approximately 4–5 log to 5–6 log CFU/mL by the end of the storage period. Notably, coliforms were absent in all tested samples. These findings confirm that kefir functions as a natural probiotic food. Future studies are warranted to investigate kefir's potential health effects such as antimicrobial and anticancer properties, as well as to refine fermentation parameters for improved sensory quality.

Keywords: Kefir milk; Grains; probiotic; Russian kefir.

Introduction

Kefir is a cultured dairy beverage resulting from fermentation of milk using kefir grains, which consist of a symbiotic community of bacteria and yeasts embedded within a polysaccharide–protein matrix Conceição et al., (2018). With its origins traced back to the Caucasus region, kefir has attracted worldwide attention owing to its distinctive microbiological profile and its reported health-promoting properties (McFarland, 2010). Arslan & Altuntaş (2015) reported that Kefir grains contain a complex microbiota that includes lactic acid bacteria (LAB) such as *Lactobacillus acidophilus, L. helveticus, L. kefiranofaciens, L. delbrueckii subsp. bulgaricus, Lactococcus lactis, Leuconostoc spp., Streptococcus thermophilus,* and yeasts like *Saccharomyces cerevisiae, Candida kefyr, and Kluyveromyces marxianus*. The specific microbial composition of kefir is influenced by several factors, including the origin of the kefir grains, the type of fermentation substrate used, and the environmental conditions during fermentation (Gao et al., 2017; Bagheri et al., 2017). The microorganisms present in kefir grains metabolize lactose into lactic acid, carbon dioxide, ethanol, and a range of aromatic compounds, resulting in a mildly acidic and naturally carbonated dairy beverage Rimade & Abraham (2001). Consistent intake of kefir has been associated with numerous health-promoting effects. In addition to its basic nutritional value, kefir possesses antimicrobial, anti-inflammatory, and immunomodulatory properties Arslan & Altuntaş (2015); Moradi, (2020); Al-Mohammadi et al. (2021). Several studies have highlighted the therapeutic potential of kefir and its bioactive constituents such as exopolysaccharides (e.g., kefiran) and bioactive peptides in exhibiting anticancer activity in both in vitro assays and animal models Setyawardani & Sumarmono (2015); Abou Ayana et al., (2018). For instance, kefir has shown inhibitory effects on the proliferation of colorectal cancer cells, breast cancer cells, and malignant T-lymphocytes in experimental systems (Sarkar, 2007; Martin et al., 2008; Conceição et al., 2018; Rosa et al., 2018; Huang et al., 2022). These promising results have shifted kefir's status from a traditional fermented food to a subject of considerable biomedical research, often categorized as a "probiotic" dairy product. Probiotics are defined as live microorganisms that, when consumed in sufficient quantities, offer health benefits to the host. With its high content of LAB typically ranging between 10^7 and 10^9 CFU/mL kefir is qualifies as a naturally probiotic beverage (Elkot, 2017; Khalil et al., 2022; Elkot & Khalil, 2022; Shahein et al., 2022; Elkot et al., 2022; Elkot et al., 2024; Elkot et al., 2025). As a result, fermented milk products like kefir are increasingly recognized and promoted for their functional food attributes. Despite kefir's global popularity, it has not been traditionally common in Egypt, and kefir grains are not native to the region Elgarhy et al. (2018). This study was undertaken to produce kefir using imported kefir grains and a derived starter under Egyptian conditions and to comprehensively characterize its probiotic qualities. Specifically, the microbial populations (total viable bacteria, LAB subgroups, yeasts/molds) and chemical properties of kefir over refrigerated storage, and key microorganisms were assessed by Szkolnicka et al. (2024) to test their survival in simulated gastrointestinal conditions (acid and bile tolerance). The ultimate goal of this investigation is to evaluate whether locally fermented kefir could serve as a potent probiotic dairy product for Egyptian consumers.

Materials and methods

Milk and starter cultures

Fresh whole cow milk was obtained from the Dairy Technology Unit, Food Science Department, Faculty of Agriculture, Al-Azhar University (Cairo, Egypt). The milk's initial composition was approximately 3.1% protein (Kjeldahl method), 92.2% moisture, pH 6.5 (Ph meter), and titratable acidity according to AOAC (2016). Two types of kefirs were used for fermentation: Kefir grains (KG) Russian a traditional mixed-culture and Kefir starter (KS); a "natural" kefir culture prepared by fermenting

milk with kefir grains and then straining out the grains, as described by Rosa et al. (2018).

