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RESEARCH PAPER

Assessment of propolis-fermented Kombucha tea's microbiological, physicochemical and sensory characteristics

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Abstract

The functional properties of fermented foods have a significant impact on human health. Kombucha tea is a functional food that provides numerous prophylactic and therapeutic benefits. This study investigated the effects of adding 0.5%, 1%, 1.5%, and 2% propolis to kombucha tea on the microbiological, physicochemical, and sensory characteristics during the 10th and 14th day incubation periods. The study concluded that adding propolis to Kombucha tea improved acidity, color, and total phenolic substance content ($p < 0.05$) but not pH value or dry matter amount ($p > 0.05$). As a result of yeast and mold analysis, no growth was detected on the 10th day of incubation, while the lowest yeast count was 4.08 log CFU/ml, and the highest was 5.46 log CFU/ml on the 14th day of incubation. The amount of propolis added to Kombucha tea was demonstrated to have a statistically significant effect on *Labtobacillus* spp., total aerobic mesophilic bacteria, and acetic acid bacteria ($p < 0.05$). *Streptococcaceae* species could not be detected in Kombucha tea. The panelists rated the most propolis-rich kombucha tea as being particularly enjoyable. As a result, physicochemical and microbiological analyses have shown that Kombucha tea can be fermented with propolis. Novel studies on the consumption of kombucha tea in combination with bee products should be performed.

Keywords

Fermentation, Functional beverage, Kombucha tea, Propolis

Introduction

Throughout human history, food has fascinated people (Henry 2010). Ecosystems, agriculture, and global health face threats due to causes such as the expanding global population and climate change (Patz et al. 2014). Consumers' growing concern for food and health has received attention in recent years (Paris et al. 2022). Functional foods can boost the immune system and positively impact human health. As a result, they are advantageous in preventing and managing diseases (Damián et al. 2022). Fermented meals are acknowledged for including advantageous microorganisms and the ability to offer health advantages. Fermented foods provide properties that contribute to their extended shelf life while also containing antioxidants that provide therapeutic and immunological advantages (Melini et al. 2019).

Kombucha tea is a beverage made by fermenting sweetened tea with yeast and acetic bacteria (Battikh et al. 2012). Yeast and bacteria that have collected in the cellulose layer offer fermentation. Kombucha tea, fermented from diverse types, is historically brewed mostly from sweetened black tea (Coelho et al. 2020). The flavor of fermented kombucha tea varies with fermentation time, but it has an a little sour, refreshing flavor similar to vinegar and apple cider (El-taher 2011). According to studies, ingesting kombucha tea inhibits cancer spread, lowers cholesterol and blood pressure, and enhances immunological and gastrointestinal functioning (Bergström 2018; Kaewkod et al. 2019; Hiremath et al. 2002). Kombucha tea possesses antioxidant, antidiabetic, anticancer, antibacterial, and probiotic effects. (Chakravorty et al. 2016; D'Angelo et al. 2017; Ivanišová et al. 2020; Bueno et al. 2021). Its qualities have lately piqued the interest of researchers as a functional beverage (Sinir et al. 2019).

Bee products have important effects on human health with bioactive components (Giampieri et al. 2022). Bees produce propolis from plants as a dark-colored component (Santos-Buelga and Paramás 2017). It has been reported that the chemical content of propolis contains 300 different components (Anjum et al. 2019). Bees use propolis to close holes in the hive, prevent airflow, and protect the hive from external threats (Pietta et al. 2002). Propolis, an intense biological activity, has recently been used in health care, food, and cosmetics (Lotfy 2006; de Groot 2013). The present study aimed to determine the effects of propolis on the fermentation of kombucha tea.

Materials and methods

Providing materials

The black tea from the local market (Lipton Turkey), sugar (Torku, Turkey), a commercially available kombucha fungus (Kombucça, Turkey), and water-soluble propolis (Fer, Turkey) were used to produce kombucha tea.

