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Fortification of cow and soymilk kefir with pomegranate peel extracts: Impact on physicochemical, microbial viability, and sensory properties

Imam Adriansyah[1,*], Mohamed Said Boulkrane[2], Sri Handayani Nofiyanti[3], Darus Dina Imama[4], and Laila Fathiyaturrahmi[5]

[1]Study Program of Food Technology, Faculty of Engineering, Universitas Bumigora, Mataram, Indonesia
[2]Study Program of Food Technology, Faculty of Biotechnology, ITMO University, Saint Petersburg, Russia
[3]Study Program of Agricultural Engineering and Biosystem, Faculty of Agricultural Technology, Universitas Udayana, Bali, Indonesia
[4]Department of Food and Agricultural Product Technology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Yogyakarta, Indonesia
[5]Study Program of Statistics, Faculty of mathematics and natural science, Universitas Mataram, Mataram, Indonesia

Abstract

Kefir is a fermented beverage valued for its probiotic-associated properties, although its antioxidant capacity remains relatively moderate. Pomegranate peel, a phenolic-rich agro-industrial by-product, offers potential as a sustainable fortifying ingredient for functional beverage development. This study investigated the effects of pomegranate peel extract (PPE) addition (0–20%, w/v) on the physicochemical, microbiological, antioxidant, and sensory characteristics of cow milk and soymilk kefir. Kefir samples were prepared using a commercial starter culture and evaluated for total phenolic content (TPC), antioxidant activity, pH, viscosity, viable lactic acid bacteria (LAB) counts, and sensory acceptance. PPE fortification significantly increased ($p < 0.05$) TPC and antioxidant activity in both kefir matrices. TPC increased from 55.31 to 182.87 mg GAE/g in cow milk kefir and from 60.91 to 199.29 mg GAE/g in soymilk kefir. LAB counts also increased substantially, with more than a two-fold increase in cow milk kefir and an approximately five-fold increase in soymilk kefir. PPE significantly reduced viscosity in cow milk kefir, while soymilk kefir remained rheologically stable, indicating a matrix-dependent response. Sensory evaluation revealed no statistically significant differences ($p > 0.05$) in taste, aroma, or color, although higher PPE levels tended to show lower numerical sensory scores, particularly in soymilk kefir. These findings indicate that PPE can enhance the phenolic content, antioxidant potential, and LAB viability of both dairy- and plant-based kefir. Moderate inclusion levels (5–15%) appeared to provide the most balanced functional and sensory profile, supporting the potential use of PPE as a natural fortifying ingredient in fermented beverage formulations.

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1. Introduction

Kefir is a fermented beverage produced from kefir grains containing lactic acid bacteria and yeasts, and it is well known for its probiotic and health-promoting properties Kefir, traditionally originating from the Caucasus region, has been historically associated with

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* Correspondence : **Imam Adriansyah** imamadriansyah@universitasbumigora.co.id

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human longevity and is valued as a source of essential nutrients and beneficial microorganisms that contribute to health maintenance (1). While Kefir provides essential nutrients and beneficial microorganisms, its antioxidant capacity has received limited attention compared to its probiotic potential. Enhancing the antioxidant capacity in a dairy product such as kefir is critically important, not only for providing functional health benefits to the consumer but also to mitigate the likelihood of oxidation processes occurring, thereby lengthening the shelf-life and preserving the quality of the fermented dairy product, which is often susceptible to damage induced by lipid peroxidation and oxidation (2).

One of the good sources of natural antioxidants that can be obtained easily and considered food waste is pomegranate peel. Pomegranate peel, constituting 26–30% of the fruit weight, is often discarded but is rich in bioactive compounds such as tannins, flavonoids, phenolic acids, dietary fibers, alkaloids, minerals, and vitamins, serving as a significant source of natural antioxidants (3). Previous research also reported that the pomegranate peel addition in curd enables to boost antioxidant activity and lengthens shelf-life by reducing the formation of spoilage microorganisms, maintaining acidity, and whey syneresis (4).These findings highlight the potential of pomegranate peel as a functional fortifying ingredient in fermented food applications.

Therefore, the main objective of the present study was to produce functional dairy products from cow and soymilk kefir fortified with pomegranate peel extracts (PPE). The outcomes are expected to contribute to the development of novel functional foods derived from dairy and plant-based substrates, while utilizing fruit processing by-products as a source of natural bioactives.

2. Materials and Methods

2.1. Materials

Pomegranate peels were obtained from a pomegranate juice seller in Senaya ploschad district, Fresh pasteurized cow milk (Prostokvashino, Russia), soymilk (Zdorovoye menyu, Russia), kefir starter containing *Lactococcus lactis* subsp. *diacetilactis, Lactococcus lactis* subsp. *lactis, Lactobacillus plantarum, Leuconostoc mesenteroides* and *Lactobacillus casei* (Kefir, Svoy yogurt Russia), MRS agar and buffered peptone (Oxoid); Folin Ciocalteu reagent, gallic acid; sodium carbonate (Na_2CO_3); 2,2-diphenyl-1-picrylhydrazyl (DPPH); and ethanol (Merck).

