Çukurova Tarım Gıda Bil. Der. Çukurova J. Agric. Food Sci.
39(1):35-44
doi: 10.36846/CJAFS.2024.131

Research Article

Investigation of Microbiological, Physicochemical, and Sensory Properties of a Kefir Drink Fortified with Propolis

Elif Şeyma BAĞDAT[1], Özge KAHRAMAN ILIKKAN*[2]

ABSTRACT
The effect of propolis fortification on kefir microbiota and kefir properties was investigated. Kefir samples were fortified with 0.5% (20 mg) (P1) or 1% (40 mg) propolis (P2) to improve the nutritional value of kefir. Similar studies on dairy products were evaluated to determine these ratios. After the first day and 7th day of storage, microbial enumerations, and physicochemical properties were determined. Total phenolic content and antioxidant capacity were analyzed with 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity assay after the 7th day of storage. 0.5% propolis fortification slightly changed the physicochemical properties of kefir within acceptable ranges compared to the control. Propolis exhibited a prebiotic effect on lactococci after the 7th day of storage. Sensory analysis revealed 0.5% propolis-fortified kefir was more acceptable. Total phenolic content as well as radical scavenging activity increased compared to the control. The results show that propolis can be used to improve the nutritional value of kefir products and develop an innovative functional product, but 0.5% fortification is more acceptable in terms of sensory properties.
Keywords: Propolis, kefir, 2,2-difenil-1-pikrilhidrazil (DPPH), total phenolic

Propolisle Zenginleştirilmiş Kefir Içeceğinin Mikrobiyolojik, Fizikokimyasal ve Duyusal Özelliklerinin Araştırılması

ÖZ
Propolis takviyesinin kefir mikrobiyotası ve kefir özellikleri üzerindeki etkisi araştırılmıştır. Kefir örnekleri, kefirin besin değerini artırmak için %0.5 (20 mg) (P1) veya %1 (40 mg) propolis (P2) ile zenginleştirilmiştir. Bu oranlar seçilirken literatürde süt ürünleriyle yapılan benzer çalışmalardan yararlanılmıştır. Depolamanın ilk günü ve 7. gününden sonra, mikrobiyal sayımlar ve fizikokimyasal özellikler belirlenmiştir. Toplam fenolik içerik ve antioksidan kapasite, depolamanın 7. gününden sonra 2,2-difenil-1-pikrilhidrazil (DPPH) radikal süpürme aktivitesi testi ile analiz edilmiştir. %0.5 propolis takviyesi, kefirin fizikokimyasal özelliklerini kontrole kıyasla kabul edilebilir aralıklarda hafifçe değiştirmiştir. Propolis, depolamanın 7. gününden sonra laktokoklar üzerinde prebiyotik bir etki göstermiştir. Duyusal analiz, %0,5 propolisle güçlendirilmiş kefirin daha kabul edilebilir olduğunu ortaya koymuştur. Toplam fenolik içeriğin yanı sıra radikal süpürme aktivitesi de kontrole kıyasla artmıştır. Sonuçlar, propolisin kefir ürünlerinin besin değerlerini iyileştirmek ve yenilikçi fonksiyonel bir ürün geliştirmek için kullanılabileceğini, ancak %0,5'lik zenginleştirmenin duyusal özellikler bakımından daha kabul edilebilir olduğunu göstermektedir.
Anahtar kelimeler: Propolis, kefir, 2,2-difenil-1-pikrilhidrazil (DPPH), toplam fenolik

ORCID ID (Yazar sırasına göre)
0000-0001-6627-7270, 0000-0001-5843-6868

Yayın Kuruluna Geliş Tarihi: 12.08.2023
Kabul Tarihi: 03.01.2024
[2] Başkent University, Kahramankazan Vocational School, Food Technology Program, 06980, Ankara
[2]Başkent University, Kahramankazan Vocational School, Food Quality Control and Analysis Program, 06980, Ankara
*E-posta: okilikkan@baskent.edu.tr

Investigation of Microbiological, Physicochemical, and Sensory Properties of a Kefir Drink Fortified with Propolis