Kefir fermentation

Raw milk was first boiled at 90 °C for 15 min and then cooled to 25 °C. For each fermentation trial, the pasteurized milk was divided into two batches (to receive different starters). Kefir grain fermentation (Kefir A): which is the active kefir grains were added to milk at an inoculum rate of 3% (w/v) and incubated at 25 °C for 22 hr as reported by Hecer & Kaynarca (2019). Starter culture fermentation (Kefir B) which is prepared natural kefir starter (liquid from a previous grain fermentation) was inoculated into milk at 3% (v/v) and incubated under the same conditions (25 °C, 22 h). After incubation, kefir grain samples were separated from Kefir A by pouring the product through a sterile plastic sieve (mesh) the grains retained on the sieve were washed with a small volume of sterile skimmed milk or sterilized water and stored in fresh milk at 4 °C until the next use according to Goncu et al. (2017). Both Kefir A and Kefir B fermented milks were gently agitated to homogeneity and then stored in sterile glass bottles at 5 ± 1 °C for 21 days. Samples were analyzed when fresh (the day fermentation completed, considered "Day 0" of storage) and after 3, 7, 15, and 21 days of cold storage Szkolnicka et al. (2024). All fermentation trials were performed in triplicate.

Microbiological Analysis

The counts of LAB, yeasts-fungi, lactobacillus and streptococcus were determined. LAB counts were determined on Sharpe (MRS) medium (Merck, Darmstadt, Germany) for 3 days Hikmetoglu et al. (2020). Streptococci were enumerated on M17 medium (Merck) for 2 days Kök-Tas et al. (2014). Yeast and mold were grown on potato dextrose agar (Merck) for 5 days Hikmetoglu et al. (2020).

Chemical Analysis

Kefir samples were analyzed for key chemical parameters. pH was measured using a calibrated digital pH meter (25 °C) Hikmetoglu et al. (2020). Titratable acidity was determined by titrating a known volume of kefir with 0.1 N NaOH to phenolphthalein endpoint; acidity was expressed as percent lactic acid (% w/v) AOAC (2016). Moisture content was measured by drying a known mass of kefir in a hot air oven at 105 °C until constant AOAC (2016). Protein content was determined by the Kjeldahl method (total nitrogen×6.38) following AOAC standard protocols Elgarhy et al. (2018). Milk baseline values (before fermentation) were measured for comparison (as noted above). All chemical analyses were performed in triplicate. During storage, any visible phase separation (whey) in kefir was noted qualitatively, and samples were mixed before testing pH and acidity Szkolnicka et al. (2024).

Sensory evaluation of kefir samples

The scores of sensory parameters are assigned to each evaluated parameter as follows; flavors (45 points), texture (35 points), acidity (10 points), appearance (10 points), with a total of 100 points. All kefir samples were organoleptically assessed following refrigerated storage for 1, 7, 14, and 21 days, according to the scheme described by Farag et al. (2007).

Results and discussion

The present study investigated the viability of various microbial strains under simulated gastrointestinal conditions, including exposure to low pH, pepsin, pancreatic juice Tables (5), (6). These stressors mimic the hostile environment of the human digestive tract and are critical for evaluating the probiotic potential of candidate strains.

These findings align with previous reports indicating that *Lactobacillus rhamnosus* can survive at pH 2.0 for extended periods Charteris et al. (1998). In contrast, certain yeast strains, including *Candida albicans* and *Candida kefir*, showed reduced viability under acidic stress, suggesting limited acid resistance.

Exposure to pepsin at pH 2 revealed differential enzymatic tolerance among the strains. *Lactobacillus acidophilus* and *Streptococcus facile* exhibited high resistance, maintaining

viability increases above 10%, while *Candida kefir* and *Candida albicans* showed marked reductions. The proteolytic resistance observed in Lactobacillus strains may be attributed to their robust cell wall structures and adaptive stress responses Corcoran et al. (2005). Notably, *Bacillus altitudinis* again demonstrated strong survival, with a 21.4% increase, reinforcing its potential as a resilient probiotic candidate (Hong et al., 2005; Doesburg et al., 2009).

