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Characterization of Rosemary (*Salvia rosmarinus*) Essential Oil Obtained by Solvent-Free Microwave Extraction with Kombucha Tea *(Anthriscus sylvestris* L.) Produced by Adding Guava (*Psidium guajava* L.) Peel and Pulp

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ABSTRACT
The study aimed to evaluate the potential of creating a functional beverage by enriching *Anthriscus sylvestris* (L.) kombucha production with guava peel and pulp, showcasing the variety and richness of the product formulations. This was done due to kombucha being a fermented beverage rich in various bioactive compounds that have significant effects on health and the possibility of enhancing product diversity through different formulations. Thus, the kombucha tea was physicochemical, phytochemical, antioxidant activity, and sensory evaluation during the 15-day fermentation period. The increased acetic acid bacteria and yeast count indicates that *Anthriscus sylvestris* is a viable substrate for the proliferation of Kombucha symbiotic microbes. Adding guava peel and rosemary oil to kombucha significantly increased its total phenolic, flavonoid, and vitamin C content ($P<0.05$). *Anthriscus sylvestris*-guava kombucha has antibacterial properties (zones of inhibition of 18.32 and 17.31 mm against *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922, respectively). Moreover, it also provided an inhibitory effect on α-glucosidase and α-amylase, enzymes associated with diabetes. The DPPH, ABTS^{*+}, FRAP, CUPRAC, and ORAC capacities were found to be 66.89-78.90%, 70.83-97.25%, 367-723.15 µmol Trolox/mL, 381.40-460.45 µmol Trolox/mL, and 486.50-737.50 µmol Trolox/mL, respectively. *Anthriscus sylvestris* kombucha has well-balanced and pleasant sensory properties. These findings indicate that preparing *Anthriscus sylvestris* kombucha tea with guava may have health effects and that further research is needed to determine potential health benefits.

Keywords: Kombucha, *Anthriscus sylvestris*, α-glucosidase, Guava peel and pulp, Rosemary essential oil, Functional beverage

1. Introduction

Kombucha is a beneficial beverage created by fermenting dried green tea (*Camellia sinensis*) and sugar with a symbiotic culture of yeast and bacteria called SCOBY, also referred to as "fungi tea" or "tea fungus" (Kitwetcharoen et al. 2023). Kombucha's phenolic and flavonoid content, antioxidant activity, antibacterial properties, and anti-diabetic effects have all been extensively studied (Morales 2020). These beverages include bioactive substances that can block enzymes involved in glucose digestion, potentially benefiting diabetics (Gao et al. 2013; Permatasari et al. 2021; Oliveira et al. 2023). Diversifying kombucha fermentation substrates entails using a variety of ingredients, such as fruits, vegetables, herbs, spices, tea blends, coconut water, honey, coffee, and alternative sweeteners, to create distinct and flavorful kombucha variations with potential health benefits (de Miranda et al. 2022). *Anthriscus sylvestris* (L.) Hoffm. is commonly referred to as wild chervil or cow parsley and is part of the Anthriscus genus (Umbelliferae). It can be found primarily across Asia, Europe, North America, Africa, and New Zealand (Olaru et al. 2015; Liu et al. 2023). Also recognized by the names wild chervil or cow parsley, this plant is classified under the Scandiceae tribe within the Apiaceae family (Olaru et al. 2016; Janković et al. 2024). Additionally, *Anthriscus sylvestris* is a significant source of lignan compounds, particularly highlighted in the fields of pharmacy and nutrition following the identification of the aryl tetralin lignan podophyllotoxin (Koulman et al. 2001; Berežni et al. 2024). Utilized in traditional medicine, *Anthriscus sylvestris* is recognized for its antibiotic, soothing diuretic, and cough-relieving properties (Chen et al. 2014).

Rosemary (*Rosmarinus officinalis* L.) represents a significant industrial crop within the realm of medicinal and aromatic vegetation (Sakar et al. 2023). The polyphenols derived from rosemary have garnered noteworthy attention due to their conspicuous anti-inflammatory properties, including the inhibition of inflammatory cell infiltration, suppression of inflammation-associated signalling pathways, reduction in the synthesis of inflammatory cytokines, and modulation of the

composition of intestinal microbiota (Mengoni et al. 2011; Li et al. 2016; Xia et al. 2017; He et al. 2022; Du et al. 2023; Zhang & Lu 2024). Notably, rosemary essential oil finds widespread utility across various sectors, particularly in food, phytomedicines, nutraceuticals, cosmetics, agrochemicals, and aromatherapeutic formulations (Martin-Piñero et al. 2020; Araya et al. 2022; Song et al. 2023).

The demand for kombucha as a functional beverage is rapidly growing due to its exceptional nutritional properties, which boost product diversity (Kim & Adhikari 2020). Guava (*Psidium guajava* L.) fruits are a sustainable supply of antioxidant compounds, polyphenols, and colorants, as well as raw protein, fiber, carbs, vitamin C, potassium, calcium, and phosphorus (Kamath et al. 2008; Kaur & Ghosh 2023). Guava has been proven to reduce the risk of cancer in body parts such as the abdomen (Chakrabortya & Athmaselvi 2014; Ninga et al. 2018). Many pharmacological investigations have shown that guava contains antioxidant, hepatoprotective, antibacterial, antidiabetic, anti-inflammatory, and anticancer properties, which support its traditional usage (Jaiarj et al. 1999; Oh et al. 2005; Burgin et al. 2010; Correa & Couto 2016; König et al. 2019; Zou & Liu 2023). Because of its biological qualities, guava fruit can also be used to make kombucha.

This study looked into *Anthriscus sylvestris* (L.)-based kombucha with guava pulp and peel, as well as rosemary essential oil. This study sought to identify a viable functional kombucha beverage, thereby growing the food market by introducing items with superior qualities.

2. Material and Methods

2.1. Materials

Anthriscus sylvestris (L.) plants were hand-picked in the Erzurum province of Turkey in May 2023, when the plants had grown to a height of 10 cm. Identification of the *Anthriscus sylvestris* L. was done at Department of Biology, Faculty of Science and Arts, Erzincan Binali Yıldırım University, Erzincan, Turkey. The kombucha starting cultures, SCOBY from medical companies (CENTRAGEN Laboratory Products Informatics and Consultancy Company), Guava (*Psidium guajava* L.), and Rosemary (*Salvia rosmarinus*) were ordered from an online purchasing site via the internet. Clinical isolates of pathogenic bacteria (*Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATTC 27853) were obtained from the Faculty of Medicine Microbiology Laboratory at Erzincan Binali Yıldırım University.