2.1. Methods

2.1.1. Preparation of PP Powder

Pomegranate peels were collected from a pomegranate juice seller in the Senaya ploschad district, Saint Petersburg. Peels were manually sorted and cleaned under running water. Pomegranate peels were prepared based on the method by Kaderides et al., with modifications, where pomegranate peels were first cut into small pieces and oven dried at 40^{o}C for 48 hours(5). After that, dried pomegranate peel was grounded using a laboratory mill (GM200, RETSCH, Germany) and sieved using mesh sieve number 20. The powder was then kept at 4^{o}C before further processing (5).

2.1.2. Preparation of Pomegranate Peel Extracts

Pomegranate peel extracts were produced based on Zivkovic et al. with slight modification; A quantity of 24 grams of pomegranate peel powders were combined with 300 mL of water, which served as the solvent. The mixture was then subjected to sonication using an ultrasonic bath (SAPPHIRE, Russia) with a maximum capacity of 4 L (35 kHz, 130 W) for 25 minutes at a temperature of 80°C. Subsequently, the sample was subjected to centrifugation at a rate of 4000 revolutions per minute for a duration of 20 minutes, followed by filtration utilizing the number 1 Whatman filter paper (6). The extract was then kept at 4°C before further use.

2.1.3. Preparation of Cow and Soymilk

Fresh pasteurized cow milk (Prostokvashino, Russia) and soymilk (Zdorovoye menyu, Russia) were used.

2.1.4. Manufacture of Cow and Soymilk Kefir Fortified with PP Extracts

Fermentation was carried out using a commercial kefir starter culture (SvoyYogurt, своййогурт). Cow and soymilk were first heated on a hotplate until its temperature reached 40°C, and kefir starters 5% (w/v) were added under a stirring process for several minutes (7). Subsequently, cow and soy milk were subjected to incubation at a temperature of 40°C for a duration of 12 hours within an oven. This incubation temperature was selected based on the manufacturer's recommended fermentation conditions for this specific starter culture to ensure optimal microbial activity and stable fermentation performance. PP extracts were then added to cow and soymilk kefir separately at a concentration of 0% (control), 5, 10, 15, and 20% (w/v) under continuous stirring. Fortified kefir samples were stored in the refrigerator at 4°C for further analysis (Figure 1).

2.1.5. Determination of pH and Viscosity Values

The pH values of the samples were determined using the pH meter device in accordance with the instructions provided by the manufacturer. While the viscosity of samples was carried out using a Brookfield viscometer at room temperature.

2.1.6. Microbial Viable Cell Count (VCC) in Kefir

Microbiological viable cell count in kefir was measured with some modifications. Each of the samples was performed in sterile 0.15% buffered peptone water with 10-fold serial dilutions. An appropriate diluted sample (1 mL) was then poured as duplicates in sterile Petri dishes then 15mL molten MRS agar was added. These plates were allowed to incubate at 37°C and for 48 h (8). Colony counts (25 to 250 CFU range) were described as:

$$\text{CFU/mL} = ((\text{Colony numbers x factor of dilution}) / \text{sample volume plated (mL)}) \qquad (1)$$

2.1.7. Antioxidant Activity (DPPH Assay)

The assay implemented the Bobo-Garcia et al., method with some modifications (9). The sample was diluted to appropriate concentration and 20 µL of sample was added to 180 µL of DPPH solution (150 µMOL/L in methanol water 80:20 v/v) in 96-well plates. Absorbance of the mixture was measured after 60 seconds mixing and 40 min incubation in the dark and room temperature in 515 nm by SPECTROstar Nano spectrophotometer (BMG LABTECH, Germany) (9). Percentage radical scavenging was obtained by formula:

$$\%\ \text{Inhibition} = [((A_{control}-A_{sample}))/A_{control}] \times 100 \qquad (2)$$

2.1.8. Total Phenolic Compounds

Determined by Folin–Ciocalteu with modifications, 20 µL of each diluted sample was mixed with 100 µL of Folin–Ciocalteu reagent (1:10 v/v with water) and 80 µL Na_2CO_3 (7.5% w/v). After a minute of mixing, in a microplate incubator, samples are then incubated for half an hour at 43°C before reading at a wavelength of 750 nm. The results were expressed as milligram gallic acid equivalents (GAE)/g, based on a gallic acid standard curve (10–100 mg/L) (10).

2.1.9. Sensory Determination

The samples of kefir were assessed by 30 semi-trained assessors (students and staff of ITMO University) according to taste, aroma, and color using a 5-point hedonic scale (1 = dislike extremely; 5 = like extremely). All the samples were coded with random three-digit numbers and shown in randomized order. Sensory assessment was performed in single booths in white light. The average score obtained was then calculated and analyzed using statistical software.

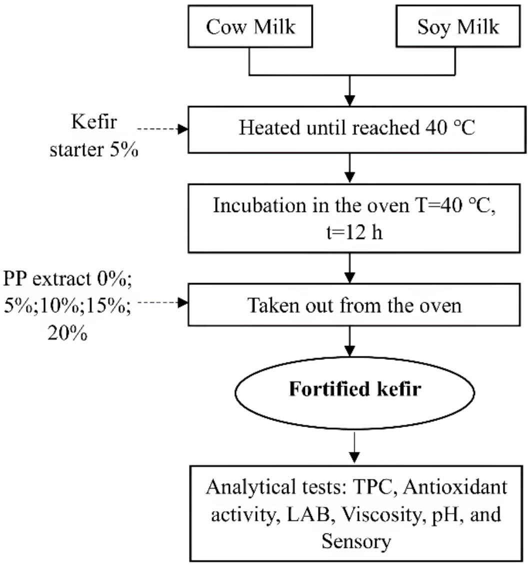

Figure 1. Flow diagram of the preparation of cow milk and soymilk kefir fortified with pomegranate peel extract (PPE).