Preparation of Kombucha tea

The production of kombucha tea required the addition of 5 grams of black tea leaves to one liter of water that had been boiled before. That was followed by the addition of 100 g/L of sucrose, which was then incubated for ten minutes. Subsequently, the tea was filtered and allowed to reach room temperature before being served. After that, 100 ml of kombucha tea that had been fermented in previously, the kombucha tea fungus, and water-soluble propolis were added and incubated at a temperature of 28±1 °C for 10 and 14 days, respectively (Steinkraus et al. 1996; Essawet et al. 2015; Degirmencioglu et al. 2019). The propolis concentrations in the groups were prepared as the control group (K1), 0.5% propolis (K2), 1% propolis (K3), 1.5% propolis (K4), and 2% propolis (K5).

Determination of pH, titratable acidity, and total dry matter

The pH values of the obtained kombucha teas were monitored with a pre-calibrated pH measuring device (Testo, Australia), and the Association of Official Analytical Chemists (AOAC) method was used to test the titratable acidity and total dry matter (TA) (AOAC 2000).

Color measurement

Color measurements were analyzed by colorimeter (Konika Minolta, CR 400, Osaka, Japan) with the Hunter scale's lightness (L*), red/green (a*), and yellow/blue (b*) parameters.

Determination of total phenolic content

The total phenolic content of samples of Kombucha tea was assessed using the Folin-Ciocalteu method. 50 µl samples of each sample of kombucha tea were taken, and 2 mL of 2% sodium carbonate was added. The mixture was vortexed and allowed to incubate for two minutes. Folin reagent was added and vortexed once more in 100 µl. After 30 minutes of shaking incubation, it was placed in a quartz cuvette, and the absorbance was assessed using a UV–visible spectrophotometer the absorbance at 750 nm. The calibration curve converted the total phenolic content into gallic acid equivalents (GAE, mM) (Chu and Chen 2006).

Determination of antioxidant activity

The DPPH (2,2-difenil-1-pikrilhidrazil) test was used to measure the antioxidant activity of kombucha tea samples. This method consists of adding 0.1 ml of diluted sample extracts (20 µL-100 µL) and Trolox to 6 ml of DPPH (Sigma-Aldrich, USA) ethanol solution (Merck, Germany) (6×10^{-5} M), mixing in a vortex, and performing the reaction. It stabilized at room temperature in the dark after 30 minutes. Dark purple radical DPPH. After receiving a proton from the antioxidant, it forms the colorless α, α-diphenyl-β-picrylhydrazyl molecule. Due to antioxidant reduction, its color lightens (Kalkan et al. 2020).

Microbiological analysis

Kombucha tea samples were incubated for 5 days under aerobic conditions at 25 °C using Rose-Bengal (RB) (Oxoid, CM0549) chloramphenicol (Oxoid, SR0078E) agar for yeast and mold isolation (Degirmencioglu et al. 2019). For the isolation of *Lactobacillus* species, Man Rogasa and Sharpe Agar (MRS) (Difco, 288210) were incubated at 37 °C for 5 days in anaerobic conditions (Akarca and Tomar 2020). Plate Count Agar (PCA) (Oxoid, CM0325) was used for the isolation of total mesophilic aerobic bacteria, and it was cultured for 5 days at 37 °C under aerobic conditions (Akarca and Tomar 2020). To enumerate acetic acid bacteria (AAB), yeast extract peptone mannitol agar (YPM) reported by Gullo and Giudici (2008) was used. For that purpose, 0.5% yeast extract (LP0021B, Oxoid), 0.3% peptone (LP0037, Oxoid), 2.5% mannitol (M4125, Sigma-Aldrich), and 1.2% agar (LP0011, Oxoid) were combined to prepare YPM agar. Spread plate inoculations were prepared on the prepared media and cultured for 5 days at 30 °C under aerobic conditions. (Sengun and

Table 1. Results of the pH, titratable acidity, and total dry matter in kombucha tea fermented with various amounts of propolis.