Figure 1- The Solvent-free microwave extraction (SFME)

2.2. Methods

2.2.1. The extraction of Rosemary (Salvia rosmarinus) essential oil

The microwave extraction system (Ethos X) was used for the extraction. Rosemary (*Salvia rosmarinus*) was placed in a Pyrex disc and exposed to microwave irradiation at 622 W (50% power) for 30 minutes at 2450 MHz. No solvent or water was added. The Clevenger apparatus was positioned through an aperture on the top of the microwave device. The opening around the neck

of this apparatus is sealed with polytetrafluoroethylene to prevent microwave leakage. This procedure is carried out under atmospheric pressure. Vapour generated during the process was condensed in an external condenser. Essential oil and water were separated by decantation. Anhydrous sodium sulfate was used to dry the rosemary essential oil (REO), which was stored in amber-capped vials at 4 °C. The Solvent-free microwave extraction (SFME) is given in Figure 1.

2.2.2. Preparation of kombucha

The washed *Anthriscus sylvestris* (L.) plant samples were dried at 50 ºC and 1 m/s air flow rate. The drying process was performed until the moisture content of the plant leaves reached to approximately <7% (w/w). The dried plant leaves were then packed and stored at 4 ºC until analysis. The pulp and the seeds were separated from the peel by cutting. The guava peel or pulp was individually dried in a household microwave oven (Arçelik, ARMD580, Turkey) for 16 hours at 55 °C until it reached approximately 10±2% moisture content. A blender (Warning Commercial, USA) was used for ground and sieved (355 - µm mesh). The powders were stored at 4 ºC until analysis. After drying, the materials were crushed and mixed with hot water at a concentration of 10 g L^{-1} for 20 min. The tea broth was then sweetened with 6% (w/w) sugar. The produced tea samples were transferred to glass jars and pasteurization at 90 °C for 20 min. Based on the literature review and preliminary tests, once the beverages reached room temperature, we incorporated 10% SCOBY (w/v) and 1% REO. The codes and descriptions for various Kombucha samples are given in Table 1. Samples were analyzed on days 0, 5, 10, and 15 of fermentation.

Table 1- Coding and descriptions for various kombucha samples

Samples	***Descriptions***
ASK1	Control kombucha (no Guava peel/pulp, and no REO*)
ASK2	Kombucha with guava pulp
ASK3	Kombucha with guava peel
ASK4	Kombucha with guava pulp and 1% REO
ASK5	Kombucha with guava peel and 1% REO

*: REO: Rosemary essential oil

2.2.3. pH, titrable acidity, and color analyses

pH values using a pH meter (Eutech PH 150 Model), and the total acidity using the AOAC method (AOAC, 2000) were determined. The color was measured on a scale of *L**, *a**, and *b** using a HunterLab (Colorflex-EZ, Hunterlab, Virginia, USA) (Varela-Santos et al. 2012).

2.2.4. Acetic acid bacteria and yeast count

Microbial research included the detection of acetic acid bacteria (AAB) and yeast counts. While counting acetic acid bacteria in samples, dilutions of Glucose Yeast Extract Calcium Carbonate Agar (GYC, 10% glucose, 1% yeast extract, 2% calcium carbonate, 1.5% agar, pH 6.8±0.2) were inoculated onto the medium using the spread plate method and the petri dishes were placed at 30 °C. It was incubated at 30 °C for 5-10 days. Following the incubation period the colonies with a cream color and a clean sedimentation zone around the petri dishes were measured as typical AAB colonies (De Vero et al. 2006). To count the yeast, 10 mL of kombucha was mixed with 0.1% (w/v) peptone water and seeded on Dichloran Rose Bengal Chloramphenicol (DRBC) agar by the spreading method. The DRBC plates were then incubated at 25 °C for five days (AOAC 2000).

2.2.5. Analysis of antioxidant activity

Antioxidant capacity was found using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH), 2,20-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt ($ABTS^{\bullet+}$), ferric ion reducing potency method (FRAP), copper-reducing antioxidant activity (CUPRAC), and oxygen radical absorption capacity (ORAC) methods. The DPPH assay was performed according to the method used by Mazidi et al. (2012) and Asl et al. (2018), with some changes. In brief, 0.2 mL of beverage was combined with 0.8 mL of methanol and a stock solution with volumes ranging from 0.0125 to 0.2 mL/mL was prepared. Following that, 0.5 ml of the diluted sample was mixed with 1.5 mL of DPPH solution (0.1 mM). It was incubated for about an hour at room temperature in a dark atmosphere, with absorbance measurements obtained at 517 nm. The inhibitory activity was calculated using the equation:

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

The $ABTS^{\bullet+}$ value of samples was assessed according to the method proposed by Miller et al. (1993). Peroxidase, $ABTS^{\bullet+}$ and hydrogen peroxide solutions were prepared using a buffer solution of sodium phosphate (5 mM, pH 7.6). $ABTS^{\bullet+}$ was prepared by mixing 0.3 mL peroxidase and 0.3 mL $ABTS^{\bullet+}$ with 0.4 mL H_2O_2 and incubating for approximately 30 min. in a dark environment. Then, 0.01 mL of the beverage samples was added to this solution and incubated for approximately 10 minutes $ABTS^{\bullet+}$ values were calculated by reading the absorbance at 734 nm.

The FRAP values of beverages were found using the method specified by Benzie & Strain (1999). In this method, 100 µL of sample and 900 µL of FRAP solution were added into the test tubes. The tubes were incubated for approximately 4 minutes, and their absorbance was measured at 593 nm.

The CUPRAC method involves mixing 100 µL of sample, 3 mL of CUPRAC reagent, and 1 mL of pure water. The CUPRAC solution was made from $CuCl_2$, neocuproine, and $NH_4C_2H_3O_2$. These substances are blended in equal quantities. After 30 minutes of incubation, absorbance values were measured at 450 nm using a spectrophotometer (R^2=0.9981; Apak et al. 2004).

The ORAC values of the beverages were found using the method described by Chusak et al. (2014). For analysis, the drinks were first diluted and incubated with 4.8 nM sodium fluorescein in a 75 mM PBS solution at 37 °C using a 0.1 M phosphate buffered solution for approximately 10 minutes. After incubation, 25 µL of 64 mM AAPH was added to this mixture. Spectrofluorometric readings were made using the ORAC-Florescein method at 485-520 nm.