Introduction

Propolis is a substance protecting hive and is also known as "bee glue". It is collected from plants by bees as a resinous material and is transferred to a hive to form its propolis employing enzymes (Bhargava vd., 2021; Özkök vd., 2021). The most important role of propolis is to protect the hive from microorganisms and predators (Bhargava vd., 2021). Propolis can contain phenolic compounds, flavonoids, and terpenes (Irigoiti vd., 2021). Propolis has been used since ancient times and has been known to have medicinal properties such as antibacterial, antioxidant, prebiotics, anticancer, hepatoprotective, antiviral, and anti-inflammatory (Ali ve Kunugi, 2021; Bhargava vd., 2021; Özkök vd., 2021). The potential therapeutic effect of propolis has been studied and has been shown that it can modulate metabolic syndrome as well as anti-cancer effects. Additionally, propolis has been shown to have the potential to inhibit viral spike fusion in host cells by molecular simulations (Ali ve Kunugi, 2021). COVID-19 patients exhibited earlier viral clearance by consuming propolis (Ali ve Kunugi, 2021; Berretta vd., 2020). It increases the production of short-chain fatty acids (SCFA) and modulates the gut microbiota (Xue vd., 2019). Therefore, propolis has been used as a functional food ingredient or sole formulation (Irigoiti vd., 2021).

Kefir is a traditional beverage fermented by starter cultures called "kefir grains" (Ilıkkan ve Bağdat, 2021). The Caucasus, Tibetan, and Mongolian mountains are the origin of Kefir (Kazou vd., 2021). Türkiye, Argentina, Russia, Taiwan, and Brazil are the countries that consume Kefir and it has been very popular because of its refreshing effect (de Lima vd., 2018). Kefir has a symbiotic microflora consisting of yeast and lactic acid bacteria. Kefir promotes human health because its microflora has dominantly lactic acid bacteria (Bengoa vd., 2019). Kefir and its polysaccharide substance "Kefiran" have many benefits such as being antimicrobial and antiproliferative (Verce vd., 2019).

This study is the first report comprehensively investigating the effect of kefirs enriched with propolis on antioxidant capacity, kefir microbiota, total phenolic, sensory, and physicochemical properties. The fortification aimed to improve the beneficial properties of kefir and to investigate the prebiotic effect of propolis.

Material and Methods

Propolis

Food grade anatolian propolis was used in this study that is brown, water extract, and manufactured in Turkey. The propolis was purchased from an approved market (Bee'O, Sbs Bilimsel Bio Çözümler San. Tic. A.Ş., Turkey, 20 ml). Total phenolic content, total flavonoid content, phenolic compounds, and antioxidant capacity were provided by the company (https://www.beeo.com.tr/analizlerimiz#fancybox-grup-2). The pH of propolis is 5.3±0.02 and the total dry matter is 11.30±1.5 (https://www.beeo.com.tr/analizlerimiz#fancybox-grup-4). Small amounts of propolis are desired as it has a strong flavor. Concentrations of propolis have been selected according to previous studies investigating only the organoleptic character of kefir fortified with propolis (Chon vd., 2020). In this mentioned study, 0.5% of propolis exhibited a better flavor results than control group and 1% was close to the control (Chon vd., 2020). Therefore, in this present study, 0.5% corresponding to 20 mg of propolis, and 1% corresponding to 40 mg of propolis concentration have been selected.

Preparation of Kefir samples

Kefir grains used in this study were previously characterized with metataxonomic analysis (Ilıkkan ve Bağdat, 2021). 100 ml of pasteurized cow milk, which contains 3.5% milkfat, were inoculated with 3 g of kefir grains. The sample containing no propolis was prepared as control kefir (C), one of the kefirs was fortified with 0.5% propolis (10 drops, % 0.5) (P1) and the other with 1% propolis (20 drops, % 1) (P2). These ratios were selected according to a previous study that analyze the organoleptic character of kefirs fortified with propolis (Chon vd., 2020). Fermented kefir was kept at 4^{o}C until the 7th day of storage after the removal of kefir grains.

Yeast/mold, Lactobacilli and lactococci content of kefirs

Lactobacilli was detected by counting on MRS agar (Merck, Darmstadt, Germany) and agar plates were incubated for 48 h at 37^{o}C in anaerobic jars (Anaerocult System: Merck 16275, Merck KGaA). M17 was used to count lactococci (Merck, Darmstadt, Germany) and plates were incubated for 48 h at 37^{o}C aerobically. Dichloran Rose Bengal Chloram Phenicol Agar (Merck, Darmstadt, Germany) was used for yeast/molds and plates were incubated for 7 days at 25^{o}C.