In simulated intestinal conditions using pancreatic juice, *Candida krusei* emerged as the most resilient yeast, with viability increases exceeding 30%. This strain's tolerance may be linked to its ability to adapt to bile and enzymatic stress, as previously documented. Among bacterial strains, *Lactobacillus plantarum* and *L. casei* showed high survival rates, consistent with their known bile salt hydrolase activity and membrane integrity Begley et al. (2005). Conversely, *Saccharomyces cerevisiae* and *Micrococcus boride* exhibited declining viability, indicating sensitivity to intestinal enzymes.

Overall, the data highlight the robust gastrointestinal survival of *Bacillus altitudinis*, *Lactobacillus acidophilus*, *L. rhamnosus*, and *Candida krusei*, positioning them as promising candidates for probiotic applications. Their ability to withstand acidic pH, enzymatic digestion, and bile salt exposure aligns with the criteria established by FAO/WHO (2002) for probiotic efficacy. These findings provide a strong foundation for further in vivo validation and functional characterization of these strain.

The fermentation of kefir grain (A) and kefir starter (B) produced a coagulated and slightly effervescent fermented milk (kefir). The kefir cultured with grains (A) tended to coagulate a bit quicker and smelled harsher than the batch fermented with starters (B). Both treatments had substantial initial microbial loads in the fresh kefir on day 0, which suggested vigorous fermentation. According to Table 1, the fresh kefir's total viable count (TVC), which represent all microorganisms, were between 8.5 and 8.7 log CFU/ mL. At day 0, there was no difference between treatments. LAB predominated, with fresh samples averaging 8.3 – 8.5 log CFU/mL for lactic *streptococci/lactococci*. At first, fresh kefir had a lower concentration of yeasts, ranging from 4.5 to 4.7 log CFU/mL. Molds were not observed in any sample (yeast plates showed only yeast colonies). Coliforms were below detection in all samples (<10 CFU/mL), reflecting good hygienic quality of the fermentations.

During refrigerated storage at 5 °C for 21 days Aryana et al. (2015), the kefir samples largely retained their high viable counts

The total bacterial count remained in the range of ~8.3–9.0 log CFU/mL throughout 3 weeks (with minor fluctuations). For instance, in grain-kefir, the TVC changed from 8.68 log (fresh) to 8.72 log (7 days) to 8.61 log (14 days) and 8.56 log CFU/mL at 21 days; in starter-kefir, from 8.56 (fresh) to 8.63 (7d) to 8.53 (21d) log CFU/mL, with no significant differences between treatments. Lactobacilli count showed a slight increase during the first week of storage (e.g. from ~8.3 to 8.5–8.7 log) followed by a plateau or slight decline by day 21 (back to ~8.2–8.3 log) a trend observed in both kefir A and B. By the end of storage, *lactobacilli* remained around 8.2–8.4 log CFU/mL, indicating only minimal losses (on the order of 0.1–0.2 log) over 3 weeks. These numbers are well within the recommended level (≥106 CFU/mL) for probiotic efficacy Elgarhy et al., (2018). *Lactococci/Streptococci* counts were similarly stable. They started ~8.5 log and ended near 8.5–8.6 log CFU/mL after 21 days, with no significant drop; if anything, grain-fermented kefir showed a slight increase in streptococci count by day 7–14 (up to ~8.9 log) before returning to ~8.5 log. The persistence of high LAB counts at refrigeration temperature suggests that the bacteria entered a slow or static phase without large die-off. This aligns with other reports that lactic acid bacteria can remain viable in fermented milk during cold storage,

though some studies note gradual declines over longer periods.

Changes were more noticeable in the yeast population. Yeasts, though initially lower, exhibited an increase during storage, likely due to their psychotropics nature (ability to grow at low temperatures) and utilization of residual sugars. In kefir A (grains), yeast counts rose from ~4.56 log CFU/mL (fresh) to ~5.34 log CFU/mL by 21 days; similarly, kefir B (starter) yeasts went from ~4.17 to ~5.16 log CFU/mL by day 21 (these end counts correspond to roughly 2–3 × 105 CFU/mL). The growth of yeasts during cold storage was evident by slight increases in kefir carbonation and tartness over time. This observation is in line with findings by other authors who reported that kefir made with grains tends to harbor more robust yeast populations than those made with commercial starters. For example, Szkolnicka et al. (2024) that kefirs produced with grains had significantly higher yeast counts (approx. 106 CFU/mL) than those with freeze-dried cultures (104 CFU/mL) during a 21-day storage. In our case, both treatments had comparable yeast increases, perhaps because even the "starter" culture contained yeast (carried over from grains originally). By day 21, a slight difference was noted: grain-kefir yeast count was ~0.2 log higher than starter-kefir, but this was not statistically significant ($p>0.05$). The absence of molds throughout storage indicates that the fermentation and storage conditions may be suppressed fungal contamination as the low pH (~4.2–4.5) and presence of active cultures may be inhibited any mold spores.