2.2.6. Total phenolic content (TPC)

The phenolic content of the beverages was assessed using the method of Somsong et al. (2017). First, 2.4 mL of the beverage sample was diluted 10 times with pure water. Then, 150 µL of Folin Ciocalteu reagent prepared in pure water (1:10) was added and mixed for approximately 2 minutes. 7.5% (w/v) Na_2CO_3 was added and incubated at room temperature for approximately 2 hours, and the absorbance was read at 765 nm.

2.2.7. Total flavonoid content (TFC)

Flavonoids in beverages, Ahlem Rebaya et al. (2015) stated, were made according to the method. In this analysis, catechin was used at rates of 0.01-0.1 mg/mL. 25 µL of standard was added to 15 µL of 5% $NaNO_2$ solution, and after approximately 6 minutes, 30 µL of aluminum trichloride (10%) was added and incubated for 5 minutes. Then, 150 µL of NaOH was added, and after incubation for approximately 15 minutes, the color turned to a liquid pink. After this, absorbance values were read at 510 nm with a microplate reader.

2.2.8. Vitamin C extraction and determination

The ascorbic acid values of beverages were determined using the method of Zhao and Shah (2014). For this, 1 g of sample was thoroughly dissolved in 20 mL of 80% methanol, adding 0.01% trifluoroacetic acid (TFA), and agitated at 200 rpm at 50 °C for approximately 4 hours. The extract was then centrifuged at 4500 g for approximately 5 minutes. Using a rotary evaporator, the extract was evaporated at 45 °C and redissolved in 5 mL of 80% methanol (v/v) to determine vitamin C, α-glycosidase, and α-amylase inhibition activities.

The acetic acid concentration was found using the method given by Li et al. (2017). The examination of acetic acid content was performed using an Agilent 1200 HPLC system equipped with a UV detector. The separation was done using a Waters Atlantis T3 column (4.6 mm×250 mm, 5 µm) at 35 °C. The mobile phase included 0.02 mol/L NaH_2PO_4 (pH 2.7). After injecting 10 µL of the supernatant into the column. The organic acids were evacuated with a phase that moved at a flow rate of 0.9 mL/min and monitored at 210 nm. Calibration curves for the pure form containing these acids were created and assessed.

2.2.9. α-glucosidase and α-amylase inhibition activities

To test α-glucosidase inhibition, combine 200 µL of extracts or 80% methanol (control) with 100 µL of 1.0 U/mL α-glucosidase solution in 0.1 M phosphate buffer (pH of 6.9) and incubate at 37 °C for 10 min. After adding 200 µL of pNPG and incubation for 10 minutes. The action ended with 1 M Na_2CO_3, and the absorbance's were obtained at 405 nm. The initial absorbance was eliminated from both the sample and the control absorption values.

To test α-amylase inhibition in beverages, 250 µL of phenolic was dissolved by 250 µL of α-amylase in 20 mM sodium phosphate buffer (pH 6.9) and kept at 37 °C for ten minutes. Following that, 250 µL of a solution containing 1% starch was included and incubated at 37 °C for roughly ten minutes. 500 µL of 3, 5-dinitrosalicylic acid was supplied. The blend was left in boiling water for 5 minutes. After being cooled to ambient temperatures, absorbance was detected at 540 nm.

2.2.10. Antimicrobial activity

The antibacterial effect of beverages was determined using the agar well diffusion method against *Enterococcus faecalis* ATCC 29212, *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATTC 27853. A sterile copper tube was used to create 6 mm-diameter wells on Mueller-Hinton agar plates. A sterile cotton swab was used to evenly distribute bacterial solutions containing 10^6 cfu mL^{-1}.

To produce a sterile residual of fermented kombucha, the resulting infusions were centrifuged and passed through a 0.22 µm sterilized microfilter. After adding 100 µL of each sample to the wells, the plates were kept at 4 °C for 2 hours to allow pre-diffusion into the agar. The plates were then left to incubate at 37 °C for a total of 18 hours. Kanamycin (50 µg/well) was used as positive guidance, while distilled water acted as the negative standard (Khazi et al. 2024).

2.2.11. Sensory analysis

Participants used a hedonic scale to rate the color, appearance, aroma, flavor, sourness, and overall acceptability of kombucha drinks. The scale ranged from one (severe dislike) to five (great liking). The sensory acceptability tests were done by panellists of 15 untrained judges of both sexes (9 women and 6 men).

2.2.12. Statistical analysis

A factorial experimental design was used for the research trial, which included five applications and four fermentation days (0^{th}, 5^{th}, 10^{th}, and 15^{th} days). The acquired data were subjected to variance analyses, and Duncan's multiple comparison tests were used to determine the significant veracity with SPSS software package version 25.0.

3. Results and Discussion

3.1. pH and acidity

pH of kombucha is critical for determining its microbiological safety because of the antibacterial effects caused by the buildup of organic acids (Villarreal-Soto et al. 2019). In the current study, pH values taken during fermentation days showed a progressive drop along with the formation of acetic acid (Table 2). The pH of kombucha samples fluctuated significantly ($P<0.05$). The highest pH value of the samples (3.47±0.04) was observed in the ASK1 sample on the 0th day of fermentation, while the lowest value (2.37±0.04) was found in the ASK4 sample on the 15^{th} day of fermentation. Furthermore, in samples containing especially plain guava pulp and peel with added rosemary essential oil, a decrease in pH values has been observed. The mean pH values for ASK1, ASK2, ASK3, ASK4, and ASK5 were 3.07±0.36, 2.82±0.29, 2.86±0.27, 2.74±0.26, and 2.85±0.33, respectively. Yavari et al. (2011) and Ayed & Hamdi (2015) both reported similar results.

According to the findings in Table 2, the initial total acidity values of samples increased during fermentation, particularly acutely until the 15^{th} day. The concentration of acetic acid in *Anthriscus sylvestris* (L.) kombucha samples differed by 1.27% and 10.65%, respectively. The acetic acid values of the samples were found to be statistically significant both in terms of the fermentation duration and the treatments applied ($P<0.05$). The acidity value has increased in all samples depending on the fermentation time. Particularly, the highest values were observed on the 15^{th} day. Additionally, among the samples, the highest acidity value on the 15^{th} day (10.65±0.91%) was found in the ASK4 sample. The results may have been influenced by the presence of pulp and rosemary essential oil. Zubaidah et al. (2018) fermented the snake fruit juices they produced using kombucha consortium for 14 days. The acidity of the beverages ranged from 0.92 to 1.65%. These study results outperformed those of the aforementioned researchers.