Physicochemical analysis

AOAC International Method (AOAC, 2016) was used for total dry matter by the gravimetric method. A digital pH meter (Inolab wtw 720) was used to measure the pH. 10 ml of kefir was used to determine titratable acidity by titrating with 0.1 N NaOH (Sulmiyati vd., 2019). Brix meter was used to detect to Brix value (HI96801, Hanna Instruments, USA).

Total phenolic content (TPC)

The total phenolic content (TPC) was determined by using Folin–Ciocalteu reagent. 80% methanol (Merck, Darmstadt, Germany) was added to kefir sample and solution at room temperature for 4 h. Supernatants were filtered with syringe filter (0.45µm, PTFE/L) after the extracts were centrifuged at 1470 × g for 5 min. 2.5 ml of Folin-Ciocalteu reagent was mixed with the filtrate (Merck, Darmstadt, Germany) and 2 ml of Na_2CO_3 (7.5 %) solution (Merck, Darmstadt, Germany). Samples were kept for 30 min at room temperature and dark. 760 nm wavelength was used to measure absorbances and mg gallic acid equivalent/ kg was used to express results of kefir samples (Ozkan vd., 2018; Sözeri Atik vd., 2021).

Antioxidant capacity

The antioxidant properties of kefir were determined with DPPH radical scavenging activity. 4.9 ml of DPPH solution (in methanol, 99%) (Merck, Darmstadt, Germany) was mixed with 100 µl of the supernatant taken from total phenolic content analysis. The mixtures were kept for 30 min at room temperature and dark. 517 nm wavelength was used to measure absorbances of samples. The % inhibition was calculated as follows, Absorbance of blank – Absorbance of the sample / Absorbance of blank x 100 (Salar vd., 2015).

Organoleptic testing

Sensory analyses of kefir samples were carried out on the 1st day of the kefir production. Evaluation of the samples was made according to the following criteria; appearance, texture, odor, flavor, consistency, and overall acceptability. Parameters were evaluated by a panel of 13 members who are semi-educated and familiar with kefir (Nadir Işık, 1979). Panelists scored the samples on a hedonic scale from 1 to 5 indicating “poor” to “excellent”, respectively (Chon vd., 2020; Pehlivan vd., 2023). The ethical approval number was taken (E-89595671-042-223340).

Statistical Analysis

A one-way analysis of variance (ANOVA) and the Tukey Kramer honestly significant difference (HSD) was applied to samples to determine differences in the treatments based on three replicates using the JMP Pro 14. $P< 0.05$ was selected to determine the significance of differences.

Results and Discussion

Kefir grains

The microbiota of kefir grains used in this study was characterized by the metataxonomic approach in the previous study (Ilıkkan ve Bağdat, 2021). Therefore, kefir grains have been known to have dominantly, namely, 65% *Bifidobacterium longum.*

Physicochemical analysis of Kefirs

To assess and compare the quality of kefir after fortification, the physico-chemical properties of kefir were analysed (Table 1). Results were in the acceptable range for kefir quality at day 0. pH values of control, P1, and P2 were 3.74, 3.91, and 3.97, respectively. Even though the pH values of fortified samples were higher than the control, they were within the acceptable range for kefir quality. The higher pH value of P1 and P2 were probably due to the content of propolis. Accordingly, the titratable acidity (%, lactic acid) of control was 1.1%, P1 was 1.0%, and P2 was 0.9. The total dry matter of kefirs fortified with propolis was higher than the control as expected because of the propolis. Accordingly, Brix values, which indicate non-fat dry matter, were 5.7, 7.1, and 7.8 for control, P1, and P2, respectively. P1 and P2 were higher than the control due to the content of propolis.

On day 7 of storage, pH values were slightly but statistically higher than values of day 0. However, the titratable acidity of samples, namely, lactic acid, was statically the same during the storage except for control which increased slightly. Brix values were statistically the same for control and P1 except for P2 which decreased. Accordingly, also, the total dry matter of P2 decreased after the 7th day of storage.

Microbial enumeration of fermented milk kefirs

On the first day of storage, only yeast and molds were slightly affected by propolis addition, and the number of yeasts decreased compared to the control. However, counts of lactobacilli and lactococci did not statistically change. Decreasing yeast did not affect the physicochemical properties of kefirs. Fortification did not affect lactobacilli and lactococci at day 0.