2.1.10. Statistical Analysis

The one-way ANOVA using Minitab version 19 (Minitab, LLC., USA) was performed to analyze differences between samples. Tukey's Multiple Comparison Test was employed to ascertain significant differences between means at a significance level of 5% ($P<0.05$)

3. Results and Discussions

3.1. Total Phenolic Content and Antioxidant Activity

Fortification of kefir with pomegranate peel extract (PPE) resulted in a significant ($p < 0.05$) and concentration-dependent increase in total phenolic content (TPC) and antioxidant activity in both cow milk and soymilk kefir (Figure 2). TPC in cow milk kefir increased to 182.87 mg GAE/g at 20 % PPE, whereas in soymilk kefir reached higher levels (199.29 mg GAE/g). Radical scavenging activity also rose substantially, from 34.86 to 81.18% in cow milk kefir and from 38.20 to 83.09% in soy milk kefir at the highest PPE concentration (table 1). Although a formal Pearson correlation analysis was not performed in the present study, the observed increase in antioxidant activity alongside increasing TPC across treatments suggests that the phenolic compounds introduced by PPE likely contributed to the enhanced antioxidant potential of the fortified kefir samples. This finding supported by Kumar et al. (2023), where they also found a strong correlation between total phenolic content (TPC) and antioxidant capacity by exploring bioactive compounds and antioxidant properties of twenty-six Indian medicinal plant extracts (11).

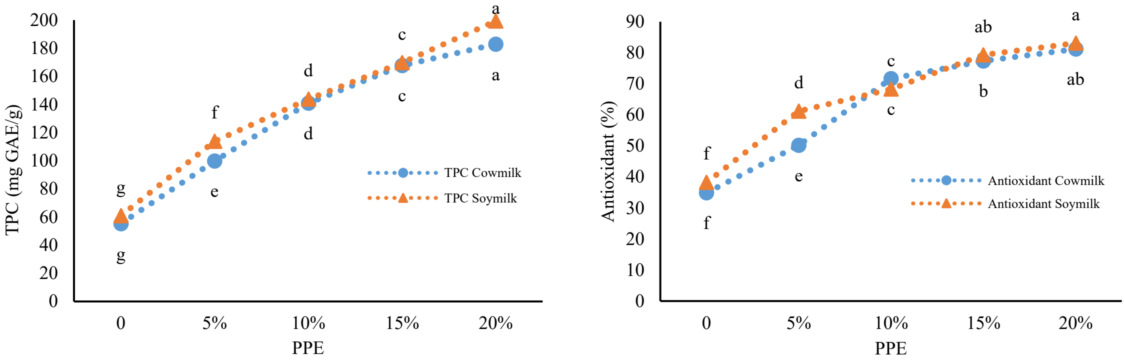

Figure 2. TPC and antioxidant activity of fortified cow milk and soymilk kefirs.

This effect may be explained by the high quantity of ellagitannins, punicalagin, gallic acid, and other phenolic compounds in PPE that are powerful hydrogen donors and free radical scavengers (12). These compounds may interfere with radical chain reactions, and thus counteract oxidative degradation, enhancing the stability of the bioactive compounds in the kefir matrix. Moreover, the soymilk kefir consistently recorded similar higher values of phenolics in comparison with cow milk kefir at all PPE levels. This difference could be explained by the reality that soy-based substrates are a natural source of isoflavones and phenolic acids which interact synergistically with PPE to enhance the overall antioxidant property of the matrix (13). In this context, the soy-based substrate may have provided a more phenolic-rich baseline onto which PPE-derived compounds were incorporated.

Protein–polyphenol interactions may also have influenced the retention and measurable behavior of phenolic compounds within the kefir systems. In dairy-based matrices, casein micelles are known to interact with polyphenols, while in soy-based systems, phenolic compounds may associate with glycinin and β-conglycinin. Such interactions can affect the solubility, stability, and analytical detectability of phenolic compounds in food matrices, and may therefore partly explain differences in measured TPC between animal- and plant-based substrates (14–16). However, in the present study, no specific binding analysis or release assay was conducted, and therefore the exact influence of these interactions on phenolic retention could not be confirmed.

From a compositional and technological perspective, fortification of kefir with PPE increased the measurable phenolic content and antioxidant activity of the product matrix. However, these results should be interpreted as evidence of matrix-level enrichment, rather than confirmed post-digestion bioavailability, since the present study did not evaluate digestive stability or bioaccessibility of PPE-derived phenolic compounds under simulated gastrointestinal conditions. This is particularly relevant because polyphenol–protein interactions, especially in dairy-based systems, may influence the release and accessibility of phenolic compounds during digestion. Therefore, although PPE fortification improved the antioxidant profile of kefir at the product level, further studies involving in vitro digestion and bioaccessibility analysis are needed to determine whether these compounds remain available for absorption after gastrointestinal transit.