3.2. Acetic acid bacteria and yeast counts

Table 2 shows the numbers of acetic acid bacteria and yeast. Following inoculation, there were substantial differences in the numbers of yeast and acetic acid bacteria between the samples. This variation could be related to the use of three different symbiotic bacterium and yeast cultures (SCOBYs), each with a different number of viable cells. The acetic acid bacteria count of the samples was found to be statistically significant both in terms of the fermentation duration and the treatments applied ($P<0.05$). The sample with the highest acetic acid bacteria count was ASK1 on the 0^{th} day of fermentation (7.47±0.03 log cfu^{-1}), while the sample with the lowest count was ASK2 on the 15^{th} day of fermentation (2.55±0.11 log cfu^{-1}). Additionally, samples with rosemary essential oil contained fewer acetic acid bacteria compared to the other samples. This result could be attributed to the antimicrobial effect of the rosemary essential oil. These findings confirm that the unusual portions of *Anthriscus sylvestris* (L.) are suitable substrates for the production of these compounds. The mean yeast count was 4.56±0.27, 4.98±0.14, 5.10±0.40, 5.22±0.14, and 4.70±0.26 log cfu/mL for samples ASK1, ASK2, ASK3, ASK4, and ASK5. These findings confirm that the leaves and stems of *Anthriscus sylvestris* (L.) are acceptable substrates, as they possess values comparable to those of typical kombuchas with a shorter fermentation time. The highest yeast count on the 5^{th} day of fermentation was observed in the ASK4 sample (5.18±0.16 log cfu^{-1}), while the lowest was in the ASK1 sample (4.46±0.42 log cfu^{-1}). Similarly, Sreeramulu et al. (2000) discovered 4.48 log cfu mL^{-1} cells of yeast and 5.3 log cfu mL^{-1} cells of bacteria in fermented liquids following six days of fermentation. Teoh et al. (2004) discovered that the counts of yeast on the sixth day of the process were between 5 and 7 log cfu/mL. Because kombucha has no established microbiological or chemical makeup, some variations in chemical parameters, process length, and cell counts are to be expected (Teoh et al. 2004). The yeast results obtained by the researchers on the 6^{th} day of fermentation align with the findings of this study. According to De Filippis et al. (2018), the most common microorganisms in kombucha culture are acetic acid bacteria and yeasts. These microorganisms' activity is complementary since yeasts hydrolyse

sucrose to produce fructose and glucose for ethanol synthesis. Acetic bacteria finally convert ethanol to acetic acid and other organic acids (Zubaidah et al. 2019).

3.3. Total phenolic content (TPC)

Secondary metabolites in plant-based products include phenolic chemicals, which have significant antioxidant properties and a positive impact on human health. These molecules affect the products' aroma, taste, and color features (Chakravorty et al. 2016; Basli et al. 2017; Delgado et al. 2019; Alara et al. 2021). As a result, alternative substrates derived from other plant species may contain phenolic chemicals (Leonarski et al. 2022). During the fermentation period, the overall phenolic content within ASK1 consistently exhibited lower levels than the other samples ($P<0.05$). Towards the conclusion of the fermentation duration, significant increments in total phenolic content were observed in ASK2, ASK3, ASK4, and ASK5 ($P<0.05$) (Figure 2A). The TPC of guava pulp and peel kombucha samples differed significantly from the control sample ($P<0.05$). The investigation found that the total phenolic content of the samples varied from 29.15±0.13 µmol GAE/100ml to 48.60±0.70 µmol GAE/100mL depending on the raw material utilized in manufacturing ($P<0.05$). ASK5 samples had a higher total phenolic content, while control samples had lower values ($P<0.05$). Furthermore, the total phenolic values of the samples, especially in the sample with rosemary essential oil and guava peel (ASK5), were found to be at the highest level. This result is likely influenced by the higher presence of phenolic compounds in the fruit peel.

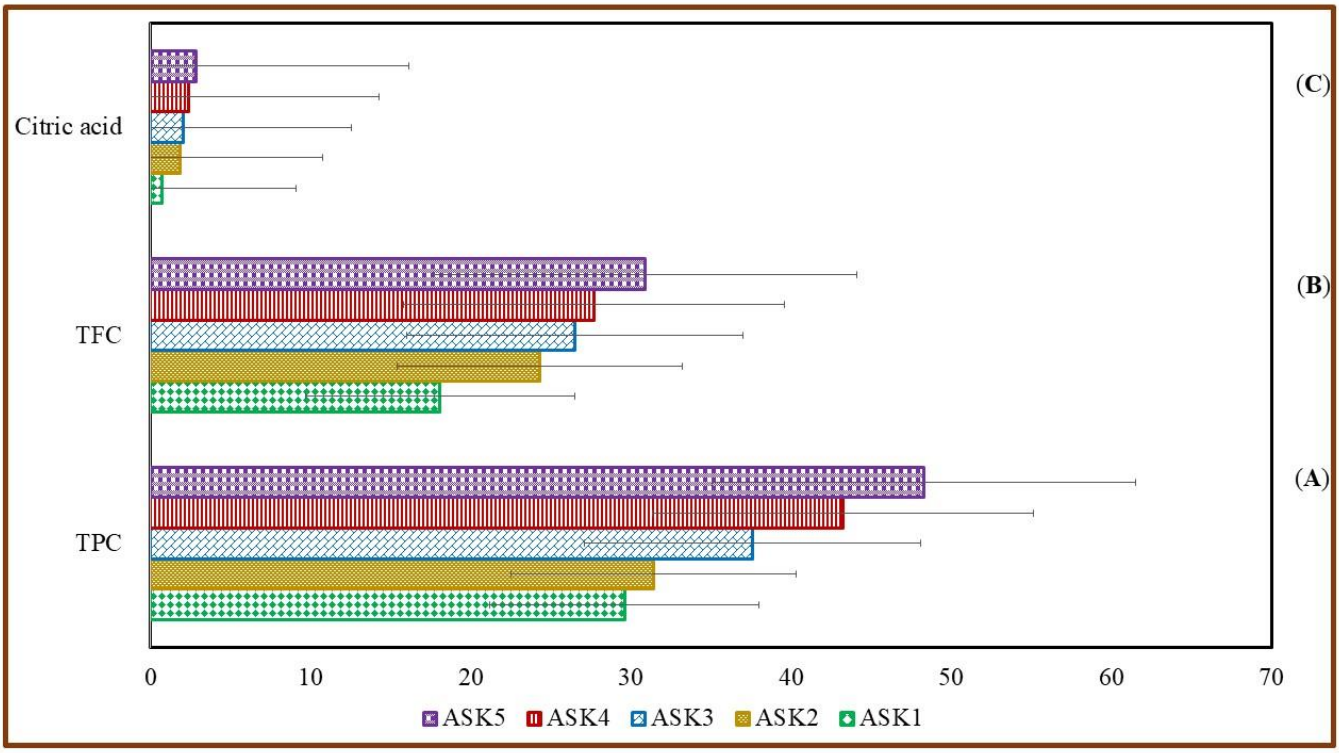

Figure 2- (A) Total phenolic content (TPC; µmol GAE/100mL); (B) Total flavonoid content (TFC; mg CE/100mL), and (C) citric acid cg/mL) values of the control kombucha (ASK1), kombucha infused with guava pulp (ASK2), kombucha infused with guava peel (ASK3), kombucha infused with guava pulp and 1% REO (ASK4), and kombucha infused with guava peel and 1% REO (ASK5)