On day 7, even though the number of lactobacilli in P1 and P2 slightly decrease compared to the control, there was no significant difference between the storage times of samples. The number of lactococci decreased on day 7 compared to day 0 but the control decreased more than P1 and P2. Yeast/mold numbers of P1 and P2 were lower than control on day 7 as at day 0, however, on day 7, yeast/mold numbers slightly increased, especially, for P1 compared to day 0 (Table 2). The preservation of lactic acid bacteria during storage may also indicate that the sensory properties of kefir are partially preserved. The product's stability is improved with the addition of propolis. At the same time, the fact that yeast and mold formation was less in both samples compared to the control was an indication that the product was better protected against undesirable microorganisms during storage.

Table 1: Physicochemical properties of kefirs fortified with propolis at day 0 and 7

Storage Time	Sample	pH	Titratable Acidity	Brix	Total Dry Matter
0	**C**	3.74±0.01^{Cb}	1.10±0.01^{Ab}	5.70±0.00^{Ca}	9.70±0.00^{Ca}
	P1	3.91±0.01^{Bb}	1.05±0.01^{Ba}	7.10±0.10^{Bb}	11.70±0.00^{Aa}
	P2	3.97±0.01^{Ab}	0.92±0.02^{Ca}	7.75±0.15^{Aa}	11.30±0.00^{Ba}
7	**C**	3.79±0.01^{Ca}	1.15±0.05^{Aa}	5.70±0.00^{Ca}	9.25±0.05^{Bb}
	P1	4.05±0.00^{Ba}	1.05±0.01^{Aa}	7.45±0.05^{Aa}	11.65±0.05^{Ba}
	P2	4.12±0.00^{Aa}	0.89±0.01^{Ba}	6.95±0.05^{Bb}	9.65±0.05^{Bb}

^{Ab}Values with different superscript letters are significantly different (ANOVA, *p<0.05) according to the Tukey HSD test (n = 3). Upper-case letters present the differences between the samples in the same storage time and lower-case letters show differences between the storage times of samples (i.e., Control 1st day and Control 7th day).

Table 2: Effect of kefir fortified with propolis on kefir microbiota (log_{10} cfu/g)

Storage Time	Sample	Lactobacilli	Lactococci	Yeast/Mold
0	**C**	7.1±0.0^{Aa}	8.6±0.2^{Aa}	6.9±0.2^{Aa}
	P1	6.8±0.1^{Aa}	8.9±0.0^{Aa}	6.5±0.3^{Bb}
	P2	7.1±0.3^{Aa}	8.8±0.4^{Aa}	6.4±0.1^{Ba}
7	**C**	7.3±0.0^{Aa}	7.2±0.1^{Bb}	7.1±0.0^{Aa}
	P1	7.0±0.1^{Ba}	7.6±0.2^{Ab}	6.7±0.1^{Ba}
	P2	7.0±0.0^{Ba}	7.8±0.3^{Ab}	6.5±0.1^{Ca}

^{Ab}Values with different superscript letters are significantly different (ANOVA, *p<0.05) according to the Tukey HSD test (n = 3). Upper-case letters present the differences between the samples in the same storage time and lower-case letters show differences between the storage times of samples (i.e., Control 1st day and Control 7th day).

Propolis has been evaluated as a food additive in yogurt in a previous study, as compatible with our research, lactobacilli in yogurt were inhibited by 0.03% propolis addition, however, streptococci increased (Güneş-Bayır vd., 2020).

Total phenolic content and antioxidant capacity

Kefir has been known to have an antioxidant capacity (Yilmaz-Ersan vd., 2018). In addition to this, propolis has also known to have high phenolic content (Özkök vd., 2021). The total phenolic concentration of the control was 46.40±0.08, and P1 and P2 had statistically higher total phenolic content than the control, 60.08±0.08 and 67.28±0.08, respectively (Figure 1). Accordingly, DPPH scavenging assay results indicated that P1 and P2 had statistically higher scavenging effects compared to the control. Control kefir had 3.1±0.08 %, P1 had 6.1±0.08 %, and P2 had 11.0±0.12 % (Figure 2). In previous studies conducted with kefir produced by goat and soy milk, antioxidant activity was stated to be 5.52±0.99 % and 8.08±1.23, respectively (Nurliyani ve Sunarti, 2015).