Overall, the findings support the use of pomegranate peel extract as a promising natural fortifying agent for improving the phenolic and antioxidant profile of both dairy- and plant-based kefir. At the same time, the differences observed between cow milk and soymilk formulations highlight the importance of food matrix composition in modulating the functional expression of added bioactive compounds.

Table 1. Tukey's multiple comparison tests on physicochemical characteristics and total LAB of fortified kefirs.

Sample	Total Phenolic Content (mg GAE/g)	Antioxidant Activity (%)	Viscosity	pH	Viable LAB counts (x10^7 CFU/mL)
CC	55.31^{g} ± 0.66	34.86^{f} ± 0.83	559.33^{a} ± 4.04	4.48^{a} ± 0.00	36.8^{e} ± 0.40
M5	99.78^{f} ± 2.90	50.14^{e} ± 0.00	343.67^{b} ± 4.04	4.45^{a} ± 0.01	41.73^{d} ± 0.61
M10	140.92^{d} ± 1.46	71.63^{c} ± 1.43	279^{c} ± 5.57	4.40^{b} ± 0.02	50.0^{c} ± 1.20
M15	167.61^{c} ± 9.05	77.36^{b} ± 1.43	245^{d} ± 4.36	4.44^{c} ± 0.00	68.93^{b} ± 2.34
M20	182.87^{b} ± 4.58	81.18^{ab} ± 1.65	156.33^{e} ± 4.04	4.31^{d} ± 0.01	84.0^{a} ± 3.02
SC	60.91^{g} ± 4.26	38.20^{f} ± 0.83	36.33^{f} ± 0.58	4.25^{e} ± 0.01	3.53^{h} ± 0.24
S5	113.8^{e} ± 0.0	61.13^{d} ± 2.19	35^{f} ± 1.00	4.25^{e} ± 0.015	4.64^{h} ± 0.14
S10	143.68^{d} ± 2.71	68.29^{c} ± 2.19	33.67^{f} ± 0.58	4.24^{e} ± 0.011	7.25^{h} ± 0.19
S15	169.78^{c} ± 1.07	79.27^{ab} ± 0.83	32^{f} ± 0.00	4.23^{e} ± 0.006	12.27^{g} ± 0.61
S20	199.29^{a} ± 4.26	83.09^{a} ± 1.43	32^{f} ± 1.00	4.23^{e} ± 0.023	16.33^{f} ± 0.34

*Values are mean ± standard deviation (n = 3).

*Different superscript letters within a column indicate significant differences ($p < 0.05$, Tukey’s test).

*CC–M20: cow milk kefir with 0–20% PPE; SC–S20: soymilk kefir with 0–20% PPE.

3.2. Viable LAB count (CFU/mL)

The viable microbial cell count, commonly referred to as the colony-forming unit (CFU) count, has become a popular method for determining the quantity of viable microorganisms within a sample. This approach entails plating a series of dilutions of the sample onto an appropriate agar medium as well as incubating the plates within adequate conditions to allow visible colonies to develop. The number of colonies is subsequently counted, and the quantity of CFUs for each unit volume of the initial sample is calculated.

The analysis of viable microbial count aimed to evaluate the effect of pomegranate peel extract on the microbial population in cow milk kefir and soymilk kefir. The addition of pomegranate peel extract (PPE) had a significant effect on the viability of lactic acid bacteria (LAB) in kefir, whereby significant increases were recorded in both cow milk and soymilk kefir ($p < 0.05$). In cow milk kefir, LAB counts increased in the control to 20% PPE, a difference of more than two-fold, to 84.0 x 10^7 CFU/mL. Although soymilk kefir started with a much smaller amount of initial growth (3.53 x 10^7 CFU/mL), it increased by five-fold to 16.33 x 10^7 CFU/mL under the same treatment. While the absolute LAB counts in soymilk kefir remained lower than those of cow milk kefir, the relative increase suggests that PPE positively influenced the LAB fraction of the fermented matrix as shown in Figure 3.

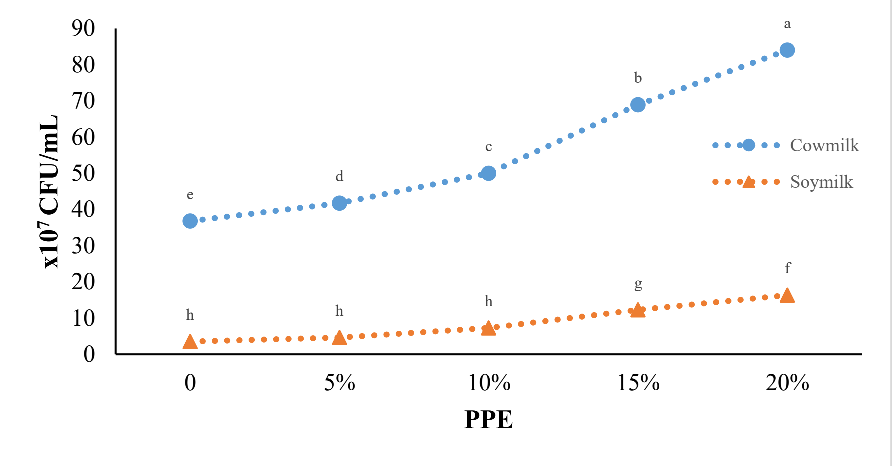

Figure 3. Viable microbial count of fortified cow milk and soymilk kefir.