3.4. Total flavonoid content (TFC)

The TFC ranged from 16.87±1.73 to 30.12±0.04 mg CE/100 mL (Figure 2B). The Kombucha samples containing guava peel and rosemary essential oil had the highest total flavonoid concentration, while the control samples had the lowest ($P<0.05$). The flavonoid content of ASK1, ASK2, ASK3, ASK4, and ASK5 was determined to be 16.87±1.73, 23.88±0.59, 26.06±0.60, 27.13±0.92, and 30.12±0.04 mg CE/100 mL, respectively. Several researchers have evaluated the total flavonoid content of kombucha made from diverse raw materials in the literature. These investigations found that flavonoid content can vary based on the raw material used in manufacture. Gamboa-Gomez et al. (2016) found that kombucha made with *Litsea glaucescens* had a lower total flavonoid content of 110.6 mg CE/L to 66.2 µg/mL, while kombucha made with *Eucalyptus camaldulensis* had a higher total flavonoid content of 29.3 µg/mL to 53.4 µg/mL after 7 days of fermentation at 25 °C. The total flavonoid contents of the samples ASK1, ASK2, ASK3, ASK4, and ASK5 on the 10th day of fermentation were determined to be 9.10±0.14, 13.54±1.53, 15.92±0.78, 20.38±1.29, and 21.86±1.31 (mg CE/100 mL) respectively. The inclusion of *Anthriscus sylvestris* (L.) plant, guava peel, and rosemary essential oil components in the formulation of sample ASK5 may have contributed to the high flavonoid content of this sample. The flavonoid content found in the kombucha sample with *Eucalyptus camaldulensis*, as

reported by Gamboa-Gomez et al. (2016) after 7 days of fermentation, was found to be slightly higher than the values obtained in this study on the 10th day of fermentation. This disparity in values is likely attributed to the differences in the materials used in the production of kombucha.

3.5. Citric organic acid

Figure 2C presents the fluctuations in citric acid concentrations in kombucha over time. Fermentation led to a considerable rise in citric acid concentration ($P<0.05$). After 15 days of storage, the acetic acid values of ASK1, ASK2, ASK3, ASK4, and ASK5 kombucha samples were 0.73±0.02, 1.82±0.04, 2.09±0.04, 2.39±0.02, and 2.83±0.09 g/mL, respectively. Fermentation resulted in a considerable rise in vitamin C levels ($P<0.05$). Generally, kombucha drinks contain the most vitamin C (15.19 mg/L for black tea and 7.88 mg/L for green tea kombucha) (Malbaša et al. 2011; Jayabalan et al. 2014; Geraris Kartelias et al. 2023). According to Hraš et al. (2000), citric acid may enhance the antioxidant properties of rosemary extract. Guava fruit also has 68 calories per 100 grams, is high in fiber, and has four times more vitamin C than oranges. The majority of the vitamin C is located in the fruits' skin, which is typically not peeled before ingestion (Mercadante et al. 1999). Citric acid can be produced by yeast and degraded by cells (Ye et al. 2014). The ASK5 sample made with guava peel, rosemary essential oil, and *Anthriscus sylvestris* (L.) had a greater citric acid value than the control sample. These findings suggest that the impact of fermentation on citric acid levels may vary depending on the specific plant species.

3.6. Antioxidant characteristics

The antioxidant capacity of various kombucha samples is shown in Figure 3A-E. Antioxidant activity increased significantly ($P<0.05$) with the addition of guava fruit, rosemary essential oil, and *Anthriscus sylvestris* (L.) compared to the control sample. The highest DPPH, ABTS^{*+}, FRAP, CUPRAC, and ORAC values were 78.90±0.08%, 97.25±1.57%, 723.15±2.61 µmol Trolox/mL, 460.45±0.58 µmol Trolox/mL, and 737.50±3.60 µmol Trolox/mL, respectively. The highest DPPH was determined in ASK5 (78.90±0.08%) and in ASK4 (77.15±0.24%). Using guava fruit and rosemary essential oil along with *Anthriscus sylvestris* (L.) further increased the DPPH value of ASK5 and ASK4 kombucha. The ASK1 sample (66.89±1.40%) had the lowest DPPH value (Figure 3A). The DPPH radical scavenging capacity of kombucha varies based on processing methods and polyphenol content, with yellow and green tea containing more physiologically active components (Jayabalan et al. 2008; Malbaša et al. 2011). Guava and rosemary essential oils have been shown to boost the bioactive potential of kombucha drinks. According to the antioxidant capacity results determined by ABTS$^{\bullet+}$, the highest values were detected in the ASK5, ASK4, ASK3, ASK2, and ASK1 samples as 97.25±1.57, 95.96±0.70, 93.05±0.72, 92.80±0.91, and 70.83±0.26%, respectively. All demonstrated a favorable link between higher guava peel and REO. Furthermore, as previously demonstrated, the observed increase in antioxidant capacity was favourably related to increased amounts of polyphenols and flavonoids (Abduh et al. 2023). Each kombucha formulation has significantly different FRAP values compared to the control ($P<0.05$). The ASK5 sample had the greatest FRAP value (723.15±2.61 µmol Trolox/mL). The ASK1 kombucha exhibited the lowest FRAP value (367±3.81 µmol Trolox/mL) (Figure 3C). The ASK1 sample was fermented with just sugar, and SCOBY was found to have the lowest bioactive potential ($P<0.05$). Significant variations were observed across kombucha tea samples in terms of ASK1 and ASK5, as well as the bioaccessibility (%) of CUPRAC values ($P<0.05$). CUPRAC values for ASK1, ASK2, ASK3, ASK4, and ASK5 samples were 381.40±1.33, 394.90±0.09, 414.80±2.43, 430.2430.08±1.02, and 460.45±0.58 µmol Trolox/mL, respectively. It was thought that the criteria impacting these changes in the composition of kombucha tea types (growing conditions, maturity duration, agricultural procedure, and climatic impact), guava fruit, rosemary essential oil, and tea leaf processing technique. Figure 3E shows the ORAC antioxidant activity. ORAC capacity was determined to be between 486.50±1.69 µmol Trolox/mL and 737.50±3.60 µmol Trolox/mL. The ORAC values of the kombucha beverages were statistically significant ($P<0.05$). The highest ORAC values were found in the ASK5 sample. Abuduaibifu & Tamer (2019) reported that DPPH, CUPRAC, and FRAP activities of black tea kombucha samples were 93.99±0.24, 56.08±0.71, and 81.77±1.48 µmol Trolox/g w.s.d.m, respectively. Previous research has demonstrated that increased quantities of polyphenols and flavonoids are connected with an increase in antioxidant capacity (Abduh et al. 2023). Furthermore, the presence of lactic acid bacteria in SCOBY may release many bioactive substances, such as exopolysaccharides, which contribute to the free radical scavenging ability of kombucha (Amjadi et al. 2023). Kombucha tea's health advantages are also due to its antioxidant content. Thus, kombucha samples, which are *Anthriscus sylvestris* (L.), guava, and rosemary oil, have the potential to be antioxidant-rich products, meaning that they may give health advantages by neutralizing free radicals.