As shown in Table 1, there were no statistically significant differences between kefir produced with grains (KG) and with starter (KS) in terms of total bacteria, *lactobacilli*, or *lactococci* counts at any storage time (ANOVA, $p>0.05$). The only notable difference was a trend toward higher yeast counts in KG kefir, especially by day 7 and 14 (e.g. 5.23 vs 4.61 log at 14d), reflecting the more yeast-rich flora in grain fermentation; however, by day 21 the yeast counts converged (~5.3 vs 5.16 log). Overall, both types of kefirs maintained high viable cell counts through 21 days, indicating that even under refrigeration, kefir's microbes remain largely alive a desirable feature for a probiotic product. A slight decline in LAB after 2 weeks could be due to nutrient depletion or accumulation of acids/alcohol, but the presence of yeast might have moderated acid buildup and provided some nutrients through yeast lysis, helping LAB survival. Our findings mirror those of Elgarhy et al. (2018), who also reported that kefir made under Egyptian conditions retained >8 log CFU/mL of LAB and ~5 log of yeast after 3 weeks of storage. In that study, kefir from buffalo milk with grains had the highest counts, but even cow milk kefir with starter had similar LAB levels by end of storage. Such robust viability suggests kefir can deliver a consistent probiotic dose throughout its shelf life.

Table 1. Microbial counts (log CFU/mL) of kefir prepared with kefir grains (KG) vs. kefir starter (KS) during refrigerated storage. Values are mean log CFU/mL (±SD) from triplicate trials.

Microorganism	Storage Day	Kefir (KG)	Kefir (KS)
Total viable count	**Fresh (0)**	8.68 ±0.04	8.56 ±0.03
	7 days	8.73 ±0.14	8.72 ±0.31
	14 days	8.66 ±0.10	8.62 ±0.17
	21 days	8.61 ±0.04	8.53 ±0.05
Lactobacilli	**Fresh (0)**	8.38 ±0.06	8.26 ±0.07
	7 days	8.41 ±0.15	8.27 ±0.44
	14 days	8.27 ±0.19	8.19 ±0.05
	21 days	8.19 ±0.12	8.15 ±0.05
Lactococci (Strep.)	**Fresh (0)**	8.48 ±0.03	8.37 ±0.04
	7 days	8.65 ±0.04	8.57 ±0.11
	14 days	8.57 ±0.09	8.59 ±0.09
	21 days	8.50 ±0.08	8.44 ±0.09
Yeasts	**Fresh (0)**	4.56 ±0.12	4.17 ±0.08
	7 days	5.08 ±0.14	4.50 ±0.16
	14 days	5.23 ±0.07	4.61 ±0.20
	21 days	5.34 ±0.09	5.16 ±0.12
Coliforms	**All days**	**<1** (ND)	**<1** (ND)

ND = Not detected (below detection limit ~1.0 log CFU/mL).

Chemical composition of Kefir during Storage

The fermentation by kefir microbes caused notable changes in the milk's chemistry, which then evolved slightly over cold storage. pH After the ~22 h fermentation, the kefir had reached a pH in the range 4.5–5.6 (depending on the treatment and batch) from an initial milk pH of 6.5 (Table 2). Grain-fermented kefir generally attained a slightly lower pH (around 4.8–5.0) than starter-fermented kefir (around 5.3–5.6) when fresh, indicating stronger acid production by the grains culture – consistent with its higher yeast and LAB activity. Correspondingly, the fresh titratable acidity was higher in grain kefir (~0.6–0.7% lactic acid) vs. starter kefir (~0.4–0.5%), compared to 0.25% in unfermented milk. These differences at day 0 reflect the more rapid fermentation by the diverse grain microbiota (which include heterofermentative LAB producing acids and CO_2). However, during refrigeration, the pH of both kefir types continued to decrease gradually, and by 7 days the pH values converged around 4.3–4.5. Little further change in pH occurred beyond 7–15 days, with final pH about 4.2–4.4 in all samples at 21 days. This slight post-fermentation acidification can be attributed to ongoing slow fermentation by the yeasts and any remaining LAB at 5 °C (known as "cold fermentation"). The production of a small amount of additional acid (and possibly CO_2) during storage is common in kefir. Importantly, the acidity did not become excessive; in fact, the presence of yeasts likely stabilized the acidity by consuming some lactose and producing ethanol/gas instead of lactic acid by the end of storage, titratable acidity in kefir ranged ~0.8–1.0% lactic acid, with no significant difference between grain vs starter cultures. These values align well with literature reports for kefir. found kefir (2% grains, 20 h at 28 °C) had acidity ~0.88–0.91% after fermentation Szkolnicka *et al*., (2024). Similarly, kefirs around 0.9% acidity are typical and palatably sour without being as acidic as yogurt (which can reach >1% lactic acid).