These findings might be due to pomegranate containing a high concentration of polyphenols, where according to previous research, polyphenols demonstrate advantageous outcomes by serving as a prebiotic substrate. Specifically, this occurs through two mechanisms. Firstly, polyphenols enhance the growth and colonization of probiotic bacterial families, namely *Bifidobacteriaceae* and *Lactobacillaceae*. Secondly, they diminish the number of pathogenic bacteria such as *Escherichia coli, Clostridium perfringens,* and *Helicobacter pylori* (14). However, in the present study, PPE was not specifically analyzed for fermentable sugars or oligosaccharides, and therefore the observed increase in LAB counts should not be interpreted as definitive evidence of a direct prebiotic effect. Instead, the enhancement in LAB viability may reflect a combination of factors, including possible residual carbohydrate fractions from the peel matrix, selective inhibition of competing microflora by tannin-rich phenolic compounds, and the antioxidant-rich phenolic composition of PPE, which may have helped create a more favorable environment for bacterial survival during fermentation.

Phenolic compounds such as punicalagin and ellagic acid may also have contributed indirectly to microbial persistence through polyphenol–microbe interactions within the fermentation matrix. In this context, the presence of phenolic compounds may support the stability or activity of selected LAB populations, although the exact mechanism remains unclear. A recent study also indicated that plant polyphenols are able to stimulate the growth of probiotics, increasing colonization capacity and metabolic activity (17). Accordingly, the present findings are more appropriately interpreted as evidence of a favourable shift in the LAB-associated fermentation environment, rather than confirmation of a classical prebiotic mechanism.

The variations in nutrient content and buffering capacity may be the reason behind differences between cow milk and soymilk kefir. Lactose, peptides, and minerals needed to boost the growth of LAB are available in cow milk but are absent in soymilk; moreover, soymilk may contain some antinutritional factors that can inhibit microbial growth (18). The inclusion of PPE into soymilk kefir probably alleviated these drawbacks by providing growth-supporting constituents and potentially decreasing oxidative stress in the fermentation environment. Nevertheless, the total LAB counts of soymilk kefir were still lower compared to cow milk kefir, as it was reported previously comparing dairy and plant-based fermentation systems (19).

The demonstrated viability of LAB in PPE-enriched kefir has significant consequences in terms of functional food. Higher populations of lactic acid bacteria (LAB), lead to better modulation of the gut microbiota and production of bioactive metabolites, which drive an increase in overall health benefits including immune and intestinal barrier effects (20). However, the present findings should be interpreted specifically in relation to LAB viability, since kefir is a symbiotic fermented system involving both LAB and yeasts, and the yeast population was not monitored in this study. Given that pomegranate peel contains phenolic compounds with reported antimicrobial and antifungal activity, it is possible that PPE may have influenced the yeast fraction differently from LAB, thereby potentially affecting the traditional microbial balance of kefir.

Therefore, the microbiological conclusions of the present study should be limited to the LAB component of the kefir system. In addition, because this study did not directly assess gut microbiota modulation, gastrointestinal survival, or in vivo health effects, the formulation is more appropriately described as a potentially functional or growth-supporting fermented product, rather than a confirmed symbiotic system. Future studies should include yeast enumeration, sugar profile analysis, and broader microbial profiling to better understand the effect of PPE on the complete kefir microbial ecosystem and the underlying mechanisms responsible for the observed enhancement in LAB viability.

3.3. pH of various samples

This analysis aimed to see how the pH of soymilk and kefir made from cow milk would change when exposed to varying quantities of pomegranate peel extracts because the pH of kefir is a significant quality that impacts the microbiological makeup of the beverage, as well as its sensory qualities and longevity. Kefir is a kind of fermented milk that is created as a result of the activity of microorganisms, including lactic acid bacteria, yeast, and acetic acid bacteria.

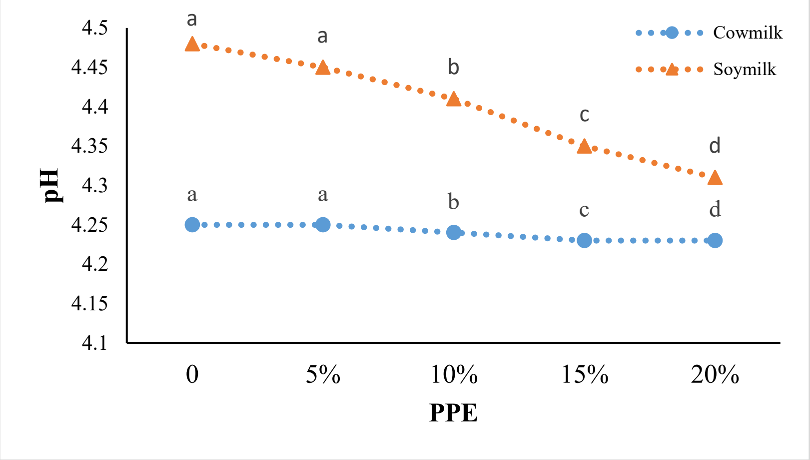

Figure 4. pH levels of fortified cow milk and soymilk kefir.

pH is one of the most important quality attributes of kefir that affects microbial stability, sensory characteristics and shelf life. An increase in PPE concentration lowered pH of soymilk kefir (4.48 to 4.31) and had no significant effects on pH of cow milk kefir (4.25 to 4.23) (Figure 4). These results can be compared with previous reports that dairy systems possess a higher buffering capacity than plant-based systems due to the availability of casein micelles, phosphates and mineral salts (21).