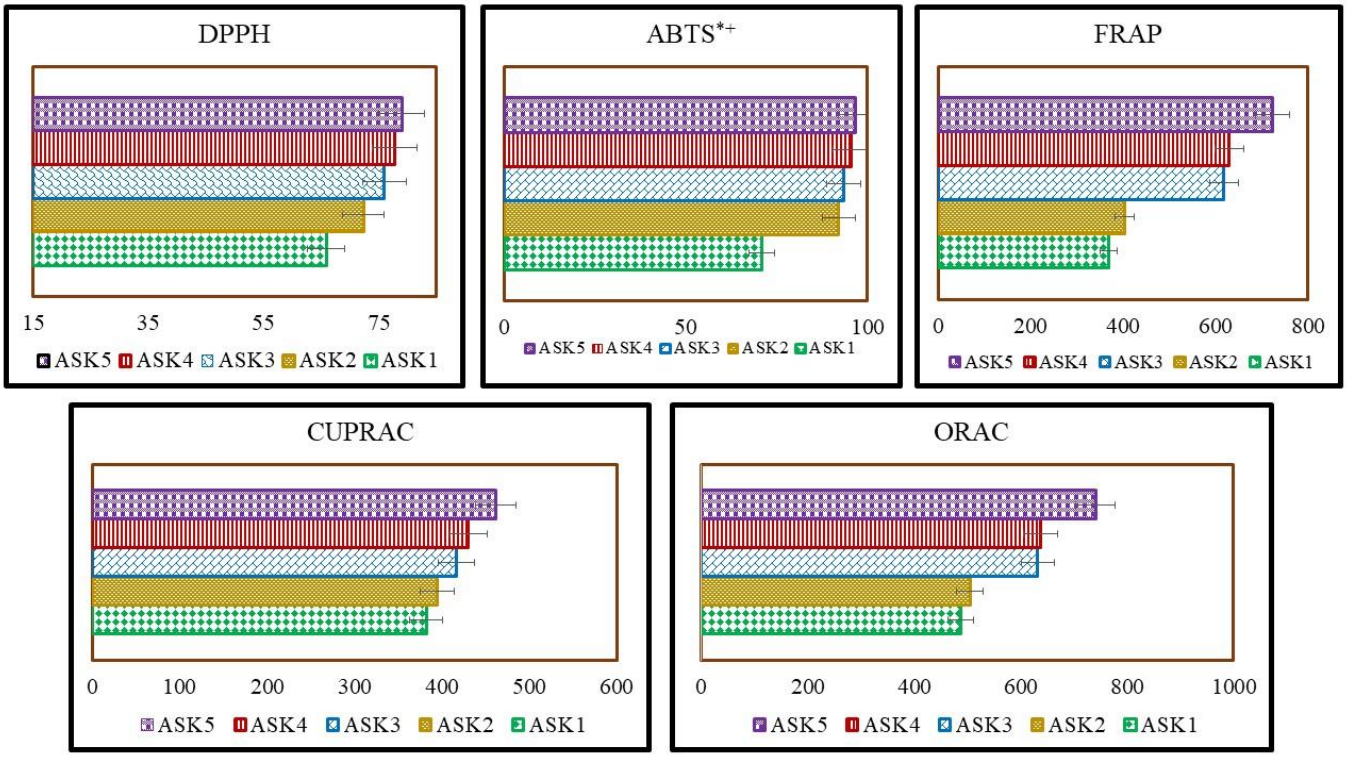

Figure 3- (A) DPPH (%); (B) ABTS*$^{+}$ (%); (C) FRAP (µmol Trolox/mL); (D) CUPRAC (µmol Trolox/mL); (E) ORAC (µmol Trolox/mL) values of the control kombucha (ASK1), kombucha infused with guava pulp (ASK2), kombucha infused with guava peel (ASK3), kombucha infused with guava pulp and % REO (ASK4), and kombucha infused with guava peel and 1% REO (ASK)

3.7. Color parameters

Table 3 displays statistically significant variations (P<0.05) in the color changes of all samples. All kombucha beverages showed statistically significant *L**, *a**, and *b** values during the fermentation process (P<0.05). The control kombucha with only *Anthriscus sylvestris* (L.) and it had the greatest *L** value after storage according to the raw material used in the production. ASK5 kombucha had the greatest *a** value after 10 days of storage. Adding guava peel and REO to kombucha teas resulted in significant differences across groups (P<0.05). On the 15th day of fermentation, the ASK2 group had the lowest *a** value (-0.53), while the ASK5 group had the highest (-4.07). Chemical alterations to phenolic molecules, particularly anthocyanins and carotenoids, affect their color diversity values. However, the inclusion of plant infusions reduced these values. According to the research of Chakravorty et al. (2016), during fermentation, thearubigin fractions might be completely or partially transformed into theaflavin, which may have been effective in converting the kombucha beverage's color to a reddish-light brown. Abuduaibifu & Tamer (2019) analyzed the *L**, *a**, and *b** values of black tea with kombucha culture after 48 h of fermentation at 28±2 °C. The results were 4.72±0.23, 5.91±0.19, and 4.52±0.27, respectively.