Propolis is a health-promoting bee product. Recently, it has a great attraction for food additives because of its properties such as antiviral, antibacterial, cariostatic, antioxidant, hepatoprotective, and anticancer (Özer, 2020). Therefore, in this present study, it was evaluated the potential to fortify kefir samples with propolis. According to the physicochemical properties of kefirs fortified with a different range of propolis, pH values of control were compatible with previous studies. In previous studies conducted with kefir, pH values were in the range between around 3.7-4.0 (Chon vd., 2020; Gürsoy vd., 2020; Ilıkkan ve Bağdat, 2021). Titratable acidity values were consistent with previous studies which detect titratable acidity between 0.71 and 1.1% (Gürsoy vd., 2020; Ilıkkan ve Bağdat, 2021; Yirmibeşoğlu ve Tefon Öztürk, 2020). In another study, Brix values were found to be between 5 and 7 as found in this study (Hong vd., 2019). After the 7th day of storage, it was observed that the total dry matter of P2 decreased, this can be due to the microbial fermentation process.

Only pH values of kefir fortified with propolis were evaluated in a preliminary study previously, but, with market kefir and on the first day of storage. Compared to this study, the pH value of the control was the same in this research, which is 3.74. However, the pH values of kefirs fortified with propolis were higher than in this mentioned study. The higher pH values were more likely because of the inhibition of lactobacilli, the decreasing of percentage of lactic acid (TA%) confirmed this (Chon vd., 2020). Microbial enumerations showed that propolis exhibited a prebiotic effect for lactococci after the 7th day of storage. Total phenolic and DPPH were higher in propolis samples. Propolis increased its scavenging effect in both kefir samples (P1 and P2) as expected.

Sensory properties

Five properties were selected for sensory evaluation, namely, appearance, texture, odor, flavor, consistency, and overall acceptability (Figure 3). 5-point scales were used by the panelists. The appearance score of control was 4.54±0.78 while P1 and P2 were 4.31±0.85 and 4.15±0.80, respectively. The consistency score of control was 4.31±0.80 while P1 and P2 were 3.92±1.19 and 3.85±1.18, respectively. The texture score of the control was 4.54±0.78 while P1 and P2 were 4.15±1.07 and 3.77±1.42, respectively. The odor score of the control was 3.69±1.11 while P1 and P2 were 3.31±1.11 and 2.62±1.45, respectively. The flavor score of the control was 4.23±0.73 while P1 and P2 were 3.69±1.25 and 2.62±1.45, respectively. The overall preferences score of control was 4.38±0.77 while P1 and P2 were 3.62±1.33 and 2.85±1.21, respectively.

According to statistical analysis of the sensory analysis of Kefir, there was a statistically significant difference in only taste and overall acceptability between the 1% propolis-fortified group and the control group ($p<0.05$). Propolis addition did not change other properties of kefir according to panelists. In a study conducted with organoleptic testing of kefir, there was a statistically significant difference in taste, flavor, color, texture, and overall acceptability in 1% propolis-fortified kefir (Chon vd., 2020). However, kefir fortified with 0.5% of propolis showed the best results compared with the control being like this study. Taste and general acceptability are also very important parameters for consumer perception.

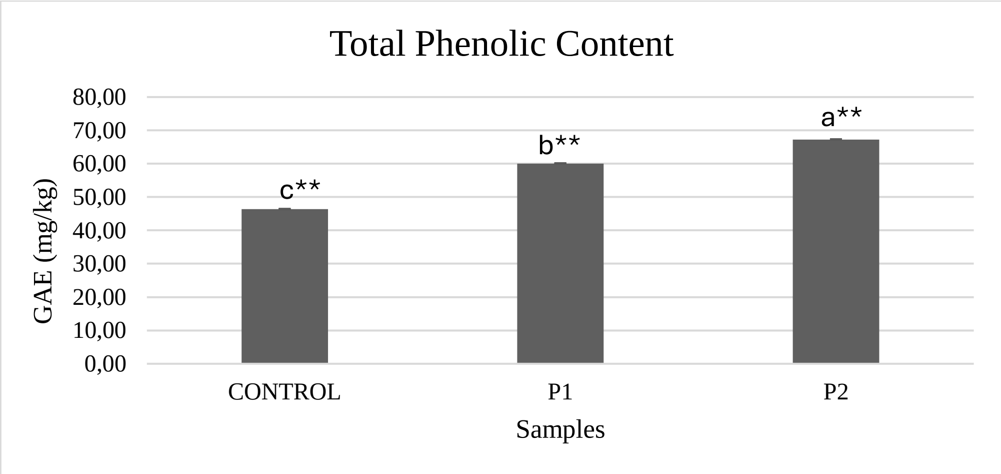

Figure 1: Total phenolic contents of kefirs fortified with propolis after 7th day of fermentation Different superscript letters significantly indicate difference (ANOVA, **p<0.01) (ANOVA, **p<0.01) according to the Tukey HSD test (n = 3).