Group	pH		Titratable acidity (g/L)		Total solid matter (%)	
	10th day	14th day	10th day	14th day	10th day	14th day
K1	2.75±0.11^{Aa}	2.75±0.15^{Aa}	8.50±0.10^{Bb}	10.03±0.03^{Ca}	1.00±0.01^{ABb}	1.02±0.00^{Aa}
K2	2.78±0.14^{Aa}	2.73±0.14^{Aa}	8.54±0.10^{Bb}	9.95±0.05^{Ca}	1.01±0.01^{ABb}	1.04±0.00^{Aa}
K3	2.74±0.16^{Aa}	2.70±0.12^{Aa}	8.33±0.10^{Cb}	9.90±0.21^{Ca}	1.02±0.02^{Aa}	1.04±0.02^{Aa}
K4	2.73±0.15^{Aa}	2.71±0.20^{Aa}	8.76±0.07^{Ab}	10.3±0.05^{Ba}	1.02±0.01^{Aa}	1.03±0.01^{Aa}
K5	2.75±0.17^{Aa}	2.71±0.21^{Aa}	8.20±0.01^{Db}	11.2±0.21^{Aa}	1.01±0.01^{Aa}	1.03±0.02^{Aa}

^{a,b,c} Differences between days shown with different letters on the same line are significant ($p < 0.05$). ^{A,B,C}Differences between groups indicated by different letters in the same column are significant($p < 0.05$).
K1: Control; K2: 0.5% propolis; K3: 1% propolis; K4: 1.5% propolis; K5: 2% propolis.

Karabiyikli 2011). To isolate *Streptococcaceae* species, M-17 agar (Oxoid, CM785) was used and incubated at 37 °C aerobic conditions for 3 days (Hansen 2002).

Sensory evaluation

Sensory evaluation was carried out with ten panelists (from 25 to 55 years of age, 5 women and 5 men) in good health. Flavor, odor, sourness, color, and overall acceptability of prepared kombucha teas were evaluated using a 1–5 hedonic scale (5 points: excellent, 4 points: good, 3 points: acceptable, 2 points: not sufficient, 1 point: not good) (Degirmencoglu et al. 2019).

Statistical analysis

In statistical analyses, the significance level was taken as $p < 0.05$, and the differences in kombucha tea samples were performed with the Minitab Statistical Software v.16 programs (Minitab Inc., State College, PA, USA) using analysis of variance (ANOVA).

Results and discussion

pH, titratable acidity, and total dry matter

The pH values obtained on the 10th and 14th days of kombucha fermentation are shown in Table 1. According to the study of Sinir et al. (2019), the pH value of kombucha tea should be 2.5 < pH < 4.2. In this study, similar results were obtained from the groups. In a study conducted to determine the antimicrobial activity of kombucha tea, the pH value of kombucha tea was determined as 2.5, which is similar to this study (Greenwalt et al. 2000). In a similar study evaluating the biochemistry of fermented kombucha tea, the pH values of kombucha teas obtained using black tea and green tea were determined as 2.7 and 2.6, respectively. This means that kombucha tea is considered a safe food because the growth of microorganisms is restricted (Sengun and Kirmizigul 2020).

The fermentation process for kombucha is carried out at 28 °C in an aerobic condition, and it ends with a flavor that is slightly acidic and similar to cider (Topuz et al. 2017). Table 1 shows the titratable acidity values of kombucha tea at the 10th and 14th days of fermentation. In similar studies, the acidity of kombucha tea was reported to be around 8 g/L on the 10th fermentation day (Chen and Liu 2000; Loncar et al. 2006; Kallel et al. 2012; Velicanski et al. 2014). The 14th day of fermentation was shown to have greater acidity levels than these studies in this study. A study on the effects of the Kombucha tea fermentation period found that the kombucha tea's acidity level was approximately 12 g/L on the 15th day of fermentation and between 15 g/L and 16 g/L on the 21st day of fermentation (Degirmencioglu et al. 2019). It can be evaluated that its acidity will increase as the fermentation time increases in kombucha tea fermentation.