3.8. Antimicrobial activities

Kombucha's antibacterial activities have been extensively explored and recorded, both in control kombucha and in those containing *Anthriscus sylvestris* (L.) extracts and guava fruit (Table 4). Kombucha's antibacterial characteristics come from the presence of organic acids, plant-derived phenolic chemicals, enzymes, proteins, and bacteriocins (Battikh et al. 2013). This study observed antibacterial effects in kombucha against gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATTC 27853) and gram-positive bacteria (*Enterococcus faecalis* ATCC 29212 and *Staphylococcus aureus* ATCC 25923). Furthermore, it was shown that samples containing rosemary essential oil (ASK4 and ASK5) exhibited higher antibacterial activity. However, kombucha made with guava peel had a stronger antibacterial impact than kombucha made with guava pulp. The activity was increased with the guava peel against both *Enterococcus faecalis* ATCC 29212 (zone of inhibition 13.05 mm) and *Pseudomonas aeruginosa* ATTC 27853 (zone of inhibition 13.67 mm) strains. This is probably due to the existence of acetic acid, which has been recognized as the primary antibacterial substance in kombucha. Numerous bioactive compounds or metabolites created through the fermentation process, such as flavonoids, bacteriocins, polyphenols, and enzymes, could potentially be the cause of kombucha's antibacterial qualities. Understanding the specific mechanisms responsible for kombucha's antibacterial properties enables the strategic use of this natural antimicrobial ability in the creation of functional foods, allowing the development of products that provide consumers with both culinary and health benefits. Furthermore, kanamycin demonstrated effectiveness against all tested bacteria. In light of these findings, it is recommended that kanamycin be considered as the antibiotic of choice for selection. In their 21-day fermentation research, Pure & Pure (2016) utilized

Escherichia coli and *Staphylococcus aureus* but did not detect antibacterial properties in the traditional kombucha and other fermented samples they examined. These results differ from the current study findings, indicating that the choice of raw materials may have influenced this outcome.

3.9. Inhibition activities of α-glucosidase and α-amylase

In dietary starch, the hydrolase inhibitors block α-amylase and α-glucosidase, decreasing glucose assimilation. These two enzymes on the small intestine's boundary are vital for carbohydrate hydrolysis. Preventing the action of these digestive enzymes decreases glucose absorption into the circulatory system by dropping the level of glucose in the body (Geraris Kartelias et al. 2023). Figure 4A-B shows the inhibitory qualities of α-glucosidase and α-amylase. The ASK5 sample inhibited α-glucosidase and α-amylase by 115.08% and 86.7%, respectively, compared to 75.3% and 67.5% in the control group. These results collectively affirm that the total acidity and pH levels of kombucha beverages are subject to variation based on the specific raw materials, SCOBY utilized in production, and the fermentation conditions employed.

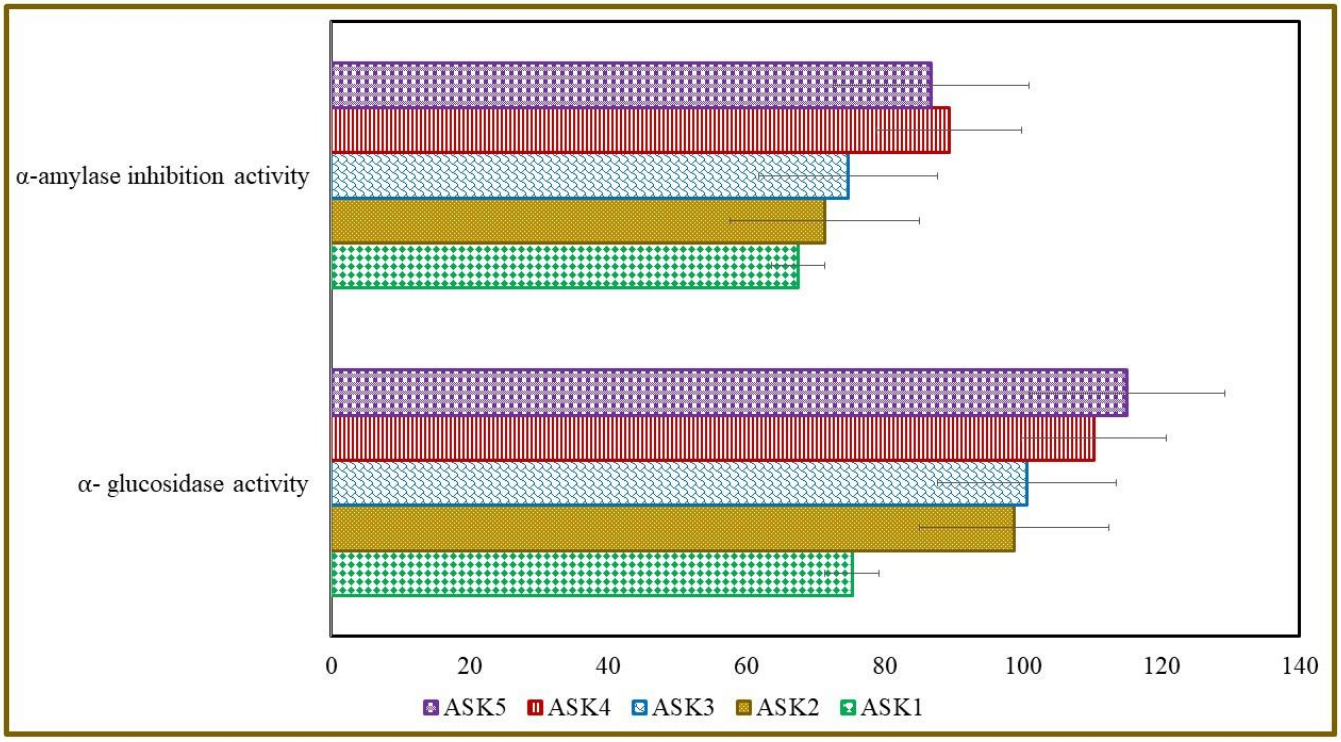

Figure 4- (A) α-glucosidase activity (%); (B) α–amylase inhibition activity (%) of the control kombucha (ASK1), kombucha inused with guava pulp (ASK2), kombucha infused with guava peel (ASK3), kombucha infused with guava pulp and 1% REO (ASK4), and kombucha infused with guava peel and 1% REO (ASK5)

The α-glucosidase enzyme inhibition assay measures the sample's inhibitory capacity against the enzyme and is expressed as a percentage of inhibition. The α-glucosidase is the most important enzyme in the absorption of carbohydrates, accelerating the decomposition of disaccharides and oligosaccharides involving maltose, maltotriose, and α-dextrins in the small intestinal tract, causing glucose (Proença et al. 2017). Both *Anthriscus sylvestris* (L.) and guava have inhibitory capabilities against the α-glucosidase enzyme. However, guava peel and rosemary essential oil outperformed kombucha in this regard. Thus, it improves glucose metabolism, glycaemic management, and insulin sensitivity (Koh et al. 2010). Wang et al. (2018) observed that fermentation can considerably boost the inhibitory efficiency of guava leaf tea against α-glucosidase. Inhibiting the carbohydrate metabolism enzymes α-glucosidase and α-amylase has been shown to effectively treat diabetes and hypertension (Ortiz-Andrade et al. 2007; Ademiluyi & Oboh 2013). Phenolic chemicals can regulate carbohydrate and lipid metabolism by inhibiting α-glucosidase and α-amylase due to their ability to chelate, modify structure, and limit enzyme performance (Lin et al. 2016; Nagappan et al. 2017).