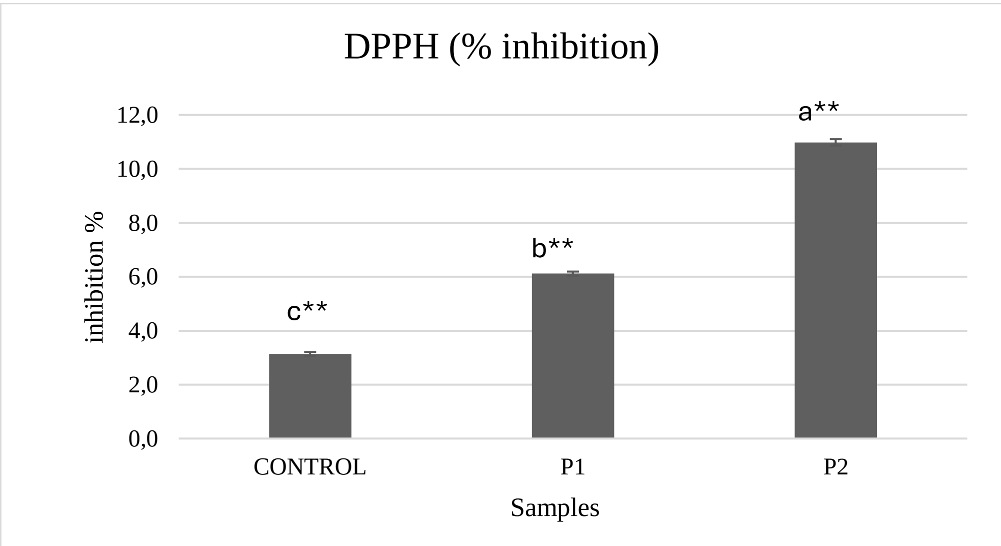

Figure 2: % inhibition with DPPH radical scavenging assay of kefirs fortified with propolis Different superscript letters indicate significantly difference (ANOVA, **p<0.01) according to the Tukey HSD test (n = 3). DPPH = 2,2-diphenyl-1-picrylhydrazyl

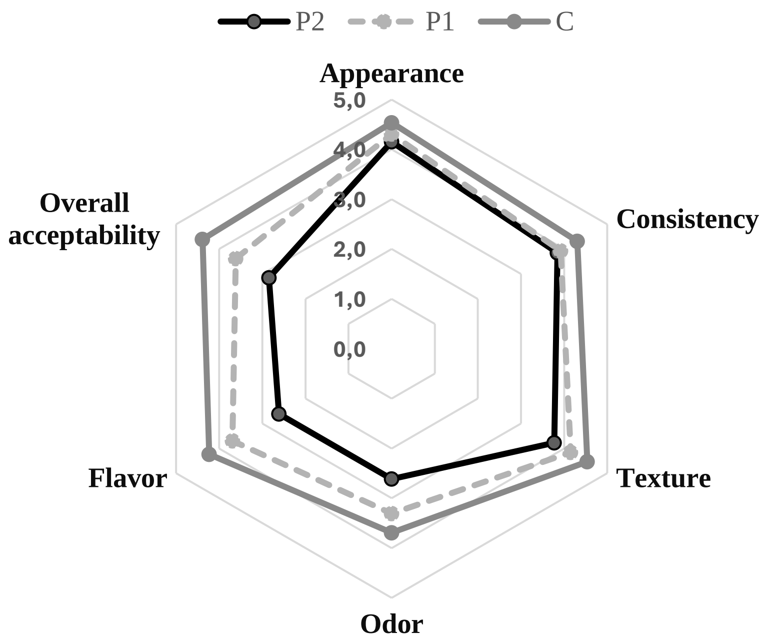

Figure 3: Sensory evaluation of kefir samples on the 1st day of production. P1: 0.5% fortified kefir, P2: 1% fortified kefir, C: Control

Kefir consumers may not be comfortable with a taste that is not like the normal, so the P1 sample, which is closer to control and has a higher level of general acceptability, was selected.