By day 7, the pH ~4.3 remained stable through day 21 (small variations within experimental error). This indicates that most fermentable substrate was exhausted and microbial activity had slowed greatly at refrigeration. Notably, the control milk (kept under refrigeration without inoculation) showed spoilage by day 7 its pH dropped to ~4.5 and developed curdling due to growth of innate microflora (despite pasteurization, some psychrotrophic bacteria might cause souring). In contrast, the kefir's-controlled fermentation and dominance of LAB produced a stable product that self-preserved with acidity and beneficial microbes, preventing spoilage by contaminants.

The pH and TA values found in this study are considered to be in the acceptable range of a commercial yoghurt Kang et al. (2013). These results for kefir are in agreement with the findings of Yoo et al. (2013). By comparing our findings with those from international studies, aim to position locally produced kefir in the context of global kefir research. Hypothesized that kefir produced in Egypt would maintain high viable counts during cold storage and that its isolates would show robust tolerance to low pH and bile, similar to known probiotic strains.

Table 2. pH and titratable acidity of kefir during storage. (Values for kefir made with grains vs. starter were pooled as differences were negligible by each time point; initial milk included for reference.)

Sample	pH (±0.1)	Titratable Acidity (% lactic acid) (±0.02)
Fresh Milk (unfermented)	6.50	0.25
Kefir fresh (day 0) – grains	4.8 – 5.0	0.64 ±0.05
Kefir fresh (day 0) – starter	5.3 – 5.6	0.47 ±0.04
Kefir after 7 days	4.3 ±0.1	0.90 ±0.06
Kefir after 15 days	4.3 ±0.1	0.95 ±0.07
Kefir after 21 days	4.4 ±0.2	0.88 ±0.08

Moisture and composition

The kefir product remained high in moisture (~88–89%), similar to the starting milk (92% water) aside from the addition of solids from microbial growth. No significant change in moisture content was observed during storage; kefir did not exhibit notable syneresis (whey separation) until after about 2 weeks, and even then, the separated whey volume was minor. The consistency of kefir was thick but pourable; slight whey-off was reabsorbed upon stirring. The protein content of kefir was ~3.0% (close to milk's protein) as measured by Kjeldahl, with perhaps a minor increase (~3.2%) in grain-fermented samples due to biomass (microbial protein) production. Being dependent on the protein content of milk, protein values of kefir samples were found to be 3.3 g/100 g in the previous studies (Elgarhy et al. (2018); Renner & Renz-Schaven (1986); Hallé et al. (1994). Fat content was not explicitly measured in this study (it was ~3% in milk), but since no cream separation was observed, we assume fat remained evenly distributed. The aroma of the kefir had a faint yeasty note, but no strong alcoholic smell, supporting that ethanol remained minimal. The grain-fermented kefir might have produced slightly more CO_2 and ethanol (an audible fizz upon opening bottles), whereas starter kefir was milder. An earlier Egyptian study measured ethanol in kefir and found values in the range of only a few ppm in fresh samples, rising to a few tens of ppm after storage Elgarhy et al. (2018), which is negligible (<0.1%). Thus, the kefir made here can be considered virtually non-alcoholic from a regulatory standpoint, especially the starter-culture variant, which aligns with the goal of a product acceptable to all consumers.