3.4. Viscosity

This analysis aimed to determine the influence of pomegranate peel extracts on the viscosity of kefir that was produced using soy and cow milk as the primary ingredients. It was expected that the inclusion of pomegranate peel extracts would result in a reduction in the viscosity of kefir, which would likely result in an improvement in the product's sensory characteristics as well as a rise in consumer acceptance. The process of viscosity determination was conducted using Brookfield viscometer.

Viscosity is an important factor of the textural and sensory qualities of kefir, which determines its appearance of creaminess and drinkability in the mind of the consumer. The current study showed a significant reduction in viscosity of cow milk kefir with PPE fortification with a value of 559.3 cP in the control and 156.3 cP at 20% PPE ($p < 0.05$). Conversely, soymilk kefir showed little alteration and did not change significantly in viscosity (32-36.3 cP) among the treatments (Figure 5). These results imply that the interaction between PPE and milk protein network is predominant in altering rheological behavior.

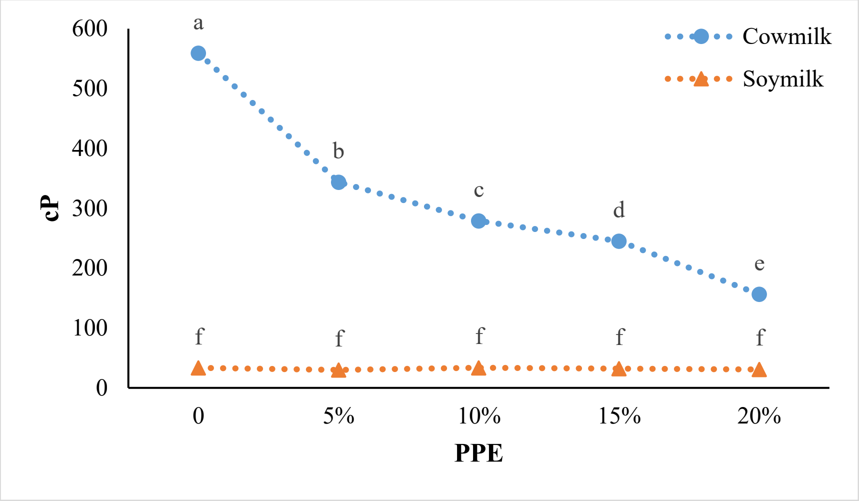

Figure 5. Viscosity levels of fortified cow milk and soymilk kefirs.

The reduction in observed viscosity of cow milk kefir can be attributed to the polyphenol-protein interaction in the gel matrix structure. PPE polyphenols, especially punicalagin and ellagitannins, may form complexes with casein micelles through hydrogen bonding and hydrophobic interactions, and such interactions may alter the structural organization and functional behavior of casein-rich systems (22). Recent studies have established that binding of polyphenols to milk proteins influences micelle aggregation and serum phase volume, potentially affecting viscosity and mouthfeel (23). Therefore, the decrease in viscosity in cow milk kefir may reflect a partial disruption or loosening of the fermented casein gel structure following PPE addition.

In contrast, soymilk kefir did not show a significant change in viscosity following PPE addition, despite the likelihood that polyphenol–protein interactions also occurred in the soy matrix. Unlike cow milk, soymilk does not contain casein micelles, but is instead composed mainly of globular storage proteins, especially glycinin (11S) and β-conglycinin (7S), which differ substantially from casein in structural conformation, surface properties, and colloidal behavior. These soy proteins may still interact with tannin-rich phenolics through hydrogen bonding and hydrophobic forces, as reported in recent studies on soy protein–polyphenol interactions (24–26). However, in the present system, such interactions may have resulted in more limited, soluble, or structurally accommodated complexes, rather than the type of network disruption or protein precipitation that would produce a measurable decrease in viscosity.

Furthermore, the lower baseline viscosity and less interconnected gel structure of fermented soymilk may have reduced the extent to which polyphenol-induced complexation translated into a detectable rheological change. Therefore, the relative rheological stability of soymilk kefir in this study was likely not due simply to the absence of casein, but rather to the distinct way in which soy globulins interact with polyphenolic compounds and organize within the fermented matrix. This suggests that the impact of PPE on viscosity depends not only on the presence of polyphenols, but also on the type, structural flexibility, and aggregation behavior of the proteins present.

These changes have considerable implications for product development. The lower viscosity of cow milk kefir could be beneficial as it would make the drink more drinkable and lighter, with an improved functional beverage positioning. Nevertheless, such changes can be seen as a negative factor among consumers who identify kefir with a thick and creamy texture. Industry solutions to this problem include the partial addition of PPE (≤10%) to reduce the loss of viscosity or the addition of natural stabilizers like pectin or inulin that can restore body and mouthfeel without losing a clean-label claim.