The total solid matter values obtained on the 10th and 14th days of kombucha fermentation are shown in Table 1. The dry matter content of kombucha teas prepared using green tea and caper buds as the tea source was evaluated. Dry matter for kombucha tea produced with green tea was 0.88%, dry matter for kombucha tea made with caper bud was 1.11%, and dry matter for kombucha tea prepared with both green tea and caper bud was 1.14% (Giritlioglu et al. 2020). This study found that the propolis addition did not significantly affect the dry matter value of Kombucha tea.

Color analysis

The process of kombucha tea fermentation involves enzymatic activity by yeasts and bacteria, which has been claimed to result in color changes due to the biotransformation of different phytochemical compounds (Haslam 2003). The color parameter values obtained in this study are shown in Table 2. Studies by different researchers show that kombucha tea has L*, b*, and a* values at various rates (Abuduaibifu 2019; Giritlioglu et al. 2020). Compared to this study's findings, Kombucha tea's substrate may change the color characteristics based on the product added and the fermentation period.

Total phenolic content

Phenolic compounds are naturally found in plants containing hydroxyl groups and aromatic rings (Giritlioglu et al. 2020). Microorganisms use phenolic compounds

Table 2. Results of the L*, b*, and a* values in kombucha tea fermented with various amounts of propolis.

Group	L* value		a* value		b* value	
	10th day	14th day	10th day	14th day	10th day	14th day
K1	23.52±0.42[Ab]	32.85±0.12[Aa]	7.57±0.05[Ab]	10.64±0.13[Aa]	7.29±0.31[Ab]	18.38±0.26[Aa]
K2	21.07±0.02[Bb]	31.53±0.25[Ba]	6.19±0.18[Cb]	8.85±0.10[Ba]	5.37±0.38[Bb]	10.32±0.27[Ba]
K3	15.51±0.31[Cb]	31.04±0.01[Ca]	6.62±0.11[Bb]	8.57±0.12[Ca]	4.56±0.10[Cb]	10.26±0.29[Ba]
K4	14.49±0.40[Db]	30.63±0.15[Da]	4.58±0.01[Db]	7.81±0.00[Da]	3.53±0.28[Db]	8.28±0.24[Ca]
K5	12.38±0.35[Eb]	22.81±0.34[Ea]	4.38±0.02[Eb]	5.13±0.29[Ea]	2.36±0.25[Eb]	6.26±0.02[Da]

[a,b,c] Differences between days shown with different letters on the same line are significant ($p < 0.05$). [A,B,C]Differences between groups indicated by different letters in the same column are significant($p < 0.05$).
K1: Control; K2: 0.5% propolis; K3: 1% propolis; K4: 1.5% propolis; K5: 2% propolis.

in tea leaves and other ingredients in kombucha tea to transform bound phenolic compounds into free phenolic compounds. This positively impacts the product's sensory quality and health effects (Degirmencioglu et al. 2019). Table 3 shows kombucha tea's total phenolic content values (mg GAE/L) on fermentation's 10th and 14th days. The total phenolic content of the Kombucha teas obtained by using black, white, and green teas was reported as 228.4 mg GAE/L, 736.1 mg GAE/L, 461.7 mg GAE/L, respectively (Topuz et al. 2017). The total phenolic content of kombucha tea produced using black tea is 574.6 mg GAE/L (Velicanski et al. 2014). According to Akarca and Tomar (2020), kombucha tea produced from red carrots and red beetroot contained total phenolic contents of 206.12 mg GAE/L and 241.19 mg GAE/L, respectively. The total phenolic content of kombucha tea made with black tea, green tea, and echinacea tea was reported by Ozyurt (2020) to be 472.09 mg GAE/L, 312.26 mg GAE/L, and 279.29 mg GAE/L, respectively. In contrast to prior investigations, Kallel et al. (2012) found higher values of total phenolic content in black tea-derived kombucha tea (1.120 mg GAE/L) and green tea-derived kombucha (1.080 mg GAE/L). A cellulose biofilm called SCOBY comprises symbiotic bacteria and yeasts (Laavanya et al. 2021). The total phenolic component was increased by SCOBY (Rahmani et al. 2019). The phenolic content of kombucha tea is also influenced by the climatic conditions in which the tea plant is grown, its stage of maturation, and the processes used in its production (Topuz et al. 2017). In the current study, it was determined that the addition of propolis to kombucha tea significantly increased the content of phenolic substances.