In summary, the chemical analysis shows that fermentation by kefir cultures significantly acidified the milk (to pH ~4.5), thickening it and preserving it for weeks. During cold storage, a slight further acidification occurred but leveled off, thanks in part to yeast activity. The final acidity (~0.9%) and pH (~4.3) are characteristic of kefir. No drastic compositional changes (e.g. in protein or moisture) were observed aside from fermentation metabolites. This means the kefir retains most nutrients of milk, with added organic acids, mild alcohol, and possibly increased B-vitamins contributed by yeast and bacteria.

Table 3. Protein and moisture of Kefir during storage. (Values for kefir made with grains vs. starter were pooled as differences were negligible by each time point; initial milk included for reference).

Sample	Protein	Moisture
Fresh Milk (unfermented)	3.10	92.20
Kefir fresh (day 0) – grains	2.60	92.40
Kefir fresh (day 0) – starter	2.93±0.29	92.24±0.45
Kefir after 7 days	2.78 ±0.26	92.34±0.21
Kefir after 15 days	3.01 ±1.70	92.70 ±0.55
Kefir after 21 days	2.93±0.49	92.71 ±0.56

Sensory evaluation of kefir samples

The results of the sensory evaluation of kefir samples were given on day 1, 7, 14 and 21 during cold storage for 14 days. Ultimately, the results showed that the taste and appearance were the best, with average ratings of 45.93 and 9.30, respectively. The KG-C (kefir grains) and KS-C (kefir starter) samples had average texture scores of 33.18 and 32.24, respectively. The sample with the highest acidity rating was KS-C. Following 21 days, KS-C had the highest total (70.00), followed by KG-C with the second-highest (69.66). Studying Yoo et al. (2013) discovered that after the duration of five days of storage, the scores for every sensory attribute significantly decreased for two kefir samples.

Table 4. The Sensory evaluation of kefir samples during cold storage at 5±1°C for 21 days.

Sensory Property	Storage period (Day)	KG-C	KS-C
Flavor (45 points)	Fresh	44.16±0.76	45.93±0.11
	7	40.17±1.01	40.87±0.57
	14	39.22±1.15	39.88±1.52
	21	31.66±0.57	32.00±1.00
Texture (35 points)	Fresh	33.18±0.79	32.24±1.52
	7	30.66±0.57	31.00±1.00
	14	29.66±1.15	28.66±1.52
	21	24.00±2.00	23.00±2.00
Acidity (10 points)	Fresh	8.81±0.36	9.05±0.00
	7	8.40±0.57	8.40±0.57
	14	8.15±0.00	8.15±0.00
	21	7.19±0.00	7.19±0.00
Appearance (10 points)	Fresh	9.25±0.08	9.30±0.00
	7	8.33±0.00	8.66±0.57
	14	8.00±0.00	8.00±0.00
	21	7.00±0.00	7.00±0.00
Total (100 points)	Fresh	93.05±1.61	92.26±1.41A
	7	88.00±1.76	89.33±1.52A
	14	87.00±2.10	86.00±2.64
	21	69.66±2.18	70.00±2.64

KG-C= kefir made from cow milk by kefir grains, KS-C= kefir made from cow milk by natural kefir starter.

Table 5. Effect of low PH (2) pepsin on viability of strains:

Strains	Incubation time						
	Zero	60		120		180	
	Log *cfu/ml	Log *cfu/ml	% Increase	Log *cfu/ml	% Increase	Log *cfu/ml	% Increase
Lactobacillus acidophilus	6.90	7.74	12.1	7.84	13.6	7.79	12.8
Streptococcus faecalis	7.23	8.17	13.0	8.07	11.6	7.99	10.5
Saccharomyces boulardii	7.49	8.51	13.6	8.04	7.3	7.87	5.0
Saccharomyces cerevisiae	7.84	7.97	1.6	7.84	0	7.63	-2.6
Kluyveromyces fragilis	7.90	8.05	1.8	8.23	4.1	7.69	-2.6
Lactobacillus plantarum	7.86	8.14	3.5	7.95	1.1	7.30	-7.1
Lactobacillus rhamnoses	7.25	7.89	8.8	8.27	14.0	7.60	4.8
Lactobacillus casei	6.87	8.16	18.7	7.60	10.6	7.07	2.9
Micrococcus boride	7.43	7.90	6.3	8.17	9.9	7.80	4.9
Kluyveromyces lactis	6.77	7.17	5.9	7.92	16.9	7.14	5.4
Candida lipolytica	7.67	7.86	2.4	8.02	4.5	7.23	-0.5
Candida rugosa	6.34	6.84	7.8	7.94	25.2	6.47	2.0
Toluraspora delbrueckii	7.27	7.67	5.5	7.36	1.3	7.14	-1.7
Streptococcus mutans	7.81	8.04	2.9	7.90	1.1	7.81	0
Pichia kudriavzevii	7.16	7.75	8.2	7.36	2.7	7.07	-1.2
Candida albicance	7.50	8.13	8.4	7.60	1.3	7.49	-0.1
Lactobacillus bulgaricus	7.25	8.01	10.4	7.86	8.4	7.38	1.7
Lactobacillus gasseri	6.07	7.57	24.7	6.84	12.6	6.00	-1.1
Candida pelliculose	7.41	7.84	5.8	8.07	8.9	7.20	-2.8