3.10. Sensory properties

ASK1, ASK2, ASK3, ASK4, and ASK5 were sensory evaluated during 15 days of fermentation. Kombucha samples were evaluated in terms of appearance, color, aroma, flavor, sourness and general acceptability. There was no significant difference in color, appearance, aroma, sourness, or overall acceptance values among the kombucha samples. Guava pulp-fermented kombucha scored considerably higher on all sensory measures compared to guava peel kombucha ($P<0.05$). ASK2 and ASK3 had better overall sensory evaluation scores than ASK4 and ASK5, as seen in Figure 5. The findings suggest that guava pulp and

peel-derived kombucha, notably ASK2 and ASK3, may appeal to consumers because of their superior sensory properties. The kombucha drink made with guava pulp and rosemary (ASK5) did not receive favourable feedback after the 10-day storage period, and the sample prepared with guava pulp and rosemary (ASK4) was also not well-received after the 15th day of fermentation. Consequently, sensory analysis was terminated at the end of the 15-day fermentation period.

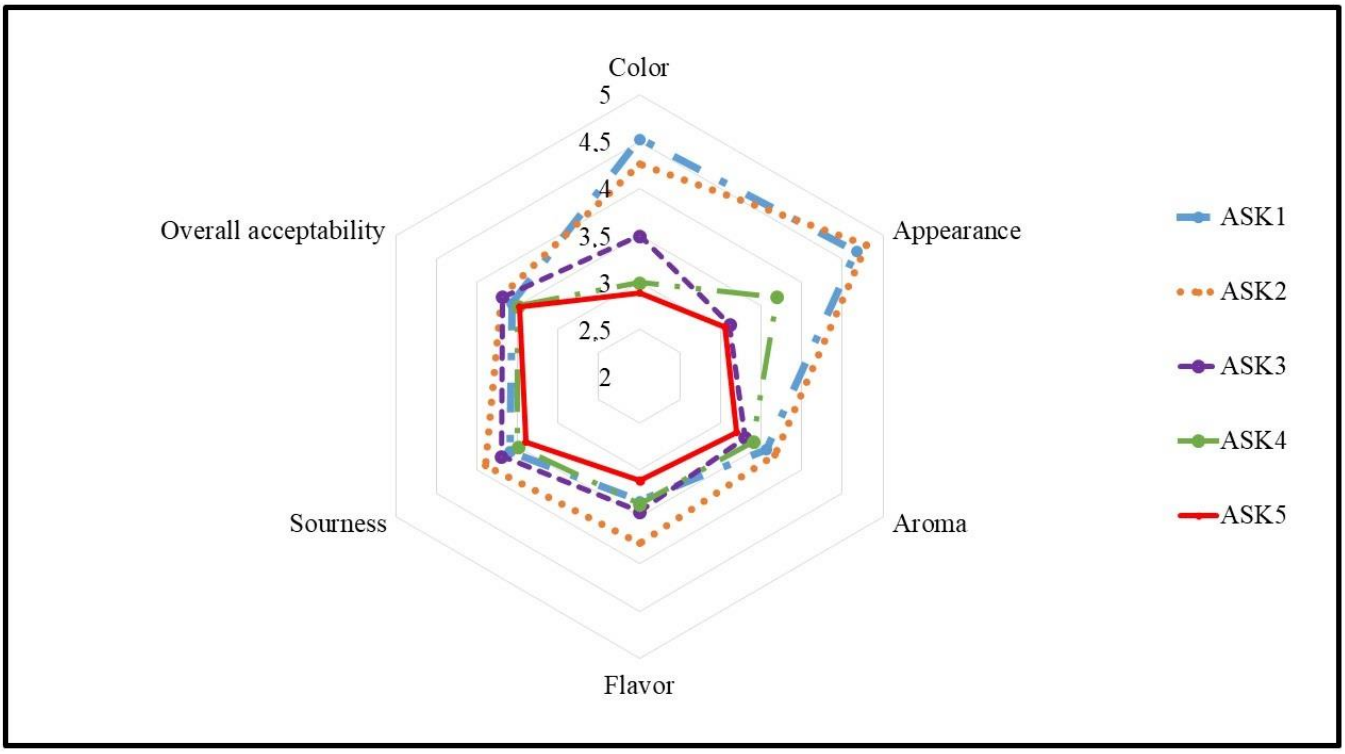

Figure 5- The sensory property assessment of the control kombucha (ASK1), kombucha infused with guava pulp (ASK2), kombucha infused with guava peel (ASK3), kombucha infused with guava pulp and 1% REO (ASK4), and kombucha infused with guava peel and 1%REO (ASK5)

4. Conclusions

Anthriscus sylvestris (L.)-based kombucha has more bioactive components and functional characteristics than ordinary kombucha. The inclusion of antioxidant and antimicrobial components in *Anthriscus sylvestris* (L.) kombucha resulted in enhanced total phenolic content and better antioxidant and antibacterial activity. Kombucha tea outperformed other samples in inhibiting the α-glucosidase enzyme, indicating medicinal promise in diabetes. Based on the higher presence of phenolic compounds in the sample (ASK5) produced with peel, it has been determined that they exhibit stronger antioxidant activity, as expected. The phenolic and flavonoid levels increased significantly, as did antioxidant capacity and a-amylase inhibitory action. Furthermore, the sensory evaluation revealed that guava pulp kombucha has a higher overall acceptance than *Anthriscus sylvestris* (L.) kombucha, emphasizing its palatability. Taken together, these findings highlight the potential of *Anthriscus sylvestris* (L.) leaves combined with guava fruit and REO as a unique and healthy ingredient for making kombucha tea with a variety of health benefits.

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Declarations

Conflict of interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Ethical approval Ethics approval was not required for this research.

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