Table 3. Results of total phenolic content in kombucha tea fermentation with various amounts of propolis.

Group	Total phenolic content (mg GAE/L)	
	10th day	14th day
K1	1,264.20±0.00[Da]	1,015.23±0.00[Eb]
K2	1,326.55±0.00[Ca]	1,240.98±0.00[Db]
K3	1,620.24±0.00[Ba]	1,376.43±0.00[Cb]
K4	1,157.13±0.00[Eb]	1,941.02±0.00[Aa]
K5	1,643.89±0.00[Ab]	1,726.02±0.00[Ba]

[a,b,c] Differences between days shown with different letters on the same line are significant ($p < 0.05$). [A,B,C]Differences between groups indicated by different letters in the same column are significant($p < 0.05$).
K1: Control; K2: 0.5% propolis; K3: 1% propolis; K4: 1.5% propolis; K5: 2% propolis.

Microbiological analysis

The process of fermenting Kombucha tea involves the utilization of a medium containing tea extract, a source of sugar, and a Symbiotic Culture of Bacteria and Yeast (SCOBY) (Leal et al. 2018; Antolak et al. 2021). The SCOBY structure contains an extensive microbial population, mainly consisting of *Gluconobacter*, *Acetobacter*, *Zygosaccharomyces*, *Saccharomyces*, and *Schizosaccharomyces* (Jayabalan et al. 2014). In addition to the mentioned microorganisms, various microorganisms may be present in SCOBY, depending on the production and geographical conditions (Mukadam et al. 2016). Table 4 indicates the findings of the microorganism analysis conducted in the present study. *Streptococcaceae* species could not be detected in Kombucha tea. In studies from Turkey, Degirmencioglu et al. (2019) determined the level of *Lactobacillus* spp. lower than this study, Şarkaya et al. (2021) reported a higher level of *Lactobacillus* spp. in the current study. The yeast count of black tea kombucha was reported as 5.92 log CFU/ml on the 10th of fermentation lower than in this study (Noronha et al. 2022). Noronha et al. (2022) reported a reduction trend of black tea kombucha during fermentation. The decrease in available nutrients

Table 4. Results of microbiological analysis in kombucha tea fermentation with various amounts of propolis.

Group	Acetic acid bacteria (log_{10} CFU/ml)		*Lactobacillus* spp. (log_{10} CFU/ml)	
	10th day	14th day	10th day	14th day
K1	5.48±0.00[Db]	5.51±0.03[Ca]	6.11±0.71[ABa]	6.56±0.25[Aa]
K2	5.46±0.00[Eb]	5.75±0.28[Ba]	5.81±0.10[Bb]	6.02±0.00[ABa]
K3	5.74±0.00[Cb]	6.43±0.10[Aa]	5.65±0.06[Bb]	5.84±0.03[ABa]
K4	5.79±0.00[Bb]	6.52±0.07[Aa]	5.40±0.57[Ba]	5.51±0.27[Ba]
K5	5.86±0.00[Ab]	6.61±0.00[Aa]	6.62±0.19[Aa]	6.41±1.13[ABa]
Group	**Yeast and Mold (log_{10} CFU/ml)**		**TMAB (log_{10} CFU/ml)**	
	10th day	**14th day**	**10th day**	**14th day**
K1	ND	5.40±0.00[Ba]	5.95±0.77[Aa]	5.75±0.25[ABa]
K2	ND	4.08±0.00[Ea]	6.00±0.45[Aa]	5.37±0.74[ABa]
K3	ND	4.78±0.00[Ca]	5.42±0.27[Aa]	5.42±0.00[ABa]
K4	ND	4.53±0.00[Da]	5.56±0.24[Aa]	5.14±0.11[Bb]
K5	ND	5.46±0.00[Aa]	5.64±0.13[Aa]	6.03±0.63[Aa]

[a,b,c] Differences between days shown with different letters on the same line are significant ($p < 0.05$). [A,B,C]Differences between groups indicated by different letters in the same column are significant($p < 0.05$).
K1: Control; K2: 0.5% propolis; K3: 1% propolis; K4: 1.5% propolis; K5: 2% propolis.