Differently, the relatively constant rheology of soymilk kefir suggests that it can be formulated with greater flexibility, with little to no textural cost of increased PPE inclusions. This strength is in line with the increasing popularity of plant-based functional beverages that tend to focus on the light texture and refreshing sensory experience. Overall, the contrasting behavior observed between cow milk and soymilk kefir emphasizes the importance of matrix-specific optimization when designing polyphenol-enriched fermented products.

3.5. Sensory Analysis

The sensory properties of cow milk kefir and soymilk kefir fortified with varying concentrations of pomegranate peel extract (PPE) were assessed based on taste, aroma, and color (Table 2; Figure 6). According to the Kruskal-Wallis test, there were no statistically significant differences ($p > 0.05$) among treatments for any of the sensory properties, suggesting that variations in PPE concentration had no perceptible effect on the sensory properties of both cow milk and soymilk. Therefore, the following interpretation is limited to descriptive tendencies observed in the mean panellist scores, rather than statistically confirmed treatment effects.

Table 2. Kruskal-Wallis test results for sensory attributes of cow milk and soymilk across pomegranate peel extracts concentration.

Attribute	Sample Type	H Statistics	p-Value	Significance
Taste	Cow milk Kefir	9.485	0.05	n.s
Taste	Soymilk Kefir	7.012	0.135	n.s
Aroma	Soymilk Kefir	0.400	0.982	n.s
Aroma	Cow Milk Kefir	7.012	0.135	n.s
Color	Soymilk Kefir	0.807	0.937	n.s
Color	Cow Milk Kefir	7.167	0.127	n.s

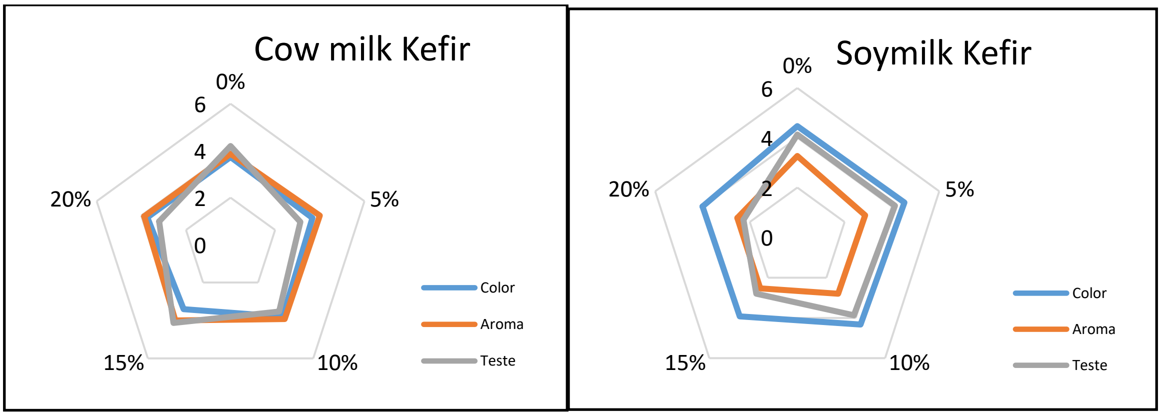

Figure 6. Sensory attributes (color, aroma, taste) of fortified cow milk and soymilk kefir.

3.5.1. Taste

PPE addition influenced the numerical taste scores of kefir samples as shown in Figure 5, although the differences among treatments were not statistically significant ($p > 0.05$). The highest mean taste score was observed in the control cow milk kefir (4.20 ± 0.86), whereas the lowest mean score was recorded in soymilk kefir fortified with 20% PPE (2.27 ± 1.28). While these numerical trends should not be interpreted as statistically confirmed treatment effects, they may still offer useful descriptive insight into panel preference patterns.

The lower taste scores observed at higher PPE concentrations may plausibly be associated with the increasing presence of polyphenolic compounds, particularly hydrolyzable tannins, which are commonly linked to bitterness and astringent mouthfeel. Tannins are known to interact with salivary proteins, thereby reducing oral lubrication and contributing to drying or puckering sensations that may reduce palatability at elevated concentrations (27). However, in the present study, the sensory panel was not specifically trained to distinguish sourness derived from fermentation acids from astringency associated with tannin-rich PPE as separate sensory dimensions. Therefore, the lower taste acceptance at higher PPE levels should be interpreted cautiously, and the contribution of tannin-related astringency should be regarded as a plausible, but not directly confirmed, explanation.

From a formulation perspective, moderate PPE levels may still be preferable if the objective is to maintain consumer acceptability while enhancing functional properties. Similar tendencies toward reduced taste acceptance at higher levels of plant-derived additives have also been reported in fermented beverage studies (28).

3.5.2. Aroma

Aroma scores also showed some numerical variation among treatments, although no statistically significant differences were detected ($p > 0.05$). The best scores in the aroma (Figure 5) were in control samples of cow milk and soymilk kefir (3.87-4.00), and the lowest scores were in the soymilk kefir with 15-20% PPE (2.53-2.80). Lower aroma preference in soymilk kefirs could also be attributed to natural beany volatiles formed by oxidation of unsaturated fatty acids catalyzed by lipoxygenase in the presence of the phenolic compounds of PPE. Moderate addition of PPE (≤ 10%), on the other hand, had a slight positive influence on aroma ratings in cow milk kefir, which may have been caused by the partial covering of volatile acids with polyphenolic compounds. However, because the observed differences were not statistically significant, these results should be interpreted only as descriptive tendencies rather than confirmed sensory effects. Previous research stated that all of the tested beverages with or without pomegranate peel extract fortification attained high sensory acceptance with an overall hedonic rating of between 7.8 and 8.0, which indicates that the addition of fruit-based extracts like pomegranate peel extract can be employed successfully to improve the overall hedonic value of fermented beverages, especially in aroma and overall acceptability (29). In general, the kefir prepared with cow milk showed a higher level of aromatic stability than the one prepared with soymilk, which suggests that the base matrix regulates volatile retention and consumer perception.