Consequently, the sensory results of two studies confirmed 0.5% propolis can be used in kefir fortification.

The present study is the first report comprehensively investigating the effect of kefir fortified with propolis on the physicochemical, microbiological, antioxidant activity, and total phenolic properties. Different studies previously conducted with different dairy products also support the results. In a study conducted with ice cream, it was recommended to add 0.6% of propolis by evaluating various ratios. Organoleptic and physicochemical parameters were not affected by these dosages, but they also allowed the enrichment of the product with biologically valuable components (Mironova vd., 2020).

In another study, when antioxidant activity analyzes and sensory analyzes were evaluated together, the ideal groups for the production of propolis-added milk products are groups containing 0.2% and 0.3% propolis, such as ice cream (Mehmetoğlu ve Tarakçi, 2023). The study showed that %0,5 propolis fortification did not extremely change the physicochemical properties as well as the microbial composition of kefir. In addition, propolis exhibited a prebiotic effect on lactococci. Even though there is no recommended daily allowance of propolis provided by food authorities, this amount was advised by the manufacturer for daily intake. The evidence from this study suggests that 0.5% propolis can be used to enhance the nutritional values of kefir.

Conclusion

Propolis, a substance protecting hives and is also known as “bee glue”, has many health promoting effect. Therefore, the consumption of Propolis has been recommended due to the content of several phenolic compounds, flavonoids, and terpenes. In this research, two healthy foods have been combined to increase health benefits of kefir. Consequently, production of kefir with propolis will provide consumers extra benefits.

References

Ali, A. M., Kunugi, H. 2021. "Propolis, bee honey, and their components protect against coronavirus disease 2019 (Covid-19): A review of in silico, in vitro, and clinical studies". Molecules, *26*(5). AOAC. 2016. "AOAC". Official Methods of Analysis. 17th ed. AOAC International, Washington, DC.

Bengoa, A. A., Iraporda, C., Garrote, G. L., Abraham, A. G. 2019. "Kefir micro-organisms: their role in grain assembly and health properties of fermented milk". Journal of Applied Microbiology, *126*(3), 686–700.

Berretta, A. A., Silveira, M. A. D., Cóndor Capcha, J. M., De Jong, D. 2020. "Propolis and its potential against SARS-CoV-2 infection mechanisms and COVID-19 disease: Running title: Propolis against SARS-CoV-2 infection and COVID-19". Biomedicine and Pharmacotherapy, *131*(June).

Bhargava, P., Mahanta, D., Kaul, A., Ishida, Y., Terao, K., Wadhwa, R., Kaul, S. C. 2021. "Experimental evidence for therapeutic potentials of propolis". Nutrients, *13*(8).

Chon, J.-W., Seo, K.-H., Oh, H., Jeong, D., Song, K.-Y. 2020. "Chemical and Organoleptic Properties of Some Dairy Products Supplemented with Various Concentration of Propolis: A Preliminary Study". Journal of Dairy Science and Biotechnology, *38*(2), 59–69.

de Lima, M. dos S. F., da Silva, R. A., da Silva, M. F., da Silva, P. A. B., Costa, R. M. P. B., Teixeira, J. A. C., … Cavalcanti, M. T. H. 2018. "Brazilian Kefir-Fermented Sheep's Milk, a Source of Antimicrobial and Antioxidant Peptides". Probiotics and Antimicrobial Proteins, *10*(3), 446–455.

Güneş-Bayır, A., Bilgin, M. G., Kutlu, S. S., Demirci, D., Gölgeci, F. N. 2020. "Microbiological, chemical and sensory analyzes of produced probiotic yoghurts added clove and propolis". Icontech International Journal, *4*(2), 1–14.

Gürsoy, O., Kocatürk, K., Güler Dal, H. Ö., Yakalı, H. N., Yılmaz, Y. 2020. "Physicochemical and Rheological Properties of Commercial Kefir Drinks". Akademik Gıda, *18*(4), 375–381.

Hong, J. Y., Lee, N. K., Yi, S. H., Hong, S. P., Paik, H. D. 2019. "Short communication: Physicochemical features and microbial community of milk kefir using a potential probiotic Saccharomyces cerevisiae KU200284". Journal of Dairy Science, *102*(12), 10845–10849.

Ilıkkan, Ö. K., Bağdat, E. Ş. 2021. "Comparison of bacterial and fungal biodiversity of Turkish kefir grains with high-throughput metagenomic analysis". Lwt, *152*(July).