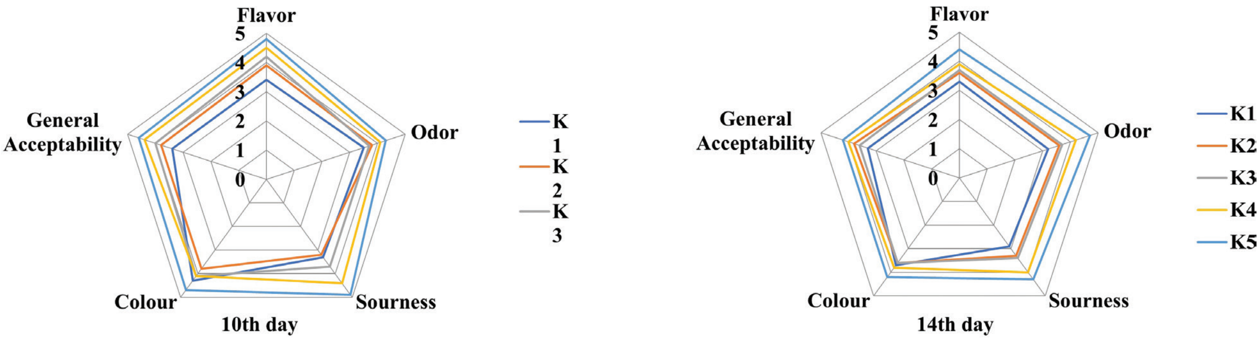

Figure 1. Sensory evaluation of kombucha tea on the 10th and 14th day of fermentation. K1: Control; K2: 0.5% propolis; K3: 1% propolis; K4: 1.5% propolis; K5: 2% propolis.

in the medium may result in the noted reduction (Tran et al. 2020). In the current study, AAB significantly increased as propolis quantity increased. Propolis shows beneficial effects on AAB, according to existing evidence. In the study conducted by Neffe-Skocińska et al. (2017), it was observed that the yeast and AAB counts in kombucha were higher, exceeding 7 log CFU/ml.

Sensory evaluation

Kombucha tea samples obtained in the study had a slightly sour, vinegar-like taste and a foamy appearance. The study's sensory analysis findings indicate increased in propolis content on the 10th and 14th days of fermentation resulted in higher scores for taste, aroma, sourness, color, and overall acceptability. The sensory evaluation scores of kombucha teas on the 10th day were found to be higher and well-received. The addition of propolis to kombucha tea has been noted to impact sensory quality favorably (Fig. 1). A study on kombucha tea using black, green, and white tea indicated that the kombucha tea produced from black tea was the most preferred (Kalkan et al. 2020). In an additional investigation, the combination of *Capparis* spp. and green tea used for making kombucha tea was found to be more favorably received by consumers (Giritlioglu et al. 2020). According to the results of the sensory analysis conducted on kombucha teas derived from black tea, red goji berry, and black goji berry, it was found that the kombucha tea made with black goji berry was the most favored among the participants (Abuduaibifu 2019). The acceptability of kombucha tea production may be improved by using different ingredients instead of the conventional method.

Conclusion

The results of physicochemical and microbiological analyses indicate that propolis can be used to ferment kombucha tea. A probiotic beverage with increased phenolic content has been obtained as a health-beneficial alternative to traditional tea consumption. Further research should be conducted on the potential effects of consuming kombucha tea combined with bee-derived products.

Conflict of competing interest

The authors have stated no conflicts of interest.

Authors' contributions

The experiments were conceived and planned by EK. EK. and RTB conducted the experiments. EK and RTB conceived and executed the simulations. RBT and EK contributed to the interpretation of the results. EK managed the composition of the manuscript. All authors provided constructive feedback and contributed to developing the research, analysis, and manuscript.

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