3.5.3. Color

Color scores showed slight numerical variation across treatments, although the Kruskal–Wallis test indicated no statistically significant differences ($p > 0.05$). The score of the color can be seen in Figure 5 where color score in cow milk kefir was between 3.40 and 3.80, with the maximum point of 10% PPE (3.80 ± 1.08). Instead, soymilk kefirs were better accepted in color with the highest score of 5% PPE (4.53 ± 0.52) and after that the control (4.47 ± 0.83). These findings may reflect the visual contribution of natural pigments present in pomegranate peel, including phenolic-derived color compounds that can influence product appearance. The slight numerical preference for certain fortified samples suggests that PPE may have contributed to a visually appealing color, particularly in the soymilk kefir matrix. However, because these differences were not statistically significant, PPE cannot be concluded to have significantly improved color acceptance under the present study conditions. This higher color acceptability concurred with the past studies in which the addition of fruit peel extracts enhanced the appearance of the fermented drinks (30).Overall, the sensory results suggest that PPE fortification did not significantly alter panelist acceptance of taste, aroma, or color in either kefir system. Although some non-significant numerical tendencies were observed, particularly at moderate PPE levels, these should be interpreted cautiously. From a formulation perspective, PPE may still be considered a promising functional ingredient, but further sensory optimization with a larger panel or more refined product development approach may be necessary to better define the most acceptable fortification level for potential commercial application. Overall, the sensory findings suggest that PPE fortification did not significantly impair consumer acceptance of kefir within the concentration range evaluated, as no sensory attribute differed significantly across treatments. At the same time, the numerical score patterns indicate that higher PPE levels, particularly in soymilk kefir, may have been associated with lower acceptance for taste and aroma, whereas moderate PPE inclusion appeared more favorable from a descriptive sensory standpoint.

These findings should be interpreted within the methodological scope of the present study. The sensory evaluation was conducted using a hedonic consumer-type panel, and not a trained descriptive sensory panel specifically designed to differentiate sensory sub-attributes such as sourness, bitterness, astringency, aftertaste, or mouthfeel. Therefore, while PPE concentration may have influenced certain sensory perceptions, the present data are best interpreted as reflecting overall consumer acceptance patterns, rather than detailed descriptive sensory mechanisms.

Collectively, the results suggest that moderate levels of PPE may offer a practical compromise between functional enrichment and acceptable sensory quality, while also highlighting the need for future studies using trained sensory panels or descriptive profiling to better characterize the sensory impact of tannin-rich fortification in fermented beverages.

4. Conclusions

The present study demonstrated that fortification of cow milk and soymilk kefir with pomegranate peel extract (PPE), particularly at low to moderate concentrations (5–15%), improved the compositional, antioxidant, and microbiological profile of the products without causing statistically significant differences in overall sensory acceptance. Although some numerical declines in taste and aroma scores were observed at higher PPE levels, especially in soymilk kefir, these differences were not statistically significant and should therefore be interpreted cautiously.

The incorporation of PPE significantly increased total phenolic content (TPC) and antioxidant activity in a concentration-dependent manner in both kefir matrices, with soymilk kefir generally showing slightly higher measured values than cow milk kefir. PPE addition also enhanced the viability of lactic acid bacteria (LAB), suggesting that pomegranate peel may provide a more favorable fermentation environment or growth-supporting components, although the exact mechanism was not determined in the present study.

From a technological perspective, PPE fortification affected viscosity and pH differently depending on the kefir matrix. A significant reduction in viscosity was observed in cow milk kefir, likely due to polyphenol–protein interactions involving the dairy protein network, whereas soymilk kefir remained relatively stable, reflecting differences in protein composition and matrix behavior. These findings highlight the importance of considering the underlying food matrix when formulating polyphenol-enriched fermented beverages.

Overall, PPE shows promising potential as a natural fortifying ingredient for improving the functional profile of kefir while simultaneously supporting the valorization of fruit-processing by-products. However, further studies are needed to evaluate the digestive stability, bioaccessibility, shelf-life, and in vivo relevance of PPE-derived bioactive compounds before broader functional or health-related claims can be established.

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Author Contributions

I.A. Conceptualization, Methodology, Investigation, Formal analysis, Visualization, Writing – original draft, Supervision. M.S.B. Conceptualization, Supervision, Project administration, Writing – review & editing. S.H.N. Methodology, Validation, Data curation, Writing – review & editing. D.D.I. Software, Resources, Statistical analysis, Writing – review & editing. L.F. Data curation, Formal analysis, Visualization, Writing – review & editing.

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Data Availability Statement

All data generated or analyzed during this study are included in this published article.

Conflicts of Interest

The authors declare no conflicts of interest.

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