Irigoiti, Y., Navarro, A., Yamul, D., Libonatti, C., Tabera, A., Basualdo, M. 2021. "The use of propolis as a functional food ingredient: A review". Trends in Food Science and Technology, *115*(June), 297–306.

Kazou, M., Grafakou, A., Tsakalidou, E., Georgalaki, M. 2021. "Zooming Into the Microbiota of Home-Made and Industrial Kefir Produced in Greece Using Classical Microbiological and Amplicon-Based Metagenomics Analyses". Frontiers in Microbiology, *12*(January), 1–17.

Mehmetoğlu, S., Tarakçi, Z. 2023.

"Investigation of The Physicochemical Properties of Propolis Added Ice Creams During Storage". Journal of Tekirdag Agricultural Faculty, *20*(2), 361–373.

Mironova, I. V., Galieva, Z. A., Konovalov, S., Bychkova, T. S., Baydan, D. V., Rozhkov, K. A. 2020. "Enrichment of milk ice cream with bee products". IOP Conference Series: Earth and Environmental Science, *613*(1).

Nadir Işık. 1979. "Gıdaların Duyusal Analizlerinde Temel İlkeler". Gıda, *4*(3).

Nurliyani, H. E., Sunarti. 2015. "Antidiabetic Potential of Kefir Combination from Goat Milk and Soy Milk in Rats Induced with Streptozotocin-Nicotinamide". Korean Journal for Food Science of Animal Resources, *35*(6), 847–858.

Özer, E. D. 2020. "Propolis and Potential Use in Food Products". Turkish Journal of Agriculture - Food Science and Technology, *8*(5), 1139–1144.

Ozkan, K., Sagcan, N., Ozulku, G., Sagdic, O., Toker, O. S., Muz, M. N. 2018. "Bioactive and bioaccessibility characteristics of honeybee pollens collected from different regions of Turkey". Journal of Food Measurement and Characterization, *12*(1), 581–587.

Özkök, A., Keskin, M., Tanuğur Samancı, A. E., Yorulmaz Önder, E., Takma, Ç. 2021. "Determination of antioxidant activity and phenolic compounds for basic standardization of Turkish propolis". Applied Biological Chemistry, *64*(1).

Pehlivan, A. D., Yadel, İ., Kılıç, N., Öztürk, H. İ. 2023. "The incorporation of Chlorella vulgaris and Chondrus crispus algae in the production of functional ayran drinks: effects on physicochemical, microbiological, and sensory characteristics". Journal of Food Measurement and Characterization, (0123456789).

Salar, R. K., Sharma, P., Purewal, S. S. 2015. "In vitro antioxidant and free radical scavenging activities of stem extract of Euphorbia trigona Miller". Tang [Humanitas Medicine], *5*(2), 14.1-14.6.

Sözeri Atik, D., Gürbüz, B., Bölük, E., Palabıyık, İ. 2021. "Development of vegan kefir fortified with Spirulina platensis". Food Bioscience, *42*(February), 40–46.

Sulmiyati, Said, N. S., Fahrodi, D. U., Malaka, R., Maruddin, F. 2019. "The physicochemical, microbiology, and sensory characteristics of Kefir Goat Milk with different levels of Kefir Grain". Tropical Animal Science Journal, *42*(2), 152–158.

Verce, M., De Vuyst, L., Weckx, S. 2019. "Shotgun metagenomics of a water kefir fermentation ecosystem reveals a novel Oenococcus species". Frontiers in Microbiology, *10*(MAR), 1–16.

Xue, M., Liu, Y., Xu, H., Zhou, Z., Ma, Y., Sun, T., … Liang, H. 2019. "Propolis modulates the gut microbiota and improves the intestinal mucosal barrier function in diabetic rats". Biomedicine and Pharmacotherapy, *118*(August), 109393.

Yilmaz-Ersan, L., Ozcan, T., Akpinar-Bayizit, A., Sahin, S. 2018. "Comparison of antioxidant capacity of cow and ewe milk kefirs". Journal of Dairy Science, *101*(5), 3788–3798.

Yirmibeşoğlu, S. S. S., Tefon Öztürk, B. E. 2020. "Comparing microbiological profiles, bioactivities, and physicochemical and sensory properties of donkey milk kefir and cow milk kefir". Turkish Journal of Veterinary and Animal Sciences, *44*, 774–781.