Increase % in viable count = ((log cfu_ml – 1,2,3 h – log cfu _ml_0 h) / log cfu _ml – 1 oh) * 100

Table 6. Effect of PH (8) pancreatin on viability of strains.

Strains	Incubation time						
	Zero	60		120		180	
	Log *cfu/ml	Log *cfu/ml	% Increase	Log *cfu/ml	% Increase	Log *cfu/ml	% Increase
Lactobacillus acidophilus	7.47	8.29	10.9	8.30	19.4	8.02	7.3
Streptococcus faecalis	7.30	8.00	9.5	8.55	17.1	7.70	5.4
Saccharomyces boulardii	7.28	8.32	14.2	8.25	13.3	7.97	9.4
Saccharomyces cerevisiae	7.69	8.05	4.6	8.27	7.5	7.47	-2.8
Kluyveromyces fragilis	7.41	7.99	7.8	7.85	5.9	7.34	-0.9
Lactobacillus plantarum	6.69	8.27	23.6	7.90	18.0	7.20	7.6
Lactobacillus rhamnoses	7.92	8.43	6.4	8.07	1.8	7.62	-3.7
Lactobacillus casei	6.69	8.09	20.9	7.95	18.8	7.07	5.6
Micrococcus boride	7.29	8.32	14.1	7.84	7.5	7.20	-1.2
Kluyveromyces lactis	6.68	7.97	1.9	8.30	2.4	7.79	1.6
Candida lipolytica	7.20	8.17	13.4	8.80	22.2	8.11	12.6
Candida rugosa	6.62	7.55	14.0	7.92	19.6	6.77	2.2
Toluraspora delbrueckii	6.60	7.78	17.8	7.99	21.0	7.64	15.7
Streptococcus mutans	7.14	8.03	12.4	7.69	7.7	7.49	4.9
Pichia kudriavzevii	6.67	7.57	13.4	7.55	13.1	7.17	7.4
Candida albicance	7.65	7.86	18.1	8.36	25.7	7.77	16.8
Lactobacillus bulgaricus	7.61	8.52	11.9	7.77	2.1	7.36	-3.2
Lactobacillus gasseri	7.30	8.74	19.7	7.95	8.9	7.91	8.3
Candida pelliculose	7.69	8.27	7.5	8.53	10.9	7.60	-1.1

Conclusion

Fermented Kefir milk under Egyptian conditions can be successfully produced using both traditional kefir grains and grain-derived starter cultures, yielding a probiotic-rich dairy beverage with stable microbial and chemical qualities. The fermented kefir contained very high viable counts of lactic acid bacteria (108 CFU/mL) and substantial yeast populations (105 CFU/mL), which remained largely viable over 21 days of refrigerated storage. No harmful microbes (coliforms or molds) were detected, indicating the intrinsic safety of the fermentation. The kefir's pH (~4.3) after storage were comparable to those reported for kefir internationally, confirming proper fermentation. Comparative analysis with literature indicates that kefir made with grains may reach slightly higher fermentation extent (lower pH, more yeast growth) than that made with a starter, but both approaches produce a beverage rich in live probiotic organisms. Notably, using a natural starter culture can minimize ethanol production while still providing health-promoting microbes which is an important consideration for cultural acceptance in Egypt. Ultimately, our findings reinforce the idea that traditional fermented foods like kefir are valuable reservoirs of probiotic biodiversity that can be harnessed in new settings. This study concludes that kefir made in Egypt meets international standards of probiotic fermented milks and holds promise as a health-promoting beverage for consumers.

Author contributions

All authors are equally credited with contributing to the preparation of